

PRIVY COUNCIL

**MEDICAL RESEARCH COUNCIL**

SPECIAL REPORT SERIES No. 255

# **CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS**

*by*

**M. VAN DEN ENDE, C. H. STUART-HARRIS, F. FULTON  
and J. S. F. NIVEN, with C. H. ANDREWES, A. M. BEGG,  
W. J. ELFORD, M. H. GLEESON WHITE, W. L. HAWLEY,  
K. C. MILLS, F. HAMILTON and C. C. THOMAS**

**LONDON : HIS MAJESTY'S STATIONERY OFFICE**

**1946**

**Price 12s. 6d. net**

**Universal Decimal Classification**

**616.922-085.7**

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## PREFACE

This report records the results of clinical and laboratory studies of typhus fever, carried out primarily in relation to investigations on the chemotherapy of the disease. The striking advances made in recent years in regard to the drug treatment of bacterial diseases had suggested the desirability of a programme of work on corresponding lines on the treatment of rickettsial infections (particularly typhus), against which the known chemotherapeutic drugs were of no value. The development of methods for the maintenance of rickettsia strains in the laboratory made possible the study of treatment in experimental animals, and investigations on the subject were undertaken by members of the Council's staff at the National Institute for Medical Research, London. An indication of the large volume of work on this subject done at the National Institute during the recent war is given in Appendix B, which lists some 238 drugs which have been tested for activity against experimental typhus infection in mice. Two of the compounds were selected for special investigation, as they were found to have a definite action in preventing the development of the disease in mice, although not when their administration was begun later than 42 hours after infection.

These were the substances designated V147 and V186, which are *para*-sulphonamidobenzamidine hydrochloride and *para*-sulphonamidobenzamidoxime hydrochloride, respectively. The former was supplied by Boots Pure Drug Company, Limited, while the latter was synthesized at the National Institute. Although they are sulphonamides in the strict chemical sense of the term, their activity is not inhibited by *para*-aminobenzoic acid, and therefore their mode of action, like their range of activity, is distinct from that of the group of drugs commonly known in clinical medicine as "sulphonamides". The activity of these new drugs, in a field where the latter are ineffective, is of considerable importance, and a study of their mode of action may lead to a better understanding of the relationship between chemical constitution and biological activity in this field. In view of the activity shown, and as the two drugs were found to have no excessive toxicity for animals or for human volunteers, it was considered justifiable to try them in cases of typhus in man.

With the co-operation of the Director-General, Army Medical Services, a British Army Typhus Research Team, including members of the Council's staff, was accordingly constituted for the purpose of carrying out these clinical trials. The work was begun in North Africa and was subsequently extended to Italy during the epidemic in Naples in 1943-44. Liaison established with members of the Medical Corps, Army of the United States, with the American Typhus Commission, and with the civil authorities in Algiers and Naples, was an essential part of the scheme, which would have been impracticable without such help. Unfortunately, under the conditions in which the trials with V147 and V186 were carried out, treatment with the drugs did not prove to be effective. Cases were rarely available for treatment early in the disease, at the stage at which, in accordance with the results obtained with the drugs in experimental animals, therapeutic value might have been expected to be shown. When used at the later stage at which most patients came under treatment, there were indications that the drugs, in some instances, may have accentuated the known liability to kidney damage in severe cases of typhus. Despite these disappointing results, it was thought advisable that a full account of the work with the drugs should be made available as a base-line for reference in future studies on the chemotherapy of typhus and other rickettsial infections.



In the course of the therapeutic trials, detailed clinical, biochemical, serological and pathological investigations were made of both the treated and control cases. The information thus obtained in the course of the studies constitutes a picture of louse-borne typhus as seen in North Africa and Naples. Typhus is a disease with which most doctors working in Great Britain and Western Europe are, fortunately, unfamiliar; but, as is well known, it is liable to occur in epidemic form under conditions of war and of famine. The second object of this publication was, therefore, to place on record an account of these observations, for the guidance of those who might be confronted with outbreaks of the disease if the abnormal conditions of the post-war period were such as to favour its spread. The account, for this purpose, is accordingly presented at some length; the records of typical cases of typhus given in Appendix A to this monograph should serve as a useful supplement to the information given in standard text-books on the subject.

In the third place, this report describes a number of important studies on the immunology of typhus fever which were made partly at the National Institute for Medical Research, and partly at the Royal Army Medical College Emergency Vaccine Laboratory, Everleigh, Wilts., close collaboration being maintained throughout between the workers at the two institutions. For security reasons, some sections of this work could not be published during the war years, and it has been thought appropriate to issue an account of them here.

The Medical Research Council wish to express their thanks to all those workers of British, American or other nationality who co-operated so generously with members of the Council's own staff in the studies here recorded. Special thanks are due also to Major-General Sir John Taylor, C.I.E., D.S.O., M.D., I.M.S. (retd.), who gave most valuable help in the task of preparing this substantial monograph for press.

MEDICAL RESEARCH COUNCIL,  
38, Old Queen Street,  
Westminster, London, S.W.1.

*24th April, 1946.*

## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### CONTENTS

	PAGE
GENERAL INTRODUCTION, by C. H. Andrewes, M.D., F.R.C.P., F.R.S. .. ..	5
GENERAL ACKNOWLEDGEMENTS .. .. .	7
I.—THE TOXICITY OF TWO SULPHONAMIDES (VI47 and VI86) HAVING ACTIVITY AGAINST EXPERIMENTAL TYPHUS IN MICE, by C. H. Andrewes, M.D., F.R.C.P., F.R.S., A. M. Begg, M.B., and M. van den Ende, M.B., Ph.D. ..	8
II.—CHEMOTHERAPEUTIC TRIALS IN TYPHUS FEVER IN MAN, AND A REPORT OF THE NAPLES TYPHUS EPIDEMIC. A REPORT OF THE BRITISH ARMY TYPHUS RESEARCH TEAM, by Major C. H. Stuart-Harris, M.D., M.R.C.P., R.A.M.C., Major M. van den Ende, M.B., Ph.D., R.A.M.C., Major M. H. Gleeson White, M.B., R.A.M.C., Capt. W. L. Hawley, M.D., M.C., A.U.S., Major J. S. F. Niven, M.D., R.A.M.C., with A. M. Begg, M.B., F. Fulton, D.M., F. Hamilton and Capt. C. C. Thomas, M.B., R.A.M.C. .. ..	12
III.—A QUANTITATIVE TEST FOR NEUTRALIZING ANTIBODIES AGAINST TYPHUS RICKETTSIAE, by M. van den Ende, M.B., Ph.D., and K. C. Mills .. ..	130
IV.—COMPARISON OF TYPHUS VACCINES IN THE LABORATORY, by F. Fulton, D.M., M. van den Ende, M.B., Ph.D., W. J. Elford, Ph.D., and K. C. Mills .. ..	146
V.—THE ANTIGENIC STRUCTURE OF TYPHUS RICKETTSIAE, by F. Fulton, D.M., and A. M. Begg, M.B. .. .. .	163
Appendix A—ILLUSTRATIVE CASE REPORTS ... .. .	192
Appendix B—LIST OF DRUGS TESTED FOR CHEMOTHERAPEUTIC ACTIVITY AGAINST EXPERIMENTAL TYPHUS INFECTION IN MICE .. .. .	242

NOTE ON THE PLATES.

*Plates I, IV, VIII and IX are reproduced in monochrome from colour photographs taken by Mr. P. G. Hennell, of the staff of the Metal Box Company Limited. They have been reproduced in monochrome to save time, but this process has necessarily involved loss of some of the detail in the originals. Copies of the original colour photographs—together with a picture of a typical typhus rash, which it was useless to reproduce in monochrome—will be deposited in the Library of the National Institute for Medical Research, London, with the summarised case histories (p. 192).*

# CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

## GENERAL INTRODUCTION.

BY

C. H. ANDREWES, M.D., F.R.C.P., F.R.S.

This report covers a large part of the work on typhus carried out since 1941 at the National Institute for Medical Research, Hampstead, London, and the Royal Army Medical College Emergency Vaccine Laboratory, Everleigh, Wilts. By agreement with workers on typhus in the U.S.A. and Canada, publication of many aspects of work in this field has been hitherto withheld for security reasons. Some work has, however, already been published from these two laboratories.

The account of an expedition to North Africa and Italy forms the largest part of this report (Section II); this expedition owed its origin to the necessity for carrying out clinical trials in man of the drugs V147 and V186, the highly promising activity of which in laboratory trials is recorded in a paper by Andrewes, King, van den Ende and Walker (1944). Section I describes studies of the toxicity of those two drugs, studies which were necessary as a preliminary to the clinical trial in typhus infections of human beings. A description of the clinical use of the drugs, however, forms only a part of the report from the Mediterranean area. A thorough clinical and clinical-pathological study was necessary of the disease in that area as it occurred in patients receiving only the usual supportive treatment; only thus could the possible value of chemotherapy be assessed. This meant that an admirable opportunity was afforded for clinical and pathological studies which were likely to be of value in themselves and not merely as a background for the chemotherapeutic trials. This report, however, does not pretend to be a comprehensive treatise on the clinical and pathological aspects of typhus. The material studied was in two areas only, and was not extensive enough to serve as a basis for an exhaustive review; moreover, parts of the work were not pursued as far as was desirable, since the members of the team were recalled for other work as soon as their primary object, that of evaluating the two drugs, was completed. Nevertheless, the accounts should provide a useful description of the clinical and pathological aspects, particularly of the Naples epidemic—an outbreak which is likely to be of historic interest.

Appendix A at the end of this monograph contains summarized case reports and temperature charts of each of the representative groups into which the patients have been divided, including treated and untreated cases.

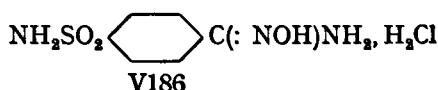
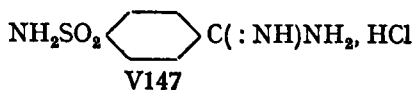
Sections III, IV and V contain accounts of laboratory investigations of typhus carried out at Hampstead. These would normally have been submitted for publication in pathological journals. Since such publication had been delayed for reasons already stated, it seemed appropriate to publish them within the same covers as the account of the chemotherapeutic trials. These three sections are not to be regarded, any more than is the clinical section, as a full account of current work in the subject. Indeed, an endeavour has been made to concentrate work on typhus in Britain into fields which were, we believed, not already under intensive study in the U.S.A. and Canada. Hence the particular attention

## 6 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

devoted to chemotherapy, and to the cultivation of rickettsiae in the mouse lung, rather than in the egg. It is now generally accepted that the intranasal inoculation of mice with material containing typhus rickettsiae offers a particularly serious hazard of accidental infection to laboratory workers. Indeed, at the inception of these studies, all five of the workers engaged in using this method at Hampstead contracted an infection (fortunately with murine, not epidemic, typhus) within a few weeks of beginning to use it (van den Ende *et. al.*, 1943). A special inoculation box (van den Ende, 1943) was then designed at the National Institute for Medical Research, for safer handling of mice and other animals used for intranasal inoculation; only one other laboratory infection occurred there in the ensuing 2½ years, though numerous fresh workers assisted in the studies. At the Military Laboratory at Everleigh, nine laboratory infections occurred prior to the introduction of an inoculation box there also; none occurred afterwards. The early murine infections had the fortunate consequence that a number of those engaged in the work had, thereafter, a solid immunity to typhus.

The laboratory work was throughout divided between the workers at the National Institute for Medical Research at Hampstead and those at the Royal Army Medical College Emergency Vaccine Laboratory at Everleigh; very close consultation was maintained between the two groups. Some strains of typhus sent from the Mediterranean and elsewhere were passaged in guinea-pigs and adapted to mice at Everleigh, others at Hampstead. The Army workers conducted immunological studies and also prepared rickettsial suspensions for sending out to the Mediterranean.

It remains to summarize briefly the laboratory findings which formed the basis of the chemotherapeutic trials in man. These have already been published (Andrewes, King, van den Ende and Walker, 1944). We found that a convenient method of test was to inoculate mice intranasally with dilute suspensions of typhus rickettsiae; such mice when killed seven days later showed discrete greyish lesions on their lung-surfaces and these could readily be counted. Details of the method are given in Section III of this report, and have also been described by Andrewes, King, van den Ende and Walker (*loc. cit.*). In earlier experiments we determined for each drug the maximal dose tolerated intraperitoneally and gave that dose in 0.5 c.c. saline daily for four days; the first dose was given two hours before infection with typhus. Results were all negative until we reached V147 (*p*-sulphonamidobenzamidine hydrochloride), which strikingly reduced the numbers of lesions to be counted on the mouse lungs. It became apparent, however, that the drug was very rapidly excreted, and that better results were achieved by administering it twice daily. When this was done, it was possible to suppress completely the development of lesions when we gave a dose of rickettsiae producing about 150 foci on the lungs of untreated mice. In later tests, including all those of substances related to V147, we injected the drug six times in all, once before infecting the mice, twice daily on each of the two following days, and once on the morning of the third following day. V186, the amidoxime corresponding to V147, was found to be equal to it in activity or perhaps a little better;



but only minor modifications in the molecule were possible without destroying the activity altogether. The activities of related compounds are shown in the tables in Appendix B. An account of the chemical side of the work, and a discussion of its possible bearing on the chemotherapy of rickettsial and virus infections, have been published separately (Andrewes, King and Walker, 1946).

V186 showed definite activity in a dose of 2 mg. twice daily, or one-twelfth of the maximum tolerated dose (24 mg.). Subcutaneous injections gave much the same results as intraperitoneal. The drug could readily be given mixed with the food, and about the same dosage was effective as with parenteral injections, provided the drug was given in the food in two divided instalments. A slight further benefit was conferred by giving the drug in four six-hourly doses. Most activity was observed with V186 or V147 given before or soon after infection, but a definite effect was still produced when treatment was delayed till 42 hours afterwards. The drugs did not appear to have any anti-rickettsial activity *in vitro*; they seemed to act by interfering with the multiplication of rickettsiae in the lungs; their action was not inhibited by *p*-aminobenzoic acid.

Nearly all tests were carried out against murine typhus (Wilmington strain); the activity of V147 and V186 was tested against epidemic typhus (Breinl and Tunis strains), and was found to be as great as against the murine infection. Murine typhus will kill mice when inoculated intraperitoneally; V147 protected against such deaths when they were due to infection, but not when massive doses produced an early "toxic" death.

Neither V147 nor V186 gave satisfactory results when tested for power to protect guinea-pigs from the fever which epidemic typhus causes when inoculated intraperitoneally.

*Papers already published:—*

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ANDREWES, C. H., KING, H., and WALKER, J. (1946) *Proc. roy. Soc., B*, **133**, 20.

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VAN DEN ENDE, M., STUART-HARRIS, C. H., HARRIES, E. H. R., STEIGMAN, A. J., and CRUICKSHANK, R. (1943.) *Lancet, Lond.*, **i**, 328.

### GENERAL ACKNOWLEDGMENTS

The authors desire to express their thanks to the many persons who have assisted in one way or another in various aspects of this work. Whenever possible, specific acknowledgments of indebtedness have been made at the appropriate position in the text. They wish, however, particularly to thank Dr. C. R. Harington, F.R.S. (Director of the National Institute for Medical Research), Major-General L. T. Poole, C.B., D.S.O., M.C. (Director of Pathology, War Office, and Chairman of the M.R.C. Typhus Committee), and Lieutenant-Colonel H. J. Bensted, O.B.E., M.C., R.A.M.C. who constantly gave their interest and support. The officers who commanded the Royal Army Medical College Emergency Vaccine Laboratory during the various phases of the work, and who themselves contributed to the research programme, were Lieutenant-Colonels H. T. Findlay and A. E. Francis, R.A.M.C. Major E. T. C. Spooner, R.A.M.C., and Captain G. K. C. Rettie, R.A.M.C., also collaborated in the work at the Army Laboratory. The British Army Typhus Research Team owed much to the good will and help of many administrative medical officers of the Staff of the Allied Force Headquarters in North Africa and of the Headquarters of the Allied Armies in Italy. Several Army pathologists, and others too numerous to mention, specifically assisted the Typhus Research Team by sending specimens for examination or inspection and by discussing the various aspects of the therapeutic problem under investigation.

## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### I.—THE TOXICITY OF TWO SULPHONAMIDES\* (V147 AND V186) HAVING ACTIVITY AGAINST EXPERIMENTAL TYPHUS IN MICE

BY

C. H. ANDREWES, M.D., F.R.C.P., F.R.S., A. M. BEGG, M.B., and  
M. VAN DEN ENDE, M.B., Ph.D.

(from the National Institute for Medical Research, London)

The two drugs most active in experimental typhus in mice, the hydrochlorides of *p*-sulphonamidobenzamidine (V147) and *p*-sulphonamidobenzamidoxime (V186), have been examined for their toxicity to laboratory animals and to human volunteers.

*Physical properties.*—Both substances are white crystalline solids. Their solubility, at room temperature, in saline +M/100 phosphate buffer at several pH levels was determined by our colleague, Dr. A. T. Fuller and is given in the following table :—

TABLE I

	V147.	V186.
pH 5.0 .. ..	5.0 per cent.	0.4 per cent.
pH 7.4 .. ..	4.0    "	0.4    "
pH 9.0 .. ..	0.2    "	0.5    "

Two per cent. solutions in water were found by our colleague, Dr. J. Walker, to have pH values of 4.99 (V147) and 2.67 (V186).

#### TOXICITY FOR MICE

Young mice weighing about 15 g. were used in these tests.

*Intraperitoneal injection.*—The drugs dissolved in 0.5 c.c. normal saline were inoculated intraperitoneally into groups mostly of 10 mice, in order to determine the 50 per cent. lethal dose (LD<sub>50</sub>). The results are shown in Table II.

TABLE II

	16 mg.	24 mg.	28 mg.	32 mg.	36 mg.	40 mg.	44 mg.	48 mg.
V147 ..	4/4	4/4	5/10	2/10	1/10	1/10	0/10	0/10
V186 ..	4/4	3/4	4/10	5/10	4/10	2/10	2/10	3/10

Numerators indicate numbers of survivors after 6 days.

Denominators indicate numbers of mice in each group.

For V147 the LD<sub>50</sub> is about 28 mg.; for V186 the slope of the dose-mortality curve is less steep, but the LD<sub>50</sub> is apparently very close to that for V147. The toxic symptoms were, however, different for the two drugs. Mice receiving toxic doses of V147 were frequently dead with convulsions in five minutes; most of those which died did so within an hour. Their respirations became gradually weaker, and they lay limply on the floor of the cage, their hind legs

\* The term sulphonamide is here used in its strictly chemical sense.

stretched out. *Rigor mortis* set in slowly, not being well-marked within 90 minutes. Mice poisoned with V186, on the other hand, looked hunched and rough soon after injection, but very few died within two hours on the doses shown in Table II. After two hours, many mice had spasticity of the limbs, but hardly any showed convulsions. *Rigor mortis* was well-developed within 20 minutes.

*Subcutaneous injection.*—The lethal dose was practically the same after subcutaneous as after intraperitoneal injection. On repeated subcutaneous inoculations, reactions were seen in the form of localized inflammation and necrosis. This is hardly surprising in the case of V186, because of the acidity of the solutions used.

*Feeding experiments.*—The drugs were well tolerated when administered in a mixture with food, and, as mentioned in the paper already published (Andrewes, King, van den Ende and Walker, 1944), were taken better when mixed with a wet mash. On a dose of 10 or 30 mg. daily (V147 and V186), mice failed to gain weight as did controls, but otherwise seemed well and showed no abnormalities when killed and examined after 10 days; on the 30 mg. daily dose a little weight was lost. When the daily dose was increased to 75 mg., mice looked definitely rough and ill. Two of six mice fed with this dose of V186 died after 11 days; they, as well as four others killed after 11 days' feeding, showed no gross post-mortem abnormalities apart from paleness of the kidneys. No histological abnormalities in the kidneys of these animals were, however, demonstrated.

#### TOXICITY FOR GUINEA-PIGS

*Subcutaneous injection.*—Tolerance for the drugs varied much from one guinea-pig to another. One animal weighing 400 g. seemed to thrive on a twice-daily dose of 200 mg. V147; this was kept up for eight days. Others died after a few days on a quarter of that dose, though most guinea-pigs would tolerate 100 mg. *per diem*. V147 and V186 had about the same toxicity. Guinea-pigs receiving excessive doses of either drug lost weight, did not eat well and looked rough. For a day or so before death they were excitable, twitched and showed spasticity of the limbs. Several died in convulsions with opisthotonos. The blood-urea in one animal, moribund after nine days' treatment, was 90 mg. per 100 c.c. No striking histological changes were found in the kidney, brain or other organs.

*Feeding experiments.*—The tolerated dose for each drug *per os* was, as with subcutaneous inoculation, about 100 mg./day for a 300–400 g. guinea-pig. Thus, in contrast to the mice, these animals could tolerate no bigger doses by mouth than parenterally. Toxic symptoms were the same as after injection. Typhus-infected guinea-pigs seemed to tolerate the drugs worse than did uninfected controls, but we did not carry out enough experiments to be certain on this point. In an attempt to discover where in the molecule of V147 ( $\text{NH}_2\text{SO}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{C}(\text{:NH})\text{NH}_2$ ) the toxicity lay, we carried out parallel feeding experiments with benzamidine ( $\text{C}_6\text{H}_5\text{C}(\text{:NH})\text{NH}_2$ , HCl) and benzene sulphonamide ( $\text{NH}_2\text{SO}_2 \cdot \text{C}_6\text{H}_5$ ). Guinea-pigs tolerated  $2 \times 300$  mg. of benzamidine daily without ill effects, but on the benzene sulphonamide they reacted much as they had to V147 in similar doses (100 mg./day).

#### TOXICITY FOR RABBITS

A rabbit, weighing 3.9 kg., received 800 mg. V147 intraperitoneally. The blood-urea, which had an initial value of 36 mg. per 100 c.c., gradually rose to a level of 58 mg. after five hours, and to 73 mg. per 100 c.c. after 24 hours. No significant changes occurred in the blood-sugar level. Dr. C. Rimington kindly made these determinations.



## 10 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Dr. F. C. McIntosh carried out for us another experiment on the acute effects of V147. A rabbit, weighing 2.1 kg., received, under "Dial" anaesthesia, 0.21 c.c. of a 10 per cent. solution of V147 intravenously (100 mg./kg.). A fall of blood-pressure occurred, but the normal level was regained within a minute. After 500 mg./kg., a bigger fall of blood-pressure occurred at each of two tests, and the normal pressure was not restored for about three minutes. A dose of 2.5 g. of V147 caused a stoppage of respiration, and a dramatic fall of blood-pressure, which was not relieved by atropine. Two minutes later, the heart stopped, but it resumed beating after intracardiac injection of 0.1 c.c. adrenaline. It continued beating for some time while artificial respiration was applied and the blood-pressure rose to 100 mm., but natural breathing had stopped permanently. Apparently, death was caused primarily by inhibition of respiration, some part being played also by peripheral vascular dilatation.

### TOXICITY FOR RHESUS MONKEYS

Rhesus 1 (female), weight 3.95 kg., received V186 subcutaneously in 10 per cent. solution, doses being given in the morning and afternoon, and progressively increased. No ill effects were seen while the doses were raised over a period of three days from 200 mg. to 1.2 g. per dose. On the following day, after doses of 1.6 and 2.4 g., the monkey crouched flat on the bottom of the cage, but seemed normal 10 minutes later. Next morning, however, the animal was found dead. No striking abnormality was found at autopsy.

Rhesus 2 (male), weight 3.2 kg., received V186 mixed with the diet and with the drinking water, and, according to its appetite, took between 0.5 and 1.25 g. daily over a period of 17 days. No ill effects were observed. The animal had been injected intraperitoneally with epidemic typhus at the beginning of this course of treatment, but showed no febrile or other obvious reaction thereto; a control, untreated, monkey also showed no evidence of infection. After 11 days without the drug, the administration of V186 was resumed, this time by stomach-tube. Ten c.c. of a 10 per cent. solution twice daily were well tolerated for three days; so were 15 c.c.—that is 2 and 3 g. a day. But after five days on 2 g. twice daily, the monkey was noted to be much quieter than before. The drug was discontinued, and the next day the animal lay on the floor of the cage hardly breathing. On the following day it was much better, though very thin; it was killed, but nothing striking was found post-mortem.

Rhesus 3 (male), weight *c.* 3 kg., was given 1 g. V186 by stomach-tube twice daily; it also received epidemic typhus material intraperitoneally. After three days of drug treatment, it became very quiet and the temperature fell from 101° F. to 99° F. Next day it was found dead; nothing abnormal was found, apart from a slight peritoneal reaction to the recently injected typhus material.

Thus, a fatal intraperitoneal dose for one animal was 1.0 g./kg., given in two divided doses in the day; a second animal died after 1.25 g./kg., given daily *per os* over several days. A third monkey died after three days when fed with half that dose.

### TOXICITY FOR MAN

Two male volunteers, D.I.S. (aged 20) and W. F. J. (aged 24), were given a careful physical examination, and their exercise-tolerance, resting blood-pressure, blood-urea and blood-counts were determined. Both were found normal by these tests. They stayed for the week, in which drugs were administered, in a private ward at the National Hospital, Queen Square, but were allowed to be up for most of the day. They were given V186 solution by mouth morning and afternoon, the successive doses rising as follows:—

	D.I.S.		W.F.J.	
	a.m.	p.m.	a.m.	p.m.
Day 1 ..	40 mg.	—	—	80 mg.
Day 2 ..	150 mg.	—	—	300 mg.
Day 3 ..	600 mg.	—	—	1 g.
Day 4 ..	2 g.	—	2 g.	2 g.
Day 5 ..	3 g.	3 g.	3 g.	4 g.
Day 6 ..	4 g.	4 g.	4 g.	4 g.

No toxic symptoms were noted on this dosage. We did not think it necessary to give more of the drugs than this, which is a usual level for effective sulphamide therapy. The drugs were dissolved in about 60 c.c. of water, and the taste was said to be pleasant till the level of 2 g. doses was reached. Blood-pressure and pulse-rates were measured half-an-hour after each dose. Differential blood-counts and blood-urea determinations were made daily, and urine examined twice daily for sugar and albumin. None of these tests showed any significant deviation from the normal. The blood-urea determinations (in mg. per 100 c.c.) were as follows:—

	Day	1	2	3	4	5	6	7	8	14	19	25
D.I.S.	..	26	24	29	35	29	29	32	31	36	38	—
W.F.J.	..	34	24	26	30	30	32	30	34	28	26	42

“Follow-up” examinations were made of blood-cells and blood-urea levels for 18 days after the subjects had left hospital; these also showed no abnormalities.

#### DISCUSSION AND SUMMARY

Studies have been made of the toxicity for mice, guinea-pigs and human beings of two substances (V147 and V186) having activity against experimental typhus in mice. Data as to oral toxicity can be summarised as follows:—

	Mouse.	Guinea-pig.	Rhesus.†	Man.†
Maximum daily dose in g./kg. tolerated <i>per os</i> over several days	2	0.3	0.25	>0.13

† Data available for V186 only.

It thus appears that the drugs are better tolerated by mice than by larger species. For all species studied the maximal dose (in g./kg.) tolerated at a single intraperitoneal or subcutaneous injection seems not very different in quantity from that which can safely be given daily *per os* over a period of several days. The action of the two drugs in lowering blood-pressure and tending to raise blood-urea levels invites comparison with that of diamidines used in trypanosomiasis (Wien, 1943). Dawes (1945) has carried out pharmacological studies of the substances.

#### ACKNOWLEDGMENTS

We wish to thank the workers mentioned in the text who helped in various parts of this study; Dr. E. A. Carmichael, who allowed us the hospitality of a ward at the National Hospital, Queen Square, for the two human volunteers; and the volunteers themselves, D. I. S. and W. F. J., members of the Friends' Ambulance Unit.

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## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### II.—CHEMOTHERAPEUTIC TRIALS IN TYPHUS FEVER IN MAN, AND A REPORT OF THE 1943-4 NAPLES TYPHUS EPIDEMIC. (A REPORT OF THE BRITISH ARMY TYPHUS RESEARCH TEAM.)

BY

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#### CONTENTS

	PAGE
History of the Organisation and Work of the British Army Typhus Research Team	12
The Preliminary Study in North Africa, July to December, 1943 .. .. .	17
The North African Cases of Typhus .. .. .	21
The Naples Study, January to March, 1944 .. .. .	33
Epidemiology .. .. .	33
Diagnostic Criteria and Differential Diagnosis .. .. .	36
Selection of Clinical Material studied during the Investigation .. .. .	36
Clinical Picture of Typhus Fever in Patients treated Non-specifically .. .. .	38
Therapeutic Trials of the Drugs, V186 and V147 .. .. .	60
Miscellaneous Chemotherapeutic Measures .. .. .	78
Penicillin Therapy .. .. .	78
Sulphathiazole Therapy .. .. .	79
Sulphaguanidine Therapy .. .. .	79
Pathological Investigations .. .. .	82
Animal Experiments .. .. .	82
Serology .. .. .	84
Blood Counts .. .. .	94
Blood Chemistry .. .. .	100
Post-Mortem Findings .. .. .	111
Discussion .. .. .	120
References .. .. .	127

#### HISTORY OF THE ORGANISATION AND WORK OF THE BRITISH ARMY TYPHUS RESEARCH TEAM

The British Army typhus research team was created in June 1943, when, at the request of the Secretary of the Medical Research Council, the Director General of the Army Medical Services agreed to organise a field trial of the treatment of typhus by the chemotherapeutic agents V186 and V147. It seemed probable at this time that, if the drugs were active in human typhus as in experimental typhus infection in mice, they might prove of particular importance in the treatment of typhus in Europeans both in the Army and in

the populations of newly liberated territories. Nevertheless, the therapeutic trial had to be undertaken upon such cases of typhus as were available in areas then under Allied administration; and in June 1943, either North Africa or the Middle East appeared to be the most suitable area in which to carry out the work. The distance involved in the journey to the Middle East, and the difficulty in the transport of the necessary laboratory equipment to this theatre, led to the selection of North Africa as the area of choice. The team, consisting of Majors M. van den Ende, R.A.M.C. and C. H. Stuart-Harris, R.A.M.C., accompanied by Sergeant J. R. Macdonald, R.A.M.C. and Private H. Proudfoot, R.A.M.C.—together with the equipment of a General Hospital laboratory, and numerous special pieces of equipment obtained either by the Army or the Medical Research Council—was, therefore, dispatched to Algiers on 21st June, 1943, by hospital ship. The terms of reference of the team laid down as the primary object of the research the therapeutic trial of the drugs, V186 and V147. The opportunity was also to be taken for pathological investigation and, in particular, strains of rickettsiae from as many widely separated areas as possible were to be isolated and despatched to England for further study in the typhus laboratories of the Council and the Army. Serum samples from the cases of typhus which were studied were to be desiccated in the most effective possible way, and were to be despatched to the laboratories in England. The equipment of the team included apparatus for carrying out these items of special work. The team was also asked to make any observations considered to be of value, relating to the prevention of typhus by the use of vaccines.

#### GENERAL ARRANGEMENTS

The team arrived in Algiers at the end of June, 1943, and began work in July, after arrangements had been made with the Director of Hygiene for Algiers, M. Grenouilleau, and with Colonel E. Benhamou, of the French Army, for the utilisation of clinical facilities in Algiers at the Civil Infectious Diseases Hospital at El Kettar and in the Civil Hospital at Mustapha. Suitable accommodation for the laboratory work could not be found; the laboratory apparatus was housed at first in a French ward of the Mustapha Hospital and later transferred to a room in the wing of the same hospital occupied by a Surgical Section of No. 96 British General Hospital. In August, 1943, a request was made for the construction of a Nissen hut in the grounds of No. 95 British General Hospital to house the laboratory apparatus and experimental animals newly arrived from England, and sanction for this was obtained. Construction of the hut began in September, but the hut was not ready for occupation until December, 1943, and it began to function as a laboratory shortly before transfer of the main activities of the team to Italy. Between July and December all the laboratory work of the team was, therefore, carried out at the Mustapha Hospital, and after this time the Nissen hut laboratory was used as described below.

Study and treatment of cases of typhus in both Algiers hospitals began in July, and continued on the small number of cases found to be available throughout the summer and autumn. The experience gained on these patients was valuable. In September, 1943, at the suggestion of Colonel F. J. Hallinan and Colonel J. S. K. Boyd, Major van den Ende paid a visit to the Middle East, which lasted for three weeks. In addition to visiting various army laboratories in Cairo and civil laboratories in Palestine, he visited the Headquarters of the American Typhus Commission and gained much valuable information regarding the general problems of typhus therapy. During this period, also, an analysis was made of the cases of typhus admitted to the El Kettar Hospital in Algiers in the winter of 1942-1943, and when Major van den Ende returned, the whole problem of the chemotherapeutic trial of

## 14 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

V186 and V147 was reconsidered in the light of the total experience then gained. It seemed doubtful whether the town of Algiers would provide the clinical material needed for the therapeutic trial during the space of one winter's season of typhus—for the following reasons:—

- (i) The drug V186 did not produce an immediately obvious clinical effect, either in small or large doses, when given to patients in the second week of the disease or slightly earlier than this. Any assessment of its effect would, therefore, have to be made on statistical grounds, by comparison of the duration of the disease and mortality in two groups of patients, only one of which received treatment with the drugs. A large number of patients would have to be studied if the benefit of the drug treatment was to be assessed on the basis of duration of disease, but assessment of effect on mortality rates would probably require a much smaller number of patients. It was also desirable that the drug treatment should be tested on patients in the earliest possible stage of typhus, because both V186 and V147 were most effective in the treatment of experimental typhus infection of mice if given as soon as possible after the initiation of infection (Andrewes, King, van den Ende & Walker, 1944).
- (ii) The clinical material which would have been available for study in Algiers during the winter of 1943 consisted almost entirely of cases in Arabs chiefly under the age of 40, and with a gross mortality rate from typhus of only 11 per cent. Only one-third of these were admitted before the 8th day of the disease, and one-quarter of the fatal cases survived for only twenty-four hours after admission. Unless the 1943–44 season of typhus in Algiers was much more severe than that of the previous year, it was obvious that there would be insufficient cases in any one season for a large therapeutic trial, and that there would be a particular paucity of the early cases, and of the patients in the older age-groups, who had a higher expected mortality rate than the younger ones.

These conclusions were discussed at a meeting of the Typhus Committee of the Medical Research Council held in London in November, 1943, which was attended by Major Stuart-Harris. In order to enable the team to make observations in any part of the Allied-occupied territory which could be reached from Algiers and where an outbreak of typhus might develop, the team was then reorganised. Two additional medical officers, Major M. H. Gleeson White, R.A.M.C. and Captain W. L. Hawley, M.C., A.U.S., were sent out to North Africa in January, 1944, accompanied by a unit of twelve nursing orderlies of the Friends' Ambulance Unit who volunteered for service in connection with typhus work. A small amount of additional equipment was also sent, in order to enable a field section of the typhus laboratory to study and treat cases of typhus at a distance from the main laboratory. In the meantime, a sharp outbreak of typhus had developed in Naples at the end of December, 1943, and Majors van den Ende and Stuart-Harris, together with Private Proudfoot and supplies of the drugs V186 and V147, were flown to Naples on the 28th December. Arrangements were made to investigate and treat patients in the Civil Infectious Diseases Hospital at Cotugno, Naples, by the courtesy of the authorities of the Allied Military Government and of the Civil Department of Health, Naples. The inadequacy of civil nursing arrangements at the hospital, and the imperative necessity of good arrangements both for the administration of the drugs and for the keeping of records, were met by the provision of six volunteer nursing officers of the Q.A.I.M.N.S. and T.A.N.S. from the 92nd and 65th British General Hospitals. In the absence of sufficient equipment, the laboratory facilities at the No. 92 General Hospital

were utilised by the courtesy of Major G. Cunningham, R.A.M.C., and specimens of serum were flown daily to Algiers by the Mediterranean Air Transport Service. Some of the serological investigations, desiccation of serum and preliminary studies of the adaptation of the strains of rickettsiae from the Naples cases to guinea-pigs were carried out at the laboratory of the typhus team at No. 95 General Hospital in Algiers. Major A. Jordan, R.A.M.C., took charge of this laboratory during January and part of February, until Major Gleeson White arrived. A considerable part of the therapeutic trial was carried out before the additional members of the typhus team and the nursing orderlies reached Naples on 30th January, but the original observers were greatly in need of assistance, and both Captain Hawley and Major Gleeson White worked in the Cotugno Hospital until 15th February, when Major Gleeson White returned to Algiers. The nursing orderlies of the Friends' Ambulance Unit took care of patients under the supervision of the Nursing Officers, and early in February a change was made in the latter, the original six officers being replaced by six other Nursing Officers of the Q.A.I.M.N.S. from the 104th British General Hospital. The laboratory equipment brought by Major Gleeson White, together with some sent from Algiers, arrived by hospital ship on the 30th January, and was at once set up in a room at the Cotugno Hospital in which all clinical laboratory tests were made. The arrangements at the hospital were very primitive during the early part of the work, because the hospital had been damaged by air-raids prior to Christmas, 1943, and was reoccupied only when the emergency caused by the epidemic arose. Medical equipment for use of the team was provided in part by A.M.G.O.T. and in part by the Army, and the entire use of one block in the hospital was given over to the team by the Superintendent of the hospital, Dottore Franza. Transport facilities to and from the hospital were provided by the courtesy of the American Field Service, which released an ambulance for use by members of the team. Two other R.A.M.C. officers, Major J. A. James and Major S. C. Truelove, visited the hospital and assisted in the recording of clinical data on several occasions, and a third, Major C. D. P. Jones, kindly gave surgical assistance on several occasions.

Apart from the numerous Allied medical officers who visited the hospital for purposes of instruction, two officers spent several days there and actually worked with the team. Major J. H. Bowie, I.M.S., arrived from Persia in January and observed the investigation for three weeks. The results then obtained did not, however, justify the organisation of a further chemotherapeutic trial of V186 or V147 in Persia. Major S. C. Ray, I.M.S., visited Naples in February, in order to continue his work on the diagnosis of typhus by skin biopsy. Much help was given to the team by the officers of the 15th American General Medical Laboratory—particularly by Major T. Mallory, M.C., A.U.S., who carried out preliminary histological examinations on some of the post-mortem material, and Captain F. Robbins, M.C., A.U.S., who carried out some of the inoculations of guinea-pigs with blood from patients in the hospital. Captain Robbins was successful in recovering several strains of rickettsiae from patients under the care of the typhus team, and animals inoculated with these strains were handed over by him for transmission to Algiers and thence to England.

An essential factor in the work of the typhus team in Naples was the organisation set up by the American Typhus Commission for the control of the epidemic. One section of the Commission was designated the "Case-Finding Scheme" and consisted of American medical officers and civilian assistants trained in the diagnosis of typhus. These officers visited the homes of all reported cases of typhus, checked the diagnosis, and searched all contacts and neighbouring families for possible typhus infection. All cases suspected of infection were sent to the Cotugno Hospital, and many patients were,

## 16 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

therefore, admitted to the hospital at an earlier stage of their disease than if they had been allowed to report their illness to a doctor in the ordinary way. Any lack of success experienced by the case-finding organisation was entirely due to immense difficulties encountered by them in carrying out their task, and particularly to the concealment of illness by the Italian community. The liaison established with the officers of the American Typhus Commission was most helpful in the work of the typhus team and enabled several early cases of typhus to be admitted to the British ward that might, otherwise, have been overlooked.

The co-operation of the Italian civil authorities during the work of the typhus team was complete. Two Italian nurses helped in the British ward at the Cotugno Hospital, and an interpreter, Signorina V. Belli-Panni, worked at the hospital throughout the investigation, with complete disregard for her own safety. In spite of her efforts, the use of dialects by many patients, and the low level of intelligence encountered, made close contact with patients, and understanding of their feelings, a matter of great difficulty. The work of the British nursing officers, and of the nursing orderlies of the Friends' Ambulance Unit, was of the greatest importance in obtaining reliable records and in carrying out therapeutic administration. The experience of nursing cases of typhus was probably a partial recompense for their zeal.

During the entire period of the work of the typhus team, Major van den Ende was in charge of the pathological investigations and post-mortems; and Major Gleeson White took charge of the laboratory in Algiers from the middle of February onwards. Captain Hawley was given charge of patients as soon as he arrived in Naples, and, together with Major Stuart-Harris, he was responsible for clinical records and the arrangement of therapy. When the therapeutic trials of V186 and V147 had been completed, patients were admitted for trial of other therapeutic agents or for observation and study of the disease. The work was terminated in March, 1944, and the patients then occupying the ward were handed over to the civilian medical authorities. Captain Hawley and Major Stuart-Harris then returned to Algiers and assisted Major Gleeson White in the examination of laboratory specimens. The laboratory work was brought to a close in April, and the original officers and other ranks of the team were returned to England, either from Algiers or directly from Naples. The equipment of the team, excluding that provided by the Medical Research Council, was handed over for use by the Army in North Africa and in Italy.

### TYPHUS RESEARCH TEAM

<i>Original members..</i>	..	..	Major M. van den Ende, R.A.M.C. Major C. H. Stuart-Harris, R.A.M.C. Sergeant J. R. Macdonald, R.A.M.C. Private H. Proudfoot, R.A.M.C.
<i>Additional members</i>	..	..	Major M. H. Gleeson White, R.A.M.C. Captain W. L. Hawley, M.C., A.U.S.*
<i>Assisting officers ..</i>	..	..	Major A. Jordan, R.A.M.C. Major K. F. W. Hinson, R.A.M.C. Major G. Cunningham, R.A.M.C. Major S. C. Truelove, R.A.M.C. Major J. A. James, R.A.M.C. Major C. D. P. Jones, R.A.M.C.
<i>Visiting officers ..</i>	..	..	Major J. H. Bowie, I.M.S. Major S. C. Ray, I.M.S.

\* Released for service with the British Army by kind permission of the Director of Medical Services, European Theatre of Operations, United States Army.

*Friends' Ambulance Unit* . . . . . 11 Nursing orderlies and one officer with  
(members anonymous, by request) . . . . . laboratory experience.

*Attached Workers (Italy) :—*

*Nursing officers* . . . . . M. Ganter, Q.A.I.M.N.S./R.  
B. Fraser, Q.A.I.M.N.S./R.  
E. Driver, Q.A.I.M.N.S./R.  
E. Sharp, Q.A.I.M.N.S./R.  
J. Cleall, Q.A.I.M.N.S./R.  
J. Hitchin, Q.A.I.M.N.S./R.  
F. S. Smith, Q.A.I.M.N.S./R.  
B. I. Duncan, Q.A.I.M.N.S./R.  
K. Walsh, Q.A.I.M.N.S./R.  
M. Roche, Q.A.I.M.N.S./R.  
J. M. McRae, T.A.N.S. (deceased).  
B. Rimmer, T.A.N.S.

*Interpreter* . . . . . Signorina V. Belli-Panni.

**THE PRELIMINARY STUDY IN NORTH AFRICA,  
JULY TO DECEMBER, 1943**

Between July and September, 1943, numerous cases of fever in the Civil Infectious Diseases Hospital of El Kettar, Algiers, and in which the diagnosis of typhus was considered possible, were investigated, and some were treated with the drug V186. A few patients at the Civil Hospital of Mustapha, Algiers, were similarly investigated and treated, and a few patients in British hospitals in or near Algiers were referred to the team for investigation, one being treated with V147. In all, 65 patients were investigated, of whom 42 were Arabs, 14 were French, 7 were British, one Italian and one Polish. The patients seen at the French civil hospitals, who included all but the Italian and British patients, were investigated in a routine manner. If the diagnosis of typhus seemed likely on clinical and pathological grounds, the patients were allocated alternately to one or other of two categories, and treatment with V186 was given to patients in only one category. Thus, after a time, a series was formed at each hospital, some of the patients of which had received treatment with V186, and the others, comprising alternate admissions, had received symptomatic and supportive therapy only. The initial diagnosis was in some cases altered later because the case failed to fulfil the diagnostic criteria of typhus which were laid down, and the case was then withdrawn from the series retrospectively. In one or two instances the diagnosis of typhus was made after the patient had begun to enter convalescence, but these cases were not included in the series used for the evaluation of the chemotherapeutic agents.

**DIAGNOSTIC CRITERIA FOR TYPHUS**

Because it was essential that the chemotherapeutic trial of the drugs V186 and V147 should be made in patients in whom the diagnosis of typhus was not in doubt, certain rigid criteria were laid down at the beginning of the investigation which may have caused the exclusion of some cases of typhus in which the serological changes were atypical. An attempt was also made to exclude other



## 18 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

infections known to be common as an accompaniment of typhus or to cause difficulty in diagnosis. The following procedure was adopted in every new patient who was investigated :—

After the history had been taken as carefully as was possible in view of the difficulties of interpretation, a routine physical examination was made. Blood was taken for blood culture, blood counts and a number of chemical investigations. A slide agglutination (Castaneda) test was then performed at the bedside with concentrated *Proteus OX19* suspension. In all cases in which a provisional diagnosis of typhus was made, the blood count and the blood urea measurement were repeated approximately every four days, and blood was taken simultaneously for serum samples. From September onwards, guinea-pigs were inoculated intraperitoneally with blood from each case diagnosed as typhus, and attempts were made to recover rickettsiae by inoculation of sternal marrow, and also of blood, intranasally into mice. Blood and urinary chloride estimations were also added to the list of routine tests, and, whenever possible, sternal marrow smears were examined for the presence of the intracellular bodies described by Benhamou (1942). Kahn tests were made on many of the serum samples by Major K. F. W. Hinson, R.A.M.C., of the 94th British General Hospital.

*Serological.*—Agglutination tests were performed by a modified Dreyer's technique, with standard bacterial suspensions supplied by the Royal Army Medical College Emergency Vaccine Laboratory. Serial dilutions of serum were made in Dreyer tubes with graduated capillary pipettes, and an equal volume of the suspension of appropriate density added. The tubes were kept at 50° C. for 4 hours and then overnight at 37° C. Readings were taken after a total of 18 hours' incubation. The titres recorded were the highest dilutions of serum showing visible (standard) agglutination. The first serum sample from each patient was tested against 0 suspensions of *Proteus X19, X2, XK, Bact. paratyphosum* A and B, *Bact. typhosum*, *Br. melitensis* and *Br. abortus*. Subsequent samples from each patient were tested only against the *Proteus* strains if the provisional diagnosis of typhus was made, and were repeated with other bacterial suspensions as and when indicated.

Agglutinations of rickettsiae were performed with suspensions prepared from the lungs of rats or mice. An epidemic (Cairo) and a murine (Wilmington) strain were used. During the earlier part of this work, when supplies of "epidemic" suspension were limited, only some of the serum samples were tested against both suspensions, and the remainder against the murine strain only. The tests were performed in Dreyer tubes. Incubation was at 37° C. in a waterbath for 18 hours, after which readings were recorded in the same way as with bacterial suspensions.

*Blood cultures.*—Ten c.c. of blood were withdrawn with a heat sterilised all-glass syringe, and 5 c.c. amounts were cultivated in 20 c.c. each of 0.5 per cent. glucose broth and 0.5 per cent. taurocholate broth. The broth was tryptic digest broth made from sheep's heart. The cultures were incubated at 37° C. and examined daily. As soon as growth occurred, subcultures were made on blood agar and McConkey's medium. If no growth occurred in the broth media, subcultures were made after 4 days.

Organisms were identified by the usual fermentation and slide agglutination tests—using diagnostic sera provided by the Royal Army Medical College Emergency Vaccine Laboratory.

*Blood counts, etc.*—In all cases, a complete blood count was performed when the patient was first seen. When a diagnosis of typhus was made, the blood count was repeated at intervals of approximately 4 days.

During the early part of the investigations, a comparison was made between results obtained with capillary blood and oxalated (dry potassium oxalate) venous blood. Differences were insignificant, so that, later, capillary blood counts were abandoned. Although this was not entirely desirable, it afforded a great saving of time.

Haemoglobin determinations were made by Sahli's method. The method has not, in our hands, proved entirely satisfactory. The instruments were standardised by using them to determine haemoglobin levels in 20 normal British soldiers, and calculating the value obtained for blood containing 5 million r.b.c./c.mm. This figure was found to be 116·7 on the Sahli scale. The values recorded in this report are corrected accordingly (116·7 Sahli=100 per cent.).

Sedimentation rates (per hour) were determined in Wintrobe's haematocrit tubes at room temperature. The same tubes were subsequently centrifuged, and the approximate haematocrit value obtained (these values were not corrected for shrinkage due to the presence of potassium oxalate).

Total counts of red and white cells were made in the usual way. Blood films were made at the bedside. Thick and thin films to be examined for malaria parasites and spirochaetes were stained with Leishman's stain. Smears for differential counts of white cells were made on  $\frac{1}{8}$  and 1-in. cover glasses.

*Chemical investigations.*—Blood urea values were estimated by colorimetric methods—using at first a Lovibond comparator, but later by matching colours against a standard ammonium sulphate solution in an ordinary colorimeter. At first, urease tablets were used. Later, owing to irregularity of the results obtained with tablets, urease freshly prepared from Jack bean meal was substituted.

Plasma chlorides were determined by the method of van Slyke and Sendroy (from Stitt, Clough & Clough, 1938).

Urinary urea determinations were made by the hypobromite method (Harrison, 1937).

*Animal inoculations.*—As soon as guinea-pigs and mice became available, attempts were made to isolate strains of rickettsiae from blood and bone marrow of patients. Guinea-pigs received intra-peritoneally 5–10 c.c. of fresh whole blood or blood clot. Their temperatures were recorded twice daily. When pyrexia of 104° F. or over occurred, the guinea-pig was killed and a 10 per cent. emulsion of brain in horse serum broth was used for subinoculation of further animals. If no pyrexia occurred within 16–18 days of the primary inoculation, the animal was killed, its brain used for passage and the serum collected for titration of rickettsial agglutinins.

In two cases, white mice were inoculated intranasally while under ether anaesthesia with suspensions of sternal marrow. They were treated with sulphathiazole to reduce intercurrent infections. Mice were killed after 4–7 days, smears of the lungs made and the lungs emulsified for further passage. A few unsuccessful attempts were made to establish the presence of murine typhus in wild rats caught in the vicinity of an army camp where a case of murine typhus had occurred.

*Drying of serum.*—A portion of each serum sample was filtered through a "gradocol" membrane of 0·5 $\mu$ , or, in the case of cloudy sera, a 1·0 $\mu$  membrane followed by one of 0·5 $\mu$  (Elford, 1931); it was then distributed in 4, 2 and 1 c.c. amounts into ampoules for drying. (We are indebted to Dr. D. G. Evans, of the National Institute for Medical Research, for the designs of apparatus used, and for his many helpful suggestions on the drying of serum.)

## 20 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

The system adopted, after some preliminary difficulties, consisted of one or two "pyrex" manifolds connected with ground glass joints to a large, stout-walled, conical condenser containing phosphorus pentoxide, and exhausted by a "Hyvac" pump (Fig. 1). It was fitted with the required  $\frac{1}{8}$  H.P. universal motor to run on a voltage of 100 or 200.

Ampoules were attached to the manifold and so arranged that the slope gave a maximal surface for drying. The serum was frozen after attachment of the ampoules to the manifolds, by packing round them a salt-ice freezing mixture. When the serum was thoroughly frozen, the pump was turned on and the freezing mixture allowed to thaw. The degree of vacuum obtained was sufficient to maintain the frozen state. When the contents were dry (usually after 6-12 hours) the ampoules were removed, their necks partially constricted, and they were transferred to desiccators containing phosphorus pentoxide. After two or three days, the desiccators were filled with dry air and the ampoules sealed.

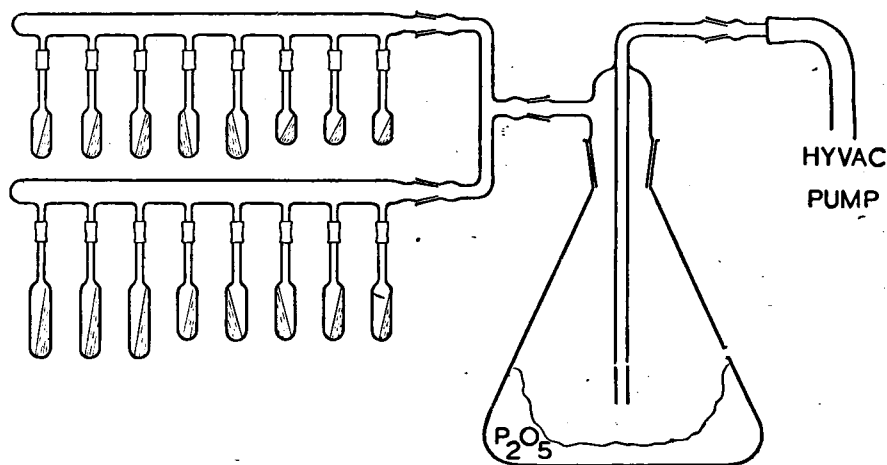


FIG. 1.—Apparatus used for drying serum.

The serum, on reconstitution, usually showed some turbidity, which in a few cases has been marked. Sera tested a year after drying have shown only slight reduction in titre of agglutinins for rickettsiae as compared with the titre of the fresh sera, and no significant fall in the agglutinating titre for bacteria (*Proteus OX* strains).

The diagnosis of typhus was considered to have been confirmed when a strain of rickettsiae serologically allied to *Rickettsia prowazeki* was recovered from the patient's blood. If no virus was recovered, or where no attempt to recover it had been made, the diagnosis of typhus was regarded as confirmed if:—

- (i) *Proteus OX19* agglutinins were present in the serum in titres of 1 : 200 or more, or if the level of *OX19* titres increased at least four-fold in amount during the course of the illness.
- (ii) Rickettsial agglutinins were present in the serum in titres of 1/25 or more.

The results of post-mortem examination in fatal cases were considered to provide an additional check of the diagnosis, but typhus was not diagnosed on post-mortem evidence alone.

### DIFFERENTIAL DIAGNOSIS OF THE NORTH AFRICAN CASES

Among the entire group of 65 patients a final diagnosis of typhus was made in 21 cases; another one was suspected but not confirmed as typhus, and one more was diagnosed as *Fièvre boutonneuse* or Mediterranean Tick Typhus.

Sixteen of the proven cases of typhus belonged to the series of patients treated with V186 or their untreated alternate controls, another three were in British soldiers previously inoculated with typhus vaccine and two more were diagnosed only when convalescence had set in. The case diagnosed as *Fièvre boutonneuse* (Algiers EK 12) resembled typhus clinically, but the patient—who was 60 years of age—was febrile but not severely prostrated. Her serum developed a high titre of agglutinin for *Proteus OX2* and only slight increase in titre for *Proteus OX19* and no rickettsial agglutinins. The rash, which was macular and petechial, was not typical of *Fièvre boutonneuse*, and there was only a doubtful primary eschar on the back. After 20 days had elapsed from the day of onset, fever subsided and she rapidly became convalescent. In most of the remaining 41 patients, the diagnosis of typhus did not appear probable on clinical grounds, chiefly because of the absence of rash, but they were investigated for typhus because they were cases of obscure pyrexia occurring in a known area of epidemic typhus. At this early stage of the investigation it seemed probable that typhus without rash would be a frequent occurrence, but, in actual fact, subsequent experience did not confirm this expectation. The 37 patients not suffering from typhus who were seen in French hospitals included 12 cases of typhoid fever proven by blood culture, and infection by an organism of the enteric group was suspected, but not proved by blood culture, in 5 more. Malaria was diagnosed 7 times, being accompanied by pneumonia in one case. Pneumonia of lobar or broncho-pneumonic type was diagnosed 5 times, being an accompaniment of dysentery once, of hepatitis once and of malaria once. Meningovascular and tertiary syphilis were each diagnosed once, acute upper respiratory infection was diagnosed twice, and relapsing fever, diphtheria, haemolytic streptococcal septicaemia (secondary to abortion) and purulent meningitis (secondary to frontal sinusitis) were each diagnosed once. One patient remained undiagnosed. Of the four British patients originally suspected to be cases of typhus, but in whom typhus was excluded, two proved to have small-pox, one had an upper respiratory tract infection and one, who also had aortic regurgitation, had an obscure pyrexia, the cause of which was not ascertained. This latter patient received treatment with V147.

#### The North African Cases of Typhus

Eighteen patients with typhus were admitted to French hospitals between July and December, and formed a series of patients receiving either V186 or symptomatic treatment. Two only were admitted to the Mustapha Hospital; one was treated with V186 and one was not. Sixteen were admitted to El Kettar Hospital; eight were treated with V186 and eight were treated symptomatically. Two of the latter patients at El Kettar were excluded from the series retrospectively, because of insufficient proof of typhus in one case and because of the diagnosis of *Fièvre boutonneuse* in the other. Of the final group of 16 patients, two were French, one was a Polish civil prisoner and the remaining 13 were Arabs. Twelve were males and four were females. Their ages ranged from 15 to 60. None of the patients treated with V186, and only one of the patients treated symptomatically, died. Table III gives the details of the patients, their ages, sex, duration of illness prior to symptoms, physical signs, course of illness, complications and treatment. The number of cases was too small for a detailed study of the symptomatology and physical findings to be profitable. In any event, the history of illness was very difficult to obtain from the Arab patients, on account of the language problem, so that the recorded symptoms were probably inaccurate. Eight gave a date of onset which indicated that they were admitted on the 5th, 6th, 7th, 9th, 10th, 10th, 10th and 15th days of illness, respectively. The others appeared to have been ill for periods of from 7 to 28 days. This lateness of hospitalisation was

22 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE  
North African Cases of Typhus—

Patient.	Sex.	Age.	Day of disease on admission.	Severity on admission.	Admission.			Rash.	Spleno enlargement.	Heart.	Lungs.
					Temperature.	Pulse.	Respiration.				
M2.*	M	59	VIII	Mod. severe	104	?	?	Macular purpuric. Haemorrhage in conjunctiva.	0	Distant heart sounds.	Basal râles ..
EK2.	F	40	? XX	Grave	98	116	40	Faded macules ..	0	Collapsed peripheral circulation.	Râles all over.
EK4.	M	19	? X	Mild	102	88	36	Macular .. ..	±	Low B.P. ..	Rhonchi ..
EK6.	F	23	X	Mild	103	112	30	Macular .. ..	±	Low B.P. ..	Rhonchi ..
EK8.	F	19	VI	Mild	102	120	40	Macular petechial	0	Low B.P. ..	Rhonchi and râles.
EK10.	M	15	? XXIX	Mild	102	110	?	None .. ..	+	Normal ..	? Right apex..
EK14.	M	? 45	? VII	Mod. severe	103	130	30	None .. ..	++	Normal ..	Rhonchi ..
M1.	M	27	X	Mod. severe	104	94	40	Petechial.. ..	0	Normal ..	Pneumonia right lower lobe.
EK1.	M	48	? X	Severe	101	?	?	Macular purpuric	0	Distant sounds Tic-tac rhythm	Nil .. ..
EK3.**	M	33	XV	Mild	101	72	?	Macular .. ..	0	Low B.P. ..	Râles ..
EK5	M	22	? IX	Mod. severe	104	120	40	Macular petechial	++	Normal ..	Râles all over
EK7.	M	30	XII	Mod. severe	101	90	26	Macular petechial	++	Normal ..	Dyspnoea only
EK9.	F	26	? XIV	Mild	100	120	32	Macular .. ..	+	Normal ..	Nil .. ..
EK11.*	M	16	V	Mod. severe	104	124	50	Macular purpuric	+	Distant sounds Tic-tac rhythm	Dyspnoea .. Rhonchi ..
EK13.	M	21	VII	Mod. severe	104	120	30	Macular haemorrhage conjunctiva	+	Normal ..	Dyspnoea .. Rhonchi ..
EK15.	M	20	X	Mod. severe	104	120	34	Macular petechial	0	Low B.P. Tic-tac rhythm	Rhonchi, râles

\* French. \*\* Polish. 0 Spleen not palpable. ± Spleen just palpable. + Spleen slightly enlarged. ++ Spleen much enlarged.

## III

*Treated Symptomatically or with V186*

<i>C.N.S.</i>	<i>Mentality.</i>	<i>Renal abnormalities (and Blood Urea figures in mg. per 100 c.c.)</i>	<i>Duration of fever in hospital (days).</i>	<i>Duration of total illness (days).</i>	<i>Complications.</i>	<i>Treatment.</i>
Nil .. ..	Confused and drowsy.	Albuminuria. Blood urea 30.	11	18	Oedema ankles, scrotum and forearms. Cystitis.	Subcutaneous saline. Hypertonic (20 per cent.) saline I.V. Suprarenal extract.
Coma and absent reflexes.	Unresponsive	No urine. Blood urea > 220.	—	Died second day.	Parotitis. Gangrene finger.	Subcutaneous saline. Ouabaine, strychnine, caffeine.
Nil .. ..	? Normal ..	Nil. Blood urea 60 ..	3	?	Nil .. ..	Subcutaneous saline. Belladonna.
Nil .. ..	Apprehensive	Retained urine one day. Blood urea 45.	6	15	Nil .. ..	Sparteine, strychnine, urotropine. Catheterisation.
Nil .. ..	Anxious ..	Albuminuria, casts. Blood urea 130. Convalescent diuresis.	3	8	Nil .. ..	Aspirin, sparteine, urotropin.
Tremulous; unequal reflexes.	Irritable ..	Nil. Blood urea 40 ..	3	?	Anaemia on admission	Iron protoxalate. Liver.
Nil .. ..	Confusion; disorientation.	Convalescent diuresis. Blood urea 150.	5	?	Icterus .. ..	NIL
Nil .. ..	Anxious ..	Nil. Blood urea 30 ..	5	4	Nil .. ..	V186.
Absent reflexes; comatose.	Unresponsive 6 days.	Nil. Blood urea 70 ..	8	?	Nil .. ..	V186. Subcutaneous saline. Ouabaine and strychnine.
Pains in limbs in convalescence.	Normal ..	Nil. Blood urea 60 ..	1	15	Oedema feet on admission.	V186.
Absent K.J. ..	Confused ..	Nil. Blood urea 80 ..	5	?	Nil .. ..	V186. Subcutaneous saline. Cupping. Ouabaine, strychnine "Syncoortyl".
Absent K.J. ..	Confused, disorientated.	Albuminuria, casts. Blood urea 80. Convalescent diuresis.	9	?	Parotitis .. ..	V186. Sulphapyridine 3 g. daily 8th to 16th days. Incision parotid.
Nil .. ..	Normal ..	Nil. Blood urea 25 ..	1	?	Nil .. ..	V186
Nil .. ..	Irritable. Confused at times.	Nil. Blood urea 45 ..	5	9	Diarrhoea. Cyanosis	V186. Belladonna and pot. iodide.
Nil .. ..	Anxious ..	Normal on admission. Developed anuria and uraemia 12th day. Blood urea 40 to 600 Albuminuria, casts.	6	12	Uraemia with prolonged vomiting after defervescence. Melæna. Parotitis.	V186. Saline subcutaneously and I.V. Hypertonic (2 per cent.) saline I.V. "Luminal", "Somnifaine."
Nil .. ..	Dull ..	Albuminuria, casts. Blood urea 55. Retained urine 1 day. Convalescent diuresis.	1½	12	Vomiting after 48 hours' therapy.	V186. Sodium bicarbonate. Subcutaneous saline. Ouabaine and strychnine.

## 24 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

common in cases of typhus in Algiers, and was due in part to a failure of the Arabs to report their disease until they were seriously ill or moribund. It resulted, first of all, in a short course of fever while the patients were under observation in hospital, with a consequent difficulty in determining the effect of treatment; and, secondly, in a high percentage of cases which were really unsuitable for chemotherapy. Though the majority of the patients, both Arab and European, were well-nourished on admission to hospital, four Arabs were emaciated. In nearly every case a considerable loss of weight occurred during the illness, partly owing to the low calorie diet which the patients received while in hospital. Lousiness was not reported to be prominent in any of them, only one or two lice usually being found on the body on entrance into the hospital *salle de dépouillage*. One or two patients did not appear lousy at all, and denied ever having been bitten by insects. It is probable that the generally low rate of lousiness may have been due to the season of the year (summer and early autumn). The Polish civil prisoner was the only patient who stated that he had frequently caught lice on his person and was in the habit of crushing them.

### GENERAL COURSE OF ILLNESS

The illnesses in these 16 patients ranged in severity from mild attacks of short fever to severe and fatal infections, and they did not differ significantly from the types of illness seen later in Naples, studied in detail and fully described later in this report. Case summaries of two patients, EK13 and M2, are given in Appendix A. Only one patient was afebrile on admission; in all the others, fever from 101°–104° F. was present, accompanied by tachycardia and raised respiration rate. The fever usually ended by slow lysis, but it terminated suddenly in four patients, all of whom were treated with V186 (EK5, 11, 13 and 15). The height of the fever did not appear to be correlated with the severity of illness.

Rashes were seen in all but two patients at the time of admission to hospital, and in 13 patients they consisted of macules, usually on the sides of the thorax, abdomen and limbs, and avoiding the face. They were accompanied by intervening petechial haemorrhages in four patients, and by actual purpura in three more. In one the rash was purely petechial (M1). The rash present in patient EK1 was particularly striking, in that the purpura took the form of irregularly shaped areas on back and front of the trunk—several millimetres across—which eventually faded and separated, leaving tiny depressed areas in the skin, apparently consisting of small areas of haemorrhagic necrosis. Two patients had haemorrhage in the bulbar conjunctiva, in the form of an irregular area some millimetres in diameter.

The abnormalities present in the various systems are summarised in Table III. The spleen was often enlarged. The blood pressure was usually low and heart sounds distant. In the one fatal case (EK2), a collapsed condition of the circulation was present. Tic-tac rhythm was encountered in three patients. Chest signs were almost invariable but usually indicative only of mild bronchitis. In only one case were signs of pulmonary consolidation present. Few demonstrable signs existed of damage to the nervous system, except for mental changes such as drowsiness and irritability, dullness, confusion and disorientation, or actual coma or stupor, with failure to respond to orders or questions. The reflexes, usually of the patellar or of the Achilles tendons, were absent in five patients (all Arabs) during the fever but returned during convalescence, and this finding is reputed by French authorities to be frequent in typhus fever in Arabs. It was not encountered to a significant extent in Naples, and may, therefore, be due to the combined effect of fever and some underlying deficiency disease, rather than to the effect of typhus alone. The renal system was not studied as fully as was desired, because of the

difficulty of obtaining satisfactory collection of specimens. Urinary changes were infrequent, consisting of albuminuria, oliguria and cylindruria only, with the exception of one case which showed red cells and in which there was a history of renal calculus. The blood urea was frequently estimated in all the patients, because nitrogen retention was regarded by the French authorities as a symptom of outstanding prognostic significance in typhus (Benhamou, Lemaire and Champeil, 1924), and also because V186 given intravenously to rabbits caused elevation of the blood urea. Six patients had normal blood ureas (25–50 mg. per 100 c.c.) throughout the course of their illness, and one of these was the man (M2) who had albuminuria during the fever, and was subsequently found to have cystitis. Seven patients had slightly elevated blood ureas (60–150 mg. per 100 c.c.) during the first few days after admission, and in two of these (EK8 and EK7) there were also albuminuria and cylindruria. The azotaemia diminished in each case as the fever progressed. Two patients had very high blood ureas; one of these (EK2) had a blood urea of more than 220 mg. per 100 c.c. just prior to death, and the urine could not be examined, because of anuria. The other patient (EK13) had a normal blood urea (40 mg. per 100 c.c.) on admission, but on the 12th day, when defervescence set in, the urea was found to have risen to more than 220 mg. per 100 c.c., and on this day he passed no urine. Following intensive parenteral therapy with normal and hypertonic saline, secretion of pale dilute urine, containing much albumin and granular and hyaline casts, began, but the blood urea value climbed still further and remained in the region of 600 mg. per 100 c.c. or more for several days. Daily outputs of 2,000 c.c. or more of urine were then maintained, and the albuminuria slowly diminished. Finally, the blood urea level slowly declined, and convalescence set in, only to be interrupted by a unilateral non-suppurative parotitis. As the development of uraemia in this patient occurred after intensive drug therapy, and differed in time relation from the azotaemia in any of the others, the phenomenon was regarded as a possible drug effect, as described below. Apart from this instance, no significant difference was noted between the blood urea levels in the treated and control groups of patients.

#### COMPLICATIONS AND SEQUELAE

In this small series of patients, complications were few. Pneumonia developed once, but as it occurred during the acute stage of the typhus it was regarded, like other respiratory tract signs, as an integral part of the infection. Gangrene occurred in the one fatal case (EK2) but involved only the skin of the index finger. Parotitis was seen in three patients (EK2, 7 and 13) and drainage of pus by incision was needed twice. Cystitis occurred in one patient (M2); oedema of the ankles, or of the lower abdominal wall, arms and legs, occurred respectively in two patients (EK3 and M2). Diarrhoea occurred in two patients, being in one (EK11) an accompaniment of the fever and in the other (EK13) an accompaniment of uraemia. In the latter case the stools on two occasions contained altered blood.

No true sequelae were seen—apart from the loss of weight which was invariable during and shortly after the fever, and was partly attributable to a low calorie intake during this period.

#### TREATMENT

The treatment of all of these patients was under the supervision of the French visiting physicians and medical students (hospital interns). No attempt was made to interfere with the symptomatic treatment of typhus which was usually given in these hospitals, such as administration of ouabaine, strychnine, caffeine and suprarenal cortical extract. Five per cent. glucose saline by the subcutaneous route was given to all patients in whom dehydration appeared, on



clinical grounds, to be present. Three patients treated by symptomatic means only, and four who received V186, were given injections of saline by this route. Hypertonic saline was administered to two patients; one of these received 40–60 c.c. of 20 per cent. sodium chloride intravenously in single daily injections, according to the method employed by Colonel Benhamou; the other received a slow intravenous drip of 2 per cent. saline. The drug V186 was administered by mouth in tablet form, and the details of dosage are given in full in Table IV. Successively bigger doses were given to the individual patients. The first patient (M1) to be treated received 0.5 g. four-hourly after an initial dose of 0.5 g., whereas patient EK13 received 3.0 and then 4.0 g. four-hourly after initial doses of 6.0 and 5.0 g. at four-hourly intervals. Treatment in each case was continued during the fever, and for a day or two after the fever had ceased. The administration of the tablets was, on the whole, well tolerated, but a few patients were troublesome and chewed the tablets, or spat them out. Sodium bicarbonate was administered to one patient (EK15) receiving V186.

#### EFFECTS OF TREATMENT WITH V186

Prior to the treatment of these patients in North Africa, all that was known about the effect of V186 in man was that it was not apparently toxic in doses of 4 g. twice daily. The first case (M1) was, therefore, treated with a very small dose of V186 and received only 11 g. altogether. No effect, either beneficial or deleterious, was observed. The next patient (EK1) was severely ill when first seen. After 24 hours' treatment, during which he received 8 g. of V186 but also full supportive and symptomatic therapy, he showed considerable clinical improvement, which continued and led to eventual complete recovery after he had received 23 g. of the drug. In this patient, although the general degree of illness appeared severe, the blood-pressure was never lower than 90/60, nor was the blood urea elevated above 70 mg. per 100 c.c. Furthermore, the improvement occurred after only a short period of treatment with V186; and, moreover, the duration of illness prior to treatment was unknown but was probably at least 10 days and may have been longer. Thus, although the change in the condition of the patient appeared to be related to V186 therapy, it was not possible to say that it would not have occurred without the drug. EK5 was a moderately severely ill patient who received 49.5 g. of V186 in six days; no effect, either beneficial or harmful, was observed. Though fever left him after 3½ days of therapy with V186, he had been ill for eight or nine days before entry into hospital, and thus had at least 12 and possibly more days of illness in all. EK7 was treated more intensively and seemed to respond to therapy, in that the temperature never rose above 102° F. and slowly diminished during the first 3½ days of treatment. Parotitis then set in acutely, and fever reappeared. Treatment with V186 was continued for a further 24 hours and then suspended in favour of sulphapyridine, at the request of the French civilian doctor. Case EK9 resembled Case EK3, and the illness had lasted for at least 14 days before admission. Fever ceased after the first 24 hours in hospital, and the patient rapidly entered convalescence. EK11 was the first patient treated with V186 in whom the onset of disease was clearly established to have been within five days of admission to hospital. He was a co-operative young man, who received intensive therapy with V186, at the rate of 2.0 and then 3.0 g. every four hours. No rapid improvement occurred; the rash became purpuric after 48 hours of therapy, prostration increased, signs in the chest continued to develop, he was cyanosed and suffered from diarrhoea. On the tenth day of disease, after four days of therapy, there was a sudden improvement and he speedily became convalescent. The fact that defervescence occurred as early as the tenth day of illness was thought to be significant, and it was believed that a shortening of the disease could be attributed to the therapy.

TABLE IV  
*Details of Treatment with V186*

<i>Patient.</i>	<i>Day of disease when treatment was begun.</i>	<i>Duration of fever after treatment (in days).</i>	<i>Initial dose (grammes).</i>	<i>Subsequent dose (grammes).</i>	<i>Days.</i>	<i>Interval.</i>	<i>Total dosage (grammes).</i>	<i>Total duration (in days).</i>
M1.	11th day of disease .. ..	3	0.5	0.5	4	Four-hourly	11.0	4
EK1.	5th hospital day .. ..	3½	2.0	1.0 0.5	3 1	Four-hourly	23.0	4
EK3.	16th day of disease .. ..	0	2.0	1.5 1.0 0.5	1½ 1 1	Four-hourly	23.5	4
EK5.	2nd hospital day .. ..	3½	2.0	1.5 1.0 0.5	4 1 ½	Four-hourly	49.5	6
EK7.	1st hospital day .. ..	8 (sepsis)	3.0	2.0 1.5 1.0 0.5	2 2 1 ½	Four-hourly	57.5	6
EK9.	1st hospital day .. ..	0	3.0	2.0	2½	Four-hourly	31.0	2½
EK11.	5th day of disease .. ..	4	4.0	3.0 2.0 3.0	} 2 4	Four-hourly	94.0	6
EK13.	8th day of disease .. ..	4	6.0	5.0 3.0 4.0	} 1½ 2	Four-hourly	86.0	4
EK15.	11th day of disease .. ..	1½	4.0	3.0	2	Four-hourly	37.0	2

## 28 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Case EK13, of approximately the same degree of clinical severity as EK11, was therefore treated similarly, but even larger doses of V186 were given during the first 24 hours, in order to achieve a saturation effect. However, the patient did not show any favourable response—he became irritable and was unco-operative. Because of a return of fever of 104° F. on the night of the ninth day of disease, the dosage of V186 was increased on the tenth day from 3·0 to 4·0 g. every four hours. Twenty-four hours later, he began to vomit persistently after the smallest administration of fluid by mouth. He then developed the condition of uraemia already described in detail above. This patient was the first to develop signs which could be interpreted as a toxic effect of the drug. None of the other patients had shown any ill effects from the treatment, and the sudden development of anuria and uraemia in this case was completely unexpected. Accordingly, case EK15 was treated with V186 more cautiously. Four grams were given initially, followed by 3 g. every four hours. Vomiting occurred after 56 hours of therapy, and was repeated on the next day, when defervescence had already occurred. Albuminuria had been present on admission and did not increase in degree, but a few granular casts were found in the urine. The blood urea, which was 35 mg. per 100 c.c. before starting therapy, was 55 mg. after 48 hours of V186 treatment, but thereafter it fell again. Some temporary diminution in urinary output occurred in this patient on the two days following defervescence, and was then followed by a diuresis. There was little evidence to support the view that V186 therapy was associated with toxic effects in this patient, except for the induction of vomiting. On the other hand, the duration of fever was only 12 days in EK15, and defervescence occurred by crisis.

From the above account it is apparent that the administration of V186 to the nine patients in the two African hospitals did not lead to any definite conclusions regarding its activity in the treatment of typhus in man. Only one patient received the drug before the end of the seventh day of the disease, and in four, the day of disease at the time of commencement of therapy was not known, because of the lack of a history of onset. Nevertheless, certain opinions were formed with regard to the action and limitations of the drug which were helpful in planning the future programme. These were:—

- (i) That V186 caused no immediate amelioration of the disease.
- (ii) That V186 had possibly shortened the duration of fever in one patient (EK11), in whom the total duration was only nine days from the onset. Because several other patients were febrile for short periods only (one to four days) after the commencement of therapy, it seemed possible that in these also V186 had shortened the duration of fever. In addition, no less than four patients treated with V186 had critical falls of temperature, which were not encountered in any of the patients not treated with the drug.
- (iii) Although it was realised that defervescence was not necessarily accompanied by cure of the disease, in most of the patients treated with or without V186 cessation of fever was accompanied by clinical recovery, and it therefore appeared possible that V186 did actually cause a slow amelioration of the disease which was shown objectively by a shortened duration of fever in those patients receiving it.

Treatment of a further large group of patients, and comparison with controls not receiving the drug, would alone determine if such a shortening of the fever was actually more frequent in patients receiving V186, and if it was statistically a significant effect. Such trials were made later at Naples (see p. 60).

- (iv) Although patients with typhus in general tolerated V186 even in large doses, one patient apparently developed kidney damage either because of the effect of the drug alone or because of the combined effect of the drug and the lesions of typhus. No effect of the drug upon the nervous or cardiovascular systems was encountered.

#### *Addendum*

As mentioned above, one patient, a British soldier in a British General Hospital, was treated with the drug V147. He was, at the time of beginning of therapy, considered to be a possible case of typhus, but the subsequent course and pathological findings caused this diagnosis to be repudiated. A certain alternative diagnosis could not be formed, except that he had an aortic regurgitation. He received 75 g. of V147 in four days of treatment, and showed no clinical change. It was observed that the urine towards the end of the period of therapy contained large numbers of granular casts, albeit there was no change in renal excretion or blood urea, and no albuminuria. Although it is conceivable that he was suffering from infective endocarditis, in view of the aortic regurgitation, and that the casts were a manifestation of embolism, it was thought probable that they were actually due to a toxic action of V147 on the kidney.

#### SEROLOGY

As the number of cases studied in Algiers was small, no attempt has been made to analyse the laboratory findings in detail. The suspension of epidemic rickettsiae was in short supply, so that it could not be used in routine tests with all serum samples. It did not always give entirely satisfactory results. All sera obtained in the Naples outbreak could be tested against freshly made suspensions, and the results of the Naples epidemic are, therefore, more satisfactory for analysis. Rickettsial agglutination tests gave no special difficulties, apart from that of obtaining adequate supplies of suspension. Zoning occurred frequently, especially with epidemic suspensions, so that it was necessary in all titrations to set up a wide range of serum dilutions.

The results showed that in all cases diagnosed as typhus on the Weil-Felix test, rickettsial agglutination was positive. In 13 there was a higher titre with epidemic rickettsiae than with the murine variety. From one of these a strain was isolated which, on subsequent investigation, was found to be epidemic in type. Several of the dried sera were later tested for complement-fixing antibodies, which confirmed that the infections were of the epidemic variety. In the one fatal case (EK2) the titres for murine and epidemic suspensions were equal. This case, like M1, was thought probably to be of epidemic type, as was subsequently proved by complement-fixation and agglutination tests with fresh antigen. M2 gave a constantly higher titre with murine suspensions, and the murine nature of the infection was confirmed by complement-fixation and "toxin" neutralisation tests. Only one case showed a persistently low OX19 titre (1/200 or less), and the rickettsial agglutinations were then of particular diagnostic value.

Agglutination of bacteria of the typhoid-paratyphoid group did not prove of great value in differential diagnosis. Agglutination tests were performed in eight of the 14 cases studied, in which the diagnosis of typhoid was firmly established. None of these patients had received T.A.B. vaccine. Unfortunately, it was not possible to test all the sera for murine and epidemic rickettsial agglutinins. The results, which are summarised in Table V, show that "O" agglutinins for *Bact. typhosum* were absent from four of the cases of typhoid, and that in a fifth agglutinins were obtained in a narrow range of dilutions on one day only, while previous and subsequent sera were negative. In five cases agglutinins were present to *Bact. paratyphosum A* or *B* in a dilution equal to, or greater than, that of the typhosum agglutinins. Agglutinins for

**TABLE V**  
*Blood Urea Values (mg. per 100 c.c.) and Agglutination Titres in Typhoid Cases.*  
*(Patients marked † are known to have died. Az on 22/9; Fo on 1/10; Or on 5/9; Ce, date unknown.)*

Patient.	Date.	Diagnosis.	Blood urea mg. %.	Typhos. "O."	Para-typh. A "O."	Para-typh. B "O."	Prot. OX19.	Prot. OX2.	Murine R.	Epidemic R.
Az. †	1943. 18/9	B.C. +	30 (20 on 21/9)	400	400	100	50	—	— (25)	—
Ca.	23/10	B.C. +	20	—	—	—	—	—	—	—
Ce. †	11/10	B.C. +	20	400	400	200	50	100	— (25)	— (25)
Fo. †	28/9	B.C. +	*210 (220 on 29/9)	— (25)	100	100	50	—	— (25)	—
Gr.	15/10	U.C. +	70	— (25)	400	50	25	25	—	—
	18/10		40	400	200	50	25	25	— (25)	— (25)
	22/10		—	— (25)	400	50	50	50	— (25)	— (25)
	26/10		—	— (25)	200	25	25	25	— (25)	— (25)
	1/11		20	— (25)	400	25	—	—	—	—
Me.	18/11	B.C. +	20	— (25)	— (25)	100	— (25)	50	— (25)	— (25)
Mo.	18/11	B.C. +	30	— (25)	— (25)	25	— (25)	50	— (25)	± in 1 dilution (1/50) only
Or. †	23/8 28/8	B.C. +	30	— (25)	— (25)	50	50	—	— (25)	—
			30	— (25)	— (25)	50	100	—	— (25)	—
X.	16/9	B.C. +	25 (35 on 21/9)	400	— (25)	25	— (25)	—	— (25)	—
Wi.	29/9	B.C. +	20	—	—	—	—	—	—	—
Ri.	29/9	F.C. +	15	—	—	—	—	—	—	—
Ha.	29/9	B.C. +	20	—	—	—	—	—	—	—
Ti.	29/9	B.C. +	15	—	—	—	—	—	—	—
Ta.	29/9	U.C. +	15	—	—	—	—	—	—	—

B.C. = Blood culture.

U.C. = Urine culture.

F.C. = Faeces culture.

\*Anuria.

*Proteus OX19* were present in six of eight cases in which the Weil-Felix test was performed. In no case was a titre of 1/200 obtained; one showed agglutinins to a titre of 1/100 and in the remaining five agglutinins were present to a dilution of 1/50 or 1/25. Repeated titrations of *OX19* agglutinins were performed in only two cases, and in neither was there a significant rise in titre.

Sera from eight patients were tested for rickettsial agglutinins. All were negative in the highest concentration of serum tested, with the exception of one case (Mo). In this case, slight ( $\pm$ ) agglutination was obtained in a single tube of a titration, in which there was a faint trace of agglutination even in the saline control. In view of the fact that the suspension showed slight spontaneous agglutinability, it is felt that this result can be disregarded.

Blood cultures were of great value in the diagnosis of typhoid fever, and positive results were obtained in 70 per cent. of the typhoid cases encountered.

#### BLOOD COUNTS

The majority of the patients with typhus showed a slight to moderate degree of anaemia, usually of normochromic or slightly hypochromic variety. In a few cases the red cell counts were below 3 million per c.mm. This anaemia may in part have been dietary in origin, or due to diseases such as malaria. Similar anaemias were, however, also encountered later in typhus patients in Italy.

White cell counts were usually approximately normal or slightly sub-normal, but with a higher proportion of polymorphs with unsegmented nuclei, during the earlier stages of the disease. During convalescence a striking lymphocytosis was sometimes encountered. Most of the patients showed at some time during the course of the illness, abnormal leucocytes of the mononuclear series. Often these cells had the appearance of plasma cells; others bore a greater resemblance to monocytes, but with deeply basophilic cytoplasm. These cells were recorded under one heading as "basophilic mononuclear cells." Polymorphonuclear leucocytosis was encountered in cases with suppurative complications.

Erythrocyte sedimentation rates were determined on oxalated blood samples in 16 cases of typhus studied in Algiers (values corrected according to haematocrit). In 15 of these the sedimentation rate was increased at some time during the illness. In one case (EK13), which was complicated by severe uraemia, the sedimentation rate was determined repeatedly, but it was never increased. As the results in this patient may have depended on the uraemia, they cannot be regarded as characteristic of typhus. In one of the remaining cases a rapid sedimentation rate was found early (fifth day), but in the others the sedimentation rate tended to reach the highest values during the early stages of convalescence. The average values for all determinations (excluding the uraemic case) in eight arbitrary time periods were as follows: 5-9th day, 8.2 mm. per hour (6 observations); 10-14th day, 13.8 mm. (18 observations); 15-19th day, 17.8 mm. (15 observations); 20-24th day, 19.5 mm. (13 observations); 25-29th day, 20.8 mm. (6 observations); 30-34th day, 12.0 mm. (4 observations); 35-39th day, 16.5 mm. (2 observations) and 40-44th day, 12.5 mm. (2 observations). These results conflict with those of many other observers, who have found the sedimentation rate to be increased early in the disease, with a return to normal values during the later stages.

#### BIOCHEMICAL STUDIES

Elevated blood ureas occurred less frequently in the small series of typhoid patients studied (Table V) than in the patients with typhus. In only one case of typhoid was the blood urea level higher than 100 mg. per 100 c.c., and the average of the highest recorded value for each of the 20 cases was 34 mg. In the typhus cases the highest figure recorded was above 100 mg. in four, and the average value, even if the excessively high figure, probably attributable to V186, is excluded, was 75 mg. per 100 c.c.

## 32 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Plasma chlorides were determined in a few cases only. This work was started in view of the apparent relationship of chloride metabolism to typhus fever, which was stressed by Julliard and Henaf (1939). Those cases in which chlorides were determined showed values in the plasma slightly below normal. Urinary chlorides were diminished in the earlier stages of several cases.

Smears of sternal marrow from a number of patients were examined for the "inclusion bodies" described by Benhamou (1942), and which he suggested as an aid to the early diagnosis of typhus. The bodies were seen in all the smears from typhus cases, sometimes in large numbers. Somewhat similar bodies, but in smaller numbers, were seen in sternal marrow smears from a case of malaria. We were unable to complete the investigation planned to determine the comparative ease of isolation of rickettsial strains from the sternal marrow and peripheral blood. Such investigations would appear to be necessary before the nature of the "inclusion bodies" in sternal marrow can be established with certainty.

### ANIMAL INOCULATIONS

Attempts to isolate rickettsiae from the blood of typhus patients by intraperitoneal injection of guinea-pigs were made in five cases. Only once was the attempt completely successful. A strain isolated from case EK11 was established by several passages in guinea-pigs, and then sent to the U.K. by air, where it was adapted to the lungs of mice. Agglutinable suspensions have since been made with this strain (Strain, Algiers EK11), and shown to be antigenically indistinguishable from those of other epidemic strains.

In two other instances, the guinea-pigs became infected with *Salmonella* or *Pasteurella* so that further passage had to be abandoned.

Attempts to isolate rickettsiae from bone marrow by intranasal inoculation of mice (Benhamou, Horrenberger and Renaux, 1943) were made in two cases. Three or four serial passages were made, and, although focal lesions were seen in the lungs of the mice and inclusion bodies in impression smears of the lungs, no rickettsiae were found. Further passages were abandoned because of intercurrent infection in the mice. These infections occurred in spite of the fact that all mice were given intraperitoneal sulphathiazole, as well as sulphathiazole in the diet.

Lice were collected from one patient (EK15) at the time of admission to hospital. Thirty lice were emulsified in horse serum broth and used to inoculate two guinea-pigs. The animals showed no febrile response, and rickettsial agglutinins did not appear in their blood.

A strain of epidemic typhus which had been isolated in mice from sternal marrow was received from Dr. Horrenberger of the Pasteur Institute, Algiers. This strain was used in a number of laboratory experiments. It could be used to prepare rickettsial suspensions for agglutination according to the method described by Fulton. Rickettsiae remained viable in lung tissue desiccated with the apparatus used for serum drying.

Subsequent work in Naples showed that isolation of rickettsial strains from the blood by guinea-pig inoculation did not offer any difficulties such as these earlier experiments had suggested. Unfortunately, the pressure of other work made it impossible to continue attempts at isolation by intranasal inoculation of sternal marrow. Furthermore, the successful use of DDT powder resulted in the disappearance of lice from the patients by the time they reached hospital, so that strain isolations from lice could not be done.

Several attempts were made to find agglutinins for murine rickettsiae in the serum of rats caught in the vicinity of army camps in the Algiers area, including one from which a patient with confirmed typhus was admitted to hospital. None of the rat sera showed the presence of such agglutinins.

## THE NAPLES STUDY—JANUARY TO MARCH, 1944

## - EPIDEMIOLOGY

When cases of typhus fever began to occur in the civilian population of Naples in March and April, 1943, they were believed by the public health authorities of the city to be the first cases of the disease to be seen there for five years. However, Naples had been the seat both of endemic and epidemic incidence of louse-borne typhus prior to 1938, but the last major epidemic before the outbreak of war was in 1924. There was no certain knowledge as to the mode of origin of the cases in the spring of 1943, but possibilities existed for the importation of the disease either from the Russian front or from the Yugoslav front where Italian troops were engaged in hostilities. The disease caused only 10 notified cases in Naples city in March to May, 1943, none in June, two in July and then a gradually increasing number each month, until

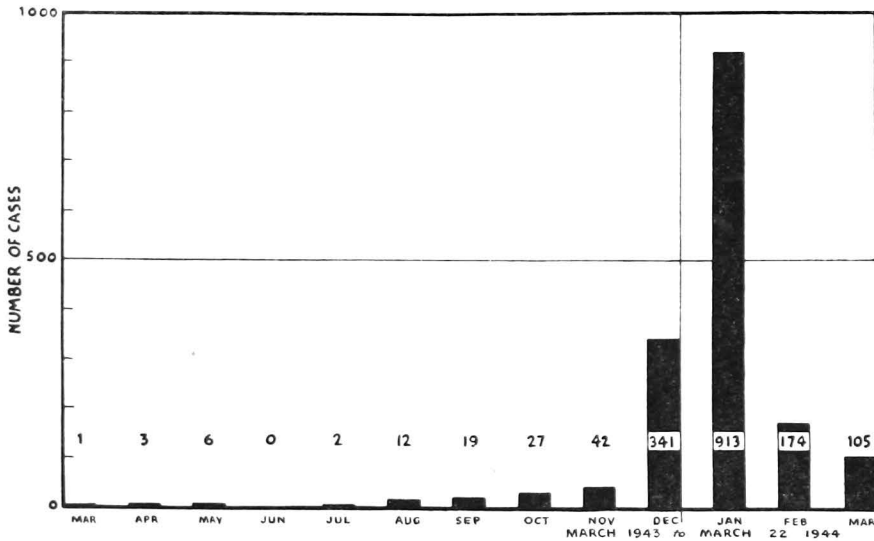


FIG. 2.—Cases of typhus reported monthly in Naples.

in December the curve of incidence rose sharply and the disease was considered epidemic. Fig. 2 records the reported cases each month, and Fig. 3 the daily reported new cases both in Naples and outside the city. It is seen that the curve for the city rose to a peak in early January, 1944, then fell sharply and underwent a further slow decline in February and March. From the standpoint of the present investigation, the form of the curve is of interest only inasmuch as the epidemic provided the clinical material which was studied. The chemotherapeutic trial of V186 was made on new cases admitted between the 2nd January and the 9th February, a period which thus included the time of maximal prevalence of the disease in the city. The later therapeutic and other studies were carried out on cases admitted between the 11th February and the 14th March. Many of the patients then came from outside Naples city, and this clinical material corresponded with the period of waning of the outbreak. Cases from outside Naples city represented groups of sporadic cases from multiple foci of infection, and in no area did the incidence become as high as in Naples itself. The case-finding scheme of the American Typhus Commission began to operate on the 2nd January, but the scheme was



### 34. CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

not fully organised until mid-January, when all the patients arriving at the hospital had been inspected, and their contacts examined for evidence of infection, by the officers of the Commission, before the patients left their houses or shelters. The factors concerned in the genesis of the outbreak will doubtless be discussed by others who had opportunity to investigate this particular problem. None could deny the importance of the crowded deep underground air-raid shelters (Plate I), from which many cases of typhus both living and dead were taken directly to hospital. In addition to the direct interference in normal activities which the war caused, the scarcity of food, of soap for washing and of opportunity for changing clothes must also have proved potent factors underlying the epidemic.

The age and sex incidence and mortality-rates during the three months of December, 1943, and January and February, 1944, for all reported cases admitted to hospital are recorded in Table VI. The figures were kindly provided by Dottore Bozzi, Civil Superintendent of the Cotugno Hospital. The table

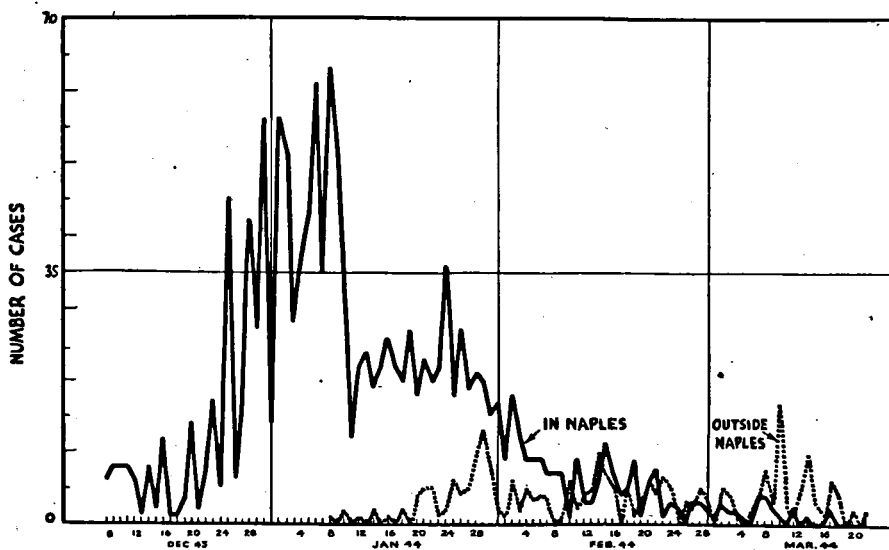


FIG. 3.—Daily reported new cases of typhus in Naples and outside the city.

shows the high incidence in children and young adults under 20, who comprised 45 per cent. of the patients. This may have been due to the fact that infection often occurred in families sheltering in the air-raid shelters and frequently involved the entire family group. It was certainly responsible in part for the remarkably low death-rate for the entire epidemic (14 per cent.), for, in general, the younger the patient, the less severe was the disease. Indeed, in babies and children the disease had an extremely mild course, with short fever, vague rash and only slight constitutional symptoms. The mortality for the whole outbreak can be judged in perspective only against the background of mortality figures for other typhus epidemics. Table VII compares the mortality by age-groups in patients admitted to the London Fever Hospital between 1848 and 1870 (Murchison, 1884), the figures for Europeans and Arabs with typhus during the epidemic in Algiers City in 1942 (by the courtesy of Major D. J. Davis of the Civil Affairs Section of the Allied Force Headquarters), and the figures for Naples. All groups show the same trend in mortality, with rising death-rate accompanying the increment in age, particularly over 40. The Naples figures, however, show a generally lower rate for each period,

PLATE I



Crowded deep underground air-raid shelter at Naples.

TABLE VI

*The Naples Epidemic—Sex and age and mortality: December, 1943–February, 1944*

Years.	Males.	Deaths.	Females.	Deaths.	Total Numbers.	Deaths.	Mortality Per Cent.
Under 3 ..	20	1	18	0	38	1	2.6
3–11 ..	133	2	91	1	224	3	1.3
12–20 ..	221	9	166	10	387	19	4.9
21–29 ..	105	11	108	10	213	21	9.8
30–38 ..	111	14	127	18	238	32	13.4
39–47 ..	59	29	116	28	175	57	32.5
48–56 ..	43	18	56	19	99	37	36.3
57–65 ..	10	7	27	15	37	22	59.4
66–74 ..	2	2	8	3	10	5	50.0
75 and over..	1	1	1	1	2	2	100.0
Gross ..	705	94	718	105	1,423	199	13.9

TABLE VII

*Comparison between typhus in the London Fever Hospital 1848–1870, Algiers 1942 and Naples 1943–1944*

Age Groups.	London, 1848–1870.		Algiers, 1942.				Naples, 1943–1944.		
	Num- bers..	Mor- tality per cent.	French.		Arabs.		Age Groups.	Num- bers.	Mor- tality per cent.
			Num- bers.	Mor- tality per cent.	Num- bers.	Mor- tality per cent.			
0–9 ..	1,430	4	20	10.0	76	6.6	0–11	262	1.5
10–19 ..	5,121	3.5	72	7.0	317	7.3	12–20	387	4.9
20–29 ..	4,127	12.3	62	17.8	620	9.2	21–29	213	9.8
30–39 ..	2,976	23.2	79	32.9	368	18.4	30–38	238	13.4
40–49 ..	2,546	35.5	79	44.5	246	26.0	39–47	175	32.5
50–59 ..	1,231	51.1	73	67.2	111	50.5	48–56	99	36.3
60–69 ..	588	65.1	} 42	} 81.0	} 54	} 53.6	57–65	37	59.4
70–79 ..	116	75.8					66 and over.	12	58.3
80 and over	3	100.0							
Gross ..	18,268	18.92	457	35.8	1,846	16.8	—	1,423	13.9

compared with the figures for British and French Europeans, and resemble more closely the Arab figures. The low fatality rate between the ages of 30 and 38 (13.4 per cent.) is particularly striking when compared with the figures for the British (23.2 per cent.) and French European patients (32.9 per cent.), and is actually lower than the Arab figure (18.4 per cent.). The generally low fatality-rate for young adults also contrasts with the death-rate for cases of typhus fever in unvaccinated British soldiers in the Middle East and North Africa, which in January to June, 1943, was 32 per cent. in 134 patients. It may be that the type of typhus experienced on the continent of Europe is less virulent than that occurring in the Middle East or North Africa. There are few reliable figures for comparison, but it is known that in Poland before 1938 the fatality varied greatly in different epidemics and was sometimes as low as 6 per cent. and sometimes as high as 20 per cent. (Mosing, 1938). Another similarity between typhus in Poland and that in Naples was the high incidence

## 36 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

in children. Mosing recorded a 61 per cent. incidence of the disease in ages under 20 in 307 cases examined and proved to be typhus in 1936; this may be compared with the figure of 45 per cent. in the Naples epidemic.

Finally, it must be emphasised that the Naples outbreak was practically confined to Italian civilian and military personnel. All patients studied at the Cotugno Hospital, and described below, were Italian and the majority were Neapolitan.

### DIAGNOSTIC CRITERIA AND DIFFERENTIAL DIAGNOSIS

The same criteria with regard to diagnosis were applied to the Naples cases as to the cases in North Africa. In every case attempts were made to exclude enteric fever, relapsing fever and malaria. The final diagnostic criterion was considered to be the recovery of rickettsiae from individual patients by animal inoculation, and of 19 patients investigated in this manner 17 produced transmissible febrile infections in the guinea-pig which were later established as rickettsial in origin (see below). In the absence of proof by the recovery of the causative organism, diagnosis rested on the demonstration of changes in the agglutination reactions to organisms of the *Proteus OX* group, and to rickettsiae as described above. There was no real opportunity to study the differential diagnosis of typhus in Naples, as was done in North Africa, because the Cotugno Hospital only admitted carefully sifted cases. Enteric fever was occurring in Naples during the epidemic, but only in small numbers of cases; only one case of possible enteric fever was seen, and in this the blood culture was negative and death occurred before final serological proof had been obtained; typhus seemed to be an equally probable diagnosis, but no post-mortem examination was made (Naples 2). Purpura accompanying a severe anaemia was seen once, and also purpura in a case of agranulocytosis following arsenical therapy for syphilis. A fatal pyaemia due to the staphylococcus also occurred in one patient who was regarded and treated as a case of typhus during life (Naples 52). A small number of cases of smallpox occurred in Naples during the outbreak, but differential diagnosis presented no difficulty. There were no other real diagnostic problems, and few patients were encountered in the selected groups in whom clinical and pathological findings were insufficient to support the diagnosis of typhus on the basis of the criteria which had been laid down. Complicating diseases were also conspicuous by their absence, as described below in the section dealing with Complications and Sequelae (p. 59).

### SELECTION OF CLINICAL MATERIAL STUDIED DURING THE INVESTIGATION

During the entire period of January to March, 1944, some 104 patients, of whom 93 satisfied the diagnostic criteria for typhus, were admitted to the British wards. They were treated in three different ways and formed three main groups of cases:—

- (1) Patients treated with the chemotherapeutic agents, V186 and V147.
- (2) Patients treated with various chemotherapeutic agents, such as sulphathiazole, sulphaguanidine and penicillin, or who had been inoculated with typhus vaccine.
- (3) Patients treated with general supportive but not chemotherapeutic measures—"control group". The patients who belonged to this category were admitted in January for study as "controls" to the patients treated by the chemotherapeutic agents V186 and V147, and later, when this trial was completed, they were admitted for study of the biochemistry and pathology of typhus.

The basis of selection must be described in detail, because the clinical material upon which these observations were made was a selected group and did not, therefore, represent a true cross-section of the individuals suffering from typhus and admitted to hospitals during the epidemic. All new patients admitted to the hospital with a diagnosis of typhus were seen initially either in the waiting rooms prior to delousing, in the bath house, or in the wards after admission. They were questioned as carefully as was possible with the aid of an interpreter, and an attempt was made, on the basis of history and physical signs, to decide upon the exact duration of illness.

During the period of the chemotherapeutic trials of V186 and V147 every effort was made to select patients in the early stages of the disease and also patients whose illnesses, if untreated, were likely to be sufficiently prolonged for assessment of the various therapeutic measures which would be applied. Patients who were at least 15 years of age, who had not been ill for longer than seven days and who appeared to be suffering from typhus on the basis of history or physical findings, were chosen. In the first week of the investigation, a few patients who had been ill for longer than seven days were selected because of an absence of other, more suitable, new patients. In the last two weeks of the drug trial, preference was given to patients more than 30 years old, in order to include as many as possible of the older patients who were more gravely ill than the younger ones, but these older patients were still chosen on the basis of the duration of the disease. The aim to include only patients in the first week of their disease was frequently frustrated by the fact that, after admission to the British wards, a different history—which placed the onset of the illness at an earlier date—was obtained. Patients were also included who could give no coherent history of illness, on account of loss of memory or of delirium, but who were thought to be at an early stage of their disease because of a history obtained from relatives or because of the physical findings. The exclusion of patients under the age of 15 was decided upon because of the mildness of the disease and the irregularity of its course in children. In consequence of this exclusion, almost no detailed observations were made on the manifestations of typhus in children.

When patients had been selected for study, they were transferred to the British wards and, upon arrival, were allotted to either "Treated" or "Control" categories on an alternate-case basis, the chemotherapeutic agents V186 and V147 being given only to the "Treated" group. Certain patients in both categories were eliminated from study after the completion of the trial, either because the diagnosis had been changed or because insufficient evidence for a diagnosis of typhus was obtained from the pathological findings. There remained 30 patients in the "Treated" group who satisfied the diagnostic criteria and who received V186 and V147, and three other patients who were treated with V186 and in whom the diagnosis of typhus, though probable, was insufficiently proved. There were 30 patients in the "Control" group who satisfied the diagnostic criteria and who were treated by non-specific means, and two more patients in whom the diagnosis, though probable, was not proven. Two further patients originally allotted to the "Control" series were treated with sulphathiazole because of pulmonary complications, and have, therefore, been withdrawn from the "Control" group.

Upon completion of the trial of V186, an interval of one week elapsed before trial of V147 was begun, and during this week and after completion of the trial of V147, patients were chosen for study on a slightly different basis. Late cases of the disease were not excluded, although preference was given to early cases. At the same time, no attempt was made to allot patients into strictly alternate "Treated" and "Control" groups, and sulphaguanidine or penicillin was administered to some of the patients. Sulphathiazole was given during the acute stages of the disease to two patients with severe

pulmonary complications in addition to those mentioned above. Also, seven patients who developed suppurative lesions of various types after the conclusion of the acute stage of the disease received sulphathiazole. Three of these were treated during the acute stage of the disease with V186, and four others received only supportive treatment during the acute stage and belong to the "Control" series. In the final stages of the study, a few late and moribund cases were transferred from the other wards of the hospital for investigation of renal function, and the inclusion of such cases in the "Control" group influenced the mortality figures for the series of patients treated by non-specific means. A total of 50 patients who received only supportive therapy during the acute stages of the disease, therefore, supplied the clinical material analysed below.

#### CLINICAL PICTURE OF TYPHUS FEVER IN PATIENTS TREATED NON-SPECIFICALLY

*The Patients.*—The patients were chiefly drawn from the lowest levels of society, and arrived at the hospital ill-clad and extremely dirty. Though few were suffering from extreme malnutrition and many were actually well nourished, the majority had probably been living on a restricted diet for some weeks. The diet had been particularly deficient in protein and fat, and had often consisted of vegetables, such as cauliflower and carrots, with a little bread. Meat and milk had been available to the privileged few who could afford to buy from the "black-market", but not to the majority. Citrus fruits were freely available in the months preceding the outbreak. The nutritional state of the babies and infants seen during the outbreak made it appear probable that a basically low level of dietary intake had existed in the area for many months. The presence of lice on the patients prior to admission was difficult to establish, owing to the treatment with insecticide powder which had been given in the homes by a member of the public health organisation at the time when the reported diagnosis was confirmed. Occasional instances of extremely lousy individuals were encountered, and nits were frequently found on the hair or on the clothes. Delousing was carried out firstly by treatment of hair, skin, and clothes with insecticide powder in the waiting room; then by stripping off the clothes, shaving the hairy parts and treating the hairy skin with paraffin (kerosene) in the delousing room. Finally, the patient was transferred to another room, where a bath was given; he was then wrapped in blankets and taken to the wards. All patients transferred to the British wards received a further blanket bath; the shaving was checked and the hairy parts were powdered with insecticide powder before the patients were put to bed. The active principle of the insecticide provided was DDT. It was obtained through the courtesy of the American Typhus Commission. During the entire period of the investigation, no lice were seen in any of the wards of the hospital, although nits were found on body hairs and on the string of a necklace on two occasions. The skins of many of the patients were sallow, pigmented and scarred from old scabietic and acneiform lesions, and scratch marks resulting from the irritation produced by the lice were frequently seen. It is extremely probable that the majority of the patients had been lousy at some time.

Table VIII indicates the age and sex distribution and the fatality-rates for the entire group of 50 "Control" patients. Comparison with the figures for the whole outbreak, in Table VI, emphasises the selected nature of the material which was studied. The ages of the patients varied from 15 to 62, and slightly more males than females were included in the group. The fatality-rate was correlated to some extent with age, but the groups were too small for statistical significance. The average age of the 12 fatal cases was 43.6; and of the 38 non-fatal cases, 31.

TABLE VIII

*Age, Sex Distribution and Fatality Rates of 50 "Control" Patients*

<i>Age Group</i>	<i>Male</i>	<i>Female</i>	<i>Total</i>	<i>Deaths</i>	<i>Fatality-rate per cent.</i>
15—19	6	3	9	0	0
20—29	11	4	15	1	6.6
30—39	4	6	10	4	40.0
40—49	2	5	7	2	28.6
50—59	5	2	7	3	42.8
60 and over	0	2	2	2	100.0
Total ..	28	22	50	12	24.0

*Symptomatology*

Emphasis on the difficulty of linguistic interpretation in these patients is needed, as it formed a serious barrier between doctor and patient. Symptoms were particularly difficult to record, histories were sometimes inaccurate, and the mental states of the patients were very hard to assess.

(a) *Onset.*—The onset of illness was acute in 24 (80 per cent.) of the 30 patients from whom a clear history was obtained, but the exact time of day when the first symptom was noted could rarely be remembered. In six patients the onset was insidious, with steadily mounting degree of illness; and in one of these, and one other with more acute onset, premonitory symptoms such as mild headache or aching of the teeth preceded the main complaint by a few days. The commonest initial symptoms were headache, cold feelings or shivering. No actual rigor occurred, but the feeling of coldness persisted, although the presence of fever was frequently verified by the patient and temperatures as high as 104° F. were reported within a day or two of the onset. Pains in the back, limbs, neck, eyes, or all over the body were soon experienced; the patient began to feel weak and ill, and took to bed. The appetite disappeared, slight nausea or vomiting occurred and constipation set in. There was often slight cough, usually without sputum, within a few days of the onset, at a time when headache, chilly sensations and fever were persisting. Towards the end of the first week of the disease, the patient became aware of increasing prostration, and by this time, when visited by a doctor, he was found to exhibit a rash, and was at once sent into hospital. Patients who were sent into hospital at this stage of the disease usually complained very little once they were put to bed, but headache, or cough, or constipation sometimes needed treatment. Mental symptoms were not usually severe at the onset or during the first week, but a few patients were confused or delirious on admission, although the relatives insisted that illness had only been present for a few days. Patients arriving at hospital after the first week were frequently confused, had lost all memory of the details of their earlier symptoms, and did not know the date or day of the week. Nevertheless, some patients could remember the onset, as may be seen by the fact that among the 30 patients who gave clear histories, three arrived in hospital on the fourth, four on the fifth, four on the sixth, eight on the seventh, four on the eighth, three on the ninth, and one each on the tenth, eleventh, twelfth and fifteenth days of illness.

(b) *Details of Symptoms.*—The history of illness was discounted in 16 of the complete group of 50 patients, because of delirium (seven patients), confusion (four), defective memory (two), coma (one), deafness (one) and lack of intelligence

TABLE IX

*Symptoms in 34 "Control" patients*

	<i>Numbers.</i>	<i>Per Cent.</i>
Onset known to be acute .. .. .	24	80
Onset known not to be acute .. .. .	6	20
Shivering .. .. .	34	100
Headache .. .. .	30	88
Malaise .. .. .	30	88
Cough .. .. .	23	67
Muscular pains .. .. .	20	59
Anorexia .. .. .	19	56
Constipation .. .. .	16	47
Deafness† .. .. .	14	41
Photophobia .. .. .	11	32
Dizziness .. .. .	10	29
Vomiting .. .. .	9	26
Sweating .. .. .	8	23
Insomnia .. .. .	6	17
Sore throat .. .. .	3	9
Abdominal pain .. .. .	2	
Ocular pain .. .. .	2	
Abdominal pain on coughing .. .. .	2	
Chest pain on coughing .. .. .	2	6
Epistaxis .. .. .	2	
Diarrhoea .. .. .	2	
Jaundice .. .. .	1	3

*Symptoms developing after admission and not present before*

Hoarseness of voice .. .. .	9	
Deafness .. .. .	6	
Expectoration, simple mucoid .. .. .	5	
Expectoration, with blood-stained sputum .. .. .	2	
Diarrhoea .. .. .	6	
Vomiting .. .. .	3	
Dysphagia .. .. .	6	
Hiccough .. .. .	2	
Haematemesis .. .. .	1	
Haematemesis and melaena (terminal) .. .. .	1	
Jaundice (terminal) .. .. .	2	

} from entire group of 50 patients.

(one). There were, therefore, 34 patients whose symptoms were recorded in detail and in whom the percentage frequency of the various symptoms could be calculated (Table IX).

The symptoms, in order of frequency, were shivering, headache, malaise or feeling of illness or weakness, cough, muscular pains (limbs, back or generalised), anorexia and constipation, all of which occurred in half or more of the patients. Deafness, photophobia, dizziness and vomiting occurred in 25 to 40 per cent., and sweating and insomnia occurred in less than one-quarter of the patients. Pain in the chest on coughing, pain in the abdomen on coughing, abdominal pain, epistaxis, pain in the eyes, and diarrhoea each occurred in only two patients (6 per cent.), and sore throat occurred in three (9 per cent.). One patient was jaundiced on admission. In addition to these symptoms, which mostly occurred before the patients were admitted to hospital, there were others which developed after admission and which could be checked. There was reason to think that diarrhoea developing only after admission was probably due to a ward infection. It was remarkable how rarely headache, severe enough to need medication, was encountered after admission. Nevertheless, it did occur in some patients, and may have been experienced by others whose complaints were not understood because of the difficulty in interpretation.

† Deafness was frequently present before admission, but appeared to get worse in several patients during the illness.



*Course of the Disease*

The course of the disease differed so much in the various patients that at first it seemed impossible to classify the cases in any way which would separate groups of varying grades of severity of illness.

We failed to devise an entirely satisfactory scheme on the basis of height or duration of fever, degree of prostration, the type of rash, or the degree of cardiovascular or renal abnormality: on the whole the mental symptoms appeared to be the most useful single factor on which to base a classification. We finally separated the 50 "Control" patients into three groups of mild, moderately severe and grave (see Tables X to XII), taking into consideration all aspects of the disease. The course of the disease differed in these three groups, and will be separately described. The ordinary case of moderately severe typhus which was representative of the great number of the non-fatal illnesses will be considered first. Examples of all three groups are summarised in Appendix A.

*Typical Course of Moderately Severe Typhus*

There were 20 patients in this group, none of whom died. The average age of the patients was 28 years. The patient who was admitted towards the end of the first week of the disease was usually mentally clear and gave a good history of his illness. There was a moderate degree of prostration, and usually a complaint of headache and feeling cold. High fever in the region of 103-104° F. was present on admission, together with tachycardia (110-120) and slightly raised respiration rate (about 30 per min.). A generalised macular eruption was present on the trunk and limbs, with particular preference for the sides of the abdomen and thorax, the deltoid and posterior axillary regions, the forearms, backs of the hands and knees. The macules were not usually palpable; they were pink in colour and faded on pressure, and the intervening skin often showed an indeterminate flush (subcuticular mottling). The face, which showed no rash, was usually flushed, the lips a little cyanosed; and the expression at this stage was reasonably alert. The eyes showed either a vague suffusion and slightly oedematous appearance, or frankly dilated vessels which were often distributed in bands radiating from the cornea. The conjunctiva of the eyelids showed general redness and perhaps knots of dilated vessels. The tongue was moist and mostly coated with white fur, but even at this stage, the central one-third was often drier and tinged with brown fur. The fauces were normal, and the voice was clear. There was often slight enlargement of the axillary or epitrochlear lymph glands. The cardiac impulse could rarely be palpated, the heart was not enlarged to percussion, but the heart sounds were fainter than normal, particularly in the case of the first sound. The pulse was regular but easily compressible, and the blood pressure was usually lowered to 80-90 systolic and 60-70 diastolic. The lungs, though resonant, showed rhonchi or perhaps a few inspiratory râles at one or other base, but the cough, which was frequent at this stage of illness, was usually non-productive. The abdomen was usually relaxed, and the spleen was often enlarged, its tip being palpable one or two fingers' breadth below the costal margin on deep inspiration. Tendon reflexes were usually normal. The urine, which was concentrated, was either normal or contained a light cloud of albumin with a few granular or hyaline casts, but no red blood cells nor excessive numbers of leucocytes.

Upon admission, the patient was generally quiet and co-operative and took fluids by mouth with readiness. Micturition was usually voluntary, but there was often constipation. During the daytime patients remained asleep unless roused, and did not complain; but at night, particularly in cases with high fever, there was sometimes mild delirium or confusion, and then the

## 42 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

patient was noisy and restless. During the first few days after admission, the rash sometimes appeared more evident and some of the macules, which ceased to fade on pressure, developed haemorrhagic centres. At the same time, petechial haemorrhages might be visible between the macules, in areas of pressure such as the axillary folds or ante-cubital fossae or in the conjunctivae of the lower eyelids. High fever with tachycardia usually continued; and by about the tenth or eleventh day, the patient appeared much more ill, lethargic and prostrated. The expression was duller, the mentality dazed and confused, and mild delirium with loss of memory for earlier events was sometimes shown. There was now an unco-operative frame of mind, with reluctance to drink; and in general the patient resented interference and desired chiefly to be left alone. Stiffness of the neck was present in some cases. The limbs appeared hypersensitive, and passive movements were painful and resented. The face now had a dusky or violaceous hue, the respirations were increased in rate (30-40 per min.) and perhaps slightly laboured, and the voice was slightly hoarse. The tongue was drier, and fur was brown in hue. The heart sounds were even softer, and the blood pressure was at its minimum (70/50) with variable systolic levels in the individual beats. The added sounds in the lungs were usually increased, but cough was often slight and expectoration minimal. The urinary output often underwent a diminution relative to the intake of fluid, but the urine had, if anything, a lower specific gravity than normal (1015). The skin often creased abnormally easily on the back and the limbs, and appeared slightly oedematous. This drowsy, confused and irritable stage in some cases developed even further, and the patient might be stuporose for a day or two, with slurred, muttering speech, dysphagia, stiffness of the neck and even tremor of the lips or jerky movements of the face. Incontinence of urine and even of faeces was common, although this sign was of little value in women, who were frequently incontinent from the day of admission. In other patients, and eventually even in the more seriously ill ones, an improvement set in, often with sudden remission of the fever either with or without sweating; and from the fifteenth day onwards, when the temperature had reached normal by lysis, all symptoms had lessened and the patient gradually became calm, rational and co-operative. The rash had often begun to fade even before the fall in temperature; the macules disappeared first, and the haemorrhagic elements faded more slowly. The tongue had become moist again and the fur was peeling off. The blood pressure had risen, and the heart sounds improved somewhat in quality. The signs in the chest, and splenomegaly if present, usually persisted during early convalescence. A particular feature at this time was an increase in urinary output, which often equalled or even exceeded the daily intake of fluid; and the urine was usually pale, of low specific gravity and without albumin or abnormal cellular constituents. The improvement in the patient's spirits was often dramatic and shown by a desire to sing, while the appetite usually returned abruptly and the patient clamoured for food. Convalescence was soon established, and it was surprising how soon even those patients who had been severely ill desired to get up. At this stage, however, wasting was usually obvious, the haemorrhagic elements of the rash were often visible, abnormal signs in the chest present, and the blood pressure lower than normal. By the time two weeks had elapsed after defervescence, most of these phenomena had disappeared and the patient, though shaky when up, was able to be discharged. Some patients developed suppuration, usually where they had received injections prior to admission to hospital, and abscesses which required drainage were formed. Such, then, was the average course of the disease, and the manner in which the various patients deviated from this is seen by reference to Table X. Attention is drawn to patients who showed severe degrees of involvement of the chest, with extensive signs of basal bronchiolitis or

## THE NAPLES, 1917

even frank consolidation, and to others in whom the heart was apparently more severely involved, as shown by the development of triple rhythm or by evidence of cardiac dilatation. Diarrhoea was also a troublesome symptom in certain patients, and the state of apparent dehydration seen in many was enhanced by this and necessitated the administration of parenteral fluid in the form of glucose-saline as a supplement to oral fluid.

### *The Course of Illness in Graver Forms of Typhus*

The course of the illness indicated a graver type of disease in 19 patients, 12 of whom died. The average age for the whole group was 40. The seven gravely-ill patients who recovered included some who passed through a course of illness not much different from that described above, but with more severe prostration or with severe nervous symptoms, such as low muttering or agitated delirium, or a period of prolonged stupor. In some, the development of severe delirium was thought to portend a fatal result, but complete recovery nevertheless occurred. Many of the fatal cases exhibited a characteristic course, which began in a similar fashion to that already described above. A sudden deterioration from the state of high fever and mental lucidity then occurred, and the patient lapsed into a groaning, muttering delirium, with frequent twitching of the muscles of the face and forearms, or gross muscle-group movements, and the upper limbs developed flexor rigidity. Simultaneously with this, there was often an accentuation of the haemorrhagic character of the rash, the appearance of slight oedema of the skin, increased suffusion of the eyes, a fall in pulse volume and blood pressure, and a diminution in urinary output. Temporary suppression of urinary excretion might occur, and, when catheterised, the bladder was in this event empty. The abdomen was often tense and perhaps actually distended. Deterioration usually continued from now on; delirium deepened into stupor or even coma, and the patient died without regaining consciousness. However, some improvement in the pulse volume and blood pressure was often seen before death, and secretion of urine often recommenced, though the urine was then of low specific gravity and albumin and casts were present. A period of prolonged, perhaps afebrile, coma ensued in some patients after the delirious stage, and then the patient might lie prostrate and extended for several days before death. Terminal pneumonia supervened in some such patients who were seen in other wards of the hospital, though not in any of the patients in this series. Other fatal cases exhibited, on admission, severe delirium or coma; and in them profound dehydration and subnormal temperature were also seen, with a grave degree of cardiovascular involvement, as evidenced by extreme feebleness of the pulse, blood pressure too low to register, faint heart sounds with tic-tac rhythm, and a generally cold cyanotic condition of the extremities suggesting peripheral vascular failure. Actual heart failure of congestive type was diagnosed in only one patient, who developed an acute dilatation first of the left side of the heart and then of both left and right sides; he had only mild delirium and confusion, and died essentially of cardiac failure (Naples 77). Two other patients, however, had sufficient dyspnoea to render a diagnosis of cardiac failure probable. Gangrene of the toes, of areas exposed to pressure such as the buttocks and sacrum, the trochanters or the scapula, was found in the type of patient with extreme cardiovascular collapse and was an extremely bad prognostic sign. The lungs did not necessarily show more extensive involvement in the gravely-ill patients than in the milder cases, and some fatal cases exhibited only rhonchi or a few râles. Extensive areas of râles, usually at the bases but spreading upwards and involving the trachea terminally, did occur, however, and were attributed to pulmonary oedema in some patients and to bronchiolitis in others. Two patients with evidence of actual consolidation, and one with severe bronchiolitis,

## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Had a complete recovery. The tachypnoea seen in many patients was not always terminal; in some cases it was attributed to a nervous system lesion, in others to cardiac or pulmonary involvement, and in some at least to acidosis or uraemia. In contrast with the deep stertorous type of breathing seen in the latter patients, rapid shallow respirations were seen in others, particularly in the comatose. Loss of moisture through the open mouth certainly exaggerated the dryness and dirtiness of the tongue in these patients, and the dried organ could not readily be protruded beyond the teeth. There was a tendency for the rash to be more haemorrhagic, although not more extensive, in the fatal cases, and one such case had only a trivial rash. Death occurred between the eighth and nineteenth days of disease in the six patients who died and whose onset of illness was known; in the other fatal cases, the time elapsing before death could be estimated only on a basis of duration of hospitalisation. Five patients died within 36 hours of admission, one died on the fourth, and the others between the fifth and eighteenth hospital days. The general correlation between mortality and age, though suggested by the findings in Table VIII, is best seen by reference to the figures for the entire epidemic. The increased fatality-rate above the age of 40 years is then obvious (Table VI).

### *The Course of Illness in the Mild Cases of Typhus*

There were 11 patients whose illnesses were milder than that of the average or moderately severe case. They probably were representative of a large group of such patients who fell ill during the outbreak but who were not usually included in the cases selected for study, because of the triviality of the illness, lateness in gaining admission to hospital or atypical nature of the rash. It is not possible to describe an average course of mild typhus; but trivial or short duration of fever, absence of marked prostration, lessened tendency to tachycardia and to involvement of the nervous system and lungs, were all noted. Most cases had a rash, but this was slight in extent in six patients. Haemorrhages in the form of petechiae, and in two patients actual purpuric spots, were, however, seen, but no patient had conjunctival haemorrhages in the lower lid. Most patients showed some rise in the pulse rate, and some had lowered blood pressure, but often the heart sounds were good in volume and did not diminish during the course of the disease. No abnormal signs in the cardiovascular system were detected in four patients, nor in the respiratory tract of six. After a few days in hospital, these patients lost their fever, and convalescence was, in general, more rapid than in the more severe types. Dehydration and mental dullness or mild confusion were all encountered in individual patients, but they were all transient signs which soon disappeared after admission and routine treatment. The average age for this group of 11 mildly-ill patients was 28 years, as in the case of the moderately-ill patients.

### *Analysis of the Physical Findings*

Tables X to XII give a classification of all the patients, and a description of some of the physical findings, and Table XIII gives the results of detailed analysis of the physical findings in all the patients.

*The fever.*—The temperature of all but the first few patients who were admitted for study was recorded rectally every four hours until convalescence was established. The fever was remittent in 16, continued in 13, continued and then remittent in 9 and irregular in 10 patients. There was no fever in two patients, one of whom died. The duration of illness from the day of onset to the day of defervescence was calculated in 23 patients in whom the day of onset was accurately known and who survived the illness. It varied from 10 to 18 days and averaged 14 days. A temperature of 104° F. or over was reached and sustained for a variable period in 26 patients (52 per cent., see Table XIII).

## THE NAPLES STUDY

The temperature subsided by lysis in 32 of 37 febrile patients who rec (86 per cent.) and by crisis in five (14 per cent.). Either a steady fall of ture occurred or sharp remissions of temperature followed by a ris on one or more days preceding the establishment of normal levels.

During the fever, the pulse rate was almost invariably raised, although not usually disproportionately to the temperature. A relative bradycardia was recorded in only two patients during the fever. True bradycardia was not commonly seen during convalescence, but two of 38 patients had pulse rates of 50 or below at this time.

The respiration showed an increased rate and other features to be expected in febrile patients, many of whom had some bronchitis. Apart from the moribund patients, 10 had dyspnoea and four orthopnoea, associated in three with cardiac dilatation, but in only one with definite evidence of congestive failure.

Cyanosis was present to a variable degree in 27 of the 50 patients. The slight cyanosis of the lips, seen on admission, was often succeeded by a dusky or violaceous tint to the facial flush, which lasted until defervescence had set in. A cold cyanosis of the extremities, of the finger-nails, fingers, toes and areas of incipient gangrene—together with a cyanotic hue of the elements of the rash—was seen in gravely ill patients, particularly those with peripheral cardiovascular failure. The livid veins of the extremities in these patients (unlike those of patients with congestive failure) were collapsed and presented extreme difficulty for venepuncture. The blood obtained from such veins was very dark and clotted rapidly.

*Facies, appearance of the eyes, tongue and rash.*—Flushing of the face was seen in 26 (52 per cent.) of the patients on admission or within a few hours of admission, and this, together with the appearance of the eyes, gave the patient a striking facies. As the disease progressed, the early bright pink flush (which often avoided the circum-oral area) became dusky in hue and ultimately faded. At this time also, the alert expression was replaced by a dull heavy one, with drooping eyelids suggesting slight ptosis. In patients with severe cerebral involvement, frequent twitchings or choreiform movements of the face sometimes developed while the face was still flushed, thus again presenting a striking picture. The expressionless facies of the comatose patient—in which the eyes were sometimes open, the lower jaw was dropped and the facial wrinkles were smoothed out—was also characteristic.

The eyes were abnormal in all but two of the 50 patients, but in 18 there was only slight glistening of the conjunctiva or a mildly oedematous appearance. Injected conjunctival vessels were seen in 27 patients (54 per cent.) and blepharitis occurred in three. The presence of haemorrhagic splashes or petechiae in the lower lids was noted in 11 patients, of whom three died and eight recovered. The sign was particularly valuable for diagnosis in patients in whom the rash was ill-developed, or who had no rash (Avtsin, 1943). The fundus oculi was examined in only a few patients; no abnormalities were found.

The condition of the tongue is usually regarded as a sign of great prognostic significance in typhus fever, and the prognosis as bad if the tongue is persistently dry (Wolbach, Todd and Palfrey, 1922). In our patients, no strict relationship seemed to exist between the prognosis and the dryness or degree of furring of the tongue, although no fewer than 10 of 18 patients who exhibited a dry tongue on admission to hospital suffered grave illnesses. This lack of correlation may have been due to the rigorous toilet of the mouth, and to the care with which a good intake of fluid was maintained. Severely ill or moribund cases not under our care exhibited the classical dry cracked or shrivelled tongue with heavy deposit of brown fur. We noted tremor of the tongue at times, but usually in patients exhibiting tremor of the face or arms.

CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE  
Moderately

Patient's number.	Sex.	Age.	Duration of fever (days).	Duration over 104° (days).	Pulse.	Respiration.	Dyspnoea.	Chest.			Cardio-vascular.				Renal.		Skin.		
								Bronchitis.	Bronchiolitis.	Pneumonia.	Low B.P.	Triple rhythm.	Dilatation.	C.V. S. collapse.	Oliguria.	Albuminuria.	Rash extent.	Rash haemorrhage.	
65	♀	52	18	1	130	30	0	+	0	0	+	0	0	0	0	0	0	++	Pet.
35	♂	18	14	0	120	30	0	0	+	0	+	0	0	0	0	+	+++	Pet.	
73	♀	15	17	7	130	40	±	0	0	+	+	0	0	0	0	0	+	Pet.	
49	♀	37	14	6	120	30	0	+	0	0	+	0	0	0	0	+	0	0	
53	♀	38	16	1	110	30	0	+	0	0	+	0	0	0	0	0	+	Pet.	
41	♂	24	15	8	120	30	0	+	0	0	+	0	0	0	0	+	+	0	
26	♂	34	14	3	120	30	0	0	+	0	+	0	+	0	0	+	+++	+	
57	♂	25	14	4	110	30	0	+	0	0	+	0	+	0	0	0	++	+	
37	♂	24	16	2	130	30	0	0	0	0	+	0	0	0	+	+	++	+	
67	♂	23	16	5	130	35	0	0	0	0	+	0	0	0	0	+	+	0	
17	♂	15	15	2	130	30	+	+	0	0	+	+	0	0	0	0	++	+	
25	♂	50	?	1	110	30	0	0	0	0	+	0	0	0	0	+		+	
98	♀	24	14	5	120	30	0	+	0	0	+	+	+	0	0	+	++	0	
75	♂	17	?	3	120	35	0	+	0	0	+	0	0	0	0	0	+++	+	
84	♀	26	?	0	120	25	0	+	0	0	+	0	0	0	0	0	++	Pet.	
85	♀	26	13	0	130	30	0	0	0	0	+	0	0	0	0	0	+++	Pet.	
88	♀	40	?	0	90	20	0	0	0	0	+	0	0	0	0	0	++	Pet.	
95	♀	19	13	0	140	35	0	+	0	0	0	0	0	0	0	0	+++	+	
23	♂	17	13	8	120	35	0	+	0	0	+	0	0	0	0	+	++	Pet.	
47	♂	46	14	2	110	30	0	+	0	0	+	0	0	0	+	+	0	0	

? = Unknown

THE 'NAPLES STUDY

X

Severe Typhus

C.N.S. signs.						Mental state.						Miscellaneous.
Incontinence urine.	Incontinence faeces.	Changes in reflexes.	Twitching.	Tremor.	Meningism.	Symptoms.						
+	0	0	0	0	0	None .. .. .	..	..	..	..	..	Severe deafness; diarrhoea; sacral sore.
0	0	0	0	0	0	Nocturnal restlessness	..	..	..	..	..	—
+	0	0	0	0	0	Simple drowsiness	..	..	..	..	..	Otitis media.
+	+	0	0	0	0	Simple drowsiness	..	..	..	..	..	Ocular petechiae; diarrhoea.
+	0	0	0	0	0	Simple drowsiness	..	..	..	..	..	—
0	0	0	0	0	0	Dazedness	..	..	..	..	..	Congenital heart disease.
0	0	0	0	0	0	Mild delirium	..	..	..	..	..	—
0	0	0	0	0	0	Mild delirium, noisy and restless	..	..	..	..	..	Abscess thigh.
+	0	0	0	+	0	Dazed, mild delirium	..	..	..	..	..	Diarrhoea.
+	+	0	0	0	0	Dazed, mild delirium	..	..	..	..	..	Diarrhoea.
0	0	0	0	0	+	Confusion, chiefly nocturnal	..	..	..	..	..	Abscess buttock.
+	0	0	0	+	0	Dull, confused	..	..	..	..	..	—
+	0	0	0	0	+	Drowsy, confused	..	..	..	..	..	Diarrhoea.
0	0	0	0	0	0	Confused, drowsy, mild delirium	..	..	..	..	..	—
+	0	0	0	0	0	Confused, mild delirium, irritable, drowsy	..	..	..	..	..	—
+	0	0	0	0	+	Confusion, mild delirium, drowsy, dull	..	..	..	..	..	—
+	0	0	0	0	+	Confusion, mild delirium, dazed	..	..	..	..	..	Dehydrated.
+	+	+	0	0	+	Confusion, mild delirium, dazed, drowsy	..	..	..	..	..	Icterus; abscess buttock.
+	0	0	0	0	+	Confusion, transient stupor, dysphagia	..	..	..	..	..	—
+	0	0	0	0	0	Confusion, transient stupor, dysphagia	..	..	..	..	..	Ocular petechiae.

Pet. = Petechiae

CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE  
Grave

Patient's number.	Sex.	Age.	Duration of fever (days).	Duration over 10 <sup>4</sup> (days).	Pulse.	Respiration.	Dyspnoea.	Chest.			Cardio-vascular.				Renal.		Skin.	
								Bronchitis.	Bronchiolitis.	Pneumonia.	Low B.P.	Triple rhythm.	Dilatation.	C.V.S. collapse.	Oliguria.	Albuminuria.	Rash extent.	Rash haemorrhage.
33	♂	22	?	2½	130	30	0	0	0	±	+	+	0	0	0	+	++	Pet.
100	♀	30	?	0	130	35	0	+	0	0	+	0	0	0	0	0	++	+
69	♂	20	13	2	110	30	0	+	0	0	+	0	0	0	+	++	+++	+
15	♂	28	10+	4	140	50	+	0	+	0	+	0	0	0	?	0	+++	+
79	♂	51	13	0	115	30	0	+	0	0	+	0	0	0	0	0	++	0
6	♀	40	10+	1	130	40	+	0	+	+	+	0	0	0	?	?	++	Pet.
66	♀	55	15	0	110	30	+	0	0	0	+	0	0	0	+	+	+	Pet.
*77	♂	20	17	5	150	40	+	+	0	0	+	+	+	0	+	0	+++	0
*64	♀	32	15	3	140	60	+	0	+	0	+	0	+	0	0	+	++	+
*9	♀	62	?	0	130	40	+	0	+E	0	+	0	+	+	?	?	+++	+
†99	♀	35	?	0	120	30	0	0	0	0	+	0	0	+	?	?	++	+
†101	♂	58	?	0	140	40	T	0	+E	0	+	0	0	+	?	?	++	+
*43	♂	52	8	1	120	30	T	+	0	0	+	0	0	T	+	+	+	Pet.
*55	♂	44	9	2	120	30	0	+	0	0	+	0	0	0	+	+	++	+
*61	♂	38	14	1	130	30	T	+	0	0	+	0	0	T	+	+	++	+
†68	♂	52	?	0	120	30	+	0	+E	0	0	0	0	0	0	+	+++	+
†102	♀	40	?	0	140	40	T	0	0	0	+	0	0	0	?	?	++	+
*39	♀	60	6	0	180	40	0	0	+E	0	+	0	0	+	0	?	+++	+
†86	♂	30	?	1	140	40	0	0	+E	0	+	0	0	+	?	?	++	+

\*Died. † Died within 36 hours of admission. E = Pulmonary oedema.  
? = Unknown.



XI

*Typhus*

<i>C.N.S. signs.</i>						<i>Mental state.</i>	
<i>Incontinence urine.</i>	<i>Incontinence faeces.</i>	<i>Changes in reflexes.</i>	<i>Twitching.</i>	<i>Tremor.</i>	<i>Meningism.</i>		
						<i>Symptoms.</i>	<i>Miscellaneous.</i>
+	0	+	+	0	+	Agitated delirium, complete disorientation	—
+	+	+	0	+	+	Mild confusion and delirium, irritable ..	Dehydration.
+	0	0	0	0	+	Low muttering delirium, dazedness ..	Haematemesis.
0	0	0	0	0	+	Mild delirium, irritable, noisy, restless ..	—
+	0	0	0	0	0	Low muttering delirium, confusion ..	Sacral sore.
+	+	0	0	0	0	Confusion and prolonged stupor .. ..	Sacral sore.
+	+	0	0	0	0	Drowsiness .. .. .	Cystitis; sacral sore.
+	0	0	0	0	0	Mild delirium, restlessness, drowsiness and stupor.	—
+	0	0	0	0	0	Mild confusion deepening into terminal stupor.	—
+	0	0	0	0	0	Mild confusion deepening into terminal stupor.	Incipient gangrene.
?	?	0	0	0	0	Agitated delirium, complete disorientation	Dehydration.
?	?	0	0	+	+	Delirious, confused, disorientated .. ..	Gangrene, ribs and sacrum.
0	0	+	+	+	0	Muttering delirium .. .. .	Terminal icterus.
+	0	0	+	0	0	Low muttering, grimacing delirium, dysphagia.	Choreiform movements; gangrene back.
+	+	0	+	+	0	Low muttering, groaning delirium, dysphagia.	Terminal convulsions.
+	0	0	+	+	0	Low muttering delirium .. .. .	Haematemesis and melaena.
+	+	0	0	+	+	Severe delirium, dysphagia .. .. .	—
+	+	0	+	+	0	Mild delirium passing to prolonged coma ..	Gangrene feet, thigh.
+	0	+	0	+	+	Quiet coma .. .. .	Dehydration; gangrene ear, buttock, sacrum.

T = Terminal. Pet. = Petechiae.

10+ = more than 10 days.

✓**CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS**

**TABLE**  
**MIII**

Patient's number.	Sex.	Age.	Duration of fever (days).	Duration over 104° (days).	Pulse.	Respiration.	Dyspnoea.	Chest.			Cardio-vascular.			Renal.		Skin.	
								Bronchitis.	Bronchiolitis.	Pneumonia.	Low B.P.	Triple rhythm.	Dilatation.	C.V.S. collapse.	Oliguria.	Albuminuria.	Rash extent.
4	♂	21	11	0	120	20	0	0	0	0	0	0	0	0	?	+	0
19	♀	37	?	0	120	25	0	0	0	+	0	0	0	?	0	++	+
31	♀	27	?	0	100	25	0	0	0	+	0	0	0	?	0	++	Pet.
45	♀	43	?	0	110	30	+	0	0	0	0	0	0	0	0	+	Pet.
59	♂	16	10	0	100	20	0	0	0	+	0	0	0	0	0	+	0
83	♂	23	?	0	115	20	0	0	+	0	0	0	0	0	0	++	Pet.
13	♀	18	?	0	120	30	0	0	0	+	0	0	0	?	+	Pet.	
71	♂	28	14	0	110	25	0	0	+	0	0	0	0	0	0	++	+
7	♂	18	11	2	110	25	0	0	0	+	0	0	0	?	0	0	0
63	♂	35	?	0	100	20	0	0	0	0	0	0	0	0	0	+	0
11	♀	45	?	0	110	30	0	0	0	0	0	0	0	?	+	0	0

? Unknown.

XII

*Typhus*

<i>C.N.S. signs.</i>						<i>Mental state.</i>						<i>Miscellaneous.</i>	
<i>Incontinence urine.</i>	<i>Incontinence faeces.</i>	<i>Changes in reflexes.</i>	<i>Twitching.</i>	<i>Tremor.</i>	<i>Meningism.</i>	<i>Symptoms.</i>							
0	0	0	0	0	0	None	..	..	..	..	..	..	—
+	+	0	0	0	0	Irritable and drowsy	..	..	..	..	..	..	Diarrhoea.
+	0	0	0	0	0	Dull	..	..	..	..	..	..	Cervicitis.
+	0	0	0	0	0	None	..	..	..	..	..	..	Dehydration.
0	0	0	0	0	0	None	..	..	..	..	..	..	—
0	0	0	0	0	0	None	..	..	..	..	..	..	—
+	0	0	0	0	0	Irritable	..	..	..	..	..	..	Deafness.
+	0	0	0	0	0	None	..	..	..	..	..	..	—
0	0	0	0	0	0	Nocturnal disorientation	..	..	..	..	..	..	—
0	0	0	0	0	0	Dull and mild confusion	..	..	..	..	..	..	—
+	0	+	0	0	0	Nocturnal confusion and mild delirium	..	..	..	..	..	..	Sacral sore.

Pet. = Petechiae.

52 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE XIII

*Physical findings in 50 "Control" cases*

<i>Fever—</i>		0-12 days—average 5.8 days	
Duration of fever in hospital .. .. .		10-18 "	14.0 "
Duration of fever from onset (23 patients who survived)		1-8 "	3.2 "
Duration of fever of 104° F. or over .. .. .			
		<i>Numbers. Per Cent.</i>	
Fever of 104° F. or over .. .. .	26	52	} 96
Remittent fever .. .. .	16	32	
Continued fever .. .. .	13	26	
Continued and later remittent fever .. .. .	9	18	
Irregular fever .. .. .	10	20	
No fever after admission .. .. .	2	4	
<i>Pulse—</i>			
Tachycardia .. .. .	48	96	
Relative bradycardia .. .. .	2	4	
Convalescent bradycardia .. .. .	2	4	
<i>Respirations—</i>			
Increased respiration rate .. .. .	40	80	
Dyspnoea .. .. .	10	20	} 28
Terminal dyspnoea .. .. .	4	8	
Shallow respirations .. .. .	3	6	
<i>General state—</i>			
Prostration (of varying degree) .. .. .	43	86	
Clinical appearance of dehydration (at some time) .. .. .	29	58	
Cyanosis .. .. .	27	54	
Icterus .. .. .	3	6	
Flushed face .. .. .	26	52	
<i>Eyes—</i>			
Suffused or glistening .. .. .	18	36	
Conjunctival injection .. .. .	27	54	
Haemorrhages into conjunctiva of lower lids .. .. .	11	22	
<i>Tongue—</i>			
Moist .. .. .	20	40	
Dry .. .. .	30	60	
Dry on admission .. .. .	18	36	
Furred (at some time) .. .. .	33	66	
<i>Rash—</i>			
None .. .. .	1	2	
No rash, but haemorrhages in eyelids .. .. .	2	4	
Macular or haemorrhagic rash in skin .. .. .	47	94	
Macular rash only .. .. .	9	18	
Macular and haemorrhagic rash .. .. .	34	68	
Haemorrhagic rash only .. .. .	4	8	
Petechiae .. .. .	23	46	
Purpura .. .. .	22	44	
Pronounced sub-cuticular mottling .. .. .	5	10	
Glandular enlargement .. .. .	25	50	
<i>Respiratory system—</i>			
No abnormal signs .. .. .	14	28	
Signs of bronchitis .. .. .	22	44	
Signs of bronchiolitis .. .. .	7	14	
Signs of pulmonary oedema .. .. .	5	10	
Signs of pneumonia .. .. .	3	6	
<i>Cardiovascular system—</i>			
No abnormal signs .. .. .	6	12	
Low blood pressure only .. .. .	7	14	
Low blood pressure, absent cardiac impulse and weak sounds .. .. .	37	74	
Triple rhythm .. .. .	4	8	
Dilatation only .. .. .	5	10	
Dilatation with congestive failure .. .. .	1	2	
Peripheral collapse .. .. .	5	10	
Gangrene of peripheral parts .. .. .	5	10	
Bed sores without gangrene elsewhere .. .. .	5	10	

*Abdomen—*

Splenomegaly .. .. .	21	42
" slight .. .. .	13	—
" moderate .. .. .	8	—
Enlargement of liver .. .. .	4	8
Abdominal distension .. .. .	8	16

*Urinary system—*

Oliguria during the fever .. .. .	8	—
Diuresis during defervescence or convalescence .. .. .	22	—
Albuminuria, light degree .. .. .	13	18—46 per cent. of
Albuminuria, moderate cloud .. .. .	5	39 patients
Casts, granular and hyaline .. .. .	19	20—51 per cent. of
" epithelial .. .. .	1	39 patients
Haematuria, microscopic .. .. .	1	
Haematuria, gross .. .. .	1	
Bile in urine .. .. .	1	

*Nervous system—*

No mental symptoms .. .. .	6	
Slight drowsiness, dullness, dazedness, irritability or nocturnal restlessness .. .. .	10	16
Mild delirium .. .. .	6	
Mild delirium and confusion .. .. .	7	21
Mild confusion .. .. .	6	42
Mild confusion and transitory stupor .. .. .	2	
Severe delirium (agitated) .. .. .	2	
Severe delirium (low muttering) .. .. .	8	13
Prolonged stupor .. .. .	1	26
Coma .. .. .	2	
Changes in reflexes .. .. .	6	12
Abnormal movements—		
Tremors .. .. .	10	20
Twitchings .. .. .	6	12
Convulsions .. .. .	1	2
Meningism (stiffness of neck) .. .. .	13	26
Incontinence of urine—female .. .. .	20	100
Incontinence of urine—male .. .. .	14	50
Incontinence of faeces—female .. .. .	8	36
Incontinence of faeces—male .. .. .	2	7

There was a definite skin rash in all but three patients. In one of these no rash was found anywhere, and in the other two there were only a few doubtful macules on the skin but haemorrhages were present in the lower eyelids. No opportunity arose to investigate the exact day of disease on which the rash appeared. Although the character of a rash often changed after admission, no patient in this series developed a rash while under observation. A macular rash was present in 43 patients, including all the fatal cases. The characteristics of this have already been described, and the only point requiring emphasis is the extreme variability in number of elements and in distribution. The macules were slightly papular in two patients; in the remainder they were not palpable. (It must be remembered that these patients under study formed a selected group, and that the numbers of patients without rash or with atypical rashes probably formed a larger proportion of the patients admitted to the other wards than of those admitted under our care.) An erythema of the skin of the trunk (subcuticular mottling) was particularly obvious in only five patients (10 per cent.); it may well, however, have been present in others but obscured by the pigmentation and scarring from scabies and acne, and by the generally dirty condition of the skin. The extent of the rash was related only in limited degree to the severity of the disease. None died of 11 patients who had only slight rashes; seven died of 23 with "medium" or "moderate" rashes; and four died of 12 with profuse or extensive rashes. Haemorrhage into the skin, or into the eyelids, was present in 41 patients.

Petechial haemorrhages only, purpuric haemorrhages only, petechiae and also larger purpuric spots, were all seen in different patients; and ocular haemorrhage without any visible haemorrhages elsewhere occurred three times. The presence of petechiae was not an indication of severity of disease, but purpuric spots were more common in the severe types of infection. Ten of 22 patients with purpuric spots died (45 per cent.), compared with two out of 25 (8 per cent.) in patients with rashes of other types. Haemorrhages were seen on any part of the body, including the face, palms of the hands, pharynx and epiglottis. Desquamation during convalescence was seen in several patients, chiefly in those who had exhibited well-marked erythema, and fine branny flakes separated all over the trunk after complete fading of the rash had occurred.

*Glandular Enlargement.*—Lymph nodes were palpable, and were considered larger than "normal", in 25 patients (50 per cent.); in 12 the lymph node enlargement was generalised. In the remainder the nodes were palpated in the axillae only. In no case was the enlargement more than slight in degree.

#### *Respiratory system*

No abnormal respiratory tract signs were found in 14 of the 50 patients. Added sounds indicating bronchitis or bronchiolitis were heard in most of the remainder. In seven patients abundant moist râles were noted over a large part of the chest. Despite this, sputum was characteristically scanty and viscid. Two gravely-ill patients had signs of patchy consolidation; this resolved within a few days. Another developed a lobar type of pneumonia on the sixteenth day of his illness; this was not indicated by the temperature chart, and the temperature was normal on and after the eighteenth day, although the signs of consolidation lasted for five days altogether. The development of pneumonia as a terminal event in comatose patients has been referred to above; and in the fatal cases of this series, areas of consolidation, although not demonstrated in life, were sometimes noted at autopsy (see below). Five patients showed the physical signs of pulmonary oedema shortly before death.

The pharynx was often abnormal during the fever and presented a dry red glazed surface upon which sordes tended to accumulate. The larynx was examined in a few patients by Major J. A. James, R.A.M.C. Aphonia was accompanied by dry, red vocal cords in one patient, and by petechial haemorrhages in the mucosa in another. Petechiae were also seen in the pharynx and epiglottis.

#### *Cardiovascular system*

The commonest abnormalities were absence of the cardiac impulse, softness of the first heart sound and a tendency to equal spacing of the heart sounds (embryo-cardia), and a progressive fall in both systolic and diastolic blood pressures, with a reduction of the pulse-pressure, during the first 10 days of the disease. The average blood pressure was charted for the various days of disease in 30 patients in whom the onset of illness was accurately determined; the result, shown in Fig. 4, indicated that the lowest systolic and diastolic pressures were experienced on the tenth and eleventh days. Thereafter, the pressures rose somewhat, but they continued abnormally low until after the third week of the disease. Even at the time of discharge many patients still had abnormally low blood pressures, but others had by then achieved normal levels. The low average diastolic pressures shown in Fig. 4 after the seventeenth day were probably unrepresentative at this time, owing to their dependence upon a small number of observations. Fatal cases usually exhibited a downward trend in pressures, but if death did not occur before the eleventh day, a rise later occurred, followed by a fall (with disappearance of Korotkow's sounds below the sphygmomanometer cuff) a short time before death. Another

combined feature of the blood pressure noted in many patients—usually during the most acute stage of prostration and mental symptoms—was a systolic pressure which varied with the individual beats and also a diastolic level whose exact point was difficult to fix with certainty, owing to extreme softness of the sounds a few points below the sounds due to the systolic wave.

Triple rhythm was heard in four patients, one of whom, a boy of 20 (Naples 77), later died suddenly of heart failure. Cardiac dilatation occurred in five others, two of them fatal cases. The fact that cardiac failure of congestive type was rare, and that the fall in blood pressure was both common and extreme in degree, suggested that the chief disorder of the cardiovascular system lay

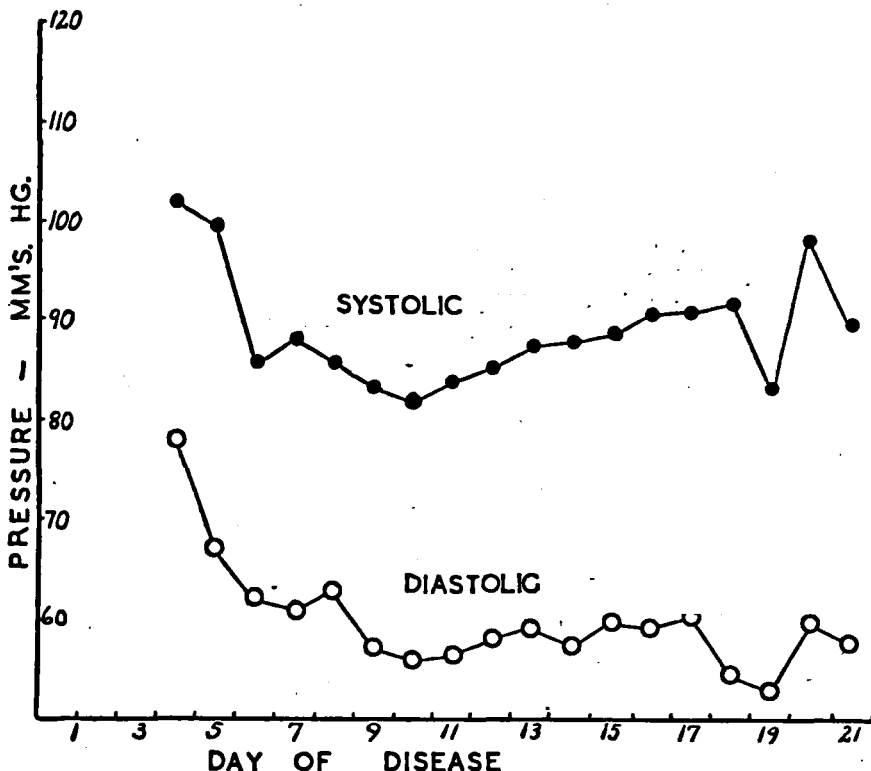


FIG. 4.—Average blood pressure at different days of disease in 30 patients.

with the peripheral vessels (Woodward and Bland, 1944). It is true that a collapsed condition of the vessels of the extremities was common, particularly in seven gravely-ill patients of this series, all of whom died; but a central failure in addition to loss of vasomotor tone seemed probable in these cases, as in others with less striking abnormalities of the vessels. (It was not possible to carry out the cardiac output, blood volume or electrocardiographic studies which would be an essential preliminary to an evaluation of the factors responsible for the observed cardiovascular phenomena.)

Gangrene of the peripheral parts, such as the nose, ears or toes, was always a bad prognostic sign and occurred in five patients, all of whom died. The affected area became blue, then cold; and later the skin over the gangrenous area became raised into bullae, filled with thin fluid, which later ruptured

In areas exposed to pressure—e.g., over the hips, scapulae or the region of the sacrum—rapidly progressive sores developed in patients with gangrene elsewhere. Ordinary bedsores, however, were found in five other patients, some of whom, though seriously ill, recovered satisfactorily. Only one patient with gangrene of the peripheral parts, in this case of the toes, was known to have recovered ultimately; a zone of demarcation developed in this instance, with presumed separation of the gangrenous area at a later date.

#### *Abdominal viscera*

The spleen was palpable at some time during the illness in 21 patients (42 per cent.); it was usually most enlarged during the fever, becoming smaller as convalescence was established. The reverse phenomenon, in which the spleen was impalpable during the fever but became palpable towards the end of the illness, was seen very occasionally. The spleen was just palpable, or enlarged to one finger's breadth below the costal margin, in 13 patients; and to two fingers' breadth or more in eight. The enlarged spleen was seldom tender, but tenderness over the splenic area in the absence of splenic enlargement was encountered once or twice. The liver was enlarged and palpable in four patients, and in two others there was tenderness over the right subcostal margin but the liver was not actually felt. Icterus was present in three patients; in two its development preceded death by only a few hours and it was trivial in degree; the third patient was jaundiced on admission but his disease was otherwise typical; there was bile pigment in the urine, and the jaundice lessened in degree during the illness, though it was still apparent even when convalescence had otherwise become established.

Abdominal distension was a troublesome symptom in eight patients, five of whom were gravely ill and three of whom died. A tense belly wall was encountered in other patients, usually when they were severely ill. The distension was apparently due to flatus in certain patients, and was relieved in part by passage of a rectal tube. Diarrhoea, with liquid, non-bloody stools, developed in six patients after admission; this symptom usually disappeared with defervescence, and there was some evidence that it was due to ward infection, although no pathogenic organisms were recovered from the stools. None of the patients with diarrhoea was gravely ill.

#### *Urinary system*

The daily output of urine was measured in 35 patients, as often as possible during the fever and early stages of convalescence. Incontinence of urine, however, which was invariable in women and was present in half of all the men, interfered with the proper estimation of the volume of the urine. Among those patients in whom the daily output could be accurately estimated, a diminished secretion of urine, usually on the eighth or ninth day of illness, was noted in eight instances. In two, the urinary secretion was apparently completely suppressed for a period of a day or two, and in the others the oliguria amounted to a reduction of daily volume to the level of 500 c.c. or less at a time when the intake of fluid averaged 2,000 c.c. Four of these patients with oliguria died, including the two who had temporary anuria; two others, who died 36 hours after admission, had passed no urine during the period of their hospitalisation.

In contrast with the oliguria which occurred during the fever, an increased secretion of urine was sometimes noted at the convalescent stage. Twenty-two patients altogether, including 16 of the 30 in whom the date of onset of the disease was accurately known, exhibited a period of mild diuresis during defervescence or convalescence. The urinary volume at this time either equalled or slightly exceeded the daily intake of fluid. The period of diuresis lasted



from one to four days, and the diuresis occurred, on the average, around the fifteenth day of disease. It happened either just before, coincidentally with or during the first five days after defervescence.

Data on the urinary findings are available for 39 patients. At the time of admission, the urine was frequently concentrated, with a specific gravity of 1020 to 1030, but subsequent specimens varied greatly as to specific gravity in individual patients. Some patients passed urine of specific gravity  $1020 \pm$  throughout the course of the fever; others passed a pale dilute urine with a specific gravity of 1015 or less. This low urinary specific gravity was also seen in instances where a temporary oliguria had been followed by secretion of normal amounts of urine. Albuminuria was encountered in 18 patients (46 per cent.), who had either moderately severe or grave illnesses. In 13 of these, albumin was present to the degree of a light cloud on heating, and in five a heavy cloud was found. Patients with albuminuria during the fever passed progressively less albumin after about the tenth day, and the urine contained no albumin during convalescence. Only traces of sugar were encountered in the urine, and these only in patients receiving large amounts of glucose either in drinks or in glucose saline. The centrifuged deposit showed granular, or granular and hyaline, casts in 19 cases. Epithelial casts were found in one. The number of casts was never very great. Red blood cells were absent from the urine of all but two patients; in one of these, a few red cells were encountered in one specimen only, and in the other frank haematuria and pyuria developed, probably as a sequel to retention of urine and frequent catheterisation. The fact that in patients with high blood urea readings the urine was frequently free of albumin, or contained only a light cloud of albumin without red cells, was striking. Albuminuria occurred as frequently in the moderately severe as among the grave cases. None of the mildly-ill patients showed albuminuria.

#### *Nervous system*

Reference to the frequent involvement of the nervous system—and to the importance of severity of mental symptoms in regard to the classification of cases—has been made above. Table XIII gives in detail the symptoms encountered in the various patients. Sixteen had no mental symptoms or no more than could be accounted for by fever and general illness. More serious cerebral symptoms occurred in the remaining 34 patients. The less serious manifestations, which consisted of mild delirious or confused states, either alone or combined, occurred in 21 patients. There were six patients with noisy, restless, dazed and slightly disorientated delirium, and eight with drowsy, dull, confused mentality, of whom two became stuporose prior to death and another two developed transitory stupor from which they recovered. Finally, there were seven patients in whom delirium and confusion were mingled. Five of these 21 patients suffered grave illnesses, and three died. The greater number, 14 in all, suffered moderately severe illnesses, and two only—who experienced mild confusion and mild delirium with confusion, respectively—had a mild degree of general illness. The mental symptoms never persisted for more than a few days, usually during the middle of the second week of their disease, in those patients who survived; and complete recovery ensued as soon as defervescence set in. Thirteen patients remain, who had a severe degree of delirium, stupor or comatose states. They were all considered on general grounds to have a grave degree of illness. Two, one of whom died, had noisy agitated delirium, and eight, six of whom died, suffered a low muttering type of delirium; stupor or coma supervened before death in all the fatal cases with delirium. Prolonged stupor was seen in one patient who ultimately recovered; and two patients, who both died, were comatose, one for a prolonged period, and one for the short period between admission to hospital and death. Hallucinations of a visual or auditory nature were not noted in these patients, but

instances of visual hallucination—causing them to make plucking movements at imaginary objects—were seen in patients in the other wards of the hospital. It is apparent, therefore, that some correlation existed between the severity of mental symptoms encountered and the gravity of the illness, though this correlation was not absolute. There is less to record in connection with actual physical signs of central nervous system involvement. There were six types of phenomena :—

- (a) *Changes in the reflexes.*—Exaggerated tendon reflexes with presence of flexor plantar response, or inequality of tendon reflexes such as absence of an Achilles jerk, occurred in six patients, of whom four were gravely ill.
- (b) *Abnormal movements.*—Three types of abnormal movements were noted. Firstly, tremor of the face, of the tongue, of the lips or of the arms was seen during actual muscle movements in 10 patients; the tremor was coarse and irregular, and was reminiscent of that seen in general paresis; only three patients who exhibited tremors recovered. Secondly, twitchings of a grimacing or choreiform type, sometimes involving large muscle groups, sometimes involving only the facial muscles, occurred in six patients, of whom only one recovered; the twitchings accompanied tremors in four patients. Thirdly, convulsions of an epileptiform character were seen in one patient just prior to death. It is clear that abnormal movements of any type were an extremely bad prognostic sign.
- (c) *Stiffness or rigidity of the limbs.*—A sign which was more difficult to evaluate than the previous one was stiffness or actual rigidity of the limbs. Most patients with tremor or exaggerated reflexes exhibited a state of partial rigidity of the limbs, in which the legs were extended but the arms were flexed at the elbows and pronated. However, this rigidity, which was certainly involuntary, was difficult to distinguish from a voluntary stiffness seen in other irritable patients who were mildly delirious. In some cases, too, limb movement was resisted because movement of the muscles or of the joints caused actual pain. Nevertheless, the rigidity which accompanied abnormal movements was probably of true nervous origin, and—like the movements themselves—it may have originated from focal lesions of the basal ganglia or cerebrum.
- (d) *Meningism.*—Stiffness or actual rigidity of the neck was present in 13 patients, but in only one was it accompanied by a positive Kernig's sign. Seven patients with meningism were gravely ill, and three died. The remaining six patients with neck stiffness suffered only moderately severe illness; neck stiffness was transient in these, and it persisted for only a few days during the period of maximal cerebral involvement as judged by mental symptoms. It was, therefore, not such a useful prognostic index as the other nervous signs recorded above.
- (e) *Incontinence.*—It has been mentioned that incontinence of urine was present at some time during the illness in all female patients. It occurred in only half of the males, and in them it coincided with the period of greatest degree of illness.
- Incontinence of faeces occurred eight times in females, but the patients were either mildly, moderately or gravely ill and no prognostic significance could be attached to the symptom, particularly because it occasionally occurred in patients with diarrhoea.

- (f) *Deafness*.—This symptom, which is dealt with under “Symptomatology”, was investigated in several patients and found to be due to a true nerve deafness, except in one patient who had an otitis media. Petechial haemorrhage of the tympanic membrane was seen in several patients.

#### *Complications and Sequelae*

The following complications occurred in this series : syphilis (two), diarrhoea, probably due to ward infection (six), haematemesis (one), terminal haematemesis and melaena associated with uraemia (one), abscesses or furuncles (four), thrombosis of brachial veins following intravenous medication (three), cystitis (one), otitis media (one); several patients had ascariasis or scabies. Parotitis, though traditionally a common complication of typhus, was seen in only two patients during the entire outbreak; it did not occur in any patient in this series. Routine pathological investigations showed no evidence of malaria, relapsing or enteric fevers.

Few sequelae were seen during or after convalescence, and none originating in the cardiovascular system, such as tachycardia, was encountered. A few patients complained of pains in the muscles or joints of the legs, or pains in the feet, but no objective signs of peripheral nerve involvement which might have been responsible for these were found. The deafness, which was most severe during the second week of the disease, had usually improved by the time the patient was fit for discharge, but it was still present in some.

#### *Treatment*

The treatment received by these 50 patients consisted of good nursing care, a fluid diet supplemented where necessary by parenteral fluid, and symptomatic treatment with analgesics or sedatives. The nursing care was that given to patients with fever, but the tendency to bedsores and gangrene necessitated particular attention to the skin. Bedsores were treated by kaolin poultice or calamine lotion dressing. Sponging with tepid water was carried out if the patient's temperature rose above 104·5° F. (rectal). Aperients or enemata were given as needed. Frequent treatment of the mouth was given, because of the tendency to sordes and dryness of the buccal mucosa, and, as a consequence of this care, the classical furring of the tongue often failed to develop. The diet consisted of oranges and bread and jam, soup and milk, supplemented with lemonade sweetened with glucose. It had a low calorific value, but the quantity was determined by the shortage of food supplies during the epidemic; by the end of January, the patients were receiving adequate amounts during the fever and, as a consequence, wasting became less marked. During convalescence the diet was always inadequate to relieve the hunger then experienced by the patient. Considerable difficulty was experienced in administering an adequate fluid intake to these patients. In spite of the fever, thirst seemed to be exceptional and even mildly-ill patients would not help themselves to drinks left by the bedside. Thus, although a daily total of 4,000 c.c. of fluid was aimed at, this was rarely achieved and most patients drank from 2,500 to 3,500 c.c. daily during the fever. When there was dysphagia, or if the patient was too delirious or stuporose to drink naturally, feeding by nasal tube was carried out, and in comatose patients a nasal catheter was inserted and then left in position. Parenteral fluid in the form of 5 per cent. glucose in normal saline was administered for various reasons, at some time in the course of the illness, to 25 patients. The reasons for administering saline were : (i) clinical evidence of dehydration as shown by a dry state of the tongue or buccal mucosa; (ii) a collapsed condition of the circulation as shown by a feeble pulse and exceptionally low blood pressure, (iii) failure to maintain fluid intake either

by mouth or by nasal tube. Saline was usually given by slow intravenous drip, and this method was found to be the most satisfactory one from the clinical point of view. Five patients received fluid subcutaneously and one patient was given fluid per rectum, but neither of these routes was satisfactory in general. Two only of the slightly ill patients received saline, one of whom received continuous therapy for three days because of clinical "dehydration". Nine of the moderately-ill patients received saline; three received a single injection of 500 c.c., five received saline on two days either continuously or in two separate injections, and one received continuous therapy for three days. Fourteen of the gravely-ill patients received saline, usually by continuous intravenous drip, which was often continued in those patients who died, until the day of death. Administration of the saline was confined to one day in four patients, two days in five, three days in three, four days in one, and six days in one. When saline was given continuously for several days, a total of 1,500 c.c. was usually given during the 24 hours. Two patients received intravenously hypertonic saline approximately 2 per cent. in strength (40 c.c. of 20 per cent. saline added to a bottle of 500 c.c. of glucose saline). The administration was carried out in connection with study of renal function. No patient who died, and had received parenteral saline, showed evidence at post-mortem of overloading of the circulation with too much fluid. Only one patient received plasma or blood, and to this patient, who developed haematemesis on the nineteenth day of disease, 1,000 c.c. of plasma were given by slow intravenous drip, followed by 1,000 c.c. of Group "O" blood. Although it was not possible to assess the value of saline as an agent for supporting the circulation or for overcoming dehydration, it seemed to be useful, and at any rate in moderately-ill patients appeared to relieve dehydration. It was not possible to employ plasma as a routine in conditions of circulatory collapse.

Sedatives were needed in 18 patients, "Luminal" and "Nembutal" being commonly used as hypnotics or for mild delirium. "Somnifaine" was used in a few patients, chiefly because it could be given by injection. Paraldehyde was found extremely valuable in the treatment of severe delirium and restlessness. Morphine was given to one patient with cardiac failure. Aspirin was used to relieve headache or muscle pains, but was needed relatively rarely.

A simple expectorant mixture containing potassium iodide and tincture of belladonna was given to patients with troublesome cough, and more rarely a linctus containing codeine was employed. Atropine was given by injection to one or two patients. Diarrhoea developing after admission to hospital was treated with kaolin. In cases in which repeated catheterisation was found to be necessary, hexamine was given three times a day. Sulphathiazole was given to four patients, but not until the ordinary febrile stage of the disease had passed. One patient with cervicitis received 40 grams of sulphathiazole in nine days; another, with severe cystitis, received 43 grams in eight days; a third, with buttock abscess, received 15 grams in three days, and a fourth, with brachial thrombosis, received 8 grams in two days.

So-called cardiac stimulants, digitalis or digitalis-like compounds, were not given to patients as a routine, in view of their questionable value in this disease. One patient with severe cardiac failure and mild oedema was given a single injection of "Mersalyl," but it failed to promote diuresis.

#### THE THERAPEUTIC TRIAL OF THE DRUGS, V186 AND V147

The preliminary results obtained in North Africa, described above, indicated that the drugs V186 and V147 produced no immediate beneficial effect when given to cases of typhus, and the plan for further trial was, therefore, to treat as many patients as possible with the drugs and to control the results by treating alternate patients with supportive or symptomatic measures only.

The method of assembly and selection of the series of patients treated with the drugs, and of the controls, has already been discussed; after elimination of four patients treated with the drugs and two controls, in whom the diagnosis was insufficiently confirmed, the series consisted of 60 patients, of whom 30 received either V186 or V147.

#### *Method of Administration*

The major portion of the trial was carried out with V186, which appeared to be slightly more active than V147 in the treatment of the experimental infection of mice. Thirty-one patients were treated with V186 (of these, four were subsequently excluded), and only three received V147. Throughout the work no data as to the retention, blood-level or excretion of the drugs could be obtained, as no method of chemical estimation was available. The work in North Africa had consisted merely of exploratory attempts to treat cases of typhus with V186, and although most patients tolerated the drug well, even when they already had a low blood-pressure or an elevated blood urea, one patient who received intensive therapy developed uraemia possibly as a result of damage to the kidney by the drug. The first few patients in the Naples study who were treated with V186, therefore, received small doses, and larger doses were given only when small doses had been found to have no therapeutic value. Patients received an initial dose of either 2 or 4 gm. in the form of tablets by mouth, followed either in four or in two hours by a smaller "holding" dose, which was repeated at regular intervals, and continued until defervescence had become established. Most patients continued to receive the drug for one day after normal temperature was reached, but the "holding" dose was usually reduced for the last two or three days of therapy.

Every effort was made to give the tablets whole, in view of the fact that the drugs had been dispensed with an excipient (stearic acid) to delay absorption and thus promote continuous entry into the body. Patients were frequently too ill to swallow, or else refused to take the tablets whole, and then the tablets were crushed in water, or powder consisting of pure drug was dissolved in water and the resulting suspension or solution was given either orally or by nasal tube. Administration of the drug was rarely easy, and was often actively resented by the patients. Only two patients received the drug intravenously, in each case as a slow drip well-diluted in normal saline containing glucose (3 grams of V186 in 500 c.c.); venous thrombosis occurred subsequently in one of them. A summary of the entire series of 34 patients treated with V186 or V147, with details of dosage and an attempted assessment of the effects of the therapy, is given in Table XIV. The most important change in administration during the progress of the study was an increase in the intensity of dosage with V186. This was achieved at first by maintaining a holding dose of 3 gm. four-hourly for one or more days after an initial dose of 4 gm., and then, when that failed to produce rapid defervescence in certain patients, two-hourly dosage of 1½ or even 2 gm. was given instead of four-hourly treatment. The change to more intensive dosage was not at first followed by an increased incidence of events of a toxic nature, but a number of the more gravely ill patients treated during the last two weeks of the study developed reactions thought to be toxic in origin, and it is possible that intensive dosage with V186 is relatively more dangerous in gravely ill than in mildly ill subjects. The subject of toxic effects of the drugs is discussed in greater detail below. The change in intensity of dosage was held to be justified because of the theoretical possibility that smaller doses were insufficient to cause a demonstrable chemotherapeutic effect, but it did not produce better therapeutic results as judged by the speed of defervescence. When the V147 trial began, in February, the method of administration was a small dose (1½ gm.) every

62 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE  
Patients treated

Number.	Sex.	Age.	Day of disease treatment begun.	Severity on admission.	Dosage.						Course of		
					Gm. Ist.	Gm. Holding.	Days.	Interval.	Drug total in gm.	Duration in days.	General severity.	Days fever after drug.	Total duration of fever.
1	M.	17	VII	Mild	2	2 1	2 4	} 4 hrly.	58	6	Mild	5	12
3	M.	20	VIII	Mod. severe	2	2 1	2 4 24 h	} 4 hrly. 4 hrly.	67	8	Grave	7	15
5	M.	39	VI	Mild	2	2 1	2 4	} 4 hrly.	54	6	Mild	5	11
8	M.	32	?	Grave	4	3 2 1	1 3 3	} 4 hrly.	78	7	Mod. severe	11	?
10	F.	63	?	Grave	4	3 Interval 2	1½ 3 days 1	} 4 hrly. 4 hrly.	32	2	Grave	1	Died 6th day
12	F.	32	?	Mild	4	2 1	1 1½	} 4 hrly.	20	2	Mild	1	?
14	M.	28	?	Mod. severe	4	3 2 1	1 2 2	} 4 hrly.	52	5	Mild	2	?
16	M.	38	?	Mild	4	3 2 1	1 2 1	} 4 hrly.	52	4	Mod. severe	3	?
18	F.	48	?	Mild	4	1 2 1	3 4 1	} 4 hrly.	76	8	Mild	10	?
20	F.	16	IV	Mild	4	3 2 1	2 3 1	} 4 hrly.	76	6	Mod. severe	5	9
21	F.	27	IX	Mod. severe	3	3 2 1	2 3 2	} 4 hrly.	78	7	Mild	7	15
22	M.	18	VI	Mod. severe	4	3 2 1	1 5 1	} 4 hrly.	91	7	Mild	8	14
24	M.	27	VII	Mod. severe	4	3 2 1	1 5 1	} 4 hrly.	91	7	Mod. severe	10	17
28	M.	39	IX	Mild	3	3 2 1	1 1 1½	} 4 hrly. 2 hrly.	60	4	Mild	3	12

## CIV

with V186

<i>Special features of illness.</i>	<i>Other therapy.</i>	<i>Possible beneficial effects of drug.</i>	<i>Possible toxic symptoms or signs.</i>
Petechial rash. Drowsy and dazed at times. Urea 35/31.	Nil	Nil	Nil
Intense purpuric rash; stuporose, meningitis, twitchings; dehydrated; bronchiolitis, transient pneumonia. Urea 73/53.	Nil	Nil	Vomiting on one day.
Macular rash; abscess groin; scabies. Urea 34/42.	Nil	Nil	Nil
Convulsions, choreiform movements on admission; bronchiolitis, petechial rash; abscess thigh. Urea 48/42.	32 g. sulphathiazole for abscess.	Rapid recovery after admission but fever and bronchiolitis for 12 days.	Nil
Twitching and tremor and uraemia on admission; collapse, bradycardia, vomiting; purpuric rash. Urea 286/278.	Saline S.C., "Nembutal," atropine.	Nil	Collapse after 24 hours' treatment; vomiting.
Transient weakness face on admission, otherwise convalescent; macular rash. Urea 61/47.	Nil	Nil	Nil
Prostrated, dysphagic, confused; petechial rash; dehydrated. Urea 57/50.	Saline S.C., aspirin.	Rapid defervescence and recovery.	Nil
Tachycardia; purpuric rash; confused. Urea 80/178/235/145/64/43.	Saline I.V.	Rapid defervescence. but slow recovery.	Uraemia after fever, vomiting. No C.V.S. collapse.
Irregular low fever; persistent purpuric rash; prostrated; bed sore, onset after broncho-pneumonia. Urea 73/33.	Nil	Nil	Nil
Macular rash. Urea 32/44/95/123/62	Nil	Early defervescence	Vomiting, uraemia after fever. No C.V.S. collapse.
No rash; drowsy and irritable; prolonged remittent fever. Urea 31/40.	Nil	Nil	Vomiting on one day.
Purpuric rash; drowsy; prolonged continued fever. Urea 44/50.	Saline I.V., "Luminal."	Nil	Nil
Purpuric rash; anxious; remittent fever and shivering attacks. Urea 38/50.	Saline I.V., aspirin.	Nil	Nil
Scanty petechial rash. Urea 42/42	Nil	Rapid defervescence and recovery.	Nil

64 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE

Number.	Sex.	Age.	Day of disease treatment begun.	Severity on admission.	Dosage.						Course		
					Gm. Ist.	Gm. Holding.	Days.	Interval.	Drug total in gm.	Duration in days.	General severity.	Days fever after drug.	Total duration of fever.
32	F.	20	IV	Mild	4	1½ 1 1 1 1½	1 1 1 1 7	} 2 hrly. 4 hrly.	128	11	Mod. severe	12	16
34	M.	23	?	Mod. severe	4	1½ 1 1½	2 1 2	} 2 hrly. 4 hrly.	73	5	Mild	4	?
36	M.	20	VI	Mild	4	1½ 1½	6 2	2 hrly. 4 hrly.	133	8	Mild	6	12
38	M.	32	VI	Mild	4	1½ 1 2	3 1 1	} 2 hrly. 4 hrly.	86.5	5	Mod. severe	4	10
42	F.	36	IV	Mild	4	1½ 2 2	1 3 2	} 2 hrly. 4 hrly.	116.5	6	Mild	3	7
44	F.	40	?	Mod. severe	4	1½ 2	1 1	} 2 hrly.	51	2	Grave	2	Died 4th day
46	M.	48	?	Mod. severe	4	1½ 1½ 3 2	1 3 1 3	4 hrly. 2 hrly. 4 hrly. Intra venous 5 times in 3 days	100	8	Grave	5	Died 11th day
48	F.	34	VII	Mod. severe	4	1½ 3	3 1	2 hrly. 4 hrly.	76	4	Grave	2	Died 5th day
50	M.	16	VIII	Mild	4	1½ 2 1½ 3	1 3 2 2	} 2 hrly. 4 hrly.	167	8	Mild	6	14
54	M.	58	?	Grave	2 (3 gm. int ravenously)	1	1	2 hrly.	11	18 hrs.	Grave	½	Died 2nd day
56	F.	24	XI	Mild	4	1½ 1 2	3 1 1	} 2 hrly. 4 hrly.	77.5	5	Mild	1	12
60	F.	24	?	Mod. severe	4	3 1½ 3	1 2 1	4 hrly. 2 hrly. 4 hrly.	65.5	4	Grave	3	Died 5th day



XIV—*contd.*

<i>of illness.</i>			
<i>Special features of illness.</i>	<i>Other therapy.</i>	<i>Possible beneficial effects of drug.</i>	<i>Possible toxic symptoms or signs.</i>
Early case. Scanty petechial rash; nocturnal delirium. Became iller without chest or cardiac signs during prolonged, continued and remittent fever. Urea 44/45.	Saline I.V., "Luminal" aspirin.	Nil	Vomiting on one day.
Prostration; dysphagia; confused and dehydrated; petechial rash. Urea 47/42.	Saline I.V.	Rapid defervescence and recovery.	Vomiting on one day.
Petechial rash, slight prostration and remittent fever. Urea 33/29.	Nil	Nil	Nil
Developed confusion, lethargy, dysphagia and hemiparesis, XI day; macular rash. Urea 32/59.	Nil	Rapid defervescence but slow recovery.	Nil
No rash; anxious; irregular fever. Urea 40/36.	Sodium bicarbonate with later doses.	Rapid defervescence and recovery.	Nil
Twitching and tremor; scanty petechial rash; collapse with delirium, anuria and pulmonary oedema. Urea 57.	Saline I.V.	Nil	Vomiting, collapse and bradycardia after 48 hours' therapy.
Agitated delirium; bronchiolitis, severe dysphagia; macular rash; diarrhoea. Treatment intermittent. Urea 47/70/80.	Saline I.V., paraldehyde, hyoscine.	Nil	Vomiting on several days.
Purpuric rash; diarrhoea; developed restlessness, dyspnoea, agitated delirium. Urea 53/200.	"Luminal" paraldehyde hyoscine.	Nil. Defervescence accompanied by collapse.	Collapse after three days' therapy.
Macular rash; remittent fever. Urea 27/32.	Nil	Nil	Nil
Purpuric rash; peripheral cardiovascular collapse; dyspnoea; gangrene back. Urea 120.	Saline I.V.	Nil	Nil
Hysteria and asthenia (paraldehyde); abscess buttock; petechial rash. Urea 25/45.	Saline I.V., sulphathiazole 25.5 gm. in 5 days.	Rapid defervescence but slow recovery.	Vomiting on several days.
Extreme purpuric rash; confusion and stupor; severe prostration; slow development cardiac distress, dyspnoea and cyanosis; incipient gangrene. Urea 171/115.	Saline I.V. "Luminal," paraldehyde.	Nil	Nil

66 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE

Number.	Sex.	Age.	Day of disease treatment begun.	Severity on admission.	Dosage.						Course		
					Gm. 1st.	Gm. holding.	Days.	Interval.	Drug total in gm.	Duration in days.	General severity.	Days fever after drug.	Total duration of fever.
62	F.	24	VI	Mod. severe	4	1½	24 hrs.	2 hrly.	20.5	1	Grave	2	Died 13th day

*Patients treated*

72	M.	18	IV	Mild	1½	1½	5	4 hrly.	46.5	5	Mod. severe	12	15
74	F.	19	VII	Mild	1½	1½	4	4 hrly.	37.5	4	Mod. severe	8	15
76	F.	37	VI	Mod. severe	1½	1½	2	4 hrly.	19.5	2	Mod. severe	11	18

*Patients treated with V186*

30	M.	32	VI	Mild	4	1½ 1 ½	1 2 2	} 2 hrly.	58	5	Mild	2	8
40	M.	57	?	Grave	4	1½	12		2 hrly.	10	8 hrs.	Grave	1
52	F.	62	?	Mod. severe	2	1 1½	1 2	} 2 hrly.	51	3	Grave	3	Died 5th day.
58	F.	40	?	Mild	4	1½	3		2 hrly.	59.5	3	Grave	2

XIV—*contd.*

<i>of illness.</i>			
<i>Special features of illness.</i>	<i>Other therapy.</i>	<i>Possible beneficial effects of drug.</i>	<i>Possible toxic symptoms or signs.</i>
Vomited before admission; scanty petechial rash. Repeated vomiting and anuria, but no collapse after defervescence. Uraemia 298/349/480/420. Abundant pale urine; haematemesis, brachial thrombosis, pyuria and pyelonephritis terminally.	Saline I.V., Plasma I.V., Blood I.V. "Luminal," paraldehyde, morphine, sulphathiazole 3 gm. in 12 hours.	Nil. Fever ceased eighth day with onset of uraemia.	Vomiting after 20 hours treatment. Development of uraemia without collapse

*with V147*

Early case. Petechial rash; mild delirium; diarrhoea. Urea 27/37.	Aspirin, paraldehyde, kaolin.	Drug stopped after 5 days because no effect.	Nil
Petechial rash; diarrhoea; nocturnal delirium. Urea 29/56.	Nil	Drug stopped after 4 days because no effect.	Vomited 2 days, 17th and 18th, after convalescence.
Rash and conjunctivitis developed after 36 hours therapy. Alarming fall of temperature and collapse of circulation; subsequent severe course with delirium; petechial and urticarial rash. Urea 28/30.	Paraldehyde.	Drug stopped after 2 days because no effect.	Collapse after 1½ days therapy.

*but excluded from Series*

Petechial rash; meningism, convulsions at onset; serological proof insufficient. Urea 36/36.	Nil	Rapid defervescence and recovery.	Nil
Purpuric rash; cardiovascular collapse, extreme cyanosis, delirium; Urea 200. No proof of typhus during life (no serum test). Presumptive typhus on P.M. results.	Nil	Nil	Nil
Delirium; septic purpuric rash; pleurisy and pneumonia. Staphylococcal pyaemia found P.M. No proof of typhus. Urea 44.	Saline I.V.	Nil	Nil
Purpuric "collar" rash; subsidence of fever accompanied by collapse, anuria, delirium and stupor. Urea 45/64/201. No proof of typhus in life (serum tests negative). Presumptive typhus on P.M. evidence.	Saline I.V., Plasma I.V., Paraldehyde.	Nil	Collapse after 72 hours therapy.

68 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

four hours at the same level for four or five days in two cases and for two days in one. The drug was then stopped—in the first two patients because of an absence of beneficial effect, and in the third because of a possible toxic reaction.

*Other Therapeutic Measures*

Apart from the administration of V186 or V147, all patients admitted during the progress of the trial received symptomatic therapy and supportive measures as indicated. Parenteral fluid was given as a supplement to fluids by mouth or by nasal tube when there was clinical evidence of dehydration or a collapsed condition of the circulation. Fourteen patients belonging to the control group of 30 not receiving V186 or V147, and accepted as cases of typhus, received parenteral fluid on one or more days during the course of their illness. Thirteen of the 27 patients treated with V186 and accepted as cases of typhus received parenteral fluid in the form of glucose saline during their illness. In the case of seven patients a single injection of 500 c.c. was

TABLE XV

*Duration of Disease at the time of Admission in Treated and Control Patients*

Group.	Clinical Severity.	Day of Disease on Admission to Hospital.								
		IV	V	VI	VII	VIII	IX	X	XI	Unknown
Treated ..	Mild .. ..	1	-	3	1	2	1	-	1	4
	Moderately severe	2	1	2	2	-	-	-	-	2
	Grave .. ..	-	1	1	1	-	-	-	-	5
	Totals ..	3	2	6	4	2	1	-	1	11
Control ..	Mild .. ..	-	-	1	2	1	-	-	-	6
	Moderately severe	-	3	2	4	1	1	-	-	2
	Grave .. ..	3	-	-	-	1	-	-	-	4
	Totals ..	3	3	3	6	2	1	-	-	12

given either subcutaneously (two cases) or intravenously; two received a total of 1,000 c.c. by intravenous drip and four patients received fluid continuously for two or more days. In contrast with the findings in all the other patients, one case treated with V186 (Naples 44) received too much fluid intravenously, and had evidence of overloading of the circulation and general water-logging of the tissues at autopsy. Fluid, in this case, was given at an exceptionally rapid rate to counteract peripheral collapse. Plasma and then Group "O" blood was given to one patient (Naples 62) after haematemesis had occurred, and this patient had received continuous saline therapy for a period of seven days prior to the haemorrhage. Sulphathiazole was given late in the course of the disease to control pyogenic infections. Among patients receiving V186, one with abscess of the thigh received 32 grams of sulphathiazole in eight days, another received 26 gm. in five days to control suppuration in the buttocks, and a third received only 3 gm. in one day because of pyuria. In no case was the sulphathiazole given until the administration of V186 and treatment of the acute stage of typhus had ceased. Among patients not given V186 or V147 three received sulphathiazole for suppuration of the buttock, brachial thrombosis and cervicitis, respectively, as already described. Again, sulphathiazole was not given until the primary fever of the typhus infection had ceased.

*General Description of the Patients Receiving V186 or V147*

A full account has already been given of the clinical condition and symptomatology of all patients with typhus who were treated purely by symptomatic or supportive measures. The severity of the illness of the patients receiving V186 or V147 had to be assessed by reference to the illnesses of the patients who acted as controls. In the following account the group of patients receiving V186 or V147 will be referred to as the "treated" group and the group of patients not receiving the drugs as the "control" group. In order to avoid redundancy, figures relating to incidence of symptoms or signs in the controls will be omitted from the text wherever possible but will be given in the tables comparing these with the treated patients.

Table XV shows the duration of disease at the time of admission to hospital for the treated and control groups of patients, and it indicates a similar distribution in the two groups both of early cases and of patients whose exact duration of illness was unknown because of loss of memory or delirium. Table XVI shows the age and sex distribution in both groups and indicates a reasonably comparable distribution in the two groups considering their small size.

TABLE XVI

*Age and Sex Incidence in Treated and Control Patients*

Age Groups.	Treated.			Control.		
	Male.	Female.	Total.	Male.	Female.	Total.
15-19 ..	4	2	6	6	2	8
20-29 ..	5	5	10	6	1	7
30-39 ..	5	4	9	3	3	6
40-49 ..	1	2	3	2	3	5
50 and over	1	1	2	2	2	4
Totals ..	16	14	30	19	11	30

Table XVII gives details of the history of onset and symptomatology in the two groups of patients. An accurate history of illness prior to admission was obtained in 19 only of the 30 treated cases, four having lost their memory and seven being delirious or confused on admission. Nine of the 19 patients had a sudden or acute onset to their illness and the remainder became gradually ill over a period of two or more days. This is a somewhat lower incidence of acute onset than in the controls, of whom 15 of the 18 who gave a history of onset believed their illness to have begun suddenly. There were, however, few other differences in the symptomatology, as may be seen by reference to Table XVII. Diarrhoea developed after admission in five patients and may have been due to a ward infection as in the case of diarrhoea in the controls. Vomiting was the only symptom obviously more frequent in the treated than in the control patients. Vomiting on one or more occasions occurred in 12 of the treated and in only three of the control patients.

The course of illness in the treated patients was, in general, similar to that in the controls, but the evolution of the disease during the second week of the fever seemed to be modified in some of the treated patients. Mental symptoms were often less severe and fewer treated patients developed signs of a severe grade of involvement of the cardiovascular or respiratory systems. This was shown by subdivision of the illnesses of the treated patients according to the criteria described above for separation of mild, moderately severe or grave

illnesses, on a combined basis of severity of mental symptoms, signs of involvement of the heart, lungs or kidneys and the outcome of the illness. Table XVIII gives in detail the numbers of patients in the various groups who exhibited the various physical signs cited in the headings. Apart from the greater number of mild illnesses among the treated group and a lessened tendency to cardiovascular involvement, the treated and control groups appeared reasonably similar when so classified. The 13 treated patients with mild illnesses had minimal evidence of cerebral involvement as judged by mental symptoms, and only slight evidence of cardiac or pulmonary involvement. None exhibited prolonged tachycardia although several had pulse

TABLE XVII  
*Symptomatology*

	<i>Treated Patients.</i>	<i>Control Patients.</i>
(a) <i>Before Admission—</i>	(19 patients)	(18 patients)
Onset known to be acute .. .. .	9	15
Onset known not to be acute .. .. .	10	3
Shivering .. .. .	18	18
Headache .. .. .	17	17
Malaise .. .. .	16	17
Muscular pains .. .. .	14	9
Constipation .. .. .	13	8
Cough .. .. .	12	14
Anorexia .. .. .	11	11
Dizziness .. .. .	7	11
Expectoration .. .. .	7	4
Deafness .. .. .	5	7
Photophobia .. .. .	5	8
Sweating .. .. .	5	7
Insomnia .. .. .	5	2
Abdominal pain .. .. .	3	3
Vomiting .. .. .	2	2
Diarrhoea .. .. .	2	1
Epistaxis .. .. .	1	1
Sore throat .. .. .	0	1
(b) <i>After admission—</i>	(30 patients)	(30 patients)
Vomiting .. .. .	12	3
Expectoration .. .. .	7	5
Expectoration (bloody) .. .. .	(4)	(3)
Diarrhoea .. .. .	5	4
Dysphagia .. .. .	5	4
Hoarseness of voice .. .. .	3	5
Hiccough .. .. .	1	0
Epistaxis .. .. .	1	0
Haematemesis .. .. .	1 (terminal)	1
Icterus .. .. .	0	1 (terminal)

rates of 120 for more than one day. The respiration rates were slightly raised but dyspnoea was absent. No rash at all was visible in two patients (Naples 21 and 42) and a third (Naples 56) had no macules but a few petechial haemorrhages were present on the abdomen. Of the eight patients who had a haemorrhagic rash, six had petechiae and only two developed purpura. None of the patients had diminution in urinary output, but six had a light cloud of albumin in the urine with a few hyaline or granular casts. None of the patients had demonstrable signs of nervous system involvement, but two of the male patients had incontinence of urine. Two patients (Naples 14 and 34) had some dysphagia and were much prostrated on admission, but both symptoms were diminished after the first 48 hours in hospital. Four patients from this group (Naples 14, 28, 34 and 42) seemed to have benefited from therapy as shown by a speedy defervescence with only 2, 3, 3 and 4 days of fever

respectively after the institution of drug treatment. Nevertheless, the other patients did not show defervescence earlier than might have been expected and the average duration of fever, from onset to defervescence, was 12 days as opposed to 11 days for the control patients with mild illnesses.

There were nine treated patients whose illness ran a more severe course as shown by greater prostration, by the occurrence of delirium or confusion, or by the occurrence of complications. These patients often had fever exceeding 104° F. for several days; the pulse-rate was sometimes higher than 120, but not usually for long, and the respiration rate was a little more elevated than that of the mildly ill patients. Dyspnoea was present in three patients (Naples 8, 32 and 76), but although seven had signs of bronchitis, only one patient developed a bronchiolitis and none had signs of pneumonia. The cardiovascular system remained normal in four patients in spite of high sustained fever, and the remaining patients showed merely lowering of the blood-pressure and faintness of the heart sounds. Three patients showed a diminution in urinary output on or about the 10th day of disease (Naples 20, 32 and 76), but four only showed albuminuria. Two patients (Naples 16 and 20), with normal blood ureas on admission, developed vomiting and raised blood ureas after the fever had ceased. Secretion of urine continued, but the urine was of low specific gravity and contained albumin and in one case many granular casts. Efforts were made to encourage diuresis and in both patients the blood urea fell after an elevation lasting for about five to seven days. One patient (Naples 8), who had a severe attack of typhus, was delirious on admission and had a typical epileptiform convulsion during examination. Convulsions did not recur subsequently, delirium steadily lessened and although meningism remained, recovery was steady and was complicated only by the development of an abscess in the thigh. Another patient (Naples 38) became confused, dysphagic and lethargic on the 10th day, when the temperature reached normal. On the following day he was found to have a hemiparesis involving face, arm and leg. The weakness and increased reflex tone slowly diminished in the course of the next four days and he made a complete recovery. Every patient in this group had a rash, which was entirely petechial in one patient (Naples 32), macular or macular and petechial in six, and macular and purpuric in only two. In one patient (Naples 76) the rash appeared for the first time on the 8th day of disease, and was both maculo-papular and urticarial in type. The illness ran a severe course, with high fever, confusion and disorientation, and diminution in urinary output; treatment, which was with V147, was discontinued prematurely after only two days, because of an apparent toxic reaction. Three patients in this group were considered clinically to have benefited by the therapy (Naples 8, 16 and 38), as shown in two patients by rapid lysis of fever three and four days respectively after the commencement of chemotherapy. However, one patient developed hemiparesis and the other uraemia after defervescence had set in. In the third patient, the degree of illness was thought to be severe because of convulsions and choreiform movements before the start of therapy, and his recovery was, therefore, thought to be significant of the beneficial effects of the drug treatment. The average duration of fever from onset to defervescence was almost exactly the same (14.3 days) in this group of treated patients, as in the controls (14.7 days).

There were eight patients who exhibited a grave degree of illness either upon admission or subsequently. One who recovered (Naples 3) was a severely ill, prostrated man of 20, who had a very extensive purpuric rash, with signs of meningitis and was stuporose. His recovery was thought, at the time, to be due to the drug therapy. The remaining seven patients, who all died, constituted a mixed group and need individual description. None of them benefited from the drug therapy. Naples 10 was a woman of 63, with a profuse purpuric rash, who had a severe degree of involvement of the nervous system on admission,

TABLE  
Classification of Treated

Category.	Severity.	Numbers.		Sex.		Onset known.	Average.				Chest.			Heart.				
		Male.	Female.	Males.	Females.		Duration of fever (days).	Duration over 104° F.	Pulse.	Respiration.	Dyspnoea.	Bronchitis.	Bronchiolitis.	Pneumonia.	Low B.P.	Triple rhythm.	Dilatation.	C.V.S. collapse.
Treated..	*Mild	13	28	8	5	9	12	1.0	112	28	0	5	0	0	7	0	0	0
Control..	*Mild	9	29	5	4	3	11	0.2	110	25	1	3	0	0	5	0	0	0
Treated..	†Moderate	9	26	5	4	7	14.3	4.4	116	32	3	7	1	0	5	0	0	0
Control..	†Moderate	13	28	10	3	11	14.7	3.6	120	31	2	8	2	1	13	1	2	0
Treated..	‡Grave	8	39	3	5	3	10.6	0.6	125	36	6	3	4	1	8	0	0	5
Control..	‡Grave	8	43	5	3	4	9.2	1.4	135	36	5	3	4	2	8	1	1	4

*Treated Cases—*

\* Numbers: 1, 5, 12, 14, 18, 21, 22, 28, 34, 36, 42, 50, 56.

† Numbers: 8, 16, 20, 24, 32, 38, 72, 74, 76.

‡ Numbers: 3, 10, 44, 46, 48, 54, 60, 62.

as shown by agitated delirium, tremor, twitching and flexor rigidity of the forearms. After only 24 hours' therapy with moderate doses of V186 there was a profound fall in temperature from 103° to 97° F., accompanied by bradycardia (pulse-rate 64) and low blood-pressure (64/40). She vomited and became semi-comatose, although capable of being roused. She later rallied and seemed to be doing well except for occasional vomiting, but respiration became increasingly noisy and distressed and she died in coma five days after admission. Her blood urea was initially high and did not fall, and the urine obtained at autopsy contained a heavy cloud of albumin and many granular casts. Naples 44 was a woman of 40 with minimal petechial rash who did not seem very ill on admission, but was confused and had twitching of the forearm muscles. She vomited and collapsed after 48 hours intensive therapy, with a fall of temperature in one day from 104° to 95° F, bradycardia with impalpable pulse and unrecordable blood-pressure. She developed anuria, twitching of the forearms continued and she became comatose with a high-pitched cry at each expiration. No rally followed the administration of parenteral fluid; she developed pulmonary oedema and died on the fourth day after admission. Naples 46 was a man of 48 who was initially severely ill as evidenced by the presence of agitated delirium, extensive bronchiolitis and rapid breathing (50 per minute). He had a macular rash only. Oral therapy with V186 was not tolerated, owing to severe dysphagia and vomiting, and he was given the drug intravenously. Some rally occurred, but after several days the mental condition deteriorated; he expectorated large amounts of viscid purulent sputum and had diarrhoea. He eventually died



XVIII

and Control Patients

Oliguria.	Renal.		Skin.				C.N.S.							Mentality.				Complications.				
	Albuminuria.	Late uraemia.	Extent of rash.				Haemorrhage in rash.	Incontinence urine.	Incontinence faeces.	Reflexes abnormal.	Twitching.	Tremor.	Meningism. †	Normal or slight symptoms.	Delirium.	Confusion.	Stupor.	Coma.	Gangrene.	Bed-sores.	Abscesses.	Deaths.
			0	+	++	+++																
0	6	0	2	7	4	0	8	5	0	0	0	0	0	11	0	2	0	0	0	1	2	0
0	0	0	1	6	2	0	4	5	1	1	0	0	0	7	1	1	0	0	0	1	0	0
3	4	2	0	6	3	0	7	6	1	2	1	1	1	2	4	3	0	0	0	0	1	0
2	8	0	2	4	2	5	10	7	1	0	0	2	2	4	5	2	2	0	0	0	2	0
2	7	3	0	3	2	2	7	7	1	2	4	3	1	1	4	1	2	0	2	0	0	7
3	4	5	0	1	4	3	8	6	3	2	5	3	2	0	5	1	1	1	3	1	0	5

Control Cases—

\* Numbers : 4, 7, 11, 13, 19, 31, 45, 59, 63.

† Numbers : 17, 23, 25, 26, 35, 37, 41, 47, 49, 53, 57, 73, 75.

‡ Numbers : 6, 9, 15, 33, 39, 43, 55, 61.

in coma 11 days after admission. Naples 48 was a woman of 34, admitted on the 7th day of disease, who did not appear ill on admission, but who had a well-marked purpuric rash. The temperature fell by slow lysis during the next two days with intensive drug therapy, but she then sank into a restless, hallucinated state which necessitated sedatives. Cardiovascular collapse slowly set in, the blood urea rose, the rash became even more haemorrhagic, and she died in a noisy, agitated delirium on the 5th day after admission. At autopsy, there was evidence of right-sided heart failure, although this had not been suspected during life. Naples 54 was a man of 58 admitted in a feeble collapsed condition, with severe cyanosis, generalised twitching movements and delirium. He had incipient gangrene of the skin over the left scapula, and an extensive purpuric rash. He did not rally during the night after admission, when he was given parenteral saline containing V186, and he died 18 hours after admission. Naples 60 was a girl of 24 who had the most extensive macular and purpuric rash seen during the whole epidemic. She was only slightly confused on admission, showed no bronchiolitis nor severe degree of cardiovascular system involvement, but was greatly prostrated. In spite of continuous parenteral saline and massive doses of V186, she steadily became worse, haemorrhages developed on the face and in the nail beds, and she became more dyspnoeic and cyanosed. Pulmonary oedema slowly developed, the systolic blood-pressure began to vary with each beat and she died on the 5th day after admission with an exceedingly livid degree of cyanosis. There was a patch of gangrene in the right subscapular area. Naples 62 was a

## 74 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

woman of 24 with a slight petechial rash, admitted on the 6th day of illness and apparently not very ill. She had vomited much before admission, but had only a slightly elevated blood urea. After nine doses of V186 she began to vomit, and after two further doses, which were each promptly vomited, drug therapy was discontinued. Her temperature fell to normal, but she became clinically worse, was drowsy, but restless when roused, and vomited at intervals. She developed temporary suppression of urine and uraemia, and in spite of prolonged parenteral administration of fluid which was followed by secretion of abundant, pale urine, the nitrogen retention persisted. Ten days after the onset of uraemia she developed fever, pyuria, and then haematemesis. She did not respond to plasma or blood intravenously and died on the 13th day after admission. There was incipient gangrene of the buttock shortly before death.

In summary, the findings in these gravely-ill patients who received V186 resembled those of the control patients with grave illnesses. In the former, death occurred between the 2nd and the 13th day after admission to hospital and in the latter between the 4th and 18th days. Fever, which had frequently subsided before death, did not serve as a basis for comparison of the two groups of patients.

The illnesses of the four patients treated with V186, whose cases were excluded from the final series, are summarised in Table XIV. Naples 30 was a mildly-ill patient who exhibited rapid defervescence after treatment but whose serum failed to show diagnostic levels of agglutinins. Naples 52 was a patient who gave no serological response in life, and who showed staphylococcal pyaemia at death. Naples 40 and 58 were definite cases of typhus on the evidence of the clinical and autopsy findings, but no serum was obtained from Naples 40 during life and the serum from Naples 58 contained no demonstrable agglutinins. The sudden deterioration of Naples 58 with anuria, delirium and cardiovascular collapse resembled that seen in Naples 44, but was more striking because the patient did not seem ill on admission.

### *Assessment of the Results of Therapy with V186 and V147*

As neither V186 nor V147 produced an immediately obvious clinical effect, either with small or large doses, it was necessary to assess the results of therapy by comparison of the outcome in the two groups of treated and control patients. The foregoing account of the clinical findings indicates that the two groups of patients were reasonably comparable in duration of illness prior to admission, in age and sex incidence, and in symptomatology. Because it is so difficult to prophesy the outcome in early cases of typhus, it was impossible to use the degree of severity of illness at the time of admission as an indication of the expected course. There was, therefore, the inevitable possibility with such small series that one or other group contained a greater proportion of patients who would suffer either a milder or a more severe illness than the average. Table XIV indicates that some patients in the treated group did, in fact, suffer a milder illness than was to be expected, but the existence of numerous other patients who showed no response whatever to therapy make it improbable that the drug treatment was responsible for the rapid lysis sometimes

TABLE XIX  
*Duration of Fever after Treatment with V186 or V147*  
*(Twenty-two patients)*

	Days.												Average.
	1	2	3	4	5	6	7	8	9	10	11	12	
Patients ..	2	1	3	2	3	2	2	2	0	2	1	2	6.0

seen. This is borne out by Table XIX, which gives the duration, in days, of fever after commencement of therapy with V186 or V147 in 22 patients who recovered and who were treated adequately. (Patients who died, and one (Naples 76) who received inadequate treatment, have been excluded.) The number of days of fever varied from 1 to 12 in individual cases and averaged 6·0. In order to compare the duration of fever after admission to hospital in the treated patients with that in the controls, Table XX was constructed. The variation was from 2 to 13 days in the treated patients, with an average of 7·2,

TABLE XX  
*Duration of Fever after Hospitalisation in Patients who recovered*

	Numbers.	Days.												Average.
		2	3	4	5	6	7	8	9	10	11	12	13	
Treated	22	2	1	3	2	2	3	1	2	1	2	1	2	7·2
Control	25	3	2	3	1	3	1	1	4	4	2	1	—	7·0

TABLE XXI  
*Total Duration of Illness (Onset to Defervescence)*

	Numbers.	Days.															Average.
		6	7	8	9	10	11	12	13	14	15	16	17				
Treated	16	—	1	—	1	1	1	4	—	2	4	1	1	12·9			
Control	14	—	—	—	—	1	2	—	1	5	2	2	1	13·8			

TABLE XXII  
*Mortality by Age Group*

Age Groups.	Treated.		Controls.	
	Numbers.	Deaths.	Numbers.	Deaths.
15-19 .. .. .	6	0	8	0
20-29 .. .. .	10	2	7	0
30-39 .. .. .	9	1	6	1
40-49 .. .. .	3	2	5	1
50 and over .. ..	2	2	4	3
Totals .. .. .	30	7	30	5

and from 2 to 12 days in the controls, with an average of 7·0 days. In order to eliminate the effect of variable duration of fever prior to admission to hospital, the duration of illness from onset to defervescence was calculated in the 16 patients who were treated and recovered and whose day of onset was known, and in the 14 similar controls (Table XXI). The average duration was 12·9 days for the treated patients and 13·8 for the controls. It is clear that there was no objective evidence of benefit to the group of patients treated with V186 or V147 as compared with the controls. Finally, the mortality in the treated and control groups in relation to age seemed to be a possible method of assessment of the value of treatment. Table XXII gives the groups of patients

classified in decades, and it indicates that the mortality neither in the young nor in the older age groups was benefited by the therapy. One last point was examined in view of the experimental finding that chemotherapy of typhus infection in mice was most successful when begun early in the disease. The duration of fever was compared in the two groups of patients admitted and treated on or before the 6th day of disease and those treated later in the illness. The average duration of fever for the eight patients treated adequately on or before the 6th day of illness was 11.7 days, and the average for the eight patients treated later than this was 14 days. There were only three patients who received treatment, on the 4th day of illness, and these had an average of 12.6 days' fever; none was treated earlier than this. There were, therefore, slight differences between patients treated early and late in this series so far as the duration of subsequent fever was concerned, which may indicate that still earlier treatment, if it could have been instituted, would have been successful in aborting the infection.

#### *Toxic Effects of V186 and V147*

During the progress of the therapeutic trial of these drugs certain phenomena were encountered which were thought to be due in all probability to a toxic action of the compounds. Indeed, the administration of the drugs was suspended as much because of this apparent toxicity as because of a lack of evidence of therapeutic effect.

(1) Retching occurred in many patients who received the drugs and persuasion was usually needed to induce the patients to continue the treatment for more than two days. Vomiting, either as a single event or on several occasions, occurred four times as often in patients treated with the drugs (12 out of 30 patients) as in controls treated symptomatically (3 out of 30 patients). It was troublesome only when it occurred in association with other phenomena of a possibly toxic nature.

(2) A peculiar collapse or shock-like state occurred in five patients altogether, including Naples 10, 44, 48 and 76 amongst those in whom the diagnosis of typhus was fully confirmed, and Naples 58, in whom the diagnosis of typhus was based purely on post-mortem evidence. There were certain features in common to all five cases, of which four were treated with V186 and one with V147. The temperature began to fall prior to the development of shock, and reached subnormal levels in three cases. Furthermore, the fall in temperature was extremely rapid in three instances. The heart rate fell, often steeply, the pulse became impalpable or very feeble and the blood pressure was either low or could not be recorded when the collapse was fully developed. Some of the patients did not appear unduly distressed, but others were restless and anxious. Two of them became anuric, and some vomited either at the commencement of the shock or subsequently. Following the institution of resuscitative measures, a rally occurred in most instances, but subsequent progress differed in the various cases. Naples 10 remained collapsed for 24 hours, after six doses of V186, but the blood pressure could always be recorded and the pulse, though slow (64), had a fair volume. Death came three days after the collapse, with increasing respiratory distress. Naples 44 lapsed into coma when the fall in temperature occurred. After two days' treatment pulse volume and blood pressure failed, she vomited and became anuric. Though the heart's action slowly improved and the rate increased from 48 to 100, anuria persisted and she died 24 hours after the original collapse. Naples 48 had a relatively slow fall of temperature on the 9th day of disease, and though she was cold, restless and agitated, her pulse could still be felt until 12 hours before death; this occurred 24 hours after collapse had set in. Naples 76, who was treated with V147, appeared collapsed and pulseless for four hours on the 7th day of

disease, during which the temperature fell from 104° to 95° F. and then again rose to 107° F. Though seriously ill, from this time onwards she made a full recovery. Naples 58 experienced a rapid deterioration in condition of the circulation on the 3rd day of treatment; the pulse rate fell to 60 and blood pressure could not be recorded. The temperature reached normal levels at this time and did not become subnormal until shortly before death, 24 hours after the original collapse. No rally occurred at any time, but though confused she did not lose consciousness until shortly before death; anuria set in 24 hours before death.

In view of the fact that four of the five patients who showed the peculiar shock state subsequently died, it might be thought that the phenomena were simply terminal manifestations of the disease. Yet the complete syndrome was not seen in fatal cases amongst those treated symptomatically without the use of the drugs V186 or V147. Nevertheless, sudden falls in temperature are not uncommon in typhus fever (Kamal and Messih, 1943), though they are not apparently associated with simultaneous bradycardia or circulatory collapse. Heimberger (1943), however, has described prolonged collapse in typhus accompanied by anomalous fall in temperature by lysis, and states that the prognosis is unfavourable in such cases. Two patients treated symptomatically (Naples 6 and 39) suffered from extreme circulatory collapse, which was not unlike that described above and developed some time after admission, but neither had bradycardia and one had actual tachycardia (180) during the period of collapse. Terminal circulatory collapse was also encountered amongst moribund patients admitted after the completion of the therapeutic trial. Thus, it was impossible to prove either that the observed phenomena in the treated patients were due to a toxic effect of the drugs or to the disease itself. Reference to the clinical records of the cases concerned demonstrates that some had received small and some large doses of V186 or V147, and that other patients received the former drug in larger doses and yet failed to show any similar condition.

(3) Clinical evidence was obtained during the trial of V186 that the drug was probably exerting a toxic action on the kidney. The chief evidence for this was that three Naples cases (16, 20 and 62) and one patient in North Africa (Algiers EK13) developed uraemia after defervescence and the uraemic state dominated the clinical picture until death (Naples 62) or eventual recovery (remaining patients). However, the fact that uraemia was also encountered in control cases of typhus not treated with V186, made it impossible to decide definitely that the uraemia, suspected in some of the treated cases as being toxic in origin, was in fact due to the drug. Post-mortem findings in fatal cases, which are described in greater detail below, showed clearly that there was a severe tubular degeneration of the kidney in those patients treated with V186. Almost complete necrosis of the first convoluted tubules was seen in many of the fatal cases in the treated group, and all showed evidence of severe tubular damage. A much milder type of tubular degeneration (cloudy swelling and hydropic degeneration) was encountered in the control series. Reference to the detailed reports of Naples case 62 and Algiers EK13 in Appendix A will show that the clinical features in these cases were essentially those attributable to tubular lesions of the kidney. The passage of large volumes of pale dilute urine containing a moderate amount of urea, together with a persistently elevated blood urea, was observed in both these patients after a phase of anuria. There thus seemed no reason to doubt that the drug therapy exerted a toxic effect on the kidneys. Yet, there was no relationship between the size of the dose of drug which was administered and the severity of renal damage. The 20 gm. of V186 received by Naples 62 was apparently sufficient to produce an irreversible degree of renal damage, whereas many patients receiving 100 gm. or more of the drug showed no clinical evidence of renal damage at all.

## 78 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

The toxic effects of V186 and V147 encountered in cases of typhus recall both the toxic properties of these drugs in animals and the toxic properties of certain allied chemicals in man. In animals, V186 and V147 had a toxic effect on both the cardiovascular and renal systems. In man, the diamidine drugs used in the treatment of trypanosomiasis and kala-azar produce shock-like states after intravenous injection. Lawson (1942) recorded that one patient developed complete collapse resembling surgical shock after 0.5 gm. of pentamidine (4:4' diamidinodiphenoxypentane) in one injection, and some other patients developed albuminuria after treatment, which was not present before administration of the drug. Stilbamidine (4:4' diamidinostilbene) was found by Devine (1940) to cause nitrogen retention in rabbits. Lourie (1942) found that diamidine drugs used in the treatment of trypanosomiasis, including stilbamidine and pentamidine, caused immediate reactions after intravenous injection, with sudden drop in the blood-pressure, faintness and perhaps epileptiform twitchings. In spite of this, no delayed toxic effects were seen. McComas & Martin (1944) described the occurrence of status epilepticus and coma in one patient with trypanosomiasis treated with pentamidine. Kirk & Henry (1944) described delayed toxic effects, with renal and hepatic lesions, following stilbamidine, but were able to relate the occurrences to the development of toxic by-products following exposure of the drug to light.

The phenomena observed in some of our patients treated with V186 or V147 clearly resemble the known toxic effects of diamidine drugs. However, the possibility exists that cases of typhus are peculiarly susceptible to toxic effects such as may be produced by these drugs, for typhus is accompanied by lesions of the cardiovascular system and often by profound disturbance of renal function. In addition to this, collapse-like states are known to occur in typhus, and it is well-nigh impossible, therefore, in individual patients to distinguish phenomena due primarily to typhus from those possibly due to drug administration. The batch of V186 in use at the time that toxic effects were particularly encountered was examined at Hampstead by animal test, but was not found to possess an excessive degree of toxicity.

### MISCELLANEOUS CHEMOTHERAPEUTIC MEASURES

In addition to the patients treated with the drugs V186 and V147 and to those receiving only symptomatic or supportive therapy, a number of other patients received treatment with other chemotherapeutic agents. There were four patients who were specially selected for penicillin therapy, four who were treated with sulphathiazole because of chest complications, and four who were given sulphaguanidine. Two of the latter patients had received a single injection of 2.5 c.c. of typhus vaccine (Cox type) during the incubation period of their disease, and one other patient, not treated by any chemotherapeutic measures at all, had also been vaccinated in the same way and at a similar period of his illness.

#### *Penicillin Therapy*

A small trial of penicillin therapy was carried out early in March, 1944, when, by the courtesy of the Director of Preventive Medicine, N.A.T.O.U.S.A., and the United States of America Typhus Commission, 4,000,000 units of penicillin were made available. Four patients were treated, in an effort to determine whether penicillin could produce an immediately beneficial effect either in a case of average severity or in a gravely-ill patient. Table XXIII shows the main outlines of the dosage, etc., and the case record of one patient who died (Naples 93) is summarised in Appendix A. The use of penicillin was suggested by reports from the United States (Greiff and Pinkerton, 1944; Moragues, Pinkerton and Grieff, 1944) that the agent exerted a beneficial effect on experimental typhus both in mice and in chick embryos.

Our own trial of penicillin therapy was on a restricted scale, and it did no more than demonstrate that penicillin produces no immediate clinical benefit and is not apparently able to limit the spread of gangrene or even of consolidation of the lungs. However, a series of patients early in the disease would have to be treated before it could be stated that penicillin was useless in the treatment of typhus or that it had no effect at all. It was not considered justifiable to continue to use penicillin for further cases at a time when it was in short supply and so urgently needed for other purposes.

#### *Sulphathiazole Therapy*

Sulphathiazole was given to four patients soon after their admission, because of the presence of chest complications (Table XXIII). Two of the patients (Naples 27 and 29) were admitted as controls during the therapeutic trial with V186 and the other two patients were admitted in March, 1944. Naples 27 was a man of 21 who had a moderately severe attack of typhus with extensive and persistent signs of bronchiolitis, though not of consolidation. Thirty-one grams of sulphathiazole were given from the 10th to the 14th day, but the temperature had begun to fall slightly before the commencement of therapy, and a slow lysis set in, with normal levels from the 16th day onwards. There was no obvious benefit from the drug in this case. Naples 29 was a girl of 22 who had a moderately severe attack of typhus, with sudden appearance of signs of consolidation of the left lung base on the 9th day. Twenty-nine grams of sulphathiazole were given in four days, and the temperature fell sharply to normal on the 13th day and remained normal. The signs of consolidation of the lung had completely disappeared three days after defervescence, and it seemed probable that the drug had produced some benefit in this case. Naples 90 was a man of 32 admitted in a very ill, delirious state after about eight days' illness. There was a cyanotic purpuric rash and the lungs were filled with bubbly râles. The blood urea was 223 mgm. per 100 c.c. Treatment with sulphathiazole was given, but after 24 hours of treatment respiratory distress had increased and the patient became comatose and died. Naples 91 was a woman of 53 admitted on the 10th day of illness, when she did not appear very ill and was thought to be recovering from her original fever. On the day following admission the temperature rose, she became dyspnoeic and signs of consolidation of the lower lobe of the right lung appeared. Sulphathiazole was given from the 12th day onwards for 10 days, a total of 81 gm. being given. She became gravely ill, was fed by a nasal tube and given glucose saline intravenously. The fever subsided on the 17th day, but tachycardia and tachypnoea persisted, though the signs of consolidation, which had by then appeared over the opposite lung base, began slowly to clear. Progress was very slow; a sacral bed sore rapidly developed into large gangrenous areas, and dyspnoea continued. She died about one month after admission, with extensive areas of gangrene. Some benefit appeared to have been conferred by the sulphathiazole in this secondary type of pneumonia.

In summary, two of the four patients who received sulphathiazole therapy for pulmonary complications appeared to benefit from the treatment. Unfortunately, bacteriological examination of the sputum in these cases was not carried out, and it could therefore not be ascertained whether they were all of pneumococcal origin.

#### *Sulphaguanidine Therapy*

A report was received during the progress of the chemotherapeutic trial with V186, that sulphaguanidine was capable of influencing the course of typhus, even in small doses. Four patients (Naples 78, 80, 81 and 82) were, therefore, treated with sulphaguanidine at the rate of 2 gm. every 4 hours (Table XXIII). The various patients received 56, 86, 72 and 74 gm. in the course of 5, 7, 6

**CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS**

**TABLE**  
*Miscellaneous*

<i>Treatment.</i>	<i>Number.</i>	<i>Sex.</i>	<i>Age.</i>	<i>Day of disease when treated.</i>	<i>Severity on admission.</i>	<i>Dosage.</i>			
						<i>First dose.</i>	<i>Subsequent dose.</i>	<i>Interval.</i>	<i>Total dose.</i>
Penicillin	92	F	16	6th	Mod. severe	25,000 U I.M.	25,000 U I.M.	3-hourly	800,000 U
	93	F	24	?	Mod. severe	20,000 U I.M.	20,000 U I.M.	3-hourly	720,000 U
	94	M	30	?	Grave	25,000 U I.M.	25,000 U I.M.	3-hourly	575,000 U
	97	F	18	10th	Mild	25,000 U I.M.	25,000 U I.M.	3-hourly	509,000 U
Sulphathiazole	27	M	21	10th	Mod. severe	4 g.	1 g.	4-hourly	31 g.
	29	F	22	9th	Mod. severe	4 g.	1 g.	4-hourly	29 g.
	90	M	32	?	Grave	1½ g.	1½ g.	4-hourly	9 g.
	91	F	53	12th	Mod. severe	3 g.	1½ g.	4-hourly	81 g.
Sulphaguanidine	78	M	17	9th	Mild	2 g.	2 g.	4-hourly	56 g.
	80	F	26	5th	Mild	2 g.	2 g.	4-hourly	86 g.
Sulphaguanidine and Typhus Vaccine (Cox)	81	F	18	4th	Mild	2 g.	2 g.	4-hourly	72 g.
	82	M	18	6th	Mild	2 g.	2 g.	4-hourly	74 g.
Typhus Vaccine (Cox)	70	M	29	—	Mod. severe	—	—	—	—



## XXIII

*Therapeutic Group*

<i>Total duration.</i>	<i>Course of Illness.</i>				<i>Other therapy.</i>
	<i>General severity.</i>	<i>Days fever after drug.</i>	<i>Total fever duration.</i>	<i>Special features of illness.</i>	
days 4	Mod. severe	8	days 14	Delirium, doubtful chest signs. Petechial rash. Urea 93/123.	Nil.
4½	Grave	9	Died 11th day	Comatose; purpuric rash. Pneumonia (bilateral). In- cipient gangrene buttock. Urea 66/70.	Nasal feeding.
3	Grave	—	Died 5th day	Delirium, coma, dehydration, gangrene, purpuric rash, pneumonia. Urea 360/250/ 190.	Continuous isotonic and hypertonic (2 per cent. and 3 per cent.) saline I.V.
3	Mild	4	14	No mental or pulmonary symp- toms, petechial rash. Urea 31.	Nil.
5	Mod. severe	5	15	Dyspnoea, bronchiolitis; macu- lopapular rash, slow lysis 14-15th day. Urea 33/26.	Expectorant (KI and belladonna).
4	Mod. severe	4	13	Purpuric rash. Consolidation left lower lobe; crisis 13th day. Urea 84/35.	Nil.
1	Grave	1	Died 2nd day	Delirium, purpuric rash, con- solidation both lower lobes. Urea 220.	Nil.
10	Grave	4	Died about 28th day	Confused, no rash, consolida- tion right lower lobe, exten- sive gangrene buttocks.	Saline I.V. Nasal feeding.
5	Mild	1	10	Mild case, normal mentality, macular rash. Urea 24.	One dose 1½ g. V147 in error.
7	Mild	7	12	Mild case, normal mentality. No rash. Urea 38.	Nil.
6	Mild	8	12	Mild case, normal mentality, macular rash. Vomited once after admission. Urea 30.	Vaccinated 2.5 c.c. 6 days before onset.
6	Mild	7	13	Mild case, normal mentality, macular rash. Urea 32.	Vaccinated 2.5 c.c. 4 days before onset.
—	Mod. severe	—	13	Confused and delirious, pete- chial rash, diarrhoea. Urea 39/38.	Vaccinated 2.5 c.c. 3 days before onset.

and 6 days, respectively. Two, 7, 8, and 7 days of fever occurred after the start of therapy, with 10, 12, 12 and 13 days of total illness respectively, but all four cases were initially mild, had poorly marked rashes and only mild cardiovascular and chest signs. In addition, Naples 81 and 82 had each received a single injection of typhus vaccine during the incubation period. None of the four patients had an elevated blood urea. No definite benefit appeared to have been conferred by the drug therapy in these cases, nor did it appear that the vaccine had greatly modified the subsequent attack in the two patients who were vaccinated; while in a third patient (Naples 70) who received vaccine during the incubation period a typical moderately severe attack of typhus with mild delirium and confusion ensued. Guinea-pigs inoculated with blood taken on the 7th and 5th days respectively, from cases Naples 70 and 81, became infected, and strains of rickettsiae were established from both patients. No harmful effect of the two sulphonamide drugs which were administered was observed, such as might have been expected from the observations quoted by Yeomans and others (1944).

## PATHOLOGICAL INVESTIGATIONS

### ANIMAL EXPERIMENTS

*Guinea-pigs.*—For the isolation of strains of rickettsiae only guinea-pigs were employed, as opportunity did not arise to continue attempts, started in Algiers, to isolate rickettsiae by inoculation of sternal bone marrow intranasally into mice. Guinea-pigs received either 5 c.c. of fresh whole blood intraperitoneally—usually at the patient's bedside—or the ground-up clot from an equivalent amount of blood. Blood clot was used for inoculation only in those cases where isolation was attempted at an advanced stage of the disease. Altogether 22 attempts were made to isolate strains from 19 patients. Members of the U.S. Typhus Commission informed us that they succeeded in isolating strains from all three cases which they attempted. Many of the remaining guinea-pig isolations were done in collaboration with the Virus and Rickettsiae Section of the 15th General Medical Laboratory, Army of the U.S.A. Guinea-pig temperatures were taken twice daily. Within 48 hours of the temperature reaching 104° F. passage was done by inoculating brain emulsion intraperitoneally into fresh animals. In a number of cases blood was collected from convalescent animals for agglutination tests with rickettsial suspensions and the epidemic nature of the typhus infection confirmed. No immunity experiments were performed.

Strains were isolated from 17 patients; in one of them, only one of two attempts at isolation succeeded. In a further two cases guinea-pig inoculation was unsuccessful, (see Table XXIV). In one of the cases in which guinea-pig isolation failed, no attempt was made at passage when the guinea-pigs receiving the primary inoculation failed to show significant temperature response (Naples 1). If passage had been done, the attempt might have succeeded. The other failure was from a moribund case well advanced in the disease (Naples 94). In two cases (Naples 23 and 72) two separate attempts were made, both of which succeeded. In the case of 72, this is significant, as the second attempt was made four days after the commencement of V147 therapy.

Three attempts were made with blood from patients who had been on V186 therapy for 24 hours or more, and all succeeded. That penicillin also is not capable of freeing the peripheral circulation of viable rickettsiae is shown by the fact that strains were isolated from two patients who had been on intensive penicillin therapy for 24 hours or more (Naples 92, 93). One attempt with blood from a moribund penicillin-treated case failed (Naples 94). Two

patients, from whom strains were isolated, had received during the incubation period (three and six days respectively, before the onset of symptoms) a single dose of 2.5 c.c. of a potent yolk-sac vaccine.

The incubation period in guinea-pigs inoculated with blood varied from 8 to 14 days. After four or five passages the incubation period became appreciably shortened, usually to five or six days and, on occasion, to three or four days. The majority of the guinea-pigs showed no scrotal reaction. In a small proportion slight swelling was noted, but it was never impossible to push the testes up out of the scrotum.

TABLE XXIV

*Showing Cases from which Attempts to isolate Strains in Guinea-pigs were made*

<i>Index No.</i>	<i>Treatment received before attempt at isolation.</i>	<i>Inoculum.</i>	<i>Day of disease on which isolation was attempted.</i>	<i>Typical temperature response in guinea-pigs receiving primary inoculum.</i>	<i>Mouse adaptation.</i>
1	—	5 c.c. whole blood	7	—	—
3	—	5 c.c. whole blood	8	+	Full in 6 passages.
15	—	Clot (5 c.c. blood)	?	+	Full in 3 passages.
17	—	Clot (5 c.c. blood)	12	+	Full in 5 passages.
23	—	5 c.c. whole blood	6	+	Partial in 3 passages.
41	—	5 c.c. whole blood	6	(2 attempts) +	Full in 3 passages.
42	—	5 c.c. whole blood	5	+	Partial in 5 passages.
67	—	5 c.c. whole blood	8	+	Full in 5 passages.
80	—	5 c.c. whole blood	6	+	—
93	—	5 c.c. whole blood	? 7	+	—
97	—	5 c.c. whole blood	9	+	Partial in 3 passages.
70	Vaccinated 3 days before onset.	5 c.c. whole blood	7	+	Partial in 3 passages.
81	Vaccinated 6 days before onset.	5 c.c. whole blood	4	+	—
92	—	5 c.c. whole blood	8	—	—
92	Penicillin ..	5 c.c. whole blood	9	+	Partial in 3 passages.
94	Penicillin ..	5 c.c. whole blood	?	—	—
72	—	5 c.c. whole blood	5	+	Full in 5 passages.
72	V147 .. ..	5 c.c. whole blood	9	+	—
8	V186 .. ..	5 c.c. whole blood	?	+	—
20	V186 .. ..	5 c.c. whole blood	5	+	Partial in 4 passages.
22	V186 .. ..	5 c.c. whole blood	? 9	+	—

Guinea-pigs of the first passage were sent by air to Algiers; there the strains were maintained in guinea-pigs until well adapted, which was usually in four to six passages. Infected guinea-pigs were then sent by air to England, where adaptation to mice was performed, either at the Royal Army Medical College Emergency Vaccine Laboratory or at the National Institute for Medical Research.

*Mice.*—Guinea-pig brain of the 3rd to 7th passage was used to inoculate mice. Mice were given, under ether anaesthesia, 0.05–0.1 c.c. of 10 per cent. brain suspension intranasally. All mice received 15 mg. of sulphathiazole intraperitoneally, as well as sulphathiazole in the diet, to reduce the incidence of secondary infections. Mice were killed after 3–7 days, lung smears examined for “inclusion bodies” (Begg, Fulton & van den Ende, 1944) and rickettsiae, and suspensions of the lungs used to inoculate further groups of mice. Serial passage at intervals of 2–6 days was continued until smears showed abundant

rickettsiae (R+++ to ++++). Stock suspensions were then prepared and stored at  $-70^{\circ}\text{C}$ . The stock was used as required to inoculate large numbers of mice for the preparation of agglutinable suspensions. Adaptation to mouse lung had previously been thought to require massive doses of rickettsiae in the primary inoculum—such as heavily infected louse gut (Durand & Sparrow, 1940). Adaptation to mouse-lung with infected guinea-pig brain was found to be possible, but at first difficult (Andrewes, King, van den Ende & Walker, 1944). It had succeeded in the hands of Giroud & Panthier (1941, 1942). In the present investigations no difficulties were encountered in adapting strains from guinea-pigs to mice and no attempt failed.

In all, 11 strains were adapted to mouse-lung (Table XXIV) and emulsions satisfactory for agglutination tests were prepared with all the strains required (6). It took 3, 3, 5, 5, 5 and 6 passages in mice, respectively, before these six strains yielded lungs rich enough in rickettsiae for the preparation of agglutinating suspensions. Mouse to mouse passages were done at various intervals and one of four days was found to be most satisfactory. In two cases yields sufficient to prepare agglutinable suspensions were obtained in 10 days after the first inoculation of guinea-pig brain.

Large round cytoplasmic inclusion bodies, such as described by Giroud & Panthier (1942) and Begg, Fulton & van den Ende (1944), were seen in smears during the early stages of adaptation; these became more scanty when rickettsiae increased in numbers.

*Eggs.*—Only two strains (Naples 1 and 17) were adapted to the yolk sacs of chick embryos (Cox, 1938). Embryos of seven or eight days were used and the primary inoculum consisted of a suspension of infected mouse-lung containing ++rickettsiae. Serial passages were performed at 6 or 7 day intervals. Rickettsiae were at first scanty in yolk-sac smears, but increased after four passages, and with one strain (Naples 17) after seven passages large yields of rickettsiae were obtained (R+++ to ++++). The heavily infected yolk sacs were found suitable for the "toxicity" tests in mice already described.

#### SEROLOGY

The serology of typhus fever has in recent years been intensively studied. The time-honoured Weil-Felix reaction is still the routine laboratory test for the diagnosis of typhus fever. The techniques and interpretation of the test have recently been again described by Felix (1942, 1944). In addition to numerous observations on the Weil-Felix reaction published during the war, there has also been a renewal of interest in the rapid bedside *Proteus* agglutination test first described by Castaneda (Castaneda & Silva, 1940; Gaud, 1941; Veintemillas, 1941; Hallmann, 1942, and others).

Recent developments in the cultivation of rickettsiae and the preparation of suspensions have stimulated the more general use of rickettsial agglutination tests in typhus. Earlier agglutination experiments (Weigl, 1930; Zinsser & Castaneda, 1932, 1933; Castaneda & Zia, 1933 and others) were limited to laboratory experiments or small scale vaccine trials. Hudson (1940) employed suspensions prepared from rat lungs in a test which was suitable for diagnostic use. Since then it has been more widely used both in the laboratory and in diagnostic tests (Stuart-Harris, Rettie & Oliver, 1943; van Rooyen & Bearcroft, 1943; Craigie, unpublished observation; Kligler & Olesnik, 1943; Giroud & Giroud, 1944). Also complement-fixation tests (Castaneda, 1936; Bengtson, 1941; Bengtson & Topping, 1942; Plotz, 1943), neutralisation of the "toxic" effect of rickettsiae (Gildemeister & Haagen, 1942; Otto & Bickhardt, 1941) and cutaneous neutralisation tests (Giroud, 1938) have become more frequently used, not only in the research laboratories, but in relation to the serological diagnosis of typhus fever. The more specific rickettsial agglutination and

complement-fixation tests have been of particular value in the differentiation between murine and epidemic typhus. Of particular interest, in view of divergent results recently published, was a comparison between the rickettsial and *Proteus OX* agglutinin responses.

Although this was not the primary object of the investigation, the epidemic in Naples afforded an opportunity of studying certain aspects of the serological responses in typhus. During the Naples epidemic 93 cases of typhus were investigated. In 59 of these the date of onset could be determined with reasonable accuracy. In the remaining 34 cases it was impossible to determine accurately the length of illness previous to admission to the Infective Diseases Hospital. The original plan, adopted in Algiers, of taking blood and serum samples at intervals of four days, had, owing to the pressure of work, to be abandoned. The interval between bleedings was in the majority of cases lengthened to approximately a week. Blood counts and chemical investigations were done immediately. The serum after separation was sent by air to Algiers, where agglutination titrations were done, and the residue of the serum samples dried as already described. Unfortunately, the supply of rickettsial suspension ran short relatively early in the investigation, so that most of the rickettsial agglutinations could not be done on fresh sera. Practically all the titrations for *Proteus OX19*, *XX* and *X2* agglutinins were, however, completed in Algiers.

When, about six months later, the dried sera were reconstituted, it was found that the agglutination titres for rickettsiae had fallen slightly with drying and storage. *Proteus* agglutinins, on the other hand, appeared to be relatively unaffected. To complete the investigation, therefore, titrations of *Proteus* agglutinins were performed on reconstituted samples of only those sera which had not been titrated fresh. Rickettsial agglutinins, on the other hand, were re-titrated on all samples of serum, except 4, of which an insufficient quantity was available for drying. In such cases, the results of fresh serum titrations are included in the results.

The rickettsial suspensions used for the final titrations were freshly made and, according to tests performed by Dr. Fulton, gave more highly specific results than similar suspensions when aged (such as those used in Algiers). They were prepared according to the method described by Fulton & Begg (p. 164). The uniform agglutinability of the suspensions was controlled by the inclusion of a standard serum of known titre with each day's titrations. The method adopted has already been described in the report on the preliminary study in Algiers.

Each serum sample was tested against a murine (Wilmington) and an epidemic (Cairo) suspension. Later, sera from 25 cases were selected for tests against seven different epidemic strains.

*Results.*—The results of tests with the murine and the Cairo (epidemic) strains are recorded in the summaries of findings in individual patients (Appendix A). In addition, a short analysis of the findings has been made.

Fig. 5 records the average agglutination titres for murine and epidemic rickettsiae, as well as *Proteus OX19*, *OX2* and *OXK*, in those 59 cases in which the date of onset was known. The values recorded are logarithms of the geometric means of titres for the following days (5 and 6, 7 and 8, 9 and 10, etc., to the 32nd day). The figure gives only an approximate indication of the agglutinin response. Serum taken during the earlier days of the disease in a number of titrations showed no agglutination with the highest concentration tested (1/20, 1/25). Such values have been recorded as 0 and may, therefore, result in too marked a reduction of the average logarithmic value. In the case of *OX2* and *OXK* agglutinin titrations the highest serum concentration tested on several occasions was 1/50. If no agglutination occurred at this dilution, it was accorded an arbitrary value of

1/25 in calculating the averages. The average titres and the numbers of observations from which the averages were calculated are given in Table XXV. The numbers of occasions in which titres higher than 1/1,000 were encountered in each period are also recorded to indicate that low titre responses were not frequent.

Taking titres of 1/200 and over as significant for *Proteus OX19*, and 1/25 or more as significant for rickettsiae, the figures show that the average values are above this level during the 5th to 6th days. The increase in rickettsial

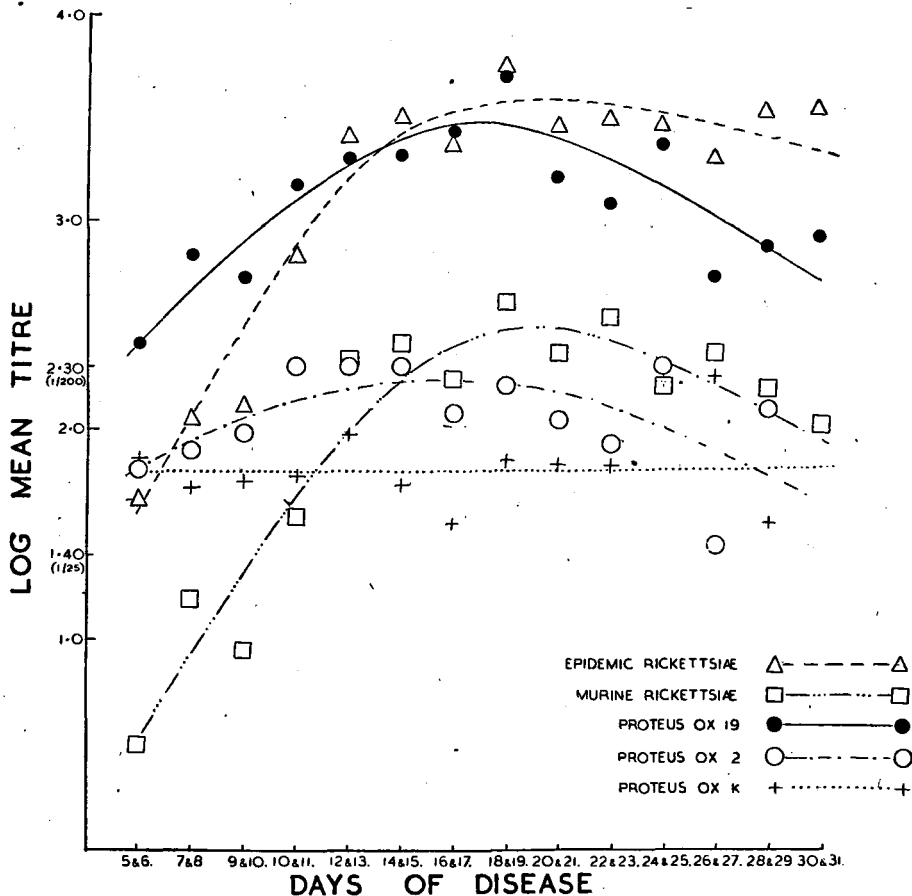


FIG. 5.—Average agglutination titres for murine and epidemic rickettsiae, and for *Proteus OX19*, *OX2* and *OXK*, in 59 cases in which the date of onset of disease was known.

agglutinins is more rapid than the *OX19* response, the agglutinins for murine rickettsiae did not reach a significant level until after the 10th day.

There is an increase in *OX2* agglutinins during the course of the disease, but the magnitude of the response is less than with *OX19*. Results with *OXK* are irregular, but there is apparently no consistent increase of agglutinins for this antigen.

In only one case were agglutination tests performed as early as the 4th day. The tests showed a diagnostic level of *OX19* agglutinins (1/400) and no agglutinins for epidemic rickettsiae. Agglutinins for both the antigens increased

TABLE XXV

Showing geometric means of agglutination titres for rickettsiae and Proteus OX antigens obtained in the study of 59 typhus cases in Naples. The table also records the number of observations from which the averages were calculated and the number of these observations showing a diagnostic agglutinating titre. (1/25 or more in the case of the rickettsial antigens, and 1/200 or more in the case of the Proteus antigens.)

Days of disease.		6-8	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32
Epidemic rickettsiae	Ave. titre .. .. .	45	113	131	674	2,583	3,179	2,367	5,628	2,874	3,061	2,985	2,029	3,414	3,535
	Number of observations ..	12	26	16	9	22	16	12	17	12	13	15	8	4	5
	Number with significant titre..	9	19	15	9	22	16	12	17	12	13	15	8	4	5
Murine rickettsiae	Ave. titre .. .. .	3	15	9	38	217	258	187	414	235	338	162	233	160	106
	Number of observations ..	12	26	16	9	22	16	12	17	12	13	15	8	4	5
	Number with significant titre..	3	14	7	7	22	16	11	17	11	12	15	8	4	4
Proteus OX19	Ave. titre .. .. .	256	681	530	1,481	1,959	2,022	2,709	4,874	1,636	1,194	2,293	545	757	841
	Number of observations ..	14	26	14	9	22	17	11	17	12	11	13	6	4	5
	Number with significant titre..	9	22	10	9	20	17	11	17	11	11	12	6	4	5
	Number with a titre of 1/1,000 or higher .. .. .	3	8	4	5	15	11	9	16	10	6	9	0	2	3
Proteus OX2	Ave. titre .. .. .	65	79	95	200	200	200	119	163	110	86	200	40	126	—
	Number of observations ..	13	24	13	9	12	10	8	8	7	9	4	3	3	—
	Number with significant titre..	1	5	5	5	6	7	3	4	3	2	3	0	1	—
Proteus OXK	Ave. titre .. .. .	73	52	56	58	94	54	35	71	67	68	168	177	40	—
	Number of observations ..	13	24	13	9	12	10	8	8	7	9	4	3	3	—
	Number with significant titre..	1	3	1	0	3	2	0	2	1	1	2	0	0	—

PATHOLOGICAL INVESTIGATIONS

later in the disease. Five of the 14 sera tested during the 5th or 6th day of the disease contained diagnostically insignificant amounts of agglutinin (1/100 or less) for *Proteus OX19*. Two of these were positive (1/40 or 1/80) and one negative with epidemic rickettsiae, while two of them were not tested with the rickettsial antigen. Of the three sera which, during the 5th or 6th day of illness, contained no rickettsial agglutinins, two were positive at dilutions of 1/200 and 1/400 respectively with *OX19*, and the remaining one did not agglutinate *OX19* even at a dilution of 1/50. For the 7th-8th day group, 4 of 26 did not agglutinate *OX19* at a dilution higher than 1/100. Of these, three were negative also with rickettsiae. In the same group, seven were negative with rickettsial antigen, but four of them agglutinated *Proteus OX19* at a dilution of 1/200 or more.

In the majority of the cases in which *OX19* titre was initially low, a significant rise occurred during the course of the illness. In three of them, however, the rise in titre was not more than two-fold (as in the low titre response described by Felix), but in only one did the titre remain below 1/200. In one of the cases, in which the titre never rose above 1/200, the diagnosis of typhus was confirmed by the isolation of the causative rickettsiae from the blood stream. Negative or weak serological reactions have been reported in severe or mild cases (Felix, 1944; Ding, 1942; Maly, 1942). Among the 21 fatal cases included in the Naples series, the maximum *OX19* titre encountered was 1/200 in one case (only one titration was done), 1/400 in two, 1/800 in a fourth and in all the rest 1/1,600 or higher. The maximum *OX19* titres obtained in each of the fatal cases are recorded in Table XXXII in the section on post-mortem findings. One case is, however, of interest, namely Naples 58, which because of negative serological findings was excluded from our series. This case, though excluded for purposes of the therapeutic trials, was nevertheless probably one of typhus in view of the characteristic histological findings in the brain. In another case there was no opportunity of retesting later sera after an early sample gave negative results with rickettsiae, but all remaining initially negative cases developed agglutinins for epidemic rickettsiae during the disease.

Agglutinins for *Proteus OX19* and epidemic rickettsiae, therefore, appear at approximately the same rate, and in the small series studied, *OX19* agglutinins were present in the serum in diagnostic amounts in 67 per cent. of sera tested during the latter half of the first week of illness, and rickettsial agglutinins in 69 per cent. The results of *OX19* agglutinin tests are in fairly good agreement with those reported (1942) by Schütz & Messerschmidt (regarding a titre of 1/200 as significant). The fact that Felix (1944) found positive reaction in 75 per cent. of cases on or before the 5th day probably depended on the reliance placed on a titre rising above that of a previous determination, even if the higher titre did not reach a level generally accepted as diagnostic. The magnitude of response and the speed with which a diagnostic titre (1/200) of *OX19* agglutinins was reached in these cases of epidemic typhus fever is in striking contrast with the results obtained in mild laboratory infections with murine typhus in vaccinated subjects (van den Ende *et al.*, 1943). Our results also differ somewhat from those of Süpfle & Fisher (1943), who found only 43 per cent. of typhus cases with a positive Weil-Felix test (1/400 or higher according to their method) in the first week, from those of Goeters (1942), who found less than 30 per cent. positive by the 6th day and 85 per cent. positive by the 7th day, those of Plotz, Wertman & Bennett (unpublished observations), who found only 54 per cent. positive (1/160 or more) by the 8th day, and of Maly (1942), who found only 42 per cent. positive (1/200 or higher) in the first week. Eyer & Dillenberg's (1943) results differ from ours only in the slightly lower percentage of *OX19* positives (taking 1/320 as significant titre) in the first week, and the better results which they obtained with rickettsial agglutinins in the



early diagnosis. The earlier appearance of rickettsial agglutinins in experimental animals has been demonstrated by Fitzpatrick & Hampil (1941) and by Zia & Liu (1938).

The striking difference in the agglutinating power of the sera for murine and epidemic rickettsiae is well shown in Fig. 5. The quantitative differences are sufficiently large to allow of differentiation between murine and epidemic forms of typhus. Rickettsial agglutination was of value also in those few cases where a significant rise of *OX2* antibodies occurred to a titre as high or higher than that of the *OX19*. The presence of rickettsial agglutinins in such cases excludes a possible diagnosis of the *Fièvre boutonneuse* group of typhus-like fevers. Apart from these functions, agglutination of rickettsiae offers no advantage over *Proteus OX19* in the diagnosis of typhus fever. Experience in Algiers was that non-specific positive *OX19* tests (1/200 or higher) did not occur in diseases such as typhoid fever as suggested, for instance, by Kunert & Buch (1942) and Goeters (1942).

The slide agglutination test with methylene blue-stained "O" suspensions of *Proteus X19* proved a reliable and convenient bedside test. In agreement with the findings of others, a very high degree of correlation was observed between the Weil-Felix test and slide agglutination. The slide test was negative in less than 2 per cent. of sera with which the Weil-Felix test was positive. The slide test can, however, not be regarded as a suitable substitute for the Weil-Felix test, as the former can obviously not show the rising titre of agglutinins which is of great importance in the diagnosis. Only strongly positive slide reactions are reliable, as delayed and partial agglutination often occurs in sera with Weil-Felix titres of less than 1/200.

The sera which were tested against a variety of epidemic strains included specimens from cases from which strains of rickettsiae, used for making the suspensions, were isolated. The results obtained with all the sera were similar, and examples are given in Table XXVI. Four of the strains used were isolated in Naples (Naples 3, 15, 41 and 72); one was isolated earlier in the year in Algiers (Algiers EK11). The adaptation of the strains to mice for the preparation of the antigens is described in another section of the report. In addition, the Cairo epidemic strain (supplied by Major C. E. van Rooyen, R.A.M.C.) which has been maintained in mice for three years, and, latterly, a newly-adapted strain (Otto) from Russia were included. The titres obtained were almost identical with all the antigens except the Algiers strain. This antigen frequently gave a slightly lower titre than the others, even with the serum of the patient from whom it was isolated. These results are in agreement with the conclusions reached in complement fixation and agglutination tests performed in this laboratory, namely, that no antigenic differences can be demonstrated between epidemic strains of rickettsiae, whether newly isolated or maintained in culture for several years. A number of sera from cases in Algiers were included in the tests with a variety of rickettsial strains. All those tested, with one exception, behaved like other epidemic sera. The murine nature of the one exception (Algiers M2) was confirmed.

All the tests with rickettsial suspensions, and more especially those tests which were performed with reconstituted dried sera and fresh rickettsial suspensions, showed well-marked zoning (Table XXVI). With the heterologous antigen (murine) usually only a single zone in the region of the more concentrated serum was noted. The epidemic antigens frequently showed a double zone. The zoning is sometimes very marked, and is of great practical importance. If an insufficient range of serum dilutions is put up, the end-point of agglutination can quite well be missed, and in some cases a strong enough pro-zone occurs to make agglutination appear negative unless the possibility of zoning is borne in mind. An explanation of the marked zoning cannot at present be offered. It may depend on the presence of different antigens which

TABLE XXVI

Showing agglutination and zoning phenomenon with one murine, one old established epidemic strain (Cairo), one more recently isolated Russian strain (Otto) and five newly isolated strains from Naples (Naples 72, 3, 41 and 15) and Algiers (Algiers EK11)

Serum.	Antigen.	20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480	40,960	Saline
Standard ..	Cairo ..		++++	++++	++++	++++	++++	++++	tr	—	—	—	—	—
	Naples 73 ..		++++	++++	++++	++++	++++	++++	tr	—	—	—	—	—
	Naples 3 ...		++++	++++	++++	++++	++++	++++	tr	—	—	—	—	—
	Naples 41 ..		++++	++++	++++	++++	++++	++++	tr	—	—	—	—	—
	Naples 15 ..		++++	++++	++++	++++	++++	++	tr	—	—	—	—	—
	Algiers EK11		++++	++++	++++	++++	++++	++	tr	—	—	—	—	—
	Murine ...		++++	++++	++++	++++	++++	+	—	—	—	—	—	—
Algiers M.2. .. 23/8 (17th day)	Cairo ..	+++	+++	++	+++	+++	+	—	+	—	—	—	—	—
	Murine ..	+++++	+++	tr	+	+++	++++	++++	++++	++++	+++	—	—	—
Naples 3. .. 12/1 (18th day)	Cairo ..	+++	+++	+++	++	tr +	+	++	+++	++	tr	—	—	—
	Naples 72 ..	+++	+++	+++	++	tr	+	++	+++	+++	—	—	—	—
	Naples 3 ..	+++	+++	+++	++	tr	+	++	+++	+++	—	—	—	—
	Naples 41 ..	+++	+++	+++	++	tr	+	++	+++	+++	—	—	—	—
	Naples 15 ..	+++	+++	++	++	tr	+	++	+++	+++	tr	—	—	—
	Algiers EK11	+++	+++	+++	++	tr	+	++	+++	+++	++	—	—	—
	Murine ..	+++	+++	+++	+++	+++	+++	+++	—	—	—	—	—	—
Naples 42. .. 2/2 (10th day)	Cairo ..	++	tr	—	tr	++	+++	+++	+	—	—	—	—	—
	Naples 72 ..	++	tr	tr	—	++	+++	+++	+++	—	—	—	—	—
	Naples 3 ..	+	tr	—	tr	+++	+++	+++	+	—	—	—	—	—
	Algiers EK11	+++	tr	—	—	+++	+++	+++	—	—	—	—	—	—
	Murine ..	—	—	+++	+++	+++	+++	—	—	—	—	—	—	—
Algiers EK11. 17/10. (14th day)	Cairo ..	++++	++++	++++	++	+++	+++	+++	—	—	—	—	—	—
	Naples 72 ..	++++	++++	+++	tr	++	+++	+++	tr	—	—	—	—	—
	Naples 3 ...	++++	++++	++	tr	++	+++	+++	—	—	—	—	—	—
	Algiers EK11	++++	++++	tr	—	tr	+++	+++	—	—	—	—	—	—
	Murine ..	tr	tr	tr	tr	++	—	—	—	—	—	—	—	—
Naples 3. 12/1 (later titration).	Cairo ..	+++	+++	+++	—	—	—	++	++	++	—	—	—	—
	Otto ..	+++	+++	+++	—	—	—	++	+++	+++	+	—	—	—
	Murine	++	+++	+++	+++	+++	++	tr	—	—	—	—	—	—
Naples 8 ..	Cairo ..	+++	+++	+++	tr	tr	tr	+++	++	tr	—	—	—	—
	Otto ..	+++	+++	+++	tr	tr	tr	+++	+++	—	—	—	—	—
	Murine ..	+++	+++	+++	++	++	++	tr	—	—	—	—	—	—

give maximal reaction at different optimal proportions. Differences in zones with different suspensions may indicate variations in the proportions of different antibody fractions (Fulton's  $M^1$ ,  $M^2$ , etc., p. 163) present. Absorption tests with a variety of antigens may help towards an explanation.

Agglutinins for one or more of the following: *B. typhosum* O, *B. paratyphosum* A O, *B. paratyphosum* B O, *Br. melitensis* O, and *Br. abortus* O were present in 23 of the 93 typhus patients investigated. In only 10 of these were agglutinins present in a titre of 1/100 or more, and only two of the seven from this group that were retested on subsequent days showed a significant rise (4-fold) during the course of the illness. In one case (Naples 58) O agglutinins for *B. paratyphosum* A increased from 1/50 to 1/800. In the other (Naples 5) agglutinins for *Br. abortus* increased from 0 at 1/25 to + at 1/200. Neither of the cases, however, gave any evidence to substantiate a diagnosis other than typhus.

In view of the frequent reports of positive results obtained in sera from typhus cases with such serological tests for syphilis as the Kahn or Meinelke (Warnecke, 1943; Ding, 1942; Reynes, 1940), a number of sera from typhus patients were kindly tested for us by Major Hinson of the 94th British General Hospital. Seventy-three serum samples from 58 cases were tested. A strongly positive Kahn reaction was encountered once; another case gave a moderately positive reaction; a further four sera gave weakly positive results; three were doubtfully positive and 63 were negative. Weak to strongly positive reactions were encountered, therefore, in 9.6 per cent. of the sera tested. This figure is slightly lower than that published by Warnecke (16.6 per cent.), and greatly different from Ding's (38 per cent. positive). Some of our sera were tested after several weeks' storage in the ice box, and a higher percentage might have been positive if it had been possible to test the sera when fresh.

#### *Complement fixation*

A number of the sera which were used in the agglutination tests with a variety of antigens, were tested for complement-fixing antibodies. The antigens used were freshly prepared suspensions of rickettsiae from rat or mouse lungs purified by the Keiselguhr method (Fulton & Begg, p. 164) and with opacity equivalent to that of  $300 \times 10^6$  *B. coli* per c.c. Three such antigens were employed: Wilmington (murine), Cairo (epidemic) and Naples 72 (epidemic). In addition, a suspension of Algiers EK11 (epidemic) which had been prepared two weeks previously and stored at  $-70^\circ$  C. was utilised.

The results are recorded in Table XXVII. They show that with the freshly prepared antigens highly specific complement-fixation occurs with the epidemic strains. The stored antigen, on the other hand, gives higher titres with all positive sera, including serum from a guinea-pig convalescent from murine typhus. The stored antigen, though more sensitive, is therefore at the same time less specific. This confirms earlier findings by Fulton. Results are expressed as the reciprocal of the highest serum dilution, giving complete fixation of 2 M.H.D. of complement in 1 hour at  $37^\circ$  C. Concentrations higher than 1/20 were not tested.

#### *"Toxin" neutralisation*

The power of neutralising the acute toxic effect of rickettsiae injected intravenously into mice was measured for 7 sera. Increasing dilutions of each serum were mixed with equal volumes of rickettsiae of appropriate concentration. This concentration was that calculated to contain twice the certainly fatal dose per 1/4 c.c. (see Fulton *et al.*, p. 154). The mixtures, after

## 92 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE XXVII

*Showing the complement-fixation obtained with one murine and three epidemic antigens. The antigen EK11 was not freshly prepared like the others and shows greater degree of nonspecific fixation*

Serum.	Date	Antigen.			
		Cairo.	Algiers EK11	Naples 72.	Murine.
Algiers M.1 .. ..	23/7	20	—	—	0
	7/8	20	160	20	0
	20/8	0	—	—	—
Naples 72 .. ..	19/2	0	0	0	0
	9/3	160	640	320	0
Naples 42 .. ..	28/1	0	—	—	0
	2/2	160	640	160	0
	8/2	160	—	—	0
Naples 17 .. ..	14/1	—	—	—	0
	27/1	80	—	—	0
	12/2	40	—	—	0
Naples 3 .. ..	12/1	160	—	—	0
	18/1	80	1,280	320	0
	26/1	40	—	—	0
Naples 32 .. ..	22/1	0	—	—	0
	3/2	40	0	0	0
	10/2	40	—	—	0
	15/2	40	—	—	0
Naples 8 .. ..	8/1	160	—	—	0
	20/1	320	640	160	0
	1/2	160	—	—	0
Algiers EK11 .. ..	8/10	20	—	—	0
	17/10	80	160	80	0
	15/11	40	—	—	0
Algiers EK7 .. ..	20/8	160	—	—	0
	30/8	160	640	160	0
	15/9	80	—	—	0
Cairo guinea-pig ..	—	160	320	160	0
Murine guinea-pig ..	—	20	80	0	40

standing  $\frac{1}{2}$  hour at 37° C. and  $\frac{1}{2}$  hour at room temperature, were used to inoculate groups of four mice, each receiving 1/4 c.c. intravenously. The number of deaths in each group was recorded after 18 hours.

The results are given in Table XXVIII. They show that neutralising power of the serum under the conditions of the test was poor. Furthermore, the difference in neutralising power for murine and epidemic strains is doubtfully significant.

It was intended to estimate the neutralising power of a number of selected sera by the pulmonary titration method (vanden Ende & Mills, p. 131). Unfortunately, an accident to the stock suspensions resulted in a gradual deterioration of titre. By the time new stocks had been prepared, a considerable interval had elapsed. The antibody content of the reconstituted sera, as judged by agglutination tests, had during this time fallen off, and the neutralisation tests were, therefore, abandoned.

TABLE XXVIII

Showing results obtained in "toxin" neutralisation tests with rickettsiae of one murine and two epidemic strains cultivated in the yolk sacs of eggs. "Cairo" and "Murine" are old established laboratory strains. Naples 17 was recently isolated during the Naples epidemic. The figures record numbers of deaths in 24 hours—a group of 4 mice being used for each serum-rickettsiae mixture.

Serum Dilutions.	Cairo "Toxin".					Naples 17 "Toxin".					Murine "Toxin".				
	1/10	1/50	1/250	1/1250	None.	1/10	1/50	1/250	1/1250	None.	1/10	1/50	1/250	1/1250	None.
Serum.															
Standard epidemic .. ..	0	0	0	4	4	0	0	0	4	4	0	4	3	4	4
Naples 72. 15/2 (4th day) .. ..	4	4	4	4	4	4	4	4	4	4	—	—	—	—	—
Naples 72. 9/3 (27th day) .. ..	0	0	3	4	4	1	0	2	4	4	—	—	—	—	—
Standard .. ..	—	—	1	4	—	—	—	—	—	—	—	—	—	—	—
Naples 8. 1/2 (37th day) .. ..	0	0	4	4	4	1	3	4	4	4	0	0	4	4	4
Standard .. ..	—	—	—	—	—	—	—	1	4	—	—	—	—	—	—
Naples 17. 14/1 (10th day) .. ..	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Standard .. ..	—	—	—	—	—	—	—	—	—	—	0	4	—	—	—
Naples 17. 12/2 (39th day) .. ..	0	1	4	4	4	0	4	4	4	4	1	3	4	4	4
Standard .. ..	—	—	—	—	—	—	—	0	4	—	—	—	—	—	—
Algiers M2. 29/10 .. ..	—	—	—	—	—	0	0	4	4	4	0	0	1	4	4
Standard .. ..	—	—	—	—	—	—	—	—	—	—	—	0	3	—	—
Algiers EK11. 10/11 (38th day) .. ..	—	—	—	—	—	0	0	4	4	4	0	0	1	4	4
Standard .. ..	—	—	—	—	—	—	—	0	4	—	—	—	—	—	—

PATHOLOGICAL INVESTIGATIONS

## BLOOD COUNTS

Accounts of the blood picture in typhus fever have been numerous, and many apparent differences in findings are encountered in the literature. These differences can probably be accounted for by the fact that great variations are seen between different cases in the same epidemic and in the same case during different stages of the disease. Thus Kracke (1941) speaks of lymphocytosis with neutropenia in typhus. Osgood & Ashworth (1937) report leucocyte counts of 4,000 to 20,000 p. cmm., with some increase of neutrophils and many immature forms. Schilling (1929) suggests that a high grade of regenerative shift, atypical lymphocytes and monocytes and irritation forms, occur in typhus as distinct from typhoid. Whitby & Britton (1944) mention relative or absolute lymphocytosis in typhus fever. Wolbach, Todd & Palfrey (1922) report that total leucocyte counts were found to vary from 6,000 to 20,000 per cmm., and that in 59 cases in which differential counts were performed during the second week, the average figures obtained were:—

Neutrophils, 75·4 per cent. (incompletely divided nuclei 50·7, nuclei with two or more lobes 24·7).

Lymphocytes, 18·6 per cent.

Large mononuclear basophils, 5·9 per cent.

Eosinophils, 0 per cent.

Van den Ende *et al* (1943) found monocytosis the most striking feature of a number of mild murine typhus cases. A striking monocytosis in many cases during the second week of the disease has been reported by Schiff (1917). The irregularity of the monocyte response has been pointed out by Elkeles (1917). Many authors agree on the occurrence of a shift to the left during the early stages of the disease, and relative lymphocytosis and return of eosinophils to the peripheral circulation during later stages (Schiff, 1917; Rabinowitch, 1913).

A detailed study of the blood picture in typhus fever has been made by Danielopolu (1918) and Danielopolu & Craciun (1939). Danielopolu not only finds the blood picture of diagnostic help, but considers that the occurrence of marked leucocytosis is of prognostic significance. These authors also report the occurrence of polycythaemia (7–8 million) in grave cases of typhus fever.

Lampert (1943) has recently reported on the blood picture of six cases of typhus. He considers the most striking features to be a polymorphonuclear leucocytosis and a lymphocytosis at the end of the febrile stage. Total counts varying between 7,200 and 26,000 p. cmm. are reported. He makes use of the differential counts in a rather elaborate calculation of an "intoxication index."

Blood counts were undertaken in our cases partly to determine whether or not the drugs V186 and V147 exerted a toxic influence on the blood-forming organs. An analysis has been made of the blood counts in 52 cases in which the date of onset of the disease was known. Thirty-three of these cases belonged to the control series, and 19 were treated with V186. The results of the two groups are recorded separately in Tables XXIX and XXX and in Figs. 6–10.

The results have been compared with values obtained in eight normal Italian hospital employees of approximately the same social status as the patients (Appendix A). The normal series is admittedly small, but the investigation of a larger group was, under the existing conditions, impossible.

The results recorded in Tables XXIX and XXX show the averages for each of the six stages of the disease chosen (5–6th day, 7–8th day, 9–10th day, 11–15th day, 16–20th and 21st–30th day). They also show the number of observations on which each average figure is based. To demonstrate the very marked variations encountered, the highest and lowest values are likewise

recorded. In the case of eosinophils, basophils and "basophilic mononuclear" cells, the number of times in which cells of these types were not encountered amongst 100-200 leucocytes, is also stated.

*Red blood corpuscles.*—A slight anaemia was present throughout the disease, and in the case of the controls was almost constant in severity. In the V186-treated group the anaemia appeared slightly less marked in the early stages of the disease, but the difference was insignificant. The average red cell counts in the two groups were within the range  $3.52$  to  $4.19 \times 10^6$ . The haemoglobin values (corrected Sahli) varied between 85 per cent. and 70 per cent., with colour indices of 1.1-0.9. Increase in the number of red blood corpuscles was not encountered in fatal cases. In eight fatal cases which had not received drug therapy and in which blood counts were performed, the highest red cell count

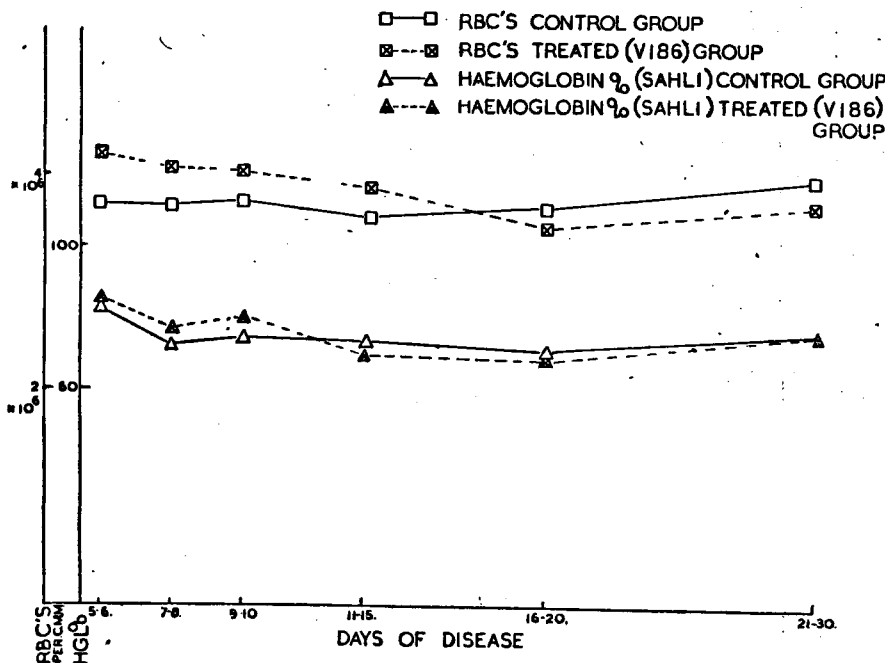


FIG. 6.—Average R.B.C. counts and haemoglobin percentages of controls and V186-treated cases.

obtained was 5.43 million per c.mm. The average red blood cell count and haemoglobin value for these eight cases were 3.93 million and 83 per cent. respectively. In seven fatal cases treated with V186 a red cell count of  $5.20 \times 10^6$  was encountered once. Average red blood cell count and haemoglobin for these seven cases were 4.17 million and 85 per cent. respectively. These findings, together with the fact that blood and plasma specific gravities were reduced, indicate that haemoconcentration was not a feature of the cases of typhus studied in Naples (Fig. 6).

*Leucocytes* (see Figs. 7-10).—The average total white cell counts in both treated and control groups were consistently slightly below normal. Individual variations were marked, and occasionally marked leucopenia or a definite leucocytosis was encountered. Danielopolu has reported the occurrence of marked leucocytosis in grave cases. We were unable to substantiate this finding. Blood counts were performed on 15 of the fatal cases in our series.

TABLE XXIX

Showing the average maximum and minimum numbers of leucocytes encountered at different stages of the disease in cases NOT receiving drug therapy. The numbers of observations on which the averages are based are stated. The table also records the counts obtained in 8 normal persons

Days of disease		5-6.	7-8.	9-10.	11-15.	16-20.	21-30.	Control Normals
Total Leucocytes	Number of observations	7	15	10	14	9	14	8
	Average	5,493	6,677	6,895	6,929	5,639	5,971	8,031
	Maximum	9,150	17,250	10,650	10,000	8,800	9,250	11,950
	Minimum	3,450	2,850	2,350	2,900	1,850	3,650	5,600
"Segmented" Polymorphs	Number of observations	7	14	9	14	7	14	7
	Average	1,790	2,292	2,445	2,837	2,468	2,310	3,378
	Maximum	5,032	8,280	4,992	4,995	4,536	4,995	5,529
	Minimum	690	315	188	1,276	148	594	1,755
"Unsegmented" Polymorphs	Number of observations	7	14	9	14	7	14	7
	Average	2,606	2,986	2,703	2,084	588	793	1,193
	Maximum	3,291	6,555	4,686	5,090	1,872	1,750	2,242
	Minimum	1,913	607	936	565	165	146	171
Lymphocytes	Number of observations	7	14	10	14	8	14	7
	Average	906	1,042	1,400	1,492	1,560	2,526	2,387
	Maximum	2,394	1,897	2,301	2,755	3,083	5,183	3,828
	Minimum	138	595	305	388	667	949	978
Monocytes	Number of observations	7	14	9	15	8	14	7
	Average	244	346	321	459	405	336	385
	Maximum	444	630	728	925	625	672	679
	Minimum	100	114	94	0	166	0	57
Eosinophils	Number of observations	7	14	9	15	8	14	7
	Average	0	4	6.5	27	43	57	98
	Maximum	0	57	59	185	72	224	291
	Minimum	0	0	0	0	0	0	0
Basophils	Number with none	7	13	8	11	2	7	1
	Number of observations	7	14	9	15	8	14	7
	Average	14	6	17	0	49	56	30
	Maximum	51	86	106	0	205	268	87
"Basophilic Mononuclears"	Minimum	0	0	0	0	0	0	0
	Number with none	5	13	7	15	3	7	4
	Number of observations	7	14	9	15	8	14	7
	Average	63	50	182	83	11	19	0
	Maximum	183	219	700	277	54	125	0
	Minimum	0	0	0	0	0	0	0
	Number with none	3	5	3	8	6	10	7



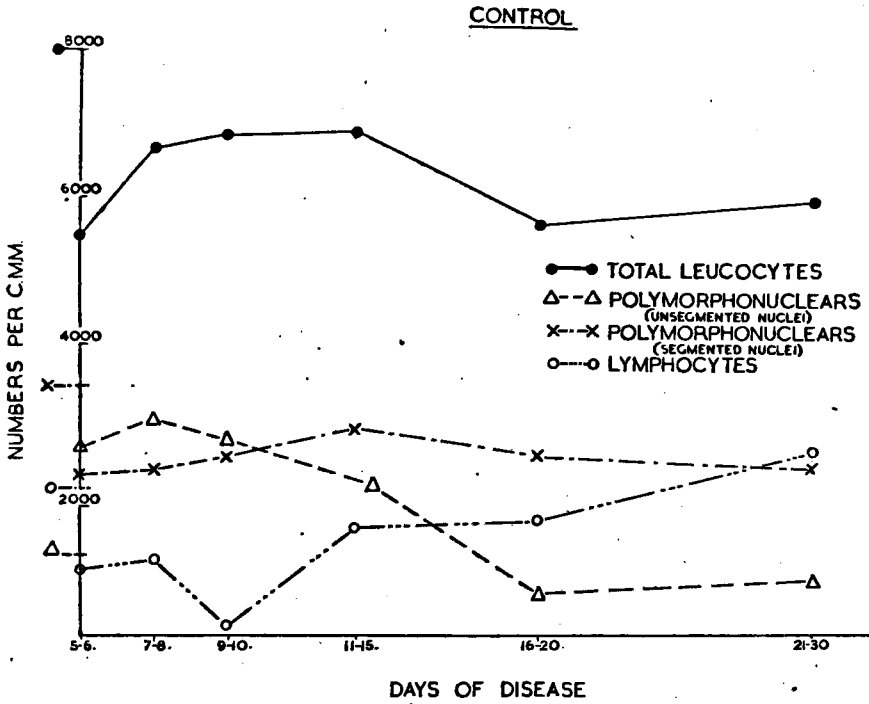


Fig. 7.—Average white cell counts of 33 control cases.

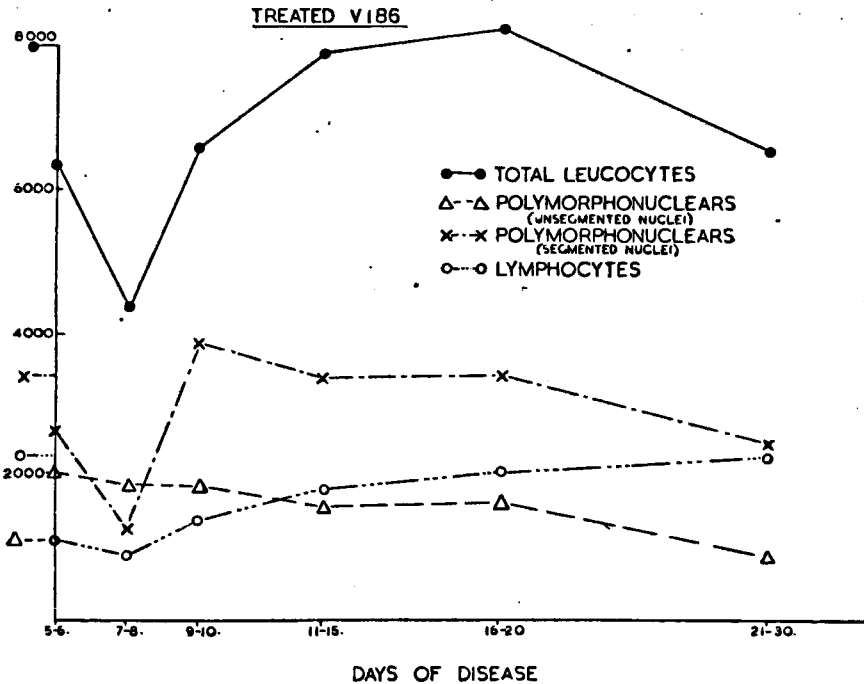


Fig. 8.—Average white cell counts of 19 cases treated with V186.

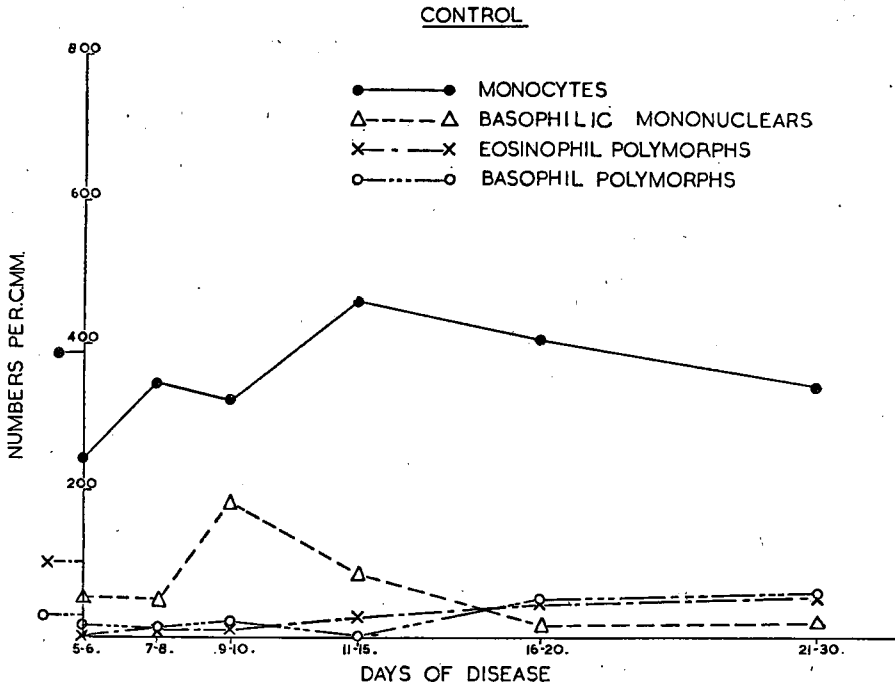


Fig. 9.—Average counts of certain white cell elements in 33 control cases.

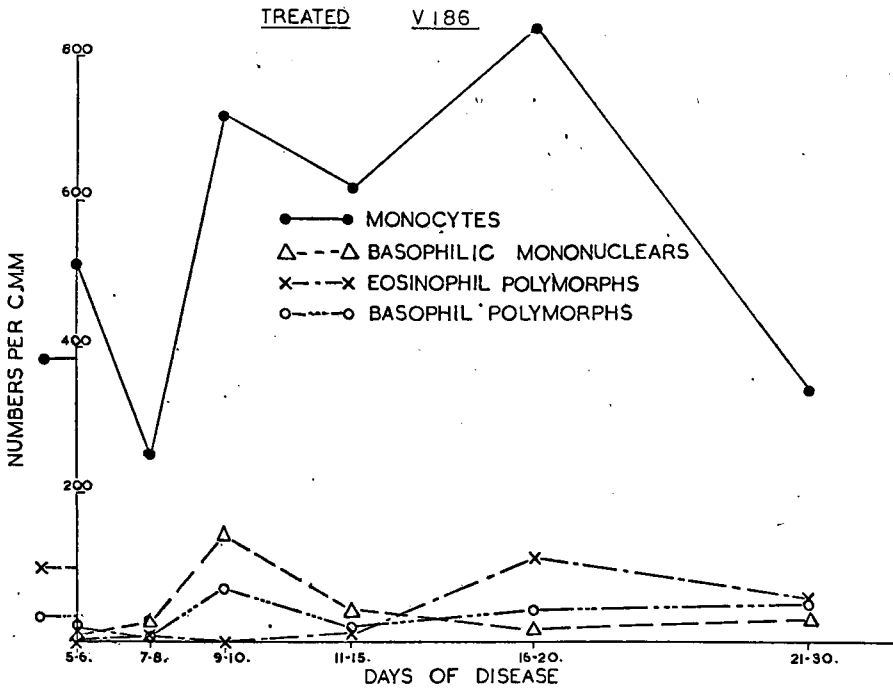


Fig. 10.—Average counts of certain white cell elements in 19 cases treated with V186.

TABLE XXX

Showing the average maximum and minimum numbers of leucocytes encountered at different stages of the disease in V186-treated cases. The numbers of observations on which the averages are based are stated.

Days of disease .. .. .		5-6.	7-8.	9-10.	11-15.	16-20.	21-30.
Total Leucocytes .. .. .	Number of observations .. .. .	4	10	5	12	9	17
	Average .. .. .	6,400	4,345	6,600	7,933	8,294	6,553
	Maximum .. .. .	9,250	6,500	10,900	12,150	11,650	11,150
	Minimum .. .. .	4,200	2,150	2,700	5,000	4,850	3,200
"Segmented" Polymorphs .. .. .	Number of observations .. .. .	4	9	3	10	7	15
	Average .. .. .	2,623	1,273	3,859	3,386	3,428	2,459
	Maximum .. .. .	5,272	2,106	6,431	5,512	6,176	6,048
	Minimum .. .. .	987	193	2,376	1,450	824	452
"Unsegmented" Polymorphs .. .. .	Number of observations .. .. .	4	9	3	10	7	15
	Average .. .. .	2,028	1,873	1,843	1,546	1,633	889
	Maximum .. .. .	3,225	3,120	2,375	2,871	2,214	2,938
	Minimum .. .. .	1,239	816	1,306	539	810	141
Lymphocytes .. .. .	Number of observations .. .. .	4	9	3	11	8	17
	Average .. .. .	1,109	899	1,387	1,800	2,066	2,296
	Maximum .. .. .	1,665	1,968	1,853	3,070	3,240	4,182
	Minimum .. .. .	885	392	857	531	1,067	1,186
Monocytes .. .. .	Number of observations .. .. .	4	9	3	11	8	17
	Average .. .. .	519	305	720	619	839	344
	Maximum .. .. .	833	714	981	954	1,497	746
	Minimum .. .. .	57	88	396	74	131	0
Eosinophils .. .. .	Number of observations .. .. .	4	9	3	11	8	17
	Average .. .. .	0	10	0	16	115	53
	Maximum .. .. .	0	51	0	100	540	141
	Minimum .. .. .	0	0	0	0	0	0
	Number with none .. .. .	4	7	3	9	4	6
Basophils .. .. .	Number of observations .. .. .	4	9	3	11	8	17
	Average .. .. .	16	5	73	18	41	53
	Maximum .. .. .	63	41	218	74	232	376
	Minimum .. .. .	0	0	0	0	0	0
	Number with none .. .. .	3	8	2	8	6	8
"Basophilic Mononuclears" .. .. .	Number of observations .. .. .	4	9	3	11	8	17
	Average .. .. .	5	23	146	43	17	30
	Maximum .. .. .	21	102	198	204	82	395
	Minimum .. .. .	0	0	109	0	0	0
	Number with none .. .. .	3	6	0	8	6	14

## 100 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

The average figures for the highest counts recorded during the fatal illnesses were 10,079 per c.mm. in the V186-treated (seven cases) and 7,638 in the control group (eight cases). The ranges of variation in the counts were:—

Treated group .. .. .	4,000–20,000
Controls.. .. .	2,350–17,250

*Differential count.*—There was in both drug-treated and control groups a definite increase in polymorphs with unsegmented nuclei (shift to the left) during the early stages of the disease. The counts of “unsegmented” polymorphs in our eight normal cases are higher than those reported by Schilling (see Whitby & Britton, 1944). The typhus cases, nevertheless, showed the increase in unsegmented polymorphs even in comparison with these normals. Myelocytes were encountered in the peripheral blood in very small numbers on two occasions only. Lymphocytes were reduced in number in the circulating blood during the early stages of the disease, but returned during convalescence. A relative lymphocytosis is a very frequent and often striking feature of convalescence in typhus.

A slight monocytosis was occasionally seen, but it was not a regular feature. The V186-treated group showed a slightly greater mononuclear increase than the untreated cases. The difference is, however, probably insignificant. “Basophilic mononuclear” cells, similar to the abnormal cells seen in the blood of typhus cases by Danielopolu, were seen in 50 of 52 cases forming the basis of this analysis. Türck or irritation cells have also been described as occurring especially during the second week in the majority of typhus cases, by Schilling, Schiff, Rabinowitch, Elkeles, etc. Many of the cells appeared to be plasma cells, or Türck cells, others had a nuclear structure more characteristic of the monocyte series. All were characterised by intense basophil staining of the cytoplasm. For convenience and because of difficulty in classification, they have been placed in a separate category of “basophilic mononuclears.” Although they were encountered in the vast majority of cases, they were not present in the blood at every examination. They were found in greatest numbers about the 9th or 10th day of the disease. Tables XXIX and XXX indicate that only approximately 40 per cent. of the films examined showed the presence of these cells.

The eosinophil and basophil polymorphs were reduced in numbers during the earlier stages of the disease, but returned to normal during convalescence.

Although the blood picture of typhus does not show any striking and characteristic features, it is of assistance in the differential diagnosis and especially in the differentiation from typhoid fever. The most valuable features are the increase of younger polymorphs, and the presence of atypical cells of the mononuclear series. The occurrence of leucopenia is not, according to our experience, against the diagnosis of typhus, as has frequently been stated. Agranulocytosis, such as in the case reported by Hoff (1943), is, however, rare.

### BLOOD CHEMISTRY

A variety of chemical tests was performed. Blood ureas were determined as a routine, not only because of their prognostic significance in typhus (Benhamou *et al.*, 1924), but also to determine whether V186 therapy produced an increase of blood urea in man as V147 did experimentally in the rabbit.

Estimations of chloride in the urine and blood plasma were done in a number of typhus cases in Algiers. The disappearance of the chloride from the urine and its diminution in the plasma (Julliard & Henaff, 1939) were confirmed. Julliard & Henaff have shown further that increase of corpuscular chloride occurs at the time of plasma chloride reduction. French physicians use the reduction of plasma and urine chloride as an indication for intensive chloride therapy. Blood plasma chloride determinations were continued in

Naples with the intention of investigating the possible relationship of the chloride changes to renal function and azotaemia. McCance & Widdowson (1937) have shown that chloride deficiency in healthy men can result in raised blood urea and diminished glomerular filtration. It was hoped also to investigate the effect of chloride therapy, in typhus, cases on blood chloride and urea levels, and on renal function.

The chemotherapeutic agents were administered as hydrochlorides. Several of the patients showed respiratory changes suggestive of acidosis, and it was thought necessary to determine whether acidosis occurred in typhus, and whether the chemotherapy aggravated it.

The clinical picture in severe typhus often suggests a state of severe dehydration, and reports of dehydration in typhus are frequently encountered in the literature. Danielopolu has reported the occurrence of polycythemia in severe typhus. Such polycythemia can be regarded as evidence of haemoconcentration. Blood and plasma specific gravities ( $\text{CuSO}_4$  method of Van Slyke and his co-workers) were determined in many severe cases in Naples, in order to discover whether or not haemoconcentration was present. Haemoconcentration would contribute to urea retention in severe typhus by diminishing glomerular filtration.

Unfortunately the circumstances and the dearth of equipment made it impossible to complete the biochemical studies. Furthermore, when the reduction in the amount of other work made a start on the biochemical investigation possible, the epidemic was waning, so that there was a scarcity of suitable severe cases for investigation. Several severely ill people in the older age groups were selected, but most of them died within 12–24 hours of admission, so that little more than preliminary diagnostic procedures could be carried out.

#### *Results*

*Blood Urea.*—During the preliminary study in Algiers the occurrence of raised blood urea in severe typhus (Benhamou *et al.*, *loc. cit.*) was confirmed. Urea estimations were continued as a routine during the Naples epidemic. The results (Table XXXI) have demonstrated the prognostic value of the blood urea level (see also Yeomans *et al.*, 1944).

In this report both the control and V186-treated groups of patients have been subdivided on clinical grounds into grave, moderately severe and mild types. This clinical subdivision has been used as a basis for the analysis of the blood urea levels.

#### *Grave typhus*

One of the patients of the control group died before blood urea estimation was done. In 13 of the remaining 18 the blood urea level was at one time or another during the illness over 100 mg. per 100 c.c. (73 per cent.). Of these 13 cases, 10 were fatal (77 per cent.), whereas of the five cases with blood ureas under 100, only 1 was fatal (20 per cent.). This patient (Naples 77) died of acute cardiac failure, showing at autopsy extensive myocardial degeneration. Two of the grave cases which had initially high ureas and yet recovered, showed a gradual fall throughout the illness. In the treated group, 5 of the grave cases (62.5 per cent.) showed blood ureas above 100 and all 5 were fatal (100 per cent.). Of the remaining 3 cases, 2 were fatal (67 per cent.) and 1 of these fatal cases showed a rising blood urea which 3 days before death had reached 80 mg. per 100 c.c. and may well have gone higher.

#### *Moderately severe typhus*

In the control cases only 4 out of 20 (20 per cent.) showed blood ureas above 100 mg. per 100 c.c. None of these cases was fatal. In the treated group 2 out of a total of 9 gave high values (22 per cent.) and in both the rise over 100 mg. per 100 c.c. was transient.



*Mild typhus*

Neither in the control group (11 cases) nor in the treated group (13 cases) was a value above 100 mg. per 100 c.c. recorded. The results are summarised in Table XXXI, which indicates a similar distribution of high and low values in patients receiving drug therapy and in controls.

It is clear that the very high urea values encountered were of great significance in prognosis. Furthermore, a search for the cause of the elevated urea may cast important light on the pathogenesis and offer suggestions for the better treatment of typhus.

Other chemical investigations (chlorides,  $\text{CO}_2$ , sp. gr.) were done in 26 patients. Control estimations carried out on normal individuals using the same techniques are shown in Fig. 11.

*Results*

Of 20 control patients investigated, seven died, all with markedly increased blood ureas, and three of these showed also marked reduction in plasma chlorides. The details of the results in seven controls and four patients who received chemotherapy are recorded in Figs. 12-22. The results recorded in these figures are representative of the results obtained in the whole group. Intravenous glucose saline was administered therapeutically to many cases, and this complicated interpretation of the results obtained.

*Plasma chlorides, urea and  $\text{CO}_2$ .*—In the majority of cases there was some reduction of plasma chloride during the early stages of the febrile illness. Of the seven control cases recorded, three were grave and four moderately severe. All recovered. One patient (Naples 66) showed an early marked reduction of plasma Cl (below 300 mg. per 100 c.c.) and high blood urea, but total  $\text{CO}_2$  of the plasma was within normal limits. In two (Naples 65, 79) there was no apparent chloride reduction, although the blood ureas were initially high. The plasma  $\text{CO}_2$  was moderately reduced during the early part of the illness. In the remaining four (Naples 67, 69, 70 and 85) there was early slight or moderate (300-340 mg. per 100 c.c.) chloride reduction. In two of these (Naples 67, 70) the  $\text{CO}_2$  level was within normal limits. Initially high blood urea levels were encountered in Naples 69 and 85, but in both the urea rapidly returned to normal.

In one fatal case treated with V186 (Naples 62) the plasma chloride early in the disease was markedly reduced, and at this time the urea was only slightly increased. The chloride level did not return to normal as in the other cases, and at the same time blood urea increased rapidly. The plasma  $\text{CO}_2$ , though initially reduced, returned to normal before death. The picture in this case is complicated by the fact that there was persistent vomiting.

Three moderately severe cases (Naples 72, 74 and 76) had received V147 therapy. Plasma chlorides during the early part of the illness were of low normal levels. All showed an early moderate or severe reduction of plasma  $\text{CO}_2$ . In two of them (74 and 76) the  $\text{CO}_2$  reached a level much lower than that reached by the control cases.

The reduction of plasma chlorides, therefore, though usually slight was present in most cases. The reduction was most marked during the first half of the febrile period, after which it returned to normal. Only occasionally was severe diminution of chloride (below 300 mg. per 100 c.c.) encountered. There appeared, however, to be no correlation between the severity of azotaemia and the reduction of plasma chloride.

Plasma  $\text{CO}_2$ , though frequently reduced during the early part of the illness, did not appear to be related to the severity of the illness or the degree of azotaemia. There was also no direct relationship between the degree of acidosis and the reduction of plasma chloride, as might have been expected if the

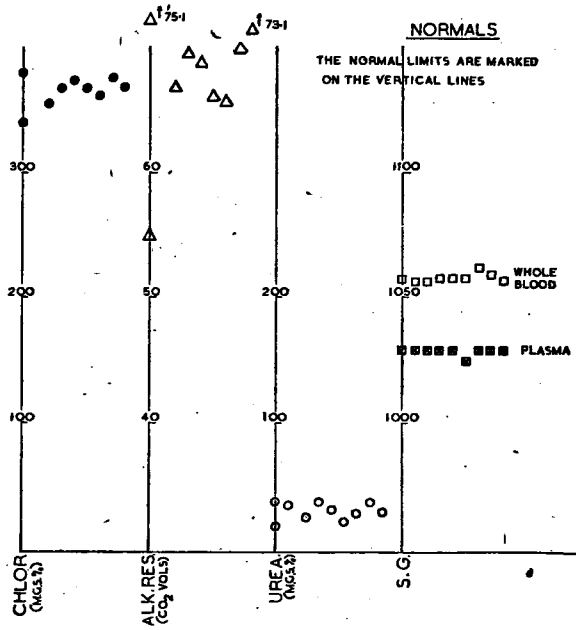


Fig. 11.—Plasma chloride and CO<sub>2</sub>, blood urea, and blood and plasma specific gravity of normal controls.

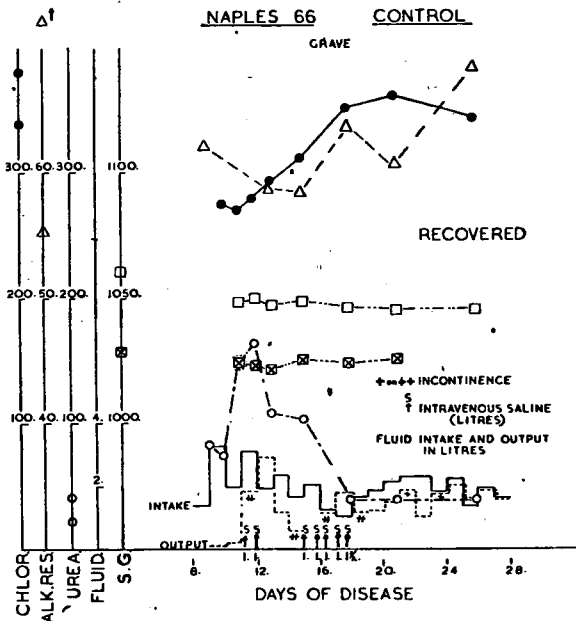


Fig. 12.—Naples 66 (control). Plasma chloride and CO<sub>2</sub>; blood urea; blood and plasma specific gravity; fluid intake and output.



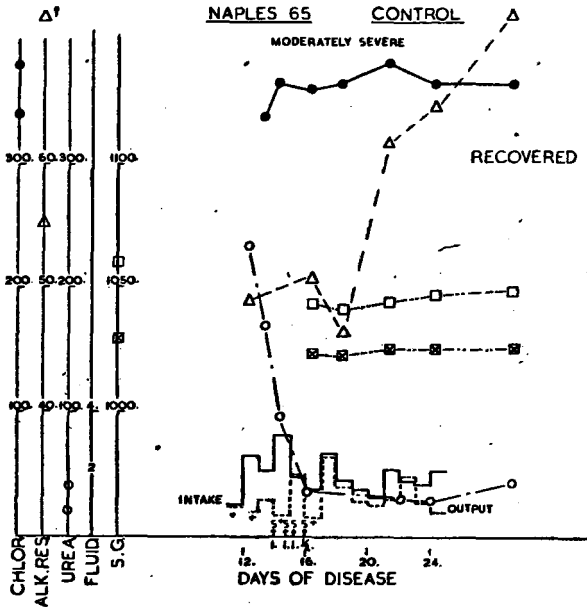


FIG 13.—Naples 65 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

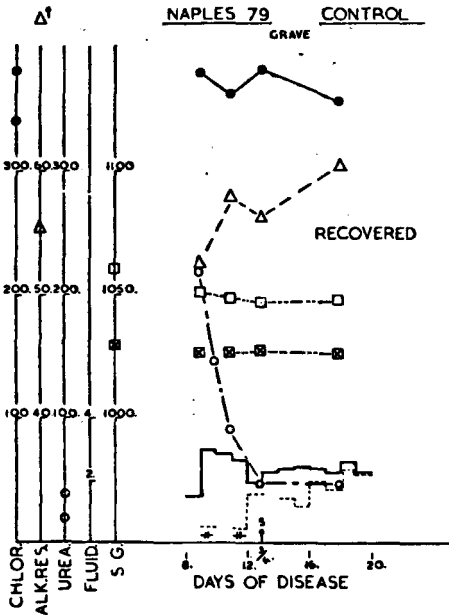


FIG. 14.—Naples 79 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

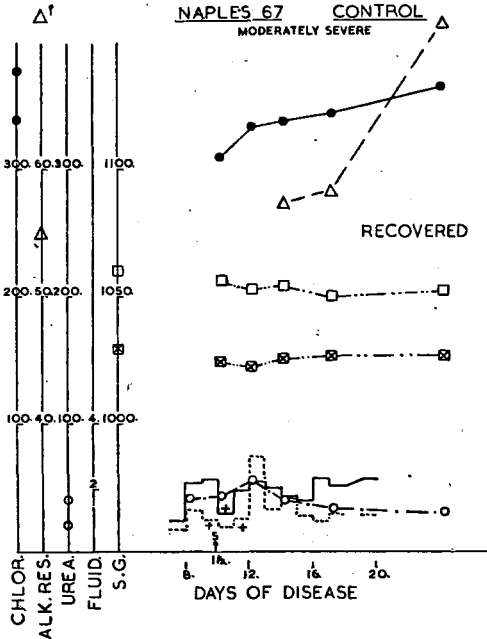


FIG. 15.—Naples 67 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

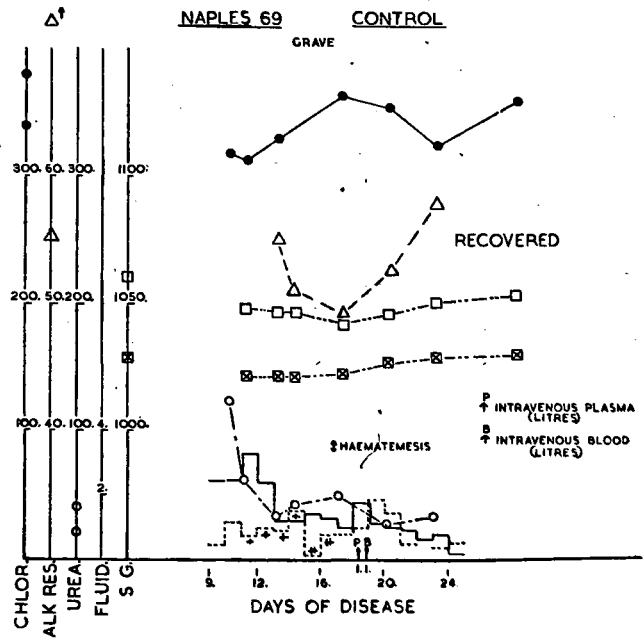


FIG. 16.—Naples 69 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

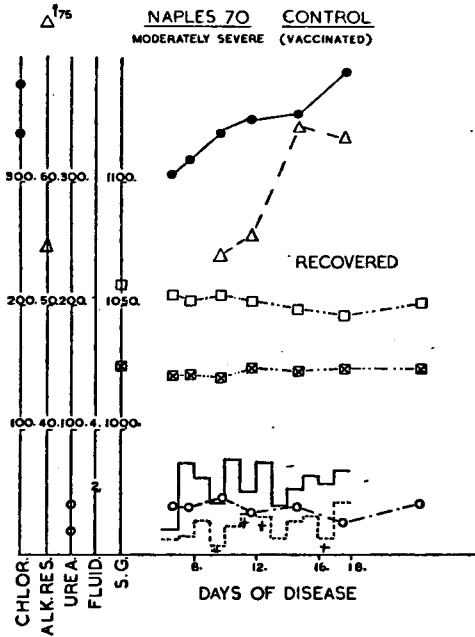


FIG. 17.—Naples 70 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

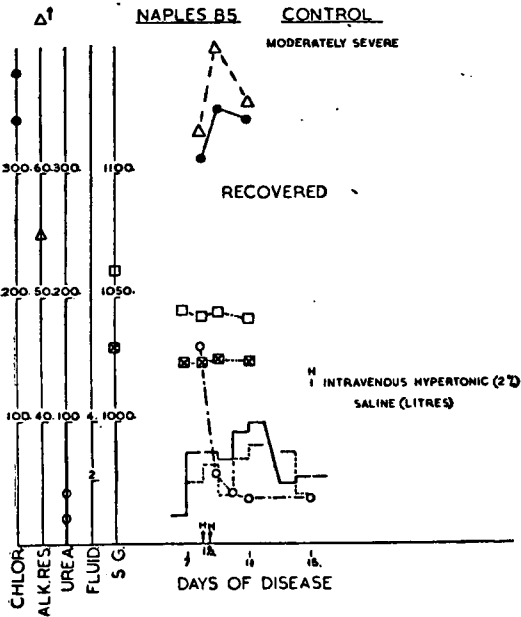


FIG. 18.—Naples 85 (control). Plasma chloride and  $\text{CO}_2$ ; blood urea; blood and plasma specific gravity; fluid intake and output.

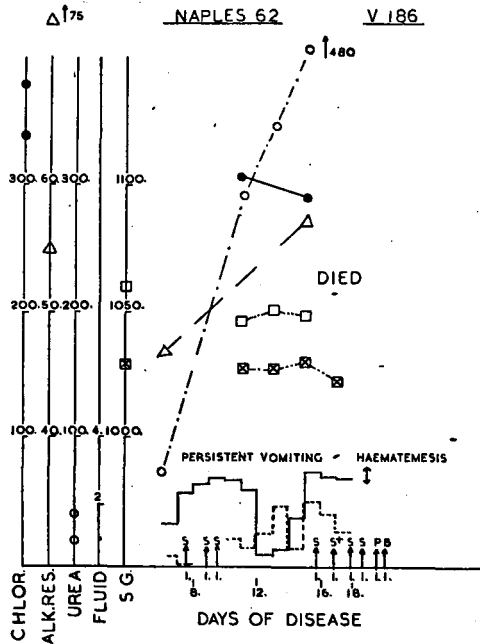


FIG. 19.—Naples 62 (treated V186). Plasma chloride and CO<sub>2</sub>; blood urea; blood and plasma specific gravity; fluid intake and output.

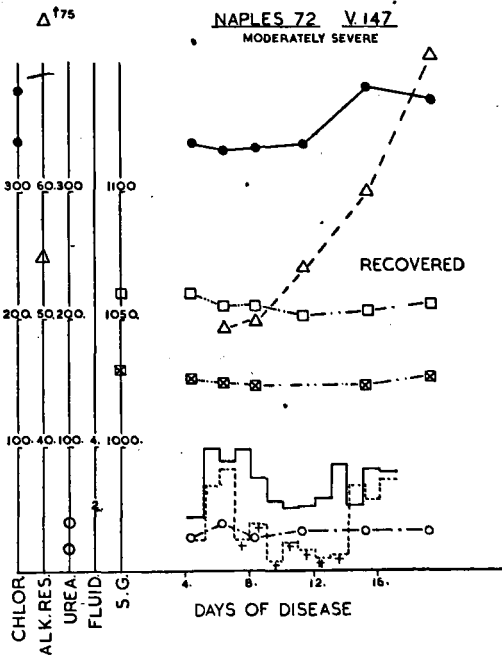


FIG. 20.—Naples 72 (treated V147). Plasma chloride and CO<sub>2</sub>; blood urea; blood and plasma specific gravity; fluid intake and output.

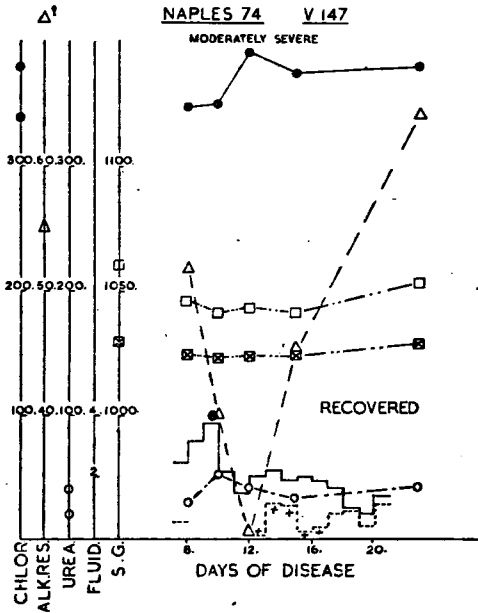


Fig. 21.—Naples 74 (treated V147). Plasma chloride and CO<sub>2</sub>; blood urea; blood and plasma specific gravity; fluid intake and output.

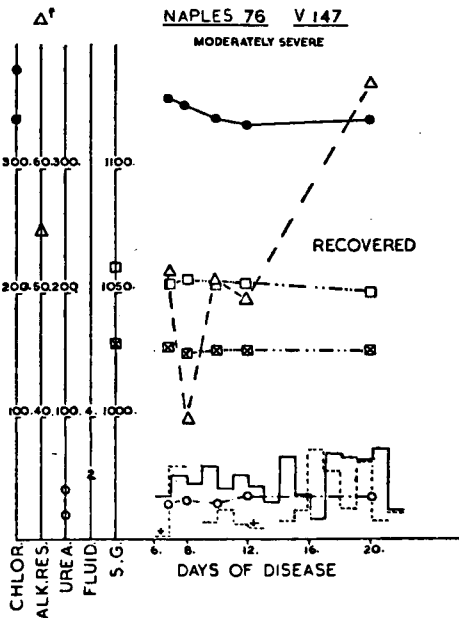


Fig. 22.—Naples 76 (treated V147). Plasma chloride and CO<sub>2</sub>; blood urea; blood and plasma specific gravity; fluid intake and output.

## 110 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

chloride reduction were caused by a chloride shift associated with increase of plasma  $pH$ . The apparent acidosis was more severe in two patients who had received V147 therapy.

Chemical investigations were also undertaken on a number of moribund patients. The results did not differ significantly from those recorded in the figures. Only single observations were obtained before the death of the patients and the results are, therefore, not reported in detail.

*Urine output and renal function tests.*—Accurate determinations of urinary output were seldom possible, as the majority of gravely-ill patients were incontinent, and were usually so during that stage of the illness when renal function tests and determination of fluid balance would have been most significant. The occurrence of oliguria in fatal cases of typhus is shown in Figs. 23–25 (Naples 43, 55 and 61). The latter show, in addition to the fluid balance, the sharp diminution of blood pressure which was almost invariable in severe typhus.

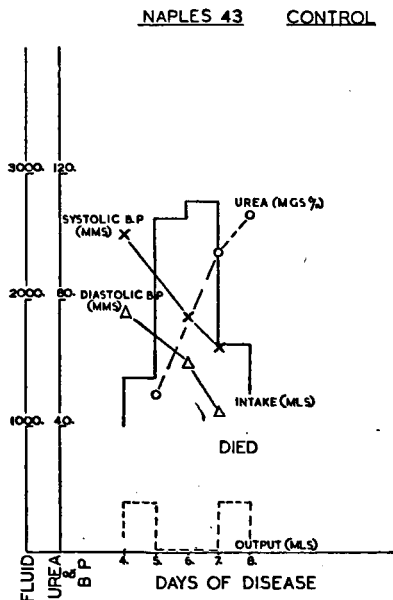


FIG. 23.—Naples 43 (control). Fluid balance; blood urea\*; blood pressure.

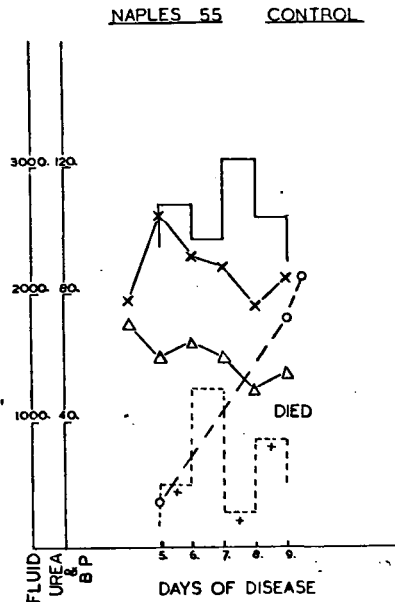


FIG. 24.—Naples 55 (control). Fluid balance; blood urea\*; blood pressure.

Albumin, and hyaline and granular casts were frequently present in the urine during the febrile stage. These abnormal constituents were not, however, present in large amounts in the control cases. Several patients who received V186 therapy, on the other hand, showed large numbers of epithelial casts and desquamated epithelial cells in the urine. That the oliguria may have been accompanied by water retention is suggested not only by diminution of blood specific gravity, but also by the rapid loss of weight which occurred during convalescence when normal urinary output or even diuresis had occurred.

Urea clearance tests were performed in eight cases. It was not possible in all cases to ensure an adequate urinary output for the tests. Satisfactory results were obtained in only five grave or moderately severe cases. In three of these there was marked reduction of maximal or standard clearance (van Slyke, quoted from Fishberg, 1939). One case, though showing marked azotaemia, and ultimately proving fatal, had no obvious diminution of renal

unction. The remaining case (Naples 85) showed marked reduction of urea clearance on admission, but normal clearance 24 hours later, after intravenous administration of  $1\frac{1}{2}$  pints of 2 per cent. saline. This patient, aged 28 years, showed only slight reduction of plasma chloride at the time the first urea clearance test was done, and probably owed her improvement to the general circulatory recovery after parenteral saline rather than to the administration of NaCl.

*Blood and plasma specific gravity.*—With the exception of two cases in which slight transitory haemoconcentration was present (not shown in the figures), all cases showed a moderate reduction in specific gravity. The specific gravities—which in the majority indicated haemoglobin concentration in the neighbourhood of 80 per cent. of the normal, and plasma proteins in the region of 6 per cent.—remained almost constant throughout the illness. Albumin-globulin ratios were not determined, but according to Woodward & Bland

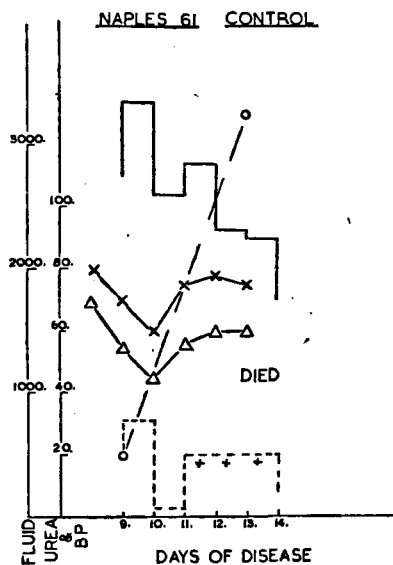


FIG. 25.—Naples 61 (control). Fluid balance; blood urea\*; blood pressure.

(1944) the reduction is due mainly to the loss of albumin. The clinical impression of dehydration was, therefore, not confirmed.

It is impossible to draw definite conclusions from the results of experiments which are as obviously incomplete as those recorded here. They do, however, emphasise the importance of further work on the biochemical aspects of typhus. There can be little doubt that severe typhus is often associated with marked reduction in renal function.

#### POST-MORTEM FINDINGS

Twenty-eight post-mortems were performed in Naples during the three months period of the investigation. Of these 21 were on patients included in the series described above. Seven others were patients in whom a diagnosis of typhus was not confirmed serologically or by guinea-pig inoculation, or in whom death occurred within a few hours of admission, before any clinical or

\* In Figs. 23, 24 and 25, the blood urea (mgm. per cent.) has been charted on a scale  $\frac{1}{10}$  that of the fluid intake.

## 112 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

pathological investigations could be made. Three of the patients (Naples 40, 52 and 58) who were excluded from the series for the reasons stated had received V186 therapy. In view of this treatment the morbid anatomical and histological findings were of importance. They are included in this part of the report, which, therefore, deals with a total of 24 cases. Post-mortems were

TABLE XXXII

*Showing the sex, age, treatment, the day of disease on which death occurred and maximum titre of agglutinins for OX19. (Figures in brackets show the day of disease, when known, on which the titre was recorded).*

Index Number.	Sex.	Age.	Day of death.	OX19	Treatment
Naples 9	F	62	?	+ 6,400	—
39	F	60	18	++++ 800 (17)	—
43	M	52	8	+++ 3,200 (8)	—
55	M	44	9	++++ 200 (5)	—
61	M	38	14	++ 5,120 (14)	—
64	F	32	15	+ 3,200 (13)	—
77	M	20	19	+++ 3,200 (17)	—
86	M	30	11+	++ 3,200	—
99	F	35	?	++++ 6,400	—
101	M	58	?9	+ 3,200	—
102	F	40	?11	+++ 1,600	—
10	F	63	?	++ 6,400	V186 32 grams.
44	F	40	?8	++++ 6,400	V186 51 grams.
46	M	48	?16	++ 6,400	V186 100 grams.
48	F	34	11	++++ 1,600 (8)	V186 76 grams.
54	M	58	9+	++ 400 (9+)	V186 11 grams.
60	F	24	11	++ 6,400 (10)	V186 65.5 grams.
62	F	24	18	+++ 10,240 (18)	V186 20.5 grams.
90	M	32	9	+ 1,600 (9)	Sulphathiazole 9 grams.
93	F	24	?18+	+++ 400	Penicillin 720,000 units.
94	M	30	?	++ 12,800	Penicillin 575,000 units.
40*	M	57	?	not done	V186 10 grams.
52*	F	62	?	- 50	V186 51.5 grams. (Staphylococcal pyaemia).
58*	F	40	?10	Repeatedly — 50	V186 59.5 grams. (Previous pneumonia. Serological tests negative for typhus, but histology typical.)

? = Date of onset unknown.

?9 = approximately 9th day.

\* Cases 40, 52 and 58 have been excluded from the chemotherapeutic trial.

not obtained on two of the cases investigated clinically; both of these belonged to the control series. One autopsy on a case with extensive gangrene of the extremities, and abdominal tuberculosis, was performed at the request of the Italian medical staff. This case was also the only one encountered in which parotitis was present. The gangrene involved the skin only and was not associated with abnormalities of the large vessels or nerves. One case which was excluded from the series showed characteristic histological findings of which a brief summary will be given (Naples 58). The case was excluded



because of persistently negative serological tests and an atypical clinical picture. Post-mortems were also performed on a patient who died before diagnosis could be confirmed serologically, and on another who proved to be a case of staphylococcal pyaemia. Both patients had received V186 therapy.

Autopsies were performed as soon after death as possible. Delay of more than 12 hours occurred when patients died in the evening, as the relatively primitive conditions produced by the war made the performance of post-mortems at night impossible. In one case only post-mortem was delayed for 24 hours and in another for 14 hours. All other post-mortems were done within 12 hours, and in 12 of the cases within 4 hours of death. Temperatures in the mortuary were often below freezing point. We felt assured, therefore, that in at least 50 per cent. of cases post-mortem autolysis was minimal.

The ages of the 24 patients, the day of the disease on which death occurred, the Weil-Felix titre and the treatment which the patients had received are given in Table XXXII. The investigations revealed differences between the control and V186-treated groups only in the renal findings. The kidney findings are, therefore, described separately, comparing the treated and control groups, but the remaining pathological features are described together.

#### *Morbid Anatomical Findings*

The gross pathological findings were very constant. Although we are in agreement with many authors who have stressed the absence of any single diagnostic feature at the autopsy table in typhus (Aschoff, 1915; Nicol, 1919; Jaffe, 1918; Ceelen, 1919; Bauer, 1916; Munk, 1916), we also agree with Jaffe (1918) that the findings as a whole, together with the clinical picture, can be of some diagnostic help even in the absence of microscopical and serological investigations.

The rash was always much less striking than during life. Only petechial elements of the exanthem persisted, and these were especially prominent in dependent parts discoloured by post-mortem lividity. Conjunctival petechiae stood out particularly well, because of the whiteness of the background and the thinness of the epithelial covering. The dryness of the muscles described by Aschoff (1915) was not a feature in these cases.

The serous cavities only rarely contained more than the normal amount of fluid; small pleural effusions were present in five cases. In an additional case (Naples 44) which had been given very large amounts of glucose saline parenterally, the effusion was more massive and was associated with subcutaneous and pulmonary oedema. In one case the deep colour of the pericardial fluid indicated an obvious jaundice. Petechial haemorrhages occurred occasionally on any of the serous surfaces.

#### *-Respiratory tract*

Some degree of congestion of the laryngeal and tracheal mucosa was a constant finding (Herzog, 1918). In some the congestion was intense, and associated with superficial shallow ulceration and numerous petechiae.

The lungs showed the almost constant presence of bronchiolitis. In 17 typhus cases there was, in addition, bronchopneumonia, which in 10 was sufficient in extent and severity to be recognisable with the naked eye. This includes one case (Naples 58) with a history of pneumonia previous to typhus, and which, at autopsy, showed an unresolved pneumonia. Bronchiolitis was almost sufficiently constant to be regarded as part of the pathological picture of typhus. The remaining abnormalities of the lungs—bronchopneumonia, congestion, oedema and emphysema—were regarded as secondary.

*Cardiovascular system*

The heart usually showed right-sided dilatation. The muscle was pale, and there was in all cases on the inner surface an irregular purplish streaking which, on histological examination, proved to be interstitial infiltration and haemorrhage.

In one patient (Naples 77) who died of acute cardiac failure, there was, in addition to marked pulmonary oedema, an intense pallor of the myocardium and a very characteristic picture of fatty degeneration of the heart. In addition, bands of muscle, large enough to be visible to the naked eye, appeared yellowish and necrotic. These areas were especially well marked in the papillary muscles.

The aorta and large vessels showed no naked eye evidence of abnormality which could be attributed to typhus. Slight fatty streaking was frequently seen, but atheromatous changes were surprisingly infrequent in this group of patients, many of whom were over 40.

*Gastro-intestinal tract*

The gastro-intestinal tract showed very slight changes. Petechial haemorrhages, on the outer and mucosal surfaces, were encountered in the stomach and small intestine. Erosions of the gastric mucous membrane were present in one case.

The liver showed a variety of changes, such as cloudy swelling and passive congestion. In one case minute focal necroses were visible to the naked eye. The pancreas, with the exception of one case, in which it was almost wooden in consistency, showed no abnormalities.

*Lymph glands*

Mesenteric lymph nodes were frequently enlarged. In some instances the enlargement was slight, in others individual glands were enlarged to the size of small almonds. They were always discrete, firm and rubbery in consistency, and uniformly fleshy in appearance on the cut surface.

*Genito-urinary tract*

The kidneys were in most cases normal in size or slightly enlarged. Several of the patients who died after V186 therapy, however, showed marked enlargement of the kidneys. Congestion was variable but in most cases petechial haemorrhages, sometimes in very large numbers, were seen on the outer and cut surfaces and in the renal pelvis. There was no distortion of the renal architecture, and demarcation between cortex and medulla was usually sharp. Pallor of the cortex was sometimes noteworthy, especially in cases which had received chemotherapy. Pale yellowish bands in the cortex were sometimes striking in the treated cases, and they proved, on histological examination, to be due to severe tubular degeneration.

The bladder mucosa in one case had a deep purple velvety haemorrhagic appearance. Many other cases showed petechiae in the mucosa of the trigone.

The testes were examined in two cases only. They showed no abnormality apart from a few petechial haemorrhages. No abnormalities attributable to typhus were found in the female genitalia.

*Suprarenal glands*

The suprarenal glands often showed moderate congestion.

*Central nervous system*

The frequency with which an apparent excess of cerebrospinal fluid was encountered was striking. Usually the pia-arachnoid in these cases had a gelatinous appearance. Petechiae on the surface of the brain were infrequent but congestion of the surface capillaries was the rule.

On cross section, petechial haemorrhages were occasionally seen in the brain stem as well as in the cerebral hemispheres (Herzog, 1918 and others). More striking and frequent were prominent blood vessels which, on scraping with the knife, did not disappear. Histologically it was confirmed that this was due to the occurrence of localised perivascular haemorrhages.

#### *Histological Findings*

The histology of typhus fever has been extensively studied. The type of lesion first studied in the skin and described by Frankel (1914, 1915) has later been shown to occur in the conjunctiva and the internal organs (Bauer, 1916; Ceelen, 1916, 1919; Chiari, 1917; Nicol, 1919; Jaffe, 1918; Spielmeyer, 1919, and Avtsin, 1943). The detailed investigations of Wolbach, Todd & Palfrey (1922) not only review the histological picture adequately, but demonstrate the aetiological agent in relation to the typical vascular lesions.

To avoid needless multiplication of work, our histological investigations were, therefore, primarily limited to a routine examination of organs to confirm the diagnosis, and to ascertain whether there was histological evidence of toxicity of the therapeutic substances used. For this reason, serial sections which are essential for the detailed study of the vascular lesions encountered in typhus, were not examined. At least two sets of tissue slices were collected at each autopsy. One was fixed in 10 per cent. formol-saline and the other either in Zenker's or in Bouin's fixative. All tissues were embedded in paraffin, and sections stained with haemalum and eosin. Other stains were used on many sections, including Giemsa's stain, Weigert's iron haemotoxylin and eosin. In some cases frozen sections were stained for fat with Scharlach R.

Tissues examined as a routine from all cases, except two in which only incomplete autopsies were performed, included lung (normal and abnormal portions), myocardium through the base of the left ventricle and through the interventricular septum, arch of the aorta, liver, spleen, pancreas, suprarenal, kidneys, and brain (parietal cortex, basal ganglia, cerebellum, midbrain, pons and medulla). Skin was examined from four cases, conjunctiva from seven, trachea from eight, stomach from five and lymph glands from nine cases. Sections of at least two slices from each situation were examined, and in many cases five or six. We were struck with the severity of the lesions sometimes encountered in the heart and kidneys, and regarded these as a further justification for recording the histological picture, in view of the assumption by some authors (Ceelen, 1916; Sturm, 1942) that the chemical changes and the fatal issue in typhus are to be attributed to lesions in the brain stem. Special attention has been paid to the renal histology by one of us (J.S.F.N.), and the renal findings are recorded in greater detail.

The typical vascular proliferative, necrotic and thrombotic changes (Abrikosov, 1941) have not been easy to demonstrate when only a small number of sections from each organ was examined. As the routine employed in post-mortem histology does not usually include the examination of serial sections, this is of importance. Kowenaar (1940), for instance, stresses that the vascular lesions in classical typhus fever differ from those in scrub typhus. In scrub typhus, lesions appear primarily perivascular while intimal lesions are secondary. Very careful histological investigations are needed to show such differences. The only situation in which vascular lesions have been found with ease is the conjunctiva. Typical lesions in this situation have been studied by Chiari (1917) and Avtsin (1943). \*Plate II, Figs. A, B, and C, show the characteristic proliferative endothelial changes and thrombosis encountered in conjunctival vessels. Like Chiari (1942) we were struck by the fact that proliferative changes (adventitial and intimal) in small arterioles were more striking than vessel necroses. The photographs show, in addition, the perivascular nodular lesions consisting of accumulations of large mononuclears,

\* Plates II to IX are to be found at the end of the volume.

plasma cells and occasionally polymorphs. Similar changes were found in the skin sections examined, but lesions were more scanty. The conjunctiva often showed, in addition, a diffuse mononuclear cell infiltration, marked congestion and many capillary interstitial haemorrhages, which account for the petechiae seen during life.

The histological changes in the larynx, trachea and large bronchi differed in that, together with more intense congestion, a more diffuse mononuclear cell infiltration was present. Secondary acute inflammatory changes in many cases masked the typhus lesions. The regular occurrence of laryngeal congestion and petechiae, and of bronchiolitis, during life, and the regular finding of intense congestion and petechiae in the respiratory mucosa at autopsy can be associated with histological changes characteristic of typhus. These observations have inclined us to regard the bronchiolitis and signs of inflammation of the upper respiratory tract (hoarseness, aphonia) as part of the disease rather than as manifestations of secondary infections (see also Ceelen, 1919). The affected mucous membranes are, however, more susceptible to secondary infection, and hence the bronchiolitis, which, in the early stages, is sharply limited to the bronchial walls (Plate II, Fig. D), frequently spreads to give rise to extensive bronchopneumonia.

Bronchopneumonia was verified histologically in all cases in which it was seen at autopsy. In addition, in seven cases it was found on histological examination, though in degree insufficient to be recognisable by the naked eye.

Bacteriological investigations could not be undertaken in all cases, but it is of interest to record that from one patient (Naples 94) who died after three days' intensive penicillin therapy, the pneumonic lesions yielded a heavy growth of pneumococci. The batch of penicillin used was subsequently shown to be active, but it was unfortunately impossible to determine whether the pneumococcus present was penicillin-sensitive.

The heart in all proven cases of typhus showed the changes which have previously been described—interstitial perivascular accumulations of large mononuclears and plasma cells, which are frequently collected into small nodules (Plate III, Figs. A and B). We do not agree that they often resemble the Aschoff nodes of rheumatic fever. An exceptional case (Naples 77) is worthy of special mention in that the subject, a male of 20, died of acute cardiac failure. His heart at autopsy presented focal necrotic (? ischaemic) changes sufficiently extensive to be seen with the naked eye (Plate III, Fig. c). Although other cases usually showed only minor degrees of myocardial degeneration, this case demonstrates that the heart muscle itself may be severely affected, and that appropriate therapeutic and nursing measures should be employed in all cases of typhus.

The aorta in all cases showed slight lymphocytic infiltration round the vasa vasorum in the adventitia and outer layers of the media. Degenerative changes in the muscle and elastic laminae when present were slight. There was no close resemblance to syphilitic aortitis, as has been suggested; in fact, there was no difficulty in differentiating the characteristic changes of the syphilitic aortitis which was present in one case (Naples 61).

The oesophagus, stomach and intestines were examined in only a few cases. In addition to petechial haemorrhages, and superficial erosions, focal perivascular mononuclear cell infiltrations were encountered.

The liver showed no characteristic histological changes. Focal lobular necrosis was seen in two cases in which there was gross venous engorgement. Accumulations of mononuclear or polymorphonuclear phagocytes in the sinusoids in excess of the normal were frequently seen, but abnormal prominence of Kupffer cells was not striking. Small round cell infiltration in the portal tracts occurred regularly, but it was of minor degree. Parenchymatous changes such as occur in many febrile illnesses were seen, but were usually slight.

The spleen was variable in appearance. In the majority of cases it showed congestion of the pulp. Mononuclears usually predominated in the pulp, but when septic complications were present, polymorphonuclears occurred in larger numbers. Myeloid transformation was not seen, and the erythrophagocytosis described by Aschoff was not much in evidence on ordinary examination. The Prussian Blue reaction, however, showed the presence of recently formed haemosiderin in the endothelial cells lining the sinuses, and in the reticulum cells of the pulp, in 90 per cent. of the cases examined.

Hyperplastic changes involving especially the sinusoidal phagocytes in the lymph glands were a striking and frequent finding.

Histological changes in the suprarenals were encountered with regularity. They were, however, always slight, consisting of perivascular foci of lymphocytes and plasma cells. Parenchymatous changes were slight, involving usually only groups of a few cells in the outer layers of the cortex. The histological changes appeared to be insufficient to account for the marked changes in blood pressure encountered in the disease.

The lesions found in the central nervous system differed in no significant degree from those previously extensively studied and described (Plate III Fig. D). It was not always possible to classify lesions according to the types described by Spielmeyer, and in no case were the so-called "Strauchartiges" lesions of the cerebellum seen. Our investigations do not allow us to enter into the controversial discussion as to the origin of the cells in the typical nodules. It is reasonable to agree that the lesions encountered in the brain substance are of neuroglial origin.

Perivascular lesions, especially round larger vessels, were, however, encountered in which the cells resembled closely those in the meninges. These cells are probably situated in the Virchow-Robin spaces, and like those of the meninges are of mesenchymal origin. Varying degrees of large mononuclear cell and plasma cell infiltration of the meninges were found in all cases.

Perivascular and petechial haemorrhages were encountered with greater frequency in our cases than was expected from our brief study of the literature (Plate III, Figs. E and F). It has been suggested by Spielmeyer that they are agonal in origin. We have, however, observed haemorrhages which were associated with evidence of focal brain necrosis and neuroglial proliferation, and occasionally haemorrhages into characteristic nodular lesions. We prefer, therefore, to regard them as manifestations of the disease rather than as agonal phenomena. An explanation of their pathogenesis is not easy—they probably depend on vascular lesions.

We have found great difficulty in demonstrating the characteristic vascular lesions of typhus in the brain by our method of study. Only occasionally have proliferative or thrombotic vascular lesions been seen.

The distribution of the nodular lesions agreed with the earlier descriptions. They were most frequent in the grey matter of the brain stem, and especially in the olivary nucleus of the medulla. They were less frequent in the cerebral hemispheres, although in this situation as well as in the basal ganglia there was often an appearance of general cellular (neuroglial) increase. We have been unable to correlate the severity of the clinical manifestations with the number and extent of the nodular lesions.

The difference in the numbers of lesions encountered in the brain in individual cases has been very striking. Variations of from three or four nodules in 10 sections to 30 or 40 nodules in a single section have been encountered. It is certainly impossible to exclude the diagnosis of typhus from the results of the examination of a single brain section. A large number of sections, including several through the medulla and other parts of the brain stem, have to be examined before a negative diagnosis in a doubtful case can be accepted.

## 18 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

One case (Naples 58) is of particular interest in that histological lesions characteristic of typhus were found in the brain (Plate III, Fig. G). The serological tests were persistently negative, and this case has accordingly been excluded from the investigation series, although we feel that a diagnosis of typhus could be made on histological grounds.

### *Kidneys*

With the exception of two cases, the renal tissue was free from obvious post-mortem change. In most instances, Zenker-fixed material was available and greater reliance has been placed on tissues so fixed than on material fixed in Bouin's fluid or formol-saline. The following staining methods were employed: haemalum and eosin, various modifications of Mallory's aniline blue stain for capillary basement membrane, Heidenhain's azocarmine and aniline blue, Lendrum & McFarlane's (1940) modification of Mallory's trichromic stain, Mallory's phosphotungstic acid haematoxylin and Weigert's modification of Gram's stain for fibrin. Staining for fatty substances, and examination for lipoids, have not been carried out, nor has it been possible to follow in serial sections the distribution and character of the periglomerular and vascular lesions referred to later.

A preliminary survey of all the kidneys was carried out by one of us (J.S.F.N.), purposely kept in ignorance of the full clinical data or details of therapy in individual cases, but knowing that azotaemia and oliguria were almost invariable in the fatal cases, that albuminuria occurred, that many patients had casts in the urine and that the blood pressure was low in almost all the cases.

Certain common features were present, some of which are the well recognised vascular and cellular lesions of typhus similar to those seen in other parts of the body. From a study of the histological changes in the specialised tissues, the kidneys, with one exception, could be placed in one of two groups. Later reference to the clinical details showed that with three exceptions, the groups corresponded to (1) those who had received V186 therapy, and (2) those who had received symptomatic treatment either alone or with penicillin or sulphathiazole; the striking feature in group (1) being the degree of tubular damage. The three exceptions included cases which had received small doses of V186 and one in which post-mortem autolysis obscured the histological picture. Some of the histological changes seen in group (1) occurred also in group (2), and these common features can, therefore, be regarded as characteristic of typhus, while the special features of group (1) must be attributed to the V186 therapy.

The one case which presented histological features different in some aspects from all the others was Naples 62. This case, in which death occurred 11 days after cessation of V186 therapy, showed, in addition to very severe tubular destruction, evidence of tubular regeneration and marked oedema of the interstitial connective tissue (Plate IV). There was also evidence of widespread focal intracapillary glomerulitis which appeared to be a later stage of the focal glomerulitis seen in varying degrees in the other cases (Plate V, Figs. A and B).

Typhus lesions such as occur in other organs were present in varying degrees in all kidneys. Thus, foci of cellular infiltration occurred irregularly throughout both cortex and medulla, being most frequent in the boundary zone, where they occurred particularly in the adventitia of the arcuate vessels. The cells were mainly of large mononuclear type, but plasma cells, lymphocytes and polymorphonuclear leucocytes also occurred; occasionally erythrophagocytosis was seen. Mitoses were fairly frequent, particularly in large cell accumulations. Capillary and arteriolar occlusion by thrombi (Plate V, Fig. c), or swollen and degenerated endothelium were sometimes found in the centre of such lesions; in others extravasated erythrocytes were observed, occasionally in obvious

relation to ruptured capillaries. Although not proved by serial sections, it is probable that the haemorrhages, like the cell infiltrations, are secondary to the endothelial lesions. Small areas of intimal thickening with degeneration of the overlying endothelium and thrombus formation unaccompanied by cellular infiltration were occasionally seen in vessels cut longitudinally. This was the earliest stage of vascular abnormality noted in this material, and it is presumably followed by proliferation of adjacent endothelium. It is considered likely, however, that while endothelial proliferation partly accounts for the cellularity of the characteristic foci and the endothelial changes are primary, the majority of the infiltrating cells are derived from the blood and from tissue histiocytes. Atrophy and degeneration of renal tubules in the vicinity of the cell infiltrations occur and proliferation of connective tissue cells rapidly takes place. Rupture of an affected vessel into a tubule in the cortex or medulla was seen fairly frequently.

In many cases there was slight but definite hyaline thickening of the intima of the small arterioles. This warrants more detailed study than has been possible for the purpose of this report, particularly in relation to possible effects on the glomeruli when their afferent or efferent arterioles are involved.

*Lesions of the glomeruli and renal parenchyma encountered irrespective of V186 therapy*

The most striking glomerular changes were :—

1. Moderate enlargement of the tufts (Plate VI, Fig. A).

2. General patency of the capillaries (Plate VI, Fig. B).

In only one subject (Naples 77) who died of cardiac failure without azotaemia was there extreme dilatation of the capillaries (Plate VI, Fig. C). Congestion of the glomeruli varied from case to case and in different parts of the same kidney.

3. Slight increase in cellularity (Plate VII, Fig. A), rarely general, never diffuse and usually confined to single loops in occasional glomeruli.

4. Occasional necrosis of single capillary loops, with pyknosis and karyorrhexis of endothelial nuclei (Plate VII, Fig. B).

5. Occasional occurrence of fibrin in individual loops.

6. Swelling of endothelial cells in some of the glomeruli. The nuclei became enlarged, the cytoplasm more abundant and the cells difficult to distinguish from the visceral epithelium of the tuft. Such swollen cells projected into the lumen of the capillaries.

7. The presence in the capsular space of coagulated albuminous material.

8. Very occasional haemorrhages from the tuft into the capsular space ; this was a rare occurrence (Plate VIII, Fig. A).

The glomerular changes were focal, in many cases involving only a few glomeruli and requiring for their recognition careful search and comparison with normal kidneys. No significant lesions have been found in the visceral or parietal epithelium of the glomerulus, although occasionally partial desquamation of the latter, and sometimes an increase in number of cells without reduplication of the layers, were found. Polymorphonuclear leucocyte infiltration was absent or scanty. The picture of acute diffuse glomerulo-nephritis described by Herzog (1935) was not confirmed.

Considerable time has been spent in examining the basement membrane of the capillaries. The occurrence of generalised swelling was doubtfully present in one or two cases, and focal thickening was seen where branching of the capillaries occurred. The acute degenerative swelling of acute nephrosis (Gray, 1933), and the changes observed by Hunter & Roberts (1933) due to metallic nephrotoxins, were, however, not encountered. Intracapillary fibrils

## 120 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

such as occur in acute glomerulo-nephritis, and the reticulate thickening of the basement membrane found in eclampsia (Bell, 1932; Baird & Shaw Dunn, 1940), were likewise absent.

Sometimes the interstitial cellular infiltrations had a periglomerular distribution (Plate VIII, Fig. B) and in such glomeruli, necrosis of loops and focal increase of cellularity were found most readily. Gross glomerular necrosis was, however, not seen. There has also been no evidence of occlusion of arcuate or interlobular vessels, although this might have been demonstrable if serial sections had been examined.

The tubules showed varying degrees of cloudy swelling with occasional necrosis of individual, or small groups of cells (Plate IX, Fig. A). These changes were in no way comparable with the condition designated acute nephrosis following toxic, metabolic or chemical poisoning (Gray, 1933), or with the tubular lesions found experimentally in animals associated with urea retention (Frothingham *et al.*, 1913; Shaw Dunn *et al.*, 1924, 1926, 1934; Underhill *et al.*, 1913). They were also in striking contrast to the more severe tubular lesions encountered in the V186-treated group.

### *Changes attributed to V186 therapy*

The kidneys of all but three subjects who died after V186 therapy showed very marked tubular degeneration (Plate IX, Fig. B). The degeneration, which occurred predominantly in the specialised tubules, varied in intensity from severe cloudy swelling and hydropic degeneration to total necrosis. The necrotic epithelium became desquamated and many epithelial casts were present in the more distal tubules. Tubular degeneration was less striking in two who had received small doses of the drug, and in a third, post-mortem autolysis obscured the histological picture.

The glomeruli of the kidneys of the treated series frequently showed a greater tendency to dilatation of the capillaries, with increased density of staining of the endothelial cells, than the control cases.

### *Summary*

It is apparent from these observations that the renal abnormalities demonstrable by ordinary histological methods were insufficient alone to account for the azotaemia of typhus.

The glomerular changes were slight, focal in distribution and involved only small portions of the glomeruli. It is possible, however, that toxic effects of the disease as well as actual infection of the renal parenchyma, as evidenced by focal, perivascular and glomerular lesions, reduced the functional reserves of the kidney, thereby hastening the onset of uraemia through extrarenal causes.

With the exception of a greater degree of capillary dilatation, glomerular changes in the treated group were similar to those in the control cases. Tubular degeneration, on the other hand, was much more severe in the treated cases. Only milder degrees of degenerative change were seen in the tubules of control cases, but it is possible that these relatively mild changes attributable to typhus predisposed to the necrotising action of V186. That the severity of tubular degeneration was probably not related to the degree of azotaemia is demonstrated by the similarity in the blood urea levels of the treated and control groups.

## DISCUSSION

Most of the observations described above were made on material provided by the epidemic of typhus in Naples, an epidemic which is likely to prove of historical interest. Nevertheless, the conditions and limitations imposed on research work by war-time conditions rendered many of the observations incomplete, and prevented final elucidation of many points of interest. It has been considered desirable to record the clinical and pathological features



of the cases which were studied as fully as possible, for the possible benefit of others who may undertake future work on the same subject. The most comprehensive recent survey of European typhus in English is the report by Wolbach, Todd & Palfrey (1922) which was based on experience in Poland after the war of 1914-1918. The account of the manifestations of typhus in Egypt by Kamal & Messih (1944) presents a picture of the disease in non-Europeans. Many of our observations agreed with the findings of both these groups of workers, and a comparison with the clinical features of typhus as given by Murchison (1884) indicates how little the disease has changed in the past 50 years. It is of interest also, that modern warfare is as potent as warfare of more ancient date in producing the conditions in which typhus may thrive. The fact that many of the cases of typhus were infected in the air-raid shelters of Naples led to the involvement of entire family groups almost simultaneously, to a relative high incidence in children, and thus to a relatively low mortality for the entire outbreak. The higher mortality in patients over the age of 40 was also noted.

We were unable to make detailed observations upon the epidemiology and control measures, because of the necessity to concentrate attention undividedly upon treatment. The Naples outbreak was in some ways excellent for the latter purpose. There were few complications or co-incidental diseases, so that the diagnosis of typhus could often be presumed on a basis of fever and history of contact with infection. Nevertheless, as Wolbach, Todd & Palfrey had found, poverty, ignorance and other difficulties led to a relatively late entry into hospital in the majority of instances, and we lacked sufficient numbers of really early cases to determine whether the drugs, V186 and V147, were potent if given within the first three days of onset. As it was, the trial indicated that the drugs were not of value at the stage of the disease at which the majority of patients arrived under medical care. The observations which have been described in this report make it possible, however, to discuss certain points in connection with the diagnosis, prognosis, clinical manifestations and pathology of typhus fever.

#### DIAGNOSIS

The three diseases, or disease groups, found to be most important in the differential diagnosis of typhus in North Africa were enteric fever, respiratory tract infections and cerebral conditions associated with coma. Typhoid fever was the most difficult condition to distinguish from typhus, in view of the fact that both conditions cause fever for several days before the development of a rash, which in both is of a rose-coloured macular type. Typhoid fever, as seen in North Africa, was a severe disease with a high mortality rate. The rash was often trivial in extent, cerebral symptoms were common, but the spleen was usually less enlarged than in typhus, the abdomen became retracted as the disease progressed, and delirium was rarely of the agitated type observed in typhus. The occurrence of diarrhoea in typhus proved a stumbling block, but it was not frequent, and the stools were not of the characteristic pea-soup or melon colour seen in typhoid. One case of severe delirium with diarrhoea was for long thought to be a case of typhus, but no serological changes indicative of typhus developed and *Bact. typhosum* was eventually recovered from the urine. A few cases of enteric fever in a Service Unit caused difficulty in differentiation from three sporadic cases of typhus encountered simultaneously. The occurrence of conjunctivitis and of haemorrhage into or between the macular elements of the rash in the cases of typhus was then of considerable assistance, and usually little difficulty was experienced in distinguishing the rashes of the two diseases. Laboratory investigations indicated the value of reliance upon the blood culture in the diagnosis of typhoid fever. Agglutination reactions with the "O" suspensions of the typhoid and paratyphoid group were often poorly developed

though the blood culture was positive. High titres of *OX19* agglutinin were not encountered in our cases of typhoid fever, though others have encountered high levels in subjects with enteric fever who had been inoculated with T.A.B. vaccine prior to infection (personal communication from Major J. C. Dick, R.A.M.C.). Nor did we encounter frequently changes in agglutinin titre to organisms of the enteric group in typhus, though these have been much emphasised in the literature. The observations on the blood urea in typhoid fever showed a further point of distinction from typhus fever. A greatly elevated blood urea was only once encountered in typhoid fever, and it was then a terminal phenomenon associated with anuria.

Respiratory tract infections, which were chiefly pneumonias, gave rise to diagnostic difficulty because pneumonia or bronchiolitis occurred frequently in proven cases of typhus. The absence of rash and presence of leucocytosis were useful signs of primary pneumonia. One case with meningism and mild delirium gave rise to difficulty in diagnosis until the signs of apical consolidation appeared; no rash developed, the blood culture was positive for the pneumococcus, and response to sulphapyridine indicated the true nature of the infection. Upper respiratory tract infection did not actually cause diagnostic difficulty, but might well have done so if influenza had been epidemic at the same time as typhus. The general resemblance of mild cases of typhus to influenza was striking, and the frequent evidence of pharyngitis and of bronchiolitis in typhus emphasised the features common to the two diseases. German authors (Mrugowsky, 1942; Jacobi & Dorschel, 1942; Lampert, 1942; Dennig, 1942; Hoff & von Brunn, 1942; Stürm, 1942) have frequently stressed the difficulty of distinguishing typhus in the pre-eruptive stage from influenza, and the latter disease must be borne in mind constantly, particularly if there are simultaneous influenza and typhus epidemics.

Cerebral conditions causing diagnostic difficulty were chiefly encountered amongst Arabs, in whom coma of variable origin is common. Coma due to drugs, vascular accidents or luetic infection led to confusion. Yet, in all these, the vascular signs typical of typhus were absent, nor were pathological findings resembling those of typhus ever seen. Meningitis was readily differentiated by examination of the cerebrospinal fluid. Cerebral malaria, though a cause of sporadic cases of coma in British troops in North Africa, never resembled typhus clinically and blood films usually indicated the true diagnosis. We did not encounter difficulty in diagnosis of typhus from appendicitis, bacillary dysentery, nephritis, measles or rubella such as others have recorded. Smallpox prior to the full eruption of the rash did, however, cause difficulty on two occasions. The earliest cases of typhus which we saw already presented an eruption in nearly every instance, and the characteristic appearance and distribution of the rash usually permitted diagnosis, particularly when conjunctivitis was also present. In a few cases the rash was purely petechial and then the presence of petechial haemorrhages in the conjunctivae of the lower eyelids was found to be a striking physical sign.

The detailed account given above of the serological findings in cases of typhus emphasises the importance of the Weil-Felix reaction in the diagnosis of typhus. The usefulness of the slide-agglutination (Castaneda) test with *OX19* suspension has already been pointed out and may be particularly evident at the time of admission of the patient to hospital or when examining contacts of cases of typhus. The rickettsial agglutination test, though useful in occasional cases such as *Fièvre boutonneuse*, or when an elevated Weil-Felix reaction of doubtful significance is present, does not greatly aid the serological diagnosis of typhus. Distinction between epidemic and murine typhus is apparently readily obtained by means of the tests employing rickettsial antigens, but may be of more interest to the epidemiologist than to the clinician. Apart altogether from serology, some diagnostic help may be gained from blood urea

estimations. The leucocyte count was rarely helpful except in differentiation from enteric fever. Even then, however, the occurrence of leucopenia did not rule out the diagnosis of typhus. Finally, the recovery of the causative rickettsiae by inoculations of guinea-pigs, would seem to be worthy of more frequent trial. Unless our experience was exceptional, recovery of strains of rickettsiae from the blood in the early or even in the later stages of typhus presents no difficulty. When all else fails, it appears to be necessary in some patients, who die at a stage of the disease when serological changes have not developed, to rely for final diagnosis upon histological examination of material obtained at autopsy. This may be of occasional importance, as in the case of one of our patients (Naples 58) who died unexpectedly.

#### PROGNOSIS

Experience frequently emphasised the difficulty in making a prognosis in a case of typhus. During the first week of the disease prognosis was impossible, and even during the second week prognosis was frequently falsified by events. Nevertheless, certain broad distinctions, based on the characteristics of the three main groups into which the illnesses were classified, were possible. It is of interest that this classification resembles that adopted by Yeomans and others (1944) on the basis of their experience of cases of typhus in Cairo. Ominous signs were severe prostration and the occurrence of severe mental aberration at an early stage of the disease. Delirium, particularly of agitated or low-muttering type, coma, and abnormal muscular movements indicated a severe degree of involvement of the nervous system and were thus bad prognostic omens. The existence of a purpuric rash, particularly in patients over 40, tachycardia in excess of 140, dyspnoea, and severe cyanosis were also unfavourable signs. At a later stage of the infection, a severe degree of collapse of the cardiovascular system, anuria, gangrene, or the development of pneumonia often heralded a fatal termination to the disease. On the other hand, absence of prostration or of mental disturbance, a relatively slow pulse and sustained blood-pressure, and a continued good output of urine usually indicated a benign type of infection. It was remarkable how complete and rapid a recovery could occur in severely-ill young adults, and equally remarkable how suddenly the condition of an elderly subject could undergo deterioration.

Our observations led us to attach considerable prognostic significance to the blood urea, thus agreeing with the findings of French workers (Benhamou *et al.*, 1924). Persistently elevated blood-urea was a bad prognostic sign. On the other hand, an elevated blood-urea immediately after admission to hospital often fell when symptomatic therapy was given, and little reliance could, therefore, be placed on a single observation unless the degree of elevation was extreme. We were unable to carry out sufficient observations to decide whether the absence of chlorides from the urine was a prognostic sign or not. The degree of lowering of the plasma chlorides was not, in fact, correlated with the azotaemia or with the severity of the infection. Changes in the alkali reserve were towards the level of acidosis, a finding which is the reverse of that of Woodward & Bland (1944). Such a change was not, however, found to be of prognostic assistance. Apart from the output of urine, the routine urine examination failed to indicate the prognosis, chiefly because a normal urine often occurred in cases with advanced azotaemia. The magnitude of the serological response was not, in our experience, indicative of the severity of illness.

#### CLINICAL MANIFESTATIONS AND PATHOLOGICAL LESIONS

The uniformity of the pathological lesions seen in the fatal cases of typhus contrasted with the variability of the disease in cases which recovered. This great clinical variation was less obvious in the cases which we studied than in the epidemic as a whole, because our cases were deliberately selected and obviously mild infections were excluded. Nevertheless, so far as the clinical

## 124 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

manifestations in the various systems were concerned, the key to the understanding of events must lie partly in the distribution of the typhus lesions, described fully and illustrated above, and partly in the functional disturbance in different organs which may be caused by rickettsial toxin. In the central nervous system, great variability in severity of lesions was encountered and little correlation could be made out between the number of demonstrable lesions and the clinical manifestations. The fact that the latter were more commonly of a psychotic nature than of the type with readily demonstrable physical signs may explain this apparent anomaly. Nevertheless, perivascular lesions and neuroglial increase were found in the cerebral hemispheres of fatal cases. The difficulty of demonstration of lesions in the nervous system from a limited examination of sections from relatively small areas must also be remembered. The fact that the nervous system manifestations of typhus were essentially those of a higher centre disorder does not mean that other nervous system lesions were unimportant. Typhus lesions were often demonstrated in the grey matter of the brain stem and the corpus striatum, and such would explain the occurrence of dysphagia, of difficulty in protrusion of the tongue, abnormal movements and muscular rigidity. Deafness, too, appeared to be a true nerve deafness in most cases and, as suggested by Seiferth (1944), may be due to a central lesion. Our observations do not enable us to decide the importance of lesions of the hypothalamus in typhus which might be connected with thermal, vascular and even biochemical changes (Stürm, 1942).

The abnormalities of the cardiovascular system in typhus have recently been studied by Woodward & Bland (1944), who made observations of the venous pressure, and the electrocardiographic changes, in addition to purely clinical observations such as were made by us. Unlike Woodward & Bland, we encountered one definite case of cardiac failure of congestive type and thought that the occurrence of cardiac dilatation and of gallop rhythm in others was indicative of myocardial damage such as was readily demonstrable at autopsy. Cardiac damage was thus as important, in our opinion, as peripheral (vasomotor) failure, and in this our views agree with those of Laurentius (1942). In the absence of detailed observation of the cardiac output, it is impossible to assess the relative importance of central or peripheral failure in the production of the observed phenomena. Even the beneficial effect of parenteral fluid in the type of case with circulatory collapse, extremely low blood pressure and feeble cardiac action might be exerted on this central cardiac mechanism by promoting an increased venous return. Nevertheless, we agree with Woodward & Bland's views on the importance of supportive therapy by free administration of large volumes of fluid, and were unimpressed by the value of so-called cardiac tonics of the strophanthus type. Cardiac arrhythmia, though recorded by Wolbach, Todd & Palfrey (1922), was not encountered by us. The demonstration of typhus lesions in the suprarenals may indicate that the disturbance of the cardiovascular system in typhus is as complicated in nature as is that of the excretory system.

The frequent occurrence of abnormal physical signs in the lungs, and the almost constant finding of bronchiolitis at post-mortem, emphasised the rickettsial nature of the pathological changes in the respiratory tract. Consolidation of the peribronchial alveoli, on the other hand, was frequently encountered at autopsy, though unsuspected during life, and at times was due to a secondary infection with organisms such as the pneumococcus. Such complications undoubtedly exerted an important effect on the outcome of the disease, particularly in elderly patients.

Abnormalities of renal excretion were encountered at an early stage of our investigation, and although no final elucidation of the mechanism of these abnormalities has emerged, it is hoped that the observations detailed above may be useful. A summary of the findings is as follows :—

- (i) Azotaemia is almost invariable in severe typhus and may be accompanied by an impaired clearance of urea from the blood. Azotaemia is often accompanied or preceded by oliguria or anuria, but the urine may be qualitatively normal though perhaps of a low specific gravity.
- (ii) The circulatory system is modified so that the blood-pressure is lowered and the pulse-pressure reduced.
- (iii) There is no demonstrable haemoconcentration, though this was sometimes assumed on clinical grounds.
- (iv) Reduction in urinary excretion of chloride, and a diminution in the level of plasma chloride, are common. No correlation exists, however, between the degree of reduction of plasma chloride and the degree of azotaemia.
- (v) The alkali reserve is sometimes lowered but may be within normal limits.
- (vi) Though tubular lesions and focal glomerular lesions in the kidney are demonstrable at autopsy, these alone are inadequate to explain the failure of urea excretion.

From these considerations it follows that the renal failure is probably a result of the combination of renal and pre-renal causes. Recent studies have elucidated the mechanism of pre-renal azotaemia (Fishberg, 1939) in intestinal intoxication (Schoenthal *et al.*, 1933), in diabetes (McCance & Lawrence, 1935), in alkalosis (Nicol, 1940), in gastric haemorrhage (Bläck, 1942), in chloride deficiency states (McCance & Widdowson, 1937) and in Addison's disease (Loeb *et al.*, 1935). Several of the factors responsible, at least in part, for the azotaemia in these conditions are present also in typhus fever.

Thus, diminished glomerular filtration due to reduction of blood pressure is likely to be operative in typhus, and may in some cases be the sole explanation of the azotaemia. Excessive tissue catabolism may also play a part in the azotaemia of typhus. It seems more probable that in the majority of cases impaired renal function is brought about by multiple circulatory and chemical factors together with focal vascular and glomerular lesions, and perhaps more generalised toxic effects of the disease on the renal parenchyma. If hypochloraemia is an important contributory cause to the azotaemia in typhus, then it must be regarded as an indication for NaCl and saline therapy. There seems to be insufficient evidence at present to suggest hypochloraemia in typhus as being of greater significance than the plasma chloride reduction that occurs in other infective conditions or in other conditions associated with oliguria. The possible part played by functional deficiency of the suprarenals must also be considered. Some analogy undoubtedly exists between the azotaemia of Addison's disease and of typhus, and the fact that lesions of the suprarenal were frequently demonstrated at autopsy in the typhus cases may indicate that functional deficiency of the suprarenals is an important factor which might be offset by suitable replacement therapy.

The toxic effect of the chemotherapeutic agent V186 on the convoluted tubules was very striking. In spite of the severity of the tubular lesions in the treated cases, there was no evidence of a greater degree of urea retention than in control cases. It seems likely that the harmful effects of the drug were felt particularly by those specialised tubules already affected by toxic products of the disease, as evidenced by cloudy swelling and other degenerative changes in those tubules in fatal control cases.

#### CHEMOTHERAPY

The reason for the disappointing failure of V186 and V147 in the treatment of typhus in man is difficult to explain. Our experiences emphasise once more that therapy, effective in laboratory animals, may not be equally effective in

the treatment of the human disease. It must again be pointed out that the therapeutic trials in man described in this report were undertaken at the earliest possible moment after the value of the drugs in laboratory animals was established, in the hope that effective chemotherapy would assist in reducing the mortality from typhus fever during the War, when outbreaks of this disease were most to be expected. The possibility of failure was fully realised. In the light of the results of our investigations it is interesting to speculate on the reasons for the failure of the drugs in man.

Thus, it is possible that the comparatively great susceptibility of the human kidney to their toxic action precludes the administration of chemotherapeutically adequate doses. The severe degenerative changes seen in the renal tubular epithelium of fatal treated cases during the Naples epidemic, and a number of unexplained cases of vascular collapse with the doses administered, made it inadvisable to increase the dosage still further. In other words, the margin of safety (therapeutic index) may have been smaller in man than in the mouse.

It must also be borne in mind that typhus in the mouse differs markedly from the disease in man. The successful chemotherapeutic trials in mice by Andrewes, King, van den Ende & Walker (1944), were performed by a pulmonary titration method. Mice infected intranasally with a sufficiently large dose of rickettsiae will die with complete pulmonary consolidation. Smaller doses are not fatal but produce small discrete focal areas of consolidation. The beneficial effect of V186 or V147 treatment was shown by the ability of the drugs to save life by preventing consolidation or by reducing the number of discrete focal lesions. It is conceivable that the drugs exerted their beneficial action not by direct effect on the rickettsiae but by reducing or preventing the host's reaction to the infecting agent. Marked reduction of the inflammatory response in the lungs would prevent gross pulmonary consolidation and therefore death by anoxia, or, in the case of smaller infecting doses, reduce the number and size of the focal lesions. A similar reduction in the inflammatory response in the peritoneum, in the case of mice infected intraperitoneally with the murine strain, could be expected to have a beneficial effect by reducing the secondary functional derangement of the gastro-intestinal tract.

It is of interest that in the guinea-pig, in which typhus infection is somewhat analogous to that in man, the drugs were ineffective in the small dose which could be administered. Larger doses could not be given, as the susceptibility to the toxic effect of the drugs is even greater in this species than in man. Attempts to assess the therapeutic value of the drugs in monkeys have so far been unsuccessful.

It has been suggested by Greiff, Pinkerton & Moragues (1944) that differences in intimate cell metabolism of different hosts may influence their natural susceptibility to rickettsial infections. Also, that "an agent which is therapeutically effective in typhus infection in animals of one species, might be ineffective in animals of another species, because of existing differences in metabolism." Para-aminobenzoic acid has been found to exert an inhibitory effect on the growth of rickettsiae in eggs, and also had a therapeutic effect on murine typhus infection in mice (Yeomans, *et al.*, 1944). Riboflavin deficiency has been found to render the peritoneal cells of the rat susceptible to typhus, and riboflavin therapy is then effective against the infection. Large amounts of riboflavin are, however, therapeutically ineffective in typhus-infected normal mice. The tissues of the mouse and of man may perhaps differ in their content of essential metabolites or enzymes, with resulting differences in the response to the therapeutic agents employed—and thus perhaps to the V186 group of drugs.

Modern chemotherapy of bacterial diseases is supposed to depend largely on the interference with essential metabolic processes of the organisms. A similar basis may be impossible in the case of virus and rickettsial diseases

where the pathogen depends for its survival and multiplication on parasitism of the enzymes and essential metabolites of the host's cells. The elucidation of the reasons for the success of V186 in the treatment of typhus in mice and its failure in man will help us to understand the mode of action of the drug. It will, moreover, assist in the rational development of virus chemotherapy by establishing whether the environment encountered by the virus in the cells can be altered sufficiently to be deleterious to the infecting agents without affecting the viability and function of the cells they parasitise.

It seems unnecessary to discuss further the subject of the treatment of typhus. In the absence of any specific treatment, reliance must be placed on symptomatic and supportive therapy. Search for specific therapy is likely to continue, however, because of the relative impotence of non-specific measures in the face of severe typhus. It seems premature to discuss the findings of Yeomans, *et al.* (1944), who found that large doses of para-aminobenzoic acid exerted a favourable effect on cases of typhus if given at a very early stage of the disease. This drug was of relatively feeble activity when compared with V147 or V186 in the treatment of experimental typhus in mice in our experience. The fact that Yeomans obtained favourable effects by treating patients at an even earlier stage of the disease than the cases of typhus treated by us suggests the possibility that V186 might also be active in man under such conditions. We feel, however, that further work, and particularly more rigorous control, is necessary before it can be assumed that para-aminobenzoic acid is an effective anti-rickettsial agent in man. At least it appears to possess none of the dangers of unexpected toxicity towards cases of typhus such as we encountered with V186. The small trial of penicillin described above did not lend encouragement to the view that this agent is of value in the treatment of typhus. As pointed out by Yeomans *et al.* (1944), penicillin may be of value in combating secondary bacterial infections in typhus even if it does not affect the primary disease. Our own experience was too limited for final decision on the possible scope of penicillin therapy in the late stages of typhus, but was not sufficiently encouraging to warrant further trial at a time when penicillin was urgently needed for other purposes.

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129

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## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### III.—A QUANTITATIVE TEST FOR NEUTRALISING ANTIBODIES AGAINST TYPHUS RICKETTSIAE.

BY

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#### INTRODUCTION

In estimating the potency of typhus vaccines various tests have been employed. These include determination of power to protect animals against infection and power of stimulating the formation of a variety of antibodies in man and animals. The antibodies investigated include agglutinins for *Proteus OX19*, agglutinins for rickettsiae of typhus (Weigl, 1930; Zinsser & Castaneda, 1931, 1933; Castaneda & Zia, 1933; and Hudson, 1940), and complement-fixing antibodies (Castaneda, 1936; Bengtson, 1941; Bengtson & Topping, 1942; and Plotz, 1943). More recently, tests for the formation of antibodies protecting against the acute toxic effect of rickettsiae injected intraperitoneally or intravenously into mice have been suggested (Gildemeister & Haagen, 1942; Otto & Bickhardt, 1941; Craigie, unpublished observation; and Henderson & Topping, 1945).

Although much valuable information has been obtained by these serological methods, there is as yet no evidence to prove which of the antibodies demonstrable *in vitro* is responsible for the resistance to infection. There seems to be room, therefore, for more direct tests of the antibodies which protect against infection, and for correlating, if possible, this biological test with one or other of the *in vitro* reactions. Zinsser & Castaneda (1933, 1934) have shown the power of hyperimmune sera to protect guinea-pigs against infection. Giroud (1938) has described a test for the protective antibodies by intracutaneous injection of rickettsiae and rickettsiae-serum mixtures into guinea-pigs or rabbits. This test has not always given satisfactory results in our hands. The difficulties encountered will be discussed in later sections of this paper.

The facts that all strains of typhus rickettsiae can be adapted quite readily to the lungs of mice (Castaneda, 1938, 1939; Durand & Sparrow, 1940; Giroud & Panthier, 1942; Andrewes, King, van den Ende & Walker, 1944), and that a simple reliable method of quantitative titration in mouse lungs exists, have suggested the quantitative measurement of neutralising antibodies which is here described. The method depends on the observation that appropriate dilutions of rickettsial suspension, when inoculated intranasally into anaesthetised mice, will produce isolated focal lesions. The quantitative titration of virus by this method was first suggested by Rudd & Burnet (1941) for the psittacosis virus, and later employed by van den Ende & Lush (1943) for the virus of *lymphogranuloma venereum*. Rudd & Burnet failed with this method to detect neutralising antibodies to the psittacosis virus in the serum of

## TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 131

immunised animals. Tests with typhus convalescent sera, however, soon showed that the neutralising antibodies could be demonstrated by their power to reduce pulmonary focal lesion counts.

It has been possible to design a fairly reliable method for measuring the approximate amount of neutralising antibodies in serum. The method is based on results obtained with sera from patients during and after mild laboratory infections with murine typhus, and from convalescent rabbits and guinea-pigs, as well as with sera from a number of vaccinated animals.

### MATERIAL

Four strains of rickettsiae were employed: a murine (Wilmington) strain, and three epidemic strains—the Breinl strain, an epidemic strain from Tunis, and one from Cairo. All strains were adapted by repeated passage through yolk sacs of chick embryos and the lungs of mice. Stock suspensions of each strain were prepared by emulsifying heavily infected mouse lungs or yolk sacs in horse serum broth, lightly centrifuging, distributing in small ampoules and storing at  $-77^{\circ}\text{C}$ . in a  $\text{CO}_2$  alcohol mixture. Each stock was titrated intranasally in mice. The titre of the stock emulsion as determined by repeated titrations remained constant for at least six months under the conditions of storage.

The human sera used were from a case (E) of mild laboratory infection with murine typhus in a vaccinated subject, and from a number of typhus cases occurring in the field. Convalescent rabbit sera were provided by 10 rabbits, each of which had received intraperitoneally a single dose of about half of a heavily infected yolk sac. They were bled at four- to seven-day intervals for five weeks after injection. In addition, a number of sera were obtained from rabbits which had received repeated (three to seven) injections of either a mouse lung or yolk sac type of killed vaccine. Convalescent sera were obtained from guinea-pigs used for routine passage of the strains, usually approximately two weeks after the peak of the febrile response. An attempt to obtain more potent anti-sera in guinea-pigs by the injection of massive doses of infected yolk sac was also made. All sera were stored at ordinary refrigerator temperatures.

### METHODS

#### INTRANASAL INOCULATION OF MICE

It was apparent that the accuracy of the intranasal titrations was very largely dependent on the quality of anaesthesia. Great care was therefore taken to ensure uniformity of anaesthesia, and any mouse which did not aspirate the infecting dose promptly and completely was discarded. A mixture of 5 c.c. chloroform and 45 c.c. ether was placed in a glass jar, 4 in. in diameter and 6 in. in height. A 2-in. thick pad of absorbent cotton wool was placed in the bottom of the jar, and the mouth of the jar covered with a petri dish. The mice were dropped into the jar in turn and removed with forceps when anaesthetised. When large numbers of mice were used it was found to be an advantage to employ two jars. Inoculations could usually be performed at the rate of 100 or more mice an hour, in spite of the fact that all inoculations were performed in a special inoculation chamber (van den Ende, 1943). A variety of methods for anaesthetising mice was tried. None was as satisfactory as the simple method which has been outlined. One week after inoculation the mice were killed in chloroform vapour and the focal lesions on the surface of the lungs counted according to the procedure previously described for the *lymphogranuloma venereum* virus.

TABLE XXXIII

Showing the neutralising power of human and rabbit sera against murine and epidemic rickettsiae

Serum sample	Focal lesion counts. <i>Murine rickettsiae</i>					Focal lesion counts. <i>Epidemic rickettsiae</i>				
	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	
Series E, 9.10.41 ..	26 34 41 21 (30·5)	142 11 200 187 (200)				7 14 17 ns (12·7)	83 80 64 97 (80·5)			
Series E, 6.11.41 ..	21 14 17 24 (18·5)	131 67 172 192 (140·5)				13 6 ns ns (9·5)	62 ns c (? 62)			
Series E, 8.12.41 ..	18 27 ns ns (21·5)	72 84 89 ns (81·7)				7 4 8 8 (6·8)	62 22 ns 24 (38)			
Series E, 13.1.42 ..	14 8 7 11 (10)	58 61 52 43 (54)				5 5 6 3 (4·8)	50 144 27 47 (67)			
Series E, 20.1.42 ..		27 29 17 21 (23·7)	200 300 187 200 (200)							
Series E, 23.1.42 ..		1 6 19 6 (8)						72 52 69 c (64·3)		
Series E, 6.2.42 ..		0 0 0 0	4 2 3 0	25 19 21 13 (19·5)						

(73196)	Series E, 17.2.42 ..			0 2	1 0	7 5	4 ns	48 (50)	52			4 1	7 c	15 c	24 28
	None, control ..	21 29 (27)	26 32	156 189	193 200					6 15 (9.5)	9 8	64 55 (66.3)	63 83		
	TR 119 Murine I			84 82 (93.7)	73 130	††				9+ 37 (33.3)	36 27	250			
	V .. .. .			0 0	0 ns	2 ns (1)	22 46 (33.3)			1 4 (1.7)	0 ns	c 26 (? 26)	c ns	150	
	VII .. .. .			0 0	0 0	0 ns	0 ns			0 0 (1.5)	3 3	ns 5 (4)	ns 3	24+ 100 (? 150)	200 ns'
	ETR 25 Epidemic I			93 130 (90.8)	85 55	†† ††	†† ††			37 33 (39.7)	48 ns	200 200	300 ††		
	V .. .. .			34 ns (34.5)	35 ns	200				1 2	1 0	?5 0	0 0	4+ 53	?100 c
	VII.. .. .			0 ns	0 ns	14 12 (11.3)	8 11	80 (115)	150	0 0	0 0	1 1	0 ns	2 2	1 1

ns = non-specific lesions.

†† = too many foci to count.  
Averages are given in brackets

c = massive consolidation.

## SERUM NEUTRALISATION TEST

## (a) Constant serum method :—

Progressive ten-fold dilutions of stock rickettsial emulsions, usually in 0.25 c.c. amounts, were mixed with an equal volume of undiluted serum, and left for one hour at room temperature. Each mixture was then used to inoculate four 12–16 g. mice. Each mouse received 0.05 c.c. intranasally while under ether and chloroform anaesthesia.

## (b) Constant antigen (rickettsiae) method :—

Two-fold or four-fold dilutions of serum were made in 10 per cent. normal horse serum broth. To each serum dilution, usually in 0.25 c.c. amounts, was added an equal volume of a horse serum broth suspension of rickettsiae, in a concentration sufficient to give approximately 50 to 100 lung foci when inoculated intranasally in 0.05 c.c. amounts. The mixtures were left in contact for the required length of time (usually one hour) at 37° C. After the preliminary incubation all the mixtures were plunged into ice to reduce any further neutralisation to a minimum, and each was then inoculated in 0.05 c.c. doses into four or more anaesthetised mice.

## RESULTS

The results obtained with all four strains of typhus rickettsiae used were similar. As a rule the results with murine typhus were the more satisfactory. With the epidemic strains there was greater tendency for the development of larger areas of pulmonary consolidation, and the occurrence of non-specific lesions was more frequent. Furthermore, there was a more obvious tendency in the case of the epidemic strains for the serum to produce reduction in the size of foci, rather than total absence of foci, which made counting in some cases difficult. Nevertheless, even with the epidemic strains, it was seldom impossible to interpret the results of an experiment. Mouse-lung suspensions gave more consistent and regular results than yolk sac suspensions.

Table XXXIII shows the results obtained by method (a) with a number of human and rabbit sera. The series E were obtained from a 29-year old research worker. The first serum was taken before vaccination and before commencing work on typhus, the second after a course of three injections of Castaneda's vaccine, the third after a similar course of Cox's type vaccine and the fourth after a similar course of Craigie's ether-purified vaccine. On January 17th, four days after the fourth sample was taken, symptoms of a mild attack of murine typhus started. The remaining serum samples were obtained during the subsequent seven months. Rabbit TR119 received a single injection on August 16th, 1942, of yolk sac infected with murine rickettsiae. Rabbit ETR25 received on September 18th, 1942, a single injection of yolk sac infected with Tunisian epidemic rickettsiae.

The results show that, in the case of E, sera obtained after three courses of vaccine possessed but little neutralising power. The fact that slight neutralisation was observed after Craigie's vaccine and not after the previous vaccines may depend on the greater potency of the Craigie-type vaccine, but may, of course, simply be the result of frequently repeated doses. The comparison of different vaccines by their power to evoke neutralising antibodies, is recorded in the next section (p. 146). Actual infection with murine typhus, on the other hand, very rapidly stimulated the formation of powerful neutralising antibodies. Unfortunately, owing to the small volume of some of the serum samples, it was impossible to test the whole series also against epidemic rickettsiae. A sufficient number were tested, however, to show that the sera were capable of neutralising the epidemic strain approximately to the

TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 135

same extent. This apparently complete cross-protection may depend on the fact that two courses of epidemic vaccine were given prior to infection, or on an antigenic similarity between the rickettsiae.

In the case of the rabbit sera marked cross protection was observed, but neutralisation of the homologous strain was more marked than that of the heterologous. The heterologous neutralisation was less marked in the case of sera from the rabbit infected with epidemic rickettsiae.

In these tests, in which countable numbers of foci occurred with more than one virus dilution, it appeared that the "percentage law" was obeyed (Andrews & Elford, 1933). The adherence to the law is not so close as in the

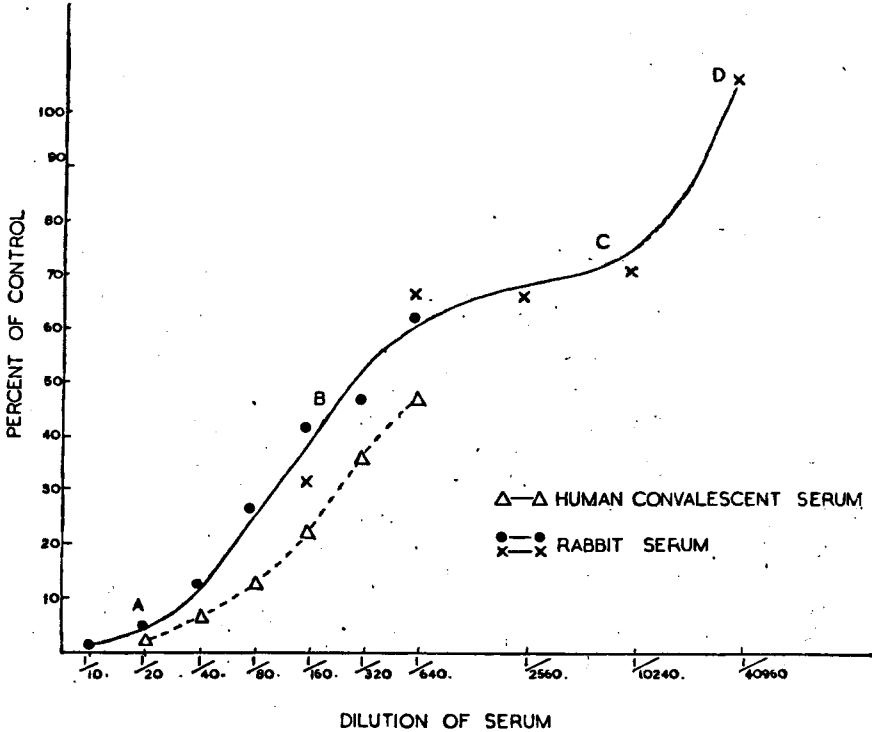


Fig. 26.—Showing the effect of dilution on the neutralising power of serum.

case of phage neutralisation, but discrepancies can probably be explained by the relatively higher counts obtained with higher virus dilutions, where there is less overcrowding of foci.

The results obtained with the constant antigen method (b), using two of the above sera—human serum E and murine typhus convalescent rabbit (No. TR119) serum—are recorded in Table XXXIV and Fig. 26. The tests were performed with a wide range of serum dilutions, using groups of animals large enough to allow of statistical analysis of the results. It will be noted that, with higher serum concentrations, represented by the part AB of the curve in Fig. 26, the reduction in focal lesion counts is proportional to the serum concentration. This part of the curve represents a reduction of 100 per cent. to approximately 50 per cent. of the count. There is, however, a wide range of serum concentrations (BC) over which serum dilution has little effect on the counts. The slope of the first part of the curve is the steepest and most constant, and, furthermore, there is, over this range of dilutions, a proportional relationship between serum

TABLE XXXIV  
Showing the effect of dilution on the neutralising power of serum

Serum	Focal lesion counts with serum dilutions																							
	1/10			1/20			1/40			1/80			1/160			1/320			1/640			None		
TR 119 VII (A)	0	3	5	5	4	4	8	18	14	17	22	24	47	69	45	62	55	27	61	65	66	134	91	111
	3	0	2	5	2	7	15	ns	20	31	28	30	51	21	47	23	56	72	73	50	49	135	121	ns
	0	2	4	8	7	4	15	12	10	44	15	35	31	34	46	52	44	41	91	50	83	104	103	ns
	2	1	1	10	8	7	10	14	20	26	33	33	48	61	41	76	47	72	56	80	76	78	103	103
	2	1	1	6	3	6	9	11	12	25	34	ns	43	48	33	31	36	50	40	81	71	108	85	86
	ns			7			ns			ns			41	53		58			ns			128		
Mean .. ..	1.8			5.7			13.4			28.4			44.6			50.1			66.1			106.5		
Per cent. of control.	1.7			5.4			12.6			26.7			41.9			47.1			62.1					
SD of mean ..	0.41			0.56			1.03			2.1			2.7			4.9								
	1/20			1/80			1/640			1/2,560			1/10,000			1/40,000			1/160,000					
(B) .. ..	1	2	2	11	9	16	35	26	20	25	39	34	41	31	27	59	40	46	44	47	50	42	46	59
	0	5	3	12	12	12	41	31	30	25	ns	29	28	37	34	43	ns	ns	56	49	60	47	37	ns
Mean .. ..	2.2			12.0			30.7			30.6			33.0			47.0			51.0			46.2		
Per cent. of control.	4.8			25.9			66.5			66.2			71.4			101.7			110.4					
	1/20			1/40			1/80			1/160			1/320			1/640								
E XII ..	3	4	5	7	4	8	13	20	7	17	27	25	13	26	64	49	68	65				127	123	99
	3	5	6	8	11	ns	11	21	12	29	27	14	45	30	34	47	51	60				108	130	135
	4	3	4	6	14	6	16	14	19	36	47	18	72	35	37	60	44	39				57	122	133
	4			9			16			34	13		66			65						125		
Mean .. ..	4.1			8.1			14.9			26.1			42.6			54.8			115.9					
Per cent. of control.	3.5			7.0			12.9			22.5			36.8			47.3								
SD of mean ..	0.31			0.99			1.37			3.12			5.86			3.18						7.28		



## TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 137

dilution and percentage reduction. It has been the aim, therefore, to determine for any one serum at least one point in the vicinity of that which gives 80 per cent. reduction in the count.

A standard serum is included in each test and, providing that constant results are obtained with the standard and that large groups of animals are used, neutralising power of different sera can be expressed by the dilutions which are capable of giving 80 per cent. reduction. Comparison of sera falling within the range BC is more difficult, unless very large groups of animals are used. In

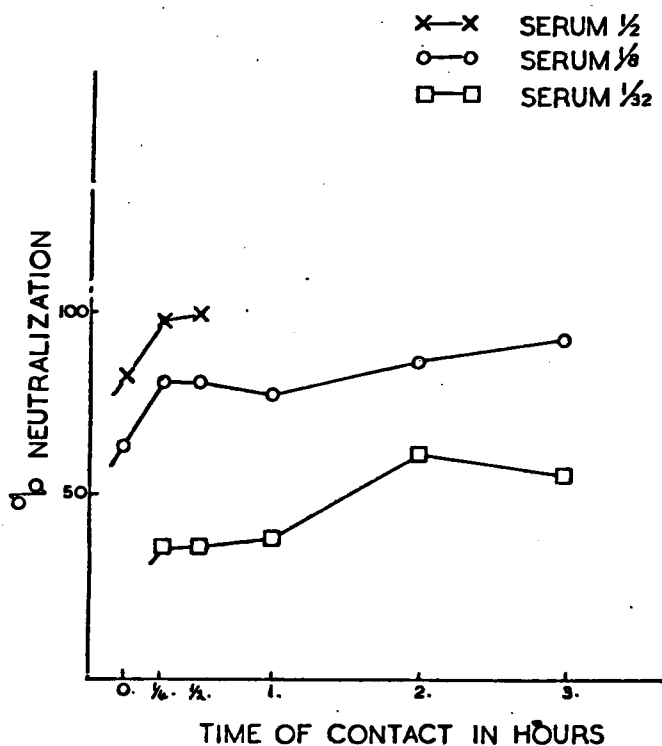


Fig. 27.—Showing the effect of preliminary incubation on the neutralising power of serum.

practice it has been found convenient to work with the range AB, and to give the sera falling within the range BC a value of less than half (the highest concentration of serum tested).

### THE EFFECT OF THE DURATION OF PRELIMINARY INCUBATION ON THE NEUTRALISING POWER OF SERUM

In earlier experiments, using the constant serum titration method, results obtained showed an apparent increase of neutralisation with increase of the time of preliminary incubation. The possibility of agglutination under the conditions of these experiments could not be ruled out. An experiment to determine the effect of preliminary incubation on neutralising power, using a constant minimum concentration of rickettsiae, is shown in Fig. 27. Dilutions of serum in 0.5 c.c. amounts were mixed with an equal volume of rickettsial suspension of such a dilution as to give rise to about 100 foci per mouse when inoculated alone. Mixtures were made three hours, two hours, one hour, half

## 138 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

an hour, and a quarter of an hour, as well as immediately before commencing mouse inoculation. Incubation was at 37° C. and all mixtures were transferred to ice immediately before the inoculations were started.

The results (Fig. 27) show that, with a final serum concentration of 1 : 2, an 80 per cent. reduction of focal lesions was shown by the sample inoculated immediately after mixing, 93 per cent. after a quarter of an hour's, and 100 per cent. after half-an-hour's incubation. With more dilute serum—1 : 8—the degree of neutralisation was less, but neutralisation after a quarter of an hour's incubation was almost as great (81 per cent.) as after two hours' incubation. Slightly greater neutralisation (93 per cent.) was observed in the mixture incubated for three hours, but the test showed that even the horse serum broth control gave a lower focal lesion count after three hours' incubation than the same material incubated for shorter periods. Prolonged incubation, therefore, appears to alter the viability and may, at the same time, increase susceptibility of the rickettsiae to antibody. With further dilution of serum—1 : 32—there is greater difference between long and short periods of incubation, but even here the greater part of the serum effect occurs during the first quarter hour.

### THE EFFECT OF COOLING AFTER PRELIMINARY INCUBATION

The majority of the experiments were designed to involve approximately 80 mice. As it took about 45 to 50 minutes to complete the inoculation of this number of mice, it was important to determine whether the reaction was inhibited at 0° C. Two identical series of serum rickettsiae mixtures were accordingly kept at 37° C. for one hour, after which they were placed on ice. One series was then inoculated into mice immediately, and the second after standing on ice for 45 minutes.

The results are recorded in Table XXXV, and show that there was no significant difference between the two. On the results of the foregoing experiments a routine test was based. All serum rickettsiae mixtures were incubated at 37° C. for one hour and placed in ice at the commencement of inoculation of the mice.

TABLE XXXV

*Showing the effect of ice in keeping neutralisation constant*

Serum dilution	Focal lesion counts.					
	1/20		1/40		1/80	
On ice 45 minutes before inoculation .. .. .	2	1 1	9	6 0	14	12 9
Mean .. .. .	3	4	3	ns	4	13
	2.2		4.5		10.4	
Inoculated immediately after placing on ice ..	1	2 2	6	3 4	11	9 16
Mean .. .. .	0	5	7	6	12	12
	2.0		5.6		12.0	

### NEUTRALISATION TESTS WITH FILTERED AND UNFILTERED RICKETTSIAL SUSPENSIONS

Merrill has shown that visible agglutination of viruses or bacteria depends on the total mass of antigen present. It was, therefore, considered possible that, if agglutination played a part in reducing the pulmonary focal lesion count in the rickettsial neutralisation test, then the reduction should be more marked when rickettsiae of large size were used than in similar experiments with small rickettsiae. It has been shown (Elford & van den Ende, 1944), that

TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 139

approximately 90 per cent. of the rickettsiae in an emulsion of infected mouse lung have an average diameter of 400 m $\mu$  or more. Only 10 per cent. or less of the rickettsiae will pass through a gradocol membrane of A.P.D. 0.73 $\mu$ . Parallel tests were, therefore, performed using crude unfiltered lung emulsions and 0.73 $\mu$  filtrate. The results, recorded in Table XXXVI, show that the neutralising power of an active serum was similar against the two suspensions. This experiment, although not disposing of the possibility of agglutination, provides evidence against its occurrence under the conditions of the test.

TABLE XXXVI

Showing the neutralising power of serum for filtered and unfiltered rickettsial suspensions

Serum TR 119 VI	Dilution of serum											
	1/8			1/32			1/128			None		
Crude lung emulsion ..	5	10	3	23	25	23	15	51	49	60	62	49
Mean ..	1	8		21	ns		49	ns		ns	ns	
	5.4			23			41			57		
0.73 filtrate ..	6	2	9	10	15	25	12	30	37	61	52	59
Mean ..	6	ns		28	ns		ns			54		
	5.8			22.7			26.3			55.2		

EFFECT OF CENTRIFUGATION ON PARTIALLY NEUTRALISED RICKETTSIAE

Further evidence against the occurrence of agglutination has been provided by centrifugation experiments. A rabbit convalescent serum of known neutralising power was used. As control, the normal serum, obtained from the same rabbit before the immunising injection, was used in identical concentration. The normal and immune serum-rickettsia mixtures were left in contact for 45 minutes at 37° C. They were then removed from the incubator and half of each mixture was centrifuged at 6,000 r.p.m. for 15 minutes. The supernatants were removed and all samples inoculated intranasally into mice in the usual way.

The results recorded in Table XXXVII show that the percentage reduction due to centrifugation of the partially neutralised rickettsial suspension was not greatly different from the reduction in the cases of rickettsial suspension

TABLE XXXVII

Showing the effect of light centrifugation on un-neutralised and partially neutralised rickettsial suspensions

	Centrifuged at 6,000 r.p.m. for 15 minutes after preliminary incubation						Not centrifuged						Reduction by centrifugation
	Rickettsial dilutions						Rickettsial dilutions						
	1/3 x 10 <sup>5</sup>		1/3 x 10 <sup>6</sup>				1/3 x 10 <sup>5</sup>		1/3 x 10 <sup>6</sup>				
Serum TR119 I ..	21	40	21	200	200	200	38	36	52	300	300	300	37 per cent.
(Normal) ..	30	19		150	150		33	48		200	200		
Mean ..	26.2						41.4						
Serum TR119 VI ..	8	6	ns	45	19	49	3	7	12	49	78	82	51 per cent.
(Immune) ..	4	5		39	26		4	ns		75	77		
Mean ..	5.8			35.6			6.5			72.2			

in normal serum. The focal lesions which, in the case of immune serum mixtures, were approximately one-tenth of the number in the control, were, therefore, evoked by particles probably not significantly larger than those in the un-neutralised suspension.

Attempts were made to determine whether agglutination occurs under the conditions of the test, by repeated microscopic examinations of serum-rickettsia mixtures with dark ground illumination. Many technical difficulties were encountered and results were not entirely satisfactory. There did not, however, appear to be any more clumping in mixtures containing immune serum than in similar mixtures with normal serum. Furthermore, visible agglutination with very much heavier rickettsial suspensions requires several hours for its appearance. It was, therefore, thought reasonable to conclude that the focal lesion test measures neutralisation and not agglutination of rickettsiae.

#### THE SPECIFICITY OF THE NEUTRALISATION TESTS

Neutralising sera were prepared in rabbits against each of the four strains of rickettsiae available. Each rabbit received a single massive dose of rickettsiae grown in the yolk sac of chick embryos. The sera were tested against the homologous strain, and the four most active sera selected. These were then tested against all four strains of rickettsiae. A standard dried serum was included in each test to form a basis of comparison. Groups of only four mice were used in the tests. The degrees of variation between individual mice, and the irregularity found with progressive serum dilutions, made it impossible to show any minor degrees of difference between the epidemic strains. The results of experiments with three of the sera are recorded in Figs. 28-30. A striking difference is seen to exist between murine and epidemic strains.

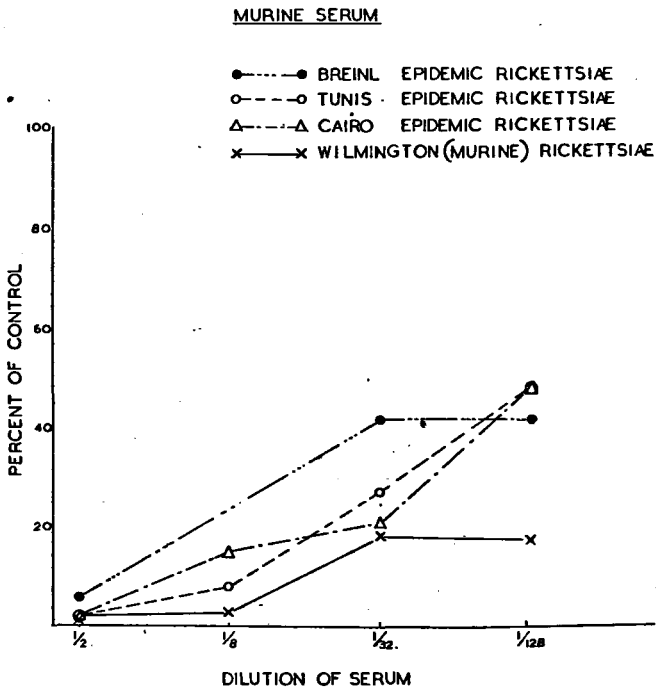


FIG. 28.—Neutralisation of epidemic and murine strains of rickettsiae by murine antiserum.

TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 141

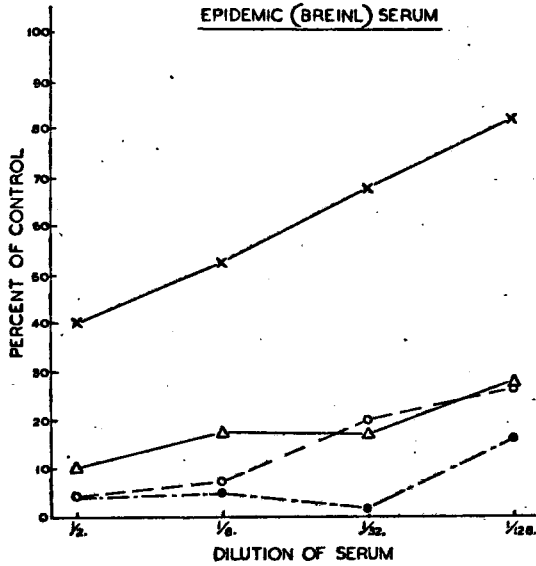


Fig. 29.—Neutralisation of epidemic and murine rickettsiae by epidemic (Breinl) antiserum.

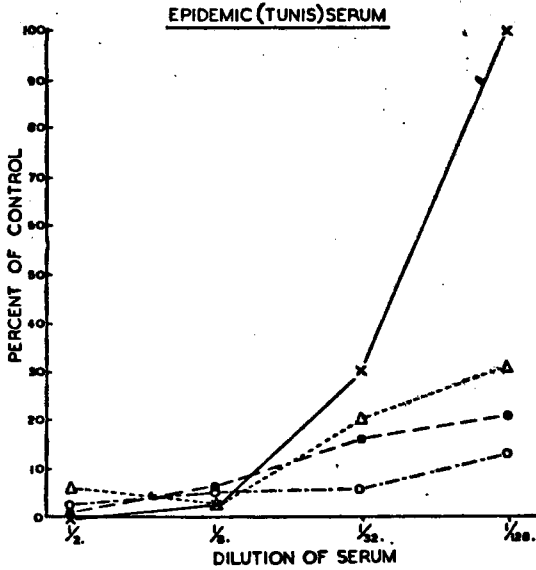


Fig. 30.—Neutralisation of epidemic and murine rickettsiae by epidemic (Tunis) antiserum.

## 142 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Between the individual epidemic strains differences are probably insignificant, although the neutralisation of the homologous strain is in all cases the strongest. The poor neutralisation of murine rickettsiae by an antiserum against the Breinl epidemic strain is particularly striking.

### COMPARISON OF THE INTRANASAL SERUM NEUTRALISATION TEST WITH THE GIROUD CUTANEOUS SERO-PROTECTION TEST

The Giroud test has an obvious advantage over the focal lesion counting method in that the results are unlikely to be affected by agglutination. A comparison was accordingly made between the intranasal and the Giroud neutralisation tests, to determine which was the more sensitive and which was the more convenient for quantitative studies.

A number of sera were tested by both methods. These sera were obtained from convalescent patients and animals, and from a number of individuals vaccinated with Craigie's vaccine. The same qualitative answer was obtained for practically all the sera in the two tests. Quantitatively on the other hand, the Giroud test was less satisfactory. It has been our experience that any effective concentration of serum will reduce in size and severity the lesions produced by a wide range of virus dilutions. It could reasonably be expected that the determination of the highest concentration of rickettsiae completely inhibited by a particular concentration of serum would provide an adequate quantitative method. In practice the determination of such an end point is not easy, as there is a very gradual reduction in the extent and severity of swelling and redness evoked by any one concentration of rickettsiae. The determination of the highest dilution of serum which will definitely reduce the size of the skin lesion is subject to the same disadvantages as were encountered with high serum dilutions in the mouse test (see Part BC of Fig. 26). Thus, there is a wide range of serum dilutions which will cause an almost equal reduction in the size of the skin lesion evoked. The result of an experiment which demonstrates this is shown in Table XXXVIII. The serum used in this experiment was the same as that used for the intranasal test recorded in Table XXXIV. These findings, when compared with those obtained in the mouse test, show that similar results are obtained by the two methods. Thus, high serum concentrations give marked reduction in the size and severity of the skin lesion. As in the mouse test, there is further a wide range of serum dilutions which give almost equal reduction of the lesions. Accurate quantitative titration, depending on small differences in the sizes of cutaneous lesions is, however, more difficult than titration by the mouse method.

The Giroud test has the additional disadvantage of requiring fully-grown rabbits with satisfactory skins, conditions which, especially in the existing circumstances, were found extremely difficult to satisfy.

### DISCUSSION

The quantitative pulmonary titration method described has given fairly accurate and consistent results with a number of sera which were sufficiently potent to reduce the focal lesion count to 20 per cent. or less of that in the controls. The potency of many sera was such that, even when they were used undiluted, the reduction was only 50 per cent. or less, and almost equal reduction was obtained over a wide range of serum dilutions. A quantitative determination of the neutralising power of each serum is impracticable because of the large groups of animals which would be required to obtain statistically significant figures. On the other hand, sera of sufficient potency to give more than 50 per cent. reduction of the focal lesion count can fairly accurately be compared quantitatively.

TABLE XXXVIII

Showing the neutralising power of human and rabbit convalescent sera as tested by the intradermal method (Giroud)

Serum	Day of reading	Dilution of serum								
		1/10	1/20	1/40	1/80	1/160	1/640	1/2,500	1/10,000	1/40,000
E XII ..	4	19fR (18+)	19fR (21+)	19fR (21+)	18fR to ± (21+)	17± (20±)	16± (21+)	16+ (21+)	15+ (18+)	16+ (19+)
	7	tr (18+)	tr (20+)	18f (20+)	18f (20+)	17± (19+)	17+ (20+)	17+ (19+)	15+ (17+)	16+ (18+)
TR 119 ..	4		14+ (19+)	17± (19+n=)	16± (18+n-)	17± (19+)	17± (19+)	18+ (19+)	17+ (19+)	18+ (18+)
VII .. ..	7		15f (18+)	17± (17+n-)	15± (17+n-)	14± (18+)	16± (18+n=)	16± (18+n-)	15+n= (18+n-)	(16+n=) (16+n=)

Figures = diameter of lesions in mm. .

R = redness.

f = faint.

± ± ± = increasing degrees of swelling.

n = n - n = increasing extent of the area of necrosis.

Readings in brackets are those of controls in the same position on opposite flank of the same rabbit.

It is hoped that the method will have a number of important applications. It has been found, for instance, that the sera from rabbits which have received three intravenous injections of formalised rickettsiae possess powerful neutralising action against homologous rickettsiae. The test may, therefore, be of value for the comparison of vaccines and the potency of immune sera in the laboratory. Sera from convalescent rabbits have been found to vary in their neutralising power for different strains of rickettsiae. The method may be of service in investigations on the antigenic relationships between these strains. Sera against the epidemic strains have been much less effective against the murine than against homologous rickettsiae. Differences between individual epidemic strains were not significant under the conditions of the tests. It is of interest to note that the serum neutralisation tests have given results roughly parallel with those obtained by Fulton (1944) using complement fixation methods.

Most of the tested sera which possessed powerful neutralising antibodies possessed also strong agglutinating power for rickettsial suspensions *in vitro*. It was considered possible, therefore, that the reduction of focal lesion counts might, especially with method (a), depend simply on agglutination. This suggestion was strengthened by the occurrence, especially in tests with the epidemic strains, of larger areas of consolidation, from which, although in many cases they appeared non-specific, no bacteria could be cultivated but rickettsiae were recovered on passage.

In method (b), on the other hand, the numbers of rickettsiae in the mixtures were reduced to a minimum, and increasing dilutions of serum were used. With this reduction in the concentration of both antigen and antibody, the chance of impact between rickettsiae to form clumps would be lessened. The results which still showed strong neutralisation are, therefore, less likely to have been dependent on agglutination.

Evidence that the test is not simply dependent on agglutination is provided by experiments which show that the same quantitative answer is given whether large-sized rickettsial particles or filtered emulsions with average diameter of less than 250 m $\mu$  are used. Furthermore, reduction in the number of foci with incompletely neutralised virus emulsions has, in centrifugation experiments, been shown to be produced by particles or aggregations of particles no larger than those in unneutralised rickettsial suspensions. Additional evidence has been provided by the finding that a number of convalescent guinea-pig sera, though possessing strong agglutinating power, showed but poor neutralising power. Filtration experiments with incompletely neutralised rickettsiae were not undertaken. Andrewes & Elford have shown that, with bacteriophage, partial neutralisation by specific antisera affects the filtration properties out of all proportion to the alteration in particle size. No additional information on the presence or absence of agglutination could, therefore, be provided by filtration experiments.

Certain quantitative aspects are of particular interest. Thus, there is neutralisation in multiple proportions when potent serum is used. With weaker sera or higher dilutions of potent sera, there is a wide range of dilution over which the focal lesion count remains almost unchanged. The reason for this is not explained. The phenomenon may depend on the inhibiting effect of small amounts of antibody on the development of focal lesions, analogous to the inhibitory effect of dilute serum on the formation of "phage plaques" (Andrewes & Elford, 1933).

#### SUMMARY

A quantitative test for the estimation of neutralising power of antirickettsial sera is described. The test depends on the determination of the extent of the reduction in the number of focal lesions produced in the lungs of mice inoculated intranasally with rickettsial suspensions mixed with progressive dilutions of anti-serum.



## TEST FOR NEUTRALISING ANTIBODIES AGAINST RICKETTSIAE 145

Evidence is provided to show that the reduction in focal lesion count is not dependent on the agglutination of rickettsiae.

A comparison is made between the Giroud sero-protection test and the mouse test. Qualitatively the tests give similar results, but quantitatively the mouse test is the more satisfactory.

It is suggested that the method may be of help in testing the potency of typhus vaccines and antisera, and in studying antigenic relationship between different rickettsiae.

### ACKNOWLEDGEMENTS

We wish to express our thanks to Dr. A. Felix for providing a number of sera from vaccinated human subjects and rabbits; and to Mr. L. Joyner and Miss D. Reynolds for their help in anaesthetising the many hundreds of mice used in this investigation. Great assistance was given during the earlier part of this study by the late Miss Dora Lush.

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## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### IV.—COMPARISON OF TYPHUS VACCINES IN THE LABORATORY.

BY

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#### INTRODUCTION

Most typhus vaccines are manufactured from rickettsiae cultivated in the yolk sacs of developing eggs (Cox, 1938) or in the lungs of rodents and larger animals (Castaneda, 1938, 1939). At the time this study was commenced little work had been reported in which the egg and lung vaccines were compared. Such a comparison is of importance to determine which type of vaccine elicits the best immunity and is thus most suitable for the protection of man. In comparisons which have been made in the past, necessary regard has not been paid to the relative numbers of the rickettsiae present in the vaccines under comparison (Plotz, unpublished observations; Topping, Bengtson & Sheer, 1945). It was the purpose of these investigations, therefore, to prepare vaccines of approximately equal rickettsial content both from mouse lung and yolk sacs. Investigations were also made on the effect of purification on the antigenicity of the rickettsiae present. The following vaccines were studied:—

- (A) was prepared from yolk sacs by Craigie's ether method (Craigie, 1945).
- (B) was an unpurified vaccine made from yolk sacs by centrifuging down the rickettsiae and re-suspending them in formalin saline without any attempt to remove the other sedimentable constituents.

In comparing these two vaccines, we hoped to discover whether the ether method of purification was in any way detrimental to the immunising antigen.

- (C) was the mouse lung equivalent of the unpurified yolk sac vaccine.
- (D) was also made from mouse lungs and purified by the kieselguhr method (Fulton & Begg, see p.165). The rickettsial suspensions so prepared are comparable in purity with bacterial suspensions.

The Wilmington strain of murine typhus rickettsia was used.

As many as possible of the known tests for the potency of typhus vaccines were employed; these included:—

(a) Tests for the ability of the vaccines to protect guinea-pigs against infection, and mice against the toxic effect of large doses of rickettsiae injected intravenously.

(b) Tests of the power of the vaccines to stimulate formation of circulating antibodies in rabbits and guinea-pigs. The antibodies investigated were agglutinins for rickettsiae and *Proteus OX19*, complement-fixing antibodies, antitoxins and neutralising antibodies.

## COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 147

### THE PREPARATION OF THE VACCINES

#### YOLK SAC VACCINES

Eggs after seven days' preliminary incubation were inoculated into the yolk sac (Cox) with a strain of murine rickettsiae which had been adapted to the yolk sac by many serial passages. The eggs were incubated at 36-37° C. and examined at frequent intervals. When the embryos became sluggish, they were opened and the yolk sacs harvested.

Thirty-three yolk sacs were collected over a period of 17 days. The yolk was drained away, but there was no attempt to wash the sacs. A film was made from each and stained by Macchiavello's method. Only yolk sacs very rich in rickettsiae were retained. A sample of each yolk sac was cultured on a blood agar slope and all were found to be sterile. As they became available, the yolk sacs were stored whole in small screw-capped bottles, without any suspending fluid. The bottles were packed in dry ice in a thermos flask. At the end of the period of collection, the sacs were thawed, ground up in a mortar and well mixed to a uniform suspension.

#### *Titration of the Suspension*

The viable rickettsiae were titrated by the mouse lung method (Andrewes, King, van den Ende and Walker, 1944). The results are recorded in Table XXXIX.

TABLE XXXIX

<i>Dilution</i> (Volume administered 0.05 ml.)	$10^{-5}$	$10^{-6}$	$10^{-7}$
Count of focal lesions .. ..	c	98	2
	c	80	5
	c	84	16
	c	84	(d)
Average .. .. .		86	8

c = indicates gross consolidation.  
d = dead at time of count.

The numbers refer to the number of discrete focal lesions on the surface of the lungs (four mice for each dilution).

#### *Vaccine A* (Craigie ether-purified type).

Twenty-one ml. of the yolk sac suspension, representing 12 yolk sacs, were used. We have followed Craigie's latest pH 7 method (unpublished). The volume of vaccine obtained was 57.5 ml.

#### *Vaccine B* (Crude yolk sac vaccine).

Seven ml. of the yolk sac suspension, representing four yolk sacs, were emulsified in 40 ml. of 10 per cent. rabbit serum broth and centrifuged at 2,000 r.p.m. for 30 minutes. This deposited much cellular debris and only a relatively small proportion of the rickettsiae (Elford & van den Ende, 1944). The deposit was discarded. The supernatant (38 ml.) was centrifuged at 10,000 r.p.m. for 20 minutes. The deposit was re-suspended in 38 ml. of 0.5 per cent. formol-saline.

#### MOUSE LUNG VACCINES

The murine typhus seed for passage was stored at -77° C. as a crude emulsion of consolidated mouse lungs in 10 per cent. horse serum broth (3 ml. per lung). This material was diluted one-third in horse serum broth, and 0.1 ml. amounts were instilled intranasally into 14-16 g. mice under light ether anaesthesia. Prior to the intranasal inoculation, each mouse received 15 mg. of sulphathiazole

## 148 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

intraperitoneally and 0.1 per cent. was added to the diet on subsequent days. Those mice which were still alive on the third day were dying with gross consolidation of the lungs. The dead mice were discarded; the living mice were killed with chloroform and their lungs harvested.

The lungs were ground up in a mortar with glass powder in 10 per cent. rabbit serum broth (1.5 ml. per lung). The suspension was centrifuged at 1,000 r.p.m. for two minutes to remove the glass powder and the coarse lung fragments. In order to avoid the danger of large batches being spoiled by the inclusion of a few lungs heavily contaminated with bacteria, the lung emulsion was made up in small quantities and stored at  $-72^{\circ}\text{C}$ . in screw capped bottles. When sterility tests had been completed the lots in which contamination was not excessive were pooled.

*Titration of the Suspension*

The viable rickettsiae were titrated by the mouse lung (spot count) method. The results are recorded in Table XL.

TABLE XL

Dilution Volume administered 0.05 ml.	$10^{-4}$	$10^{-5}$	$10^{-6}$
Count of focal lesions .. ..	c	200 ±	13
	c	54	40
	c	200 ±	42
	(d)	200 ±	(d)
Average .. ..			32

The number of aerobic bacteria present in the crude suspension as determined by growth on blood agar was  $1 \times 10^3$ /ml. Bacterial contamination is one of the disadvantages of the lung method of cultivating rickettsiae. The degree of contamination is, however, negligible from an antigenic point of view. Thus, it may be calculated that the "spot count" per ml. is  $700 \times 10^6$ , and that the total number of rickettsiae present is probably 1,000 times this number ( $7 \times 10^{11}$  per ml.). Furthermore, the bacterial contaminants can be reduced by more careful selection of the lungs.

*Vaccine C* (Crude mouse lung vaccine).

Thirty ml. of the pooled mouse-lung suspension were centrifuged at 5,000 r.p.m. for 10 minutes. The deposit was discarded and the supernatant (29.0 ml.) was centrifuged at 10,000 r.p.m. for 20 minutes. The deposit was re-suspended in 29.0 ml. of 0.5 per cent. formal-saline (*i.e.* 0.2 per cent. formaldehyde).

*Vaccine D* (purified mouse-lung vaccine).

The emulsion representing 23 consolidated lungs in 34.5 ml. of 10 per cent. rabbit serum broth was used. The suspension was centrifuged at 1,000 r.p.m. for two minutes and the deposit discarded. The supernatant S<sup>1</sup> (34 ml.) was centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant S<sup>2</sup> was discarded and the deposit was re-suspended in 34 ml. of saline. To this was added an equal volume of M/10 acetate buffer of pH 4. One per cent. of specially selected grade kieselguhr was than mixed with the buffered suspension. The kieselguhr flocculated to the bottom, carrying with it the tissue fragments. The supernatant fluid was a pure rickettsial suspension. This was rapidly neutralised with N.NaOH, and formalin was added to a final concentration of 0.5 per cent.

Since this vaccine was prepared, considerable improvement has been effected in the kieselguhr method of purification. The details of the improved method are given elsewhere in this report (Fulton and Begg, see page 165).

## COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 149

The finished vaccines were tested for sterility, and also for the absence of live rickettsiae by instilling the vaccine intranasally into groups of mice. No lung lesions were found when the mice were killed seven days later.

### STANDARDISATION OF THE VACCINES

This was undertaken to ensure that the vaccines contained approximately the same number of rickettsiae. Any method based on a measurement of opacity will, of course, be grossly misleading when purified vaccines are being compared with unpurified ones. We have used a technique which enables us to obtain approximate counts of the rickettsiae in a suspension.

Glass tubing of a suitable bore and length to fit the centrifuge cups employed was closed at one end with a well-fitting rubber bung. On the bung was placed a cover glass with 0.5 ml. of a suitable dilution of the suspension. The tube was then centrifuged at 10,000 r.p.m. for 15 minutes; in this way the sedimentable constituents of the suspension were evenly deposited on the cover glass, which was then dried and stained with hot basic fuchsin (60° C.) for five minutes. The stained preparations were dried, mounted and examined microscopically. Serial dilutions of the vaccines were examined in this way, until a dilution was attained which yielded about 40-50 rickettsiae per microscopic field, which has an area, in this case, of  $(50\mu)^2$ . A large number of fields were counted and the average number of rickettsiae recorded. The results obtained with the four vaccines are shown in Table XLI.

TABLE XLI

Dilution →	<i>Average counts of rickettsiae per (50μ)<sup>2</sup> microscopic field</i>			
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>
Vaccine A .. .. .			++	40
Vaccine B .. .. .			++	33
Vaccine C .. .. .			++	35
Vaccine D .. .. .			++	15
*Commercial epidemic typhus vaccine (batch 8.1)	++	20		

\* Recent batches of commercial typhus vaccine examined by this method have been found to contain ten times as many rickettsiae as batch 8.1.

From these counts it was inferred that Vaccines A, B and C contained approximately equal numbers of rickettsiae per ml., but Vaccine D about half the number per ml. of the others. In order to have sufficient vaccine for our purpose, Vaccines A, B and C were diluted 1/10 with 0.5 per cent. formol-saline. Vaccine D was diluted 1/5.

### ESTIMATE OF THE DEGREE OF PURITY OF THE DILUTED VACCINES

#### (i) Measurement of opacity.

The opacities of the diluted vaccines were compared in a Spekker absorptiometer with a known *Bact. coli* suspension. The results are recorded in Table XLII.

TABLE XLII

Vaccine	<i>Opacity equal to coli suspension of</i>
A .. .. .	305 × 10 <sup>6</sup> /ml.
B .. .. .	785 × 10 <sup>6</sup> /ml.
C .. .. .	390 × 10 <sup>6</sup> /ml.
D .. .. .	110 × 10 <sup>6</sup> /ml.

## 150 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

(ii) The relative purity of the vaccines.

The vaccines were diluted 1 in 5, and 0.5 ml. amounts were centrifuged down on to cover glasses in the manner previously described. Photomicrographs of representative fields are shown in Plate X, Figs. A-D. From a study of the photomicrographs it appeared that Vaccine A and Vaccine D were very similar in the degree of their purity. However, Vaccine A, not having been centrifuged during manufacture, contained a large amount of "soluble" protein derived from the egg. This difference is shown when the total nitrogen of the two vaccines is compared (Table XLIII).

TABLE XLIII

<i>Vaccine</i>	<i>Total N<sub>2</sub> per cent. by weight</i>
A .. ..	0.0838
D .. ..	0.0076

We concluded, therefore, that the vaccines could be arranged in the following order of decreasing purity: D, A, C, B.

### POTENCY TESTS

#### ACTIVE IMMUNISATION OF GUINEA-PIGS

Five groups of six guinea-pigs were used. One group received Vaccine A, a second Vaccine B, a third Vaccine C, and a fourth Vaccine D. The fifth group was kept as a control. The guinea-pigs of the vaccinated groups received one dose of 0.5 ml. of the appropriate vaccine intraperitoneally. Thirty-seven days later they were challenged by the intraperitoneal injection of 1.0 ml. of a yolk sac suspension of viable rickettsiae diluted 1/100. The rectal temperatures were recorded for the ensuing 15 days.

The temperature records are shown in Figs. 31-62. Although the experiment as a whole was unsatisfactory, the results are recorded in detail because they demonstrate a difficulty which we have frequently encountered in our work with murine typhus in guinea-pigs, viz., that they often fail to respond with a temperature which is generally accepted as significant (40° C. or 104° F.), even when large doses of viable rickettsiae are given. Results of this type indicate the difficulties encountered in attempting to evaluate the protective power of typhus vaccines by their ability to protect guinea-pigs. It is also of interest that in these experiments the three control animals which failed to show a temperature of 40° C. or more were females. The five remaining controls were males and showed significant temperatures; two of these had received only 1/100 of the dose received by each of the remaining animals in the control and vaccinated groups.

If only the response of the male animals is considered, then 4 of the 11 vaccinated guinea-pigs showed a significant fever after the challenge dose, as compared with 3 out of 3 controls. Scrotal reactions were milder in the vaccinated animals than in the controls. No difference between the protective values of the different vaccines could be seen in this experiment.

#### ACTIVE IMMUNISATION OF MICE AGAINST THE TOXIC ACTION OF RICKETTSIAE

Craigie (unpublished) has suggested that the efficacy of typhus vaccines may be estimated by actively immunising mice and then testing their immunity by the intraperitoneal injection of a dose of living rickettsiae which will kill normal mice in 2-6 hours.

PLATE X

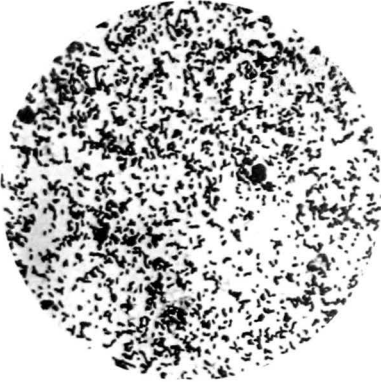


FIG. A.—Vaccine A.

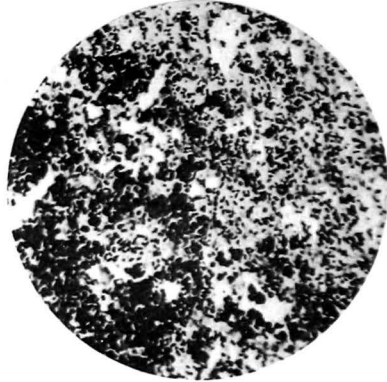


FIG. B.—Vaccine B.

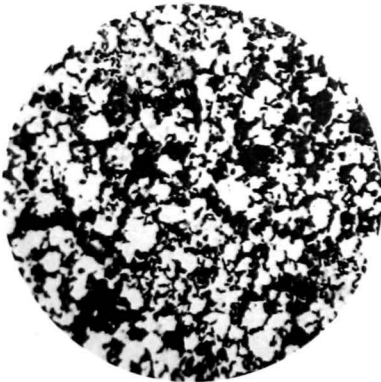


FIG. C.—Vaccine C.

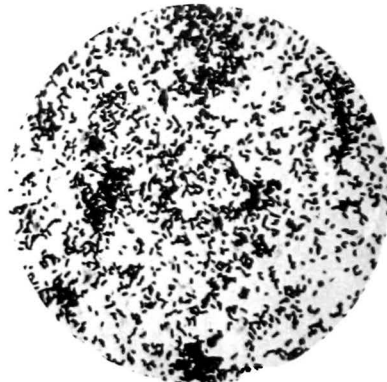
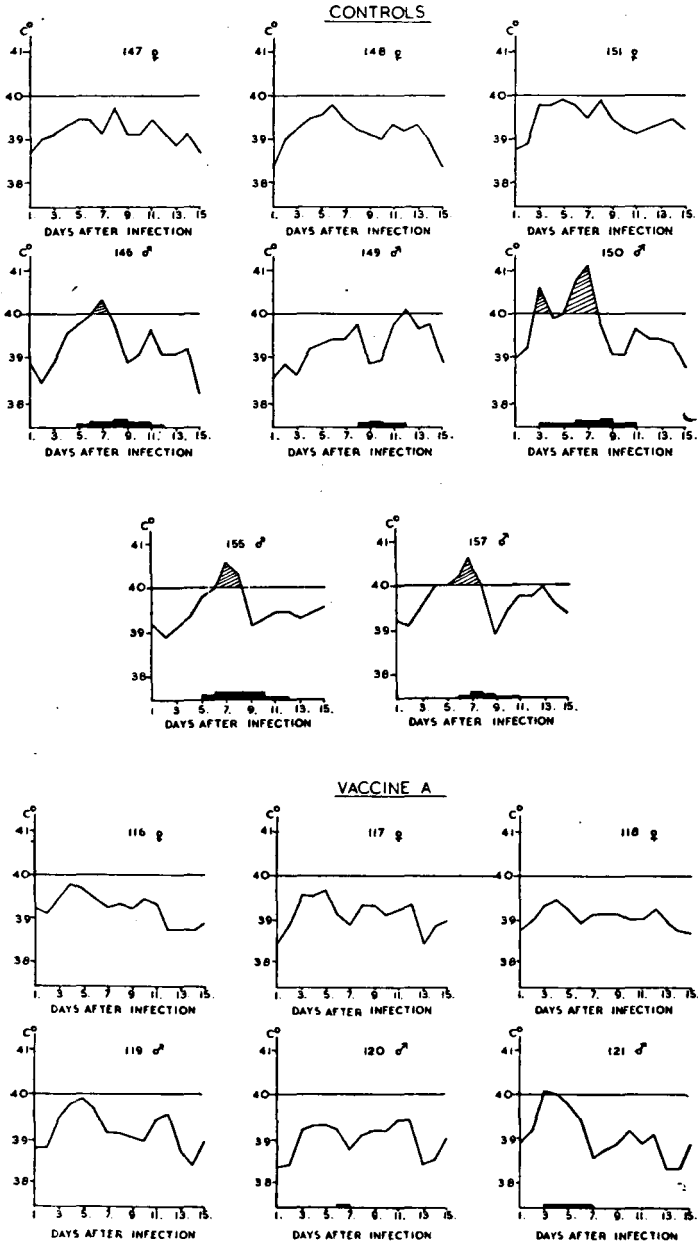


FIG. D.—Vaccine D.

Representative fields of suspensions of vaccines A, B, C and D.

# COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 151

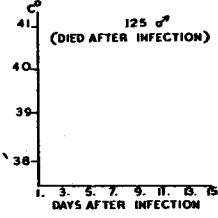
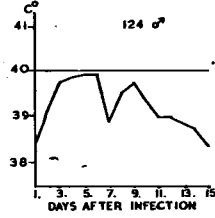
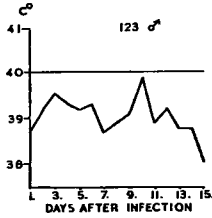
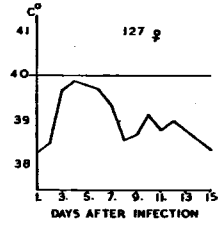
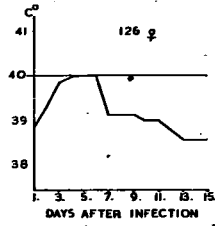
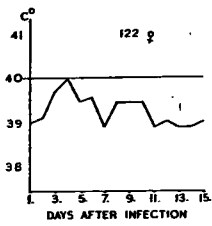
Figs. 31-62.—Temperature response of control and immunised guinea-pigs (Vaccines A, B, C and D), after challenging dose of viable rickettsiae. (Scrotal reactions are indicated as dense black areas at the foot of certain of the charts.)



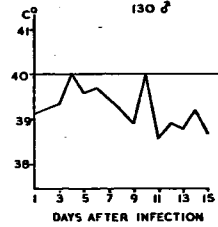
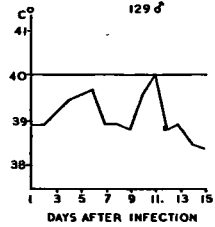
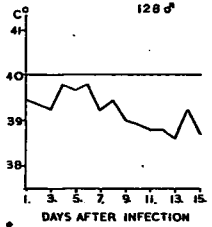
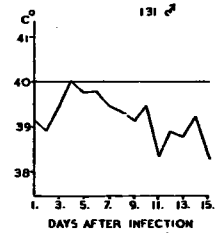
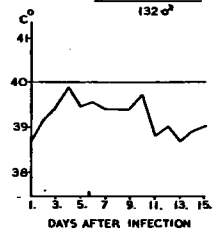
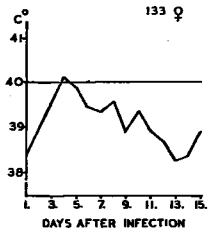


# 152. CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

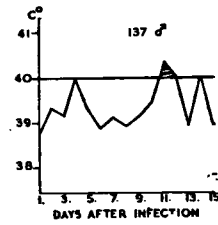
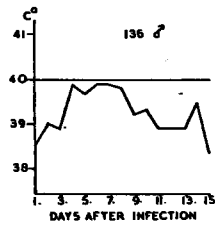
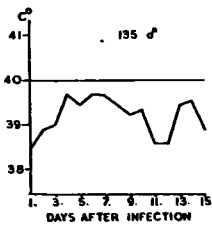
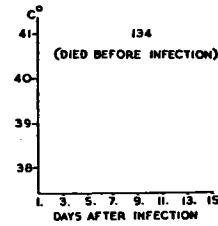
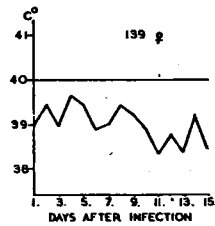
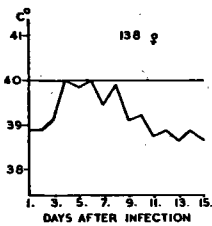
## VACCINE B



## VACCINE C



## VACCINE D



COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 153

With the use of small groups of animals, the degree of certainty that the immunised group has, in fact, been protected when the immunised group shows a proportion of survivors, will depend upon the degree of certainty that the dose of rickettsiae will kill all of an infinitely large group of normal mice. We have, therefore, defined a certainly fatal dose of such magnitude that, should a mouse in the immunised group survive, there is a reasonable degree of certainty that it has been significantly protected. In the test which has been employed and is described in detail below, the potency of a vaccine is determined by the power of a single dose of the vaccine to protect against a certainly fatal dose of toxin. As a source of rickettsial toxin we have employed (a) a suspension of selected yolk sacs in 10 per cent. horse serum broth (5 ml. per yolk sac) stored at  $-72^{\circ}$  C., or (b) in the case of the murine strain, a suspension of consolidated mouse lungs. A crude lung suspension is centrifuged and the deposit is re-suspended in 10 per cent. horse serum broth (1 ml. per lung), and stored at  $-72^{\circ}$  C. The specific toxicity is far above the toxicity of the normal lung tissue, and such a suspension behaves in every way like a yolk sac suspension, with the added advantage that it is much easier to prepare. With the epidemic strains, however, the specific toxicity is too close to the toxicity of the lung tissue to allow such suspensions to be used. Thus, for epidemic strains toxic yolk sacs are alone employed.

*Route of Injection of the Provocative Dose of Toxin*

The determination of the certainly fatal dose necessitates the drawing of a characteristic curve. Using a toxic yolk sac suspension (Wilmington strain), 0.25 ml. amounts of various dilutions in serum broth were injected intraperitoneally into groups of 30 mice. Fig. 63 shows probits plotted against log. doses (see Bliss, 1935, 1938). The toxicity of this particular batch of yolk sac suspension was low, but it is clear from the slope of the curve that the individual variation in the susceptibility of the mice to the toxin is very great.

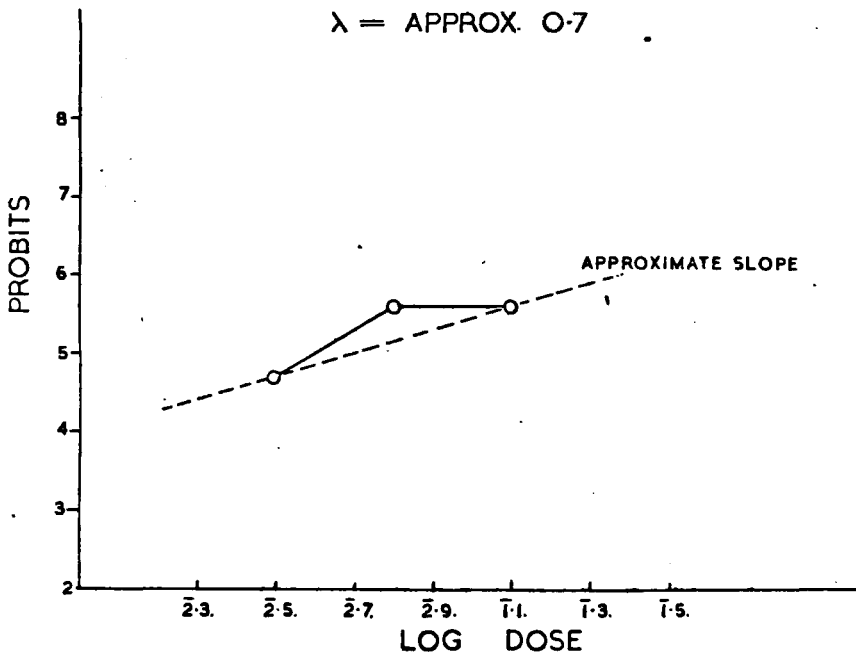


FIG. 63.—Toxicity of rickettsial suspension (murine) intraperitoneally. 30 mice per group.

## 154 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

The standard deviation of the logarithm of the individual effective dose ( $\lambda$ ) is approximately 0.7. The same "toxic" yolk sac, when injected intravenously (Bengtson, Topping & Henderson, 1945), has given more satisfactory results. By this route the material was about 10 times as toxic. Fig. 64 shows probits plotted against log. doses. In this case  $\lambda=0.15$ . We have therefore preferred to inject the provocative dose of toxin by the intravenous route.

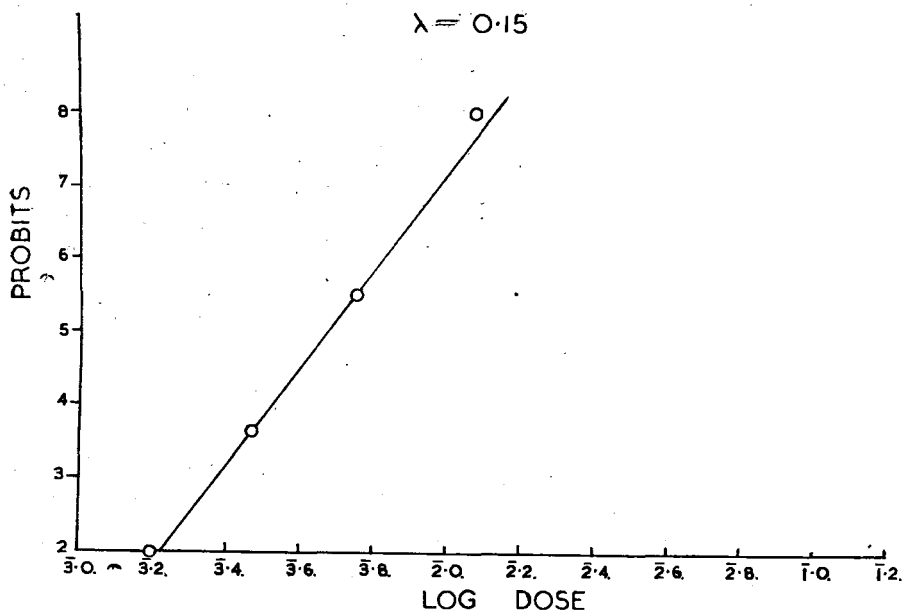


Fig. 64.—Toxicity of rickettsial suspension (murine) intravenously. 10 mice per group.

### *Determination of the Certainly Fatal Dose*

The most accurate method of defining a certainly fatal dose would be to plot a graphic provisional dosage mortality curve, and then, by giving each determination a weight proportional to its reliability, to compute the position and slope of the transformed dosage mortality curve. The certainly fatal dose could then be calculated for any degree of certainty from the variance of the expected probit at log. LD 99. Such a method would, however, be costly in mice and would hardly be practicable when consideration is taken of the small size of the batch of toxin that can reasonably be stored.

A simple graphical estimate of the certainly fatal dose is suggested as being sufficiently accurate for the purpose. We have attempted to allow for the fact that, owing to the small number of mice used for the titration, the estimate of the LD 50 is only approximate. A group of 10 mice is injected intravenously with a dose of toxin calculated by a previous small scale trial to give about 75 per cent. of deaths, and another group is injected with a dose to give about 25 per cent. of deaths. Probit values are plotted against log. doses and a straight line is drawn through these two points (see Fig. 65). On the same graph a point is plotted on the abscissa for LD 50 with a probit value of 3.6. A line is drawn through this point parallel to the dosage mortality curve. (The probit 3.6 corresponds to a mortality of 8 per cent., which is the negative limit which the LD 50 dose may be expected to give in 369 trials out of 370 when injected into 10 mice.) Where this parallel line cuts the probit for 7.5 (99.4 per cent. mortality) is read the corresponding log. dose. This dose is the certainly fatal dose to be employed and is about eight times the standard deviation of LD 50.

## COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 155

*Example:—*

Toxic suspension : Suspension of selected Breinl (epidemic) yolk sacs in 10 per cent. horse serum broth (Breinl TY-3), 5 ml. per yolk sac.

Titration : 0.25 ml. i.v. of the following dilutions made in 10 per cent. horse serum broth—1/40, 1/60, 1/80.

Injected into groups of 10 Swiss mice. The mice are not fed on the morning of the experiment.

Environmental temperature: Not controlled in summer. In the winter, temperature maintained at about 20° C.

Test is read after 18 hours. The result is recorded in Table XLIV.

TABLE XLIV

Dilution	1/40	1/60	1/80
Log dose .. ..	3.8	3.6	3.5
Mortality .. ..	10	5	2
Probit .. ..		5.0	4.1

From the graph (Fig. 65) the certainly fatal dose=0.25 ml. of 1/22 dilution.

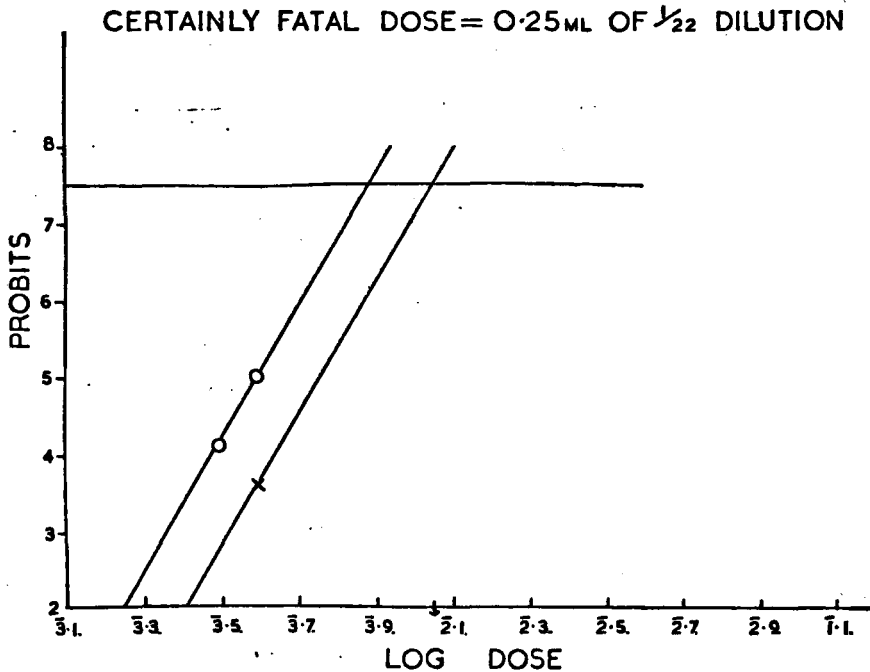


FIG. 65.—Estimation of the certainly fatal dose of epidemic rickettsiae (Breinl strain).

### *The Comparison of Vaccines by their Power to Protect against a Certainly Fatal Dose of Toxin*

Groups of 10 mice are immunised in such a way that, with the certainly fatal dose of toxin employed, at least one of the mice will die, but at least two will survive. In the comparison of vaccines no account is taken of the actual

156 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

number (2-9) which have survived. Thus, a vaccine protecting two out of 10 will be judged equal to a vaccine protecting nine out of 10. Such differences in the numbers of mice surviving are insignificant when small groups of animals (10) are used. This has been demonstrated in our experience on several occasions in which the same vaccines have been compared. If, in any test, all of the vaccinated mice survive, the test is repeated, using one dose of progressive ten-fold dilutions of the vaccine. If, in any test, none of the vaccinated mice survives, the test is repeated giving two or more doses of undiluted vaccine at weekly intervals, testing the mice on the 21st day, or one week after the last dose, if more than three doses have been used.

Owing to the relatively crude determination of the certainly fatal dose, it is necessary to include in each series a vaccine of known protective power, as otherwise the results of the different experiments will not be directly comparable.

For each of the vaccines, A, B, C and D, a group of 10 mice was immunised. Each mouse received a single dose (0.25 ml.) of the undiluted vaccine intraperitoneally. Twenty-one days later they received a certainly fatal dose of murine "toxin" (mouse lung) intravenously. Each of a control group of mice which was set aside at the commencement of the experiment received the same test dose. The result is recorded in Table XLV.

TABLE XLV

Number dying in—	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	Mice surviving at 18th hour
Control .. .. .	1	3	3	3	—	—	0
Vaccine A .. .. .	0	0	0	0	0	1	9
Vaccine B .. .. .	0	0	0	1	0	0	9
Vaccine C .. .. .	0	0	0	0	0	0	10
Vaccine D .. .. .	0	0	0	0	0	1	9

It was concluded that vaccines A, B and D were identical, but that a further test would be necessary to decide whether C was significantly more potent than the rest. A repetition of the test on two of the vaccines (C and D) gave the following results :—

	Mice surviving at 18th hour
Control .. .. .	0
Vaccine C .. .. .	3
Vaccine D .. .. .	4

It was concluded that all four vaccines were of equal immunising potency.

THE PRODUCTION OF ANTIBODIES IN VACCINATED RABBITS

Four groups of five rabbits were used. One group received vaccine A, a second vaccine B, a third vaccine C and a fourth vaccine D. The vaccine was administered intravenously in three doses of 1.0 ml. at four-day intervals.

Each rabbit was bled the day before the first dose of vaccine. The five sera of each group were pooled in aliquot amounts. This was bleeding I. A second bleeding (II) was taken two weeks after the last dose of vaccine. A third bleeding (III) was taken four weeks after the last dose of vaccine. The serum of each pool was distributed into ampoules and dried *in vacuo* in the frozen state. The dried sera were reconstituted by addition of distilled water immediately before use.

COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 157

*Rickettsial Agglutinins (M<sup>2</sup>)*

The technique used is fully described elsewhere in this report (Fulton & Begg, see p. 164). The rickettsial titres obtained with the sera of the vaccinated rabbits are recorded in Table XLVI.

TABLE XLVI

Vaccine group	Bleed	Agglutinating suspension	Final serum dilutions								Titre
			20	40	80	160	320	640	1,280	2,560	
A	I	Murine ..	0	0	0	0	-	-	-	-	< 20
	II		*T	T	T	T	T	S+	S	0	1,280
	III		T	T	T	T	S+	S-	0	0	320
B	I	Murine ..	0	0	0	0	-	-	-	-	< 20
	II		T	T	T	T	S+	S	0	0	640
	III		T	T	T	T	tr	0	0	0	160
C	I	Murine ..	0	0	0	-	-	-	-	-	< 20
	II		T	T	T	T	T	S+	tr	0	640
	III		T	T	T	T-	tr	0	0	0	160
D	I	Murine ..	0	0	0	0	-	-	-	-	< 20
	II		T	T	T	T	T	S+	tr	0	640
	III		T	T	T	T-	0	0	0	0	160

\* Dreyer's notation.

It is apparent that there was no significant difference between any of the vaccine groups.

*Agglutinins for Proteus OX19*

The technique employed in these titrations is described by Fulton & Begg (p. 185). The agglutinating titres obtained are recorded in Table XLVII.

TABLE XLVII

Vaccine group	Bleed	Agglutinating suspension	Final serum dilutions				Titre
			20	40	80	160	
A .. ..	I	OX19	0	0	0	0	<20
	II		T	S+	0	0	40
	III		tr	0	0	0	<20
B .. ..	I	OX19	0	0	0	0	<20
	II		T	S+	0	0	40
	III		tr	0	0	0	<20
C .. ..	I	OX19	0	0	0	0	<20
	II		0	0	0	0	<20
	III		0	0	0	0	<20
D .. ..	I	OX19	0	0	0	0	<20
	II		T	tr	0	0	20
	III		tr	0	0	0	<20

The OX19 agglutinins were present only in small amounts. No importance can be attached to the fact that the rabbits of vaccine group C failed to show any rise in *Proteus OX19* antibodies, in view of the low titres of OX19 elicited by A, B and D.

158 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

*Complement-fixing Antibodies (M<sup>a</sup>)*

This technique, also, is described by Fulton & Begg (p. 172). The titres obtained with the vaccinated rabbit sera are recorded in Table XLVIII.

TABLE XLVIII

Vaccine group	Bleed	Antigen	Serum dilutions						Titre
			20	40	80	160	320	640	
A	I	Murine ..	0	0	0	0	0	0	<20
	II		*4	4	4	4	1	0	160
	III		4	4	3	0	0	0	80
B	I	Murine ..	0	0	0	0	0	0	<20
	II		4	4	4	1	0	0	80
	III		4	4	1	0	0	0	40
C	I	Murine ..	0	0	0	0	0	0	<20
	II		4	4	4	3	0	0	160
	III		4	4	0	0	0	0	40
D	I	Murine ..	0	0	0	0	0	0	<20
	II		4	4	4	3	0	0	160
	III		4	4	0	0	0	0	40

\* 4 = complete fixation.

It is apparent that there was no significant difference between any of the vaccine groups.

*" Antitoxins "*

The method employed was based on that described by Henderson & Topping (1945). The source of toxin suspension used, and the method of calculating a certainly fatal dose, have been described. Twice the certainly fatal dose in 0.25 ml. 10 per cent. horse serum broth in 1.0 ml. amounts are mixed with 1.0 ml. amounts of progressive five-fold dilutions in saline of the serum to be tested (first dilution 1/5). The mixtures are kept at 37° C. for half an hour, and then at room temperature for from half an hour to two and a half hours according to the length of the particular experiment; 0.25 ml. amounts of the mixtures are injected intravenously into groups of four mice, which must be of the same breed and weight as those used in the estimation of the certainly fatal dose. A standard serum is included as a control. The number of deaths in 18 hours in each group is recorded. The results obtained with the sera from the rabbits immunised with the four vaccines under test are recorded in Table XLIX.

TABLE XLIX

Rabbit pool	Bleed	Serum dilutions			
		5	25	125	625
A .. ..	III	0	0	0	3*
B ... ..	III	0	0	0	4
C ... ..	III	0	0	0	3
D .. ..	III	0	0	1	4

Toxic suspension : Murine yolk sac suspension TY-3

One certainly fatal dose = 0.25 ml. of 1/30 dilution.

Four mice per group. \* The numbers refer to the number of mice dead at the 18th hour.

The results show no significant difference between the four vaccines.

*Neutralising Antibodies*

The technique of this test has been fully described by van den Ende & Mills (see p. 134).

A previously titrated mouse lung suspension of rickettsiae is diluted in serum broth, so that, when 0.05 ml. of this dilution is instilled intranasally into a mouse, approximately 100 focal lesions will be produced. One ml. amounts of this virus dilution are mixed with an equal volume of progressive four-fold dilutions in saline of the serum to be tested, commencing with undiluted serum. One ml. of the virus dilution is also mixed with 1 ml. of serum broth and this serves as the virus control. In addition, a standard serum is always included. The mixtures are left at 37° C. for one hour, and then cooled rapidly by placing the tubes containing the mixtures in crushed ice; 0.05 ml. of the mixtures are instilled intranasally into groups of four mice, which are killed after seven days. The focal lesions on the surface of the lungs are counted. The titre of the serum is taken as the highest serum dilution which will cause an 80 per cent. decrease in the countable focal lesions, in comparison with the control. Results obtained with the sera from the vaccinated rabbits are recorded in Table L and Fig. 66.

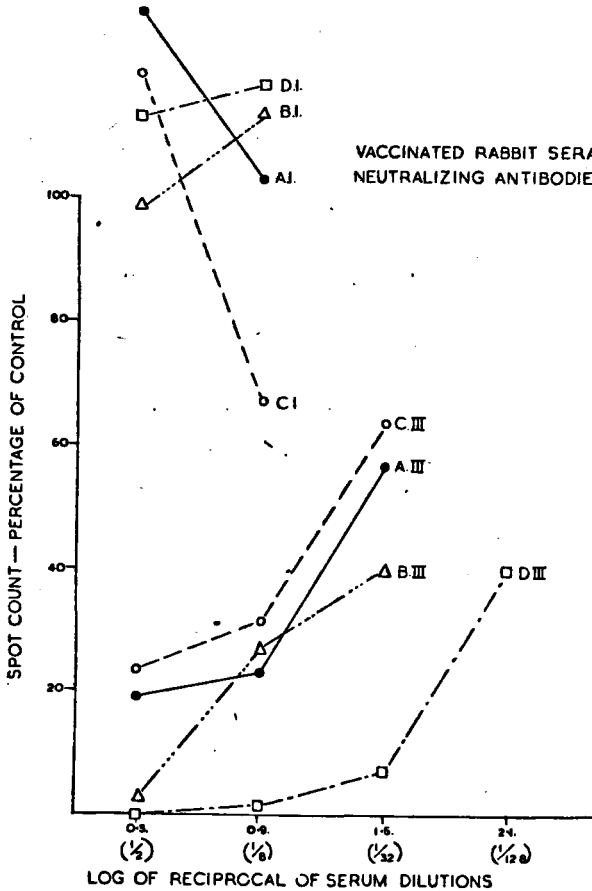


Fig. 66.—Estimation of neutralising antibodies in pools of immunised rabbits' sera by the focal lesion count method.



TABLE L

*Rickettsial suspension : Murine mouse lung suspension*

Rabbit pool	Bleed		Serum dilutions				Average control count
			1/2	1/8	1/32	1/128	
B	I	Average count ..	44.0	51.0	—	—	45
		Per cent. of control	98.0	113.0	—	—	
	III	Average count ..	1.25	12.0	18.0	—	—
		Per cent. of control	2.8	26.6	40.0	—	
D	I	Average count ..	51.0	53.0	—	—	—
		Per cent. of control	113.0	118.0	—	—	
	III	Average count ..	0	0.8	3.5	—	—
		Per cent. of control	0	1.8	7.8	—	
A	I	Average count ..	56.0	45.0	—	—	43
		Per cent. of control	130.0	103.0	—	—	
	III	Average count ..	8.0	10.0	25.0	—	—
		Per cent. of control	18.0	23.0	57.0	—	
C	I	Average count ..	52.0	29.0	—	—	—
		Per cent. of control	120.0	67.0	—	—	
	III	Average count ..	10.0	18.0	28.0	—	—
		Per cent. of control	23.0	41.0	64.0	—	
D (Repeat)	III	Average count ..	0	0.5	3.0	17.0	—
		Per cent. of control	0	1.1	7.0	40.0	

From Fig. 66, where the spot count is plotted as a percentage of the control count against the log. of the serum dilution, the neutralising titres of the sera may be calculated. The results so calculated are recorded in Table LI.

TABLE LI

*Neutralising antibodies*

Rabbit serum pool	Titre
A	3.2
B	5.0
C	1.4
D	56.0

From this table it would appear that whereas pools A, B and C are all very similar, the pool D possesses a considerably higher titre of neutralising antibodies. Whether this difference is of sufficient magnitude to have any significance is doubtful.

The guinea-pigs used in the active immunisation experiment were bled by cardiac puncture before injection of the challenge dose of rickettsiae. The sera of the guinea-pigs from each group were pooled in aliquot portions. The pooled sera were tested for their content of rickettsial agglutinins, complement-fixing antibodies, "antitoxic" and neutralising antibodies. The titres in all cases were low and no differences were encountered between the four vaccines.

COMPARISON OF TYPHUS VACCINES IN THE LABORATORY 161

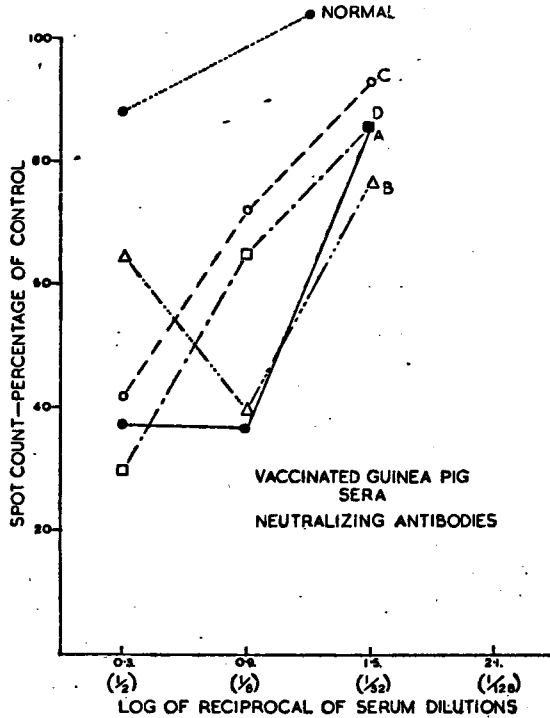


Fig. 57.—Estimation of neutralising antibodies in pools of immunized guinea-pigs' sera by the focal lesion count method.

The details of the neutralisation tests are recorded in Fig. 67. The figure shows that neutralising titres of pooled sera from the four groups of guinea-pigs were low, and that no difference was demonstrable between the four vaccines. This result suggests that the higher neutralising titre obtained with serum from rabbits immunised with vaccine D was probably due to chance.

DISCUSSION

No significant difference in immunising power could be detected between the four vaccines studied. It would appear, therefore, that vaccines are of equal potency providing they contain equal numbers of rickettsiae, as was the case in the four experimental vaccines studied. It seems, moreover, that the so-called soluble antigen present in vaccine A and absent from vaccine D is not of great immunising value. This is not surprising, in view of the fact that the "soluble antigen" appears to be a portion of the rickettsial surface antigen displaced by the ether treatment (Fulton & Begg, see p. 179). It is, therefore, possible to prepare from mouse lungs a vaccine at least equal in purity and potency to that prepared from yolk sac by Craigie's ether method. The yield of vaccine from one yolk sac (vaccine A) is, however, about nine times the yield from one mouse lung (vaccine D). In the case of epidemic typhus strains, the yield from mouse lungs is smaller than that obtained with murine typhus, but the relative yield from yolk sac and mouse lung is still probably the same. These experiments have also afforded the opportunity of comparing the

## 162 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

known potency tests for typhus vaccines. The test of a vaccine's ability to protect mice against the toxic effect of rickettsiae injected intravenously has given the most satisfactory and reproducible results, and is the method of choice. Tests for the power of protecting guinea-pigs against infection are subject to many disadvantages. Not the least of these disadvantages is the irregularity of the temperature response which, in our experience, is frequently encountered, especially with murine typhus. The ability of the vaccines to stimulate formation of agglutinins, complement-fixing antibodies and neutralising antibodies in rabbits and guinea-pigs also does not afford a suitable potency test. The individual differences in production of antibodies are so large that the method would be valueless except for the recognition of vaccines completely devoid of immunising power. The pulmonary titration method for measurement of neutralising antibodies is subject to the same disadvantages, especially when many potency tests are done and, therefore, the use of large groups of mice becomes impracticable.

### SUMMARY

The methods of preparation of four murine typhus vaccines of approximately equal rickettsial content are described. Two of the vaccines were highly purified, one from yolk sac by Craigie's ether method, the other from mouse lung by the kieselguhr method.

The potencies of the vaccines were compared by a variety of methods: (i) active immunisation of guinea-pigs; (ii) protection of mice against the toxic effect of a certainly fatal dose of rickettsiae injected intravenously; (iii) production of antibodies in rabbits and guinea-pigs. The antibodies studied were: rickettsial agglutinins, agglutinins for *Proteus OX19*, complement-fixing antibodies, "antitoxins," and neutralising antibodies.

Details of methods for the standardisation of the numbers of rickettsiae present in vaccines, and for the determination of a certainly fatal dose of "toxic" rickettsial suspension for mice, are described.

It is concluded that vaccine potency depends on the number of rickettsiae present and is independent of the "soluble antigen."

The power of a vaccine to protect mice against the "toxicity" of rickettsiae injected intravenously affords the most convenient and reliable test for vaccine potency.

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# CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

## V.—THE ANTIGENIC STRUCTURE OF TYPHUS RICKETTSIAE

BY

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### INTRODUCTION

Zinsser & Castaneda (1932) have demonstrated the serological individuality of the Mexican murine and the European louse-borne varieties of typhus fever. The more elaborate analysis of antigenic structure was at that time much hampered by the difficulty of obtaining suspensions of the louse-borne strains. Since then, the problem has been greatly simplified by the development of methods of cultivating these rickettsiae in ways other than in the gut of the louse. We have compared the antigenic structure of a murine strain of typhus rickettsiae with that of a strain of epidemic, louse-borne rickettsiae, using the techniques of agglutination and complement-fixation, and employing purified rickettsial suspensions prepared from the lungs of rats and of mice.

The murine strain we have used is the Wilmington; the epidemic strain was isolated in Egypt during 1942; it has been named the Cairo strain, and it has proved, so far as we can judge, to be identical with the classical Breinl strain of epidemic typhus rickettsiae.

We propose to adopt the unorthodox procedure of stating our working hypothesis before the presentation of the experimental data. This will enable us to define our symbols and so make possible a clearer exposition of the rather complex serological findings. The hypothesis is based upon two propositions:—

(1) That antibodies formed in response to one antigen, although adjusted to a certain structure, are not entirely uniform, but vary in specificity to some degree. Landsteiner and van der Scheer (1936) prepared immune sera to azoproteins and absorbed these sera with heterologous azoantigens. They concluded that the sera did not contain a single antibody, but antibody fractions somewhat different in their reactivity for heterologous antigens. From the constitution of the azo component, it followed that these fractions could not be specific for distinct chemical groups in the molecule.

(2) That the heating of some antigens to 100° C. creates a new antigenic specificity. Furth (1925) demonstrated that crystalline egg albumin, when heated at 100° C. for five minutes, behaved like a serologically new protein. Other methods of degrading an antigen can change its serological specificity, such as treatment with alkali (De Falco, Kazal & Arno, 1943). The assumption is made in our hypothesis that a labile antigen may be degraded *in vivo* and give rise to antibodies not only to its native form, but also to those degradation products which are antigenic.

Let the surface antigenic structure of the murine strain be represented by the letter "m" and let a degraded form of this surface antigen be represented by "m<sup>100</sup>." Similarly, let the surface antigen of the epidemic strain be represented by "c," and its degraded form by "c<sup>100</sup>."

Let us suppose that rickettsial immune sera have two main types of antibody. The one type "M" (or "C") reacts with m (or c) and the other type "M<sup>100</sup>" (or "C<sup>100</sup>") reacts with m<sup>100</sup> (or c<sup>100</sup>).

## CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Let us further suppose that M (or C) antibodies are of two grades,  $M^1$  and  $M^2$  ( $C^1$ ,  $C^2$ ).  $M^1$  ( $C^1$ ) antibodies are strictly specific, whereas  $M^2$  ( $C^2$ ) antibodies are less specific.

Let the rickettsial soluble antigens be represented by mSA (cSA) and their degraded forms by  $mSA^{100}$  ( $cSA^{100}$ ).

In the interpretation which follows the individual molecules making up the group of rickettsial antibodies are regarded as forming a continuous series. At one extreme are antibodies of maximum specificity which will react only with their exact homologue. At the other extreme are antibodies which will react with a wider range of structural types. This is shown diagrammatically in Fig. 68.

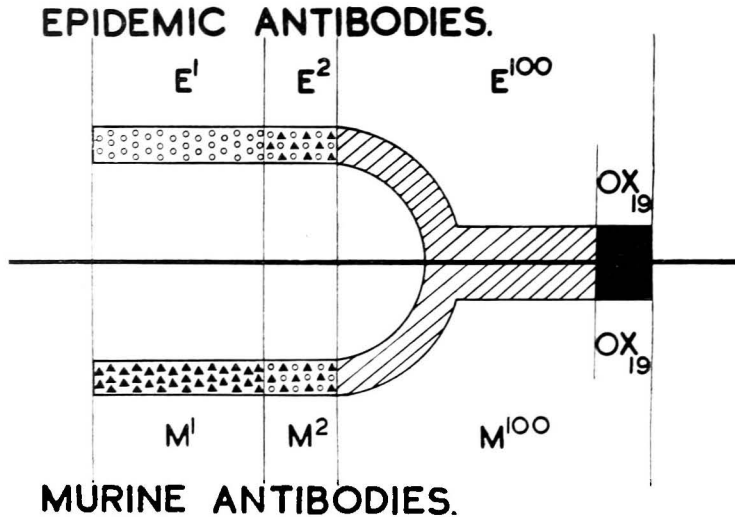


FIG. 68

### AGGLUTINATION REACTIONS

#### METHODS

##### *Agglutinating suspensions*

Following the work of Castaneda (1939) and of Hudson (1940), we have prepared suspensions of rickettsiae from the lungs of infected rats and mice. These suspensions have been purified in a new way which consists in the removal of most of the tissue debris by precipitation with kieselguhr. This technique of purification probably degrades the surface antigen to only a very slight extent as the rickettsiae are not killed by the process. Slight changes in the details of the purification have been made during the course of the work, but as these changes have only been in such minor matters as the amount of kieselguhr employed and the molarity of the suspending saline solution, we shall only give the latest method in detail.

##### *Murine suspensions*

Four consolidated rat lungs are ground in a mortar with glass powder and suspended in 60 ml. of the following mixture:—

48.0 ml. of 10 per cent. horse serum broth,

12.0 ml. of 3.8 per cent. sodium citrate.

The suspension is centrifuged at 1,000 r.p.m. for three minutes, to deposit lung debris, and the supernatant ( $S^1$ ) is pipetted off.  $S^1$  is centrifuged for

three-quarters of an hour at 5,000 r.p.m. in an angle centrifuge. The supernatant (S<sup>2</sup>) is discarded and the deposit is re-suspended in 50 ml. of physiological saline. This suspension is now centrifuged at 1,000 r.p.m. for three minutes to deposit more lung debris. The supernatant (S<sup>3</sup>) is further purified in the following way:—

Fifty ml. of distilled water are added to S<sup>3</sup> to make 100 ml. To this 5 g. of kieselguhr are added and mixed to an even suspension. [Note: Celite 244 (Johns-Manville Co.) is suitable and requires no further treatment. If this is not obtainable, the starting material is that described by manufacturers as "kieselguhr washed with acids." The heavy fraction of this is selected by suspending the kieselguhr in distilled water and centrifuging for two minutes at 500 r.p.m. The supernatant is discarded and the deposit is dried (not heated above 80° C.) and ground to a powder.]

The suspension is left to stand for 30 minutes to allow the kieselguhr to flocculate to the bottom of the tube. The tissue particles in some way combine with the kieselguhr so that the supernatant is a suspension of practically pure rickettsiae (Plate XI). In order to remove all but the last traces of kieselguhr from the supernatant, the suspension is centrifuged at 1,000 r.p.m. for 10 minutes.

The purified rickettsial suspension is now centrifuged for 30 minutes at 5,000 r.p.m. in an angle centrifuge, and the deposit from 100 ml. is re-suspended in 5.0 ml. of the following solution:—

- 4.8 ml. of physiological saline.
- 0.2 ml. of M/2 phosphate buffer pH 7.0.
- 0.05 ml. of 1 per cent. "Merthiolate".

These concentrated suspensions are stored at +4° C. and diluted as required in physiological saline. For the agglutination tests, the suspensions are diluted to match a *coli* suspension of 500 × 10<sup>6</sup>/ml.

Murine suspensions prepared from mouse lung have behaved no differently from those prepared from rat lung.

#### *Epidemic suspensions*

These have been prepared in exactly the same way, except that they have been made from mouse lung. We have not yet succeeded in adapting rickettsiae of the epidemic type to the lungs of rats. Fifty mouse lungs are equivalent to about four rat lungs.

The agglutination is conducted at 37° C. in a water bath. Ordinarily the incubation period is 18 hours, and the test is read immediately after removal of the tubes from the water bath. Round-bottomed tubes are employed, and the "standard" reading is a degree of agglutination just visible by the naked eye under suitable conditions of illumination. The agglutination is very easy to read, being as clear-cut as the *OX19* agglutination reaction. The titre of a serum is expressed as the highest serum dilution (final) giving "standard" agglutination or better.

#### *Absorbing Suspensions*

These are prepared from the concentrated suspensions described above, by diluting them in physiological saline to match a *coli* suspension of 1,000 × 10<sup>6</sup>/ml. The sera are diluted directly in the absorbing suspension, usually to one-eighth to one-sixteenth of their titre. The absorption is for one hour at 37° C., after which the rickettsiae are removed by high speed centrifugation. On some occasions the absorbed sera were also filtered through a 500-m $\mu$  membrane to make sure that all the rickettsiae had been removed. In a few instances, noted in the text, we have used suspensions of the Tunis or the Teheran strains of epidemic rickettsiae. These strains are apparently identical with the Cairo strain.

Sera

The guinea-pig sera are pooled from a number of convalescent animals infected with a single intra-peritoneal dose of yolk-sac material. The guinea-pigs were bled about 10-14 days after the peak of the fever. In a few instances, noted in the text, we have used sera from guinea-pigs infected with the Tunis strain of epidemic rickettsiae. During storage at +4° C. for a period of 6-12 months, the titres of the guinea-pig sera have shown a progressive fall.

The rabbit sera are from bleedings taken 12-16 days after infection with a single intraperitoneal dose of yolk-sac material (Y.S.), or a single intraperitoneal dose of mouse-lung material (M.L.).

All sera are stored at +4° C. without any preservative.

RESULTS

Simple Agglutination Reactions

The results of cross agglutinations show that there is no clear-cut serological difference between the murine and epidemic strains (Table LII).

TABLE LII

20.12.43			Serum dilutions									Read at 18 hours
	Serum	Suspension	20	40	80	160	320	640	1,280	2,560	5,120	Interpretation
Guinea-pig sera ..	Murine .. (146/157) ..	Murine .. Cairo ..	*T T-	T tr	T 0	S+ 0	0 0	0 0				m/M <sup>1</sup> Titre 160 c/M <sup>2</sup> Titre 20
	Cairo .. (F) ..	Murine .. Cairo ..	T T	S T	tr T	0 S+	0 tr	0 0				m/C <sup>2</sup> Titre 40 c/C <sup>1</sup> Titre, 160
Rabbit sera ..	Murine .. (127 iv ML)†	Murine .. Cairo ..	T T-	T T-	T T-	S+ S	T S	T- tr	S+ 0	S 0	0 0	m/M <sup>1</sup> Titre 2560 c/M <sup>2</sup> Titre 320
	Cairo .. (30 iv YS) ..	Murine .. Cairo ..	T T	T T	T T	T- T	S T	tr S+	0 S+	0 S	0 tr	m/C <sup>2</sup> Titre 320 c/C <sup>1</sup> Titre 2560

\* Dreyer's notation. 127 iv ML = Rabbit 127, 4th bleeding; mouse-lung antigen.  
† Sera from rabbits infected with YS material behave in the same way.

The reasons for the interpretation given will become apparent from the absorption results. The m/M<sup>1</sup> (c/C<sup>1</sup>) reaction develops much faster than the m/C<sup>2</sup> (c/M<sup>2</sup>) reaction. With guinea-pig sera more specific results may be obtained by reading the reaction after four hours' incubation, for by this time the specific reaction has fully developed while the heterologous reaction has hardly begun (Table LIII).

TABLE LIII

20.12.43			Serum dilutions									Read at 4 hrs.
	Serum	Suspension	20	40	80	160	320	640	1,280	2,560	5,120	Interpretation
Guinea-pig sera ..	Murine .. (146/157) ..	Murine .. Cairo	T- tr	T- 0	T- 0	S 0	0 0	0 0				m/M <sup>1</sup> Titre 160 c/M <sup>2</sup> Titre <20
	Cairo .. (F) ..	Murine .. Cairo ..	tr T-	0 T-	0 T-	0 S	0 0	0 0				m/C <sup>2</sup> Titre <20 c/C <sup>1</sup> Titre 160

With rabbit sera, reading at four hours does not improve the specificity of the test, for the ratio M<sup>1</sup>/M<sup>2</sup> is the same at four as at 18 hours. However, by observing the speed with which the reaction develops during the first two hours of incubation, it is often possible to decide whether the m/M<sup>1</sup> reaction or the c/M<sup>2</sup> reaction is being observed. This is shown in Table LIV.

PLATE XI

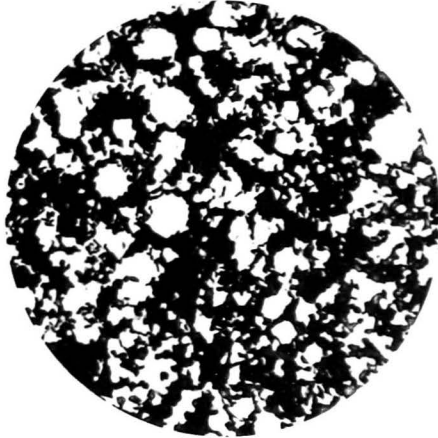


FIG. A



FIG. B

FILMS OF RICKETTSIAL SUSPENSIONS

(These films are prepared in the following manner: A short length of 9 mm. glass tubing is closed at one end by a rubber bung. A suitable dilution of the rickettsial suspension is prepared and 0.5 ml. placed in the tube, which is centrifuged at 10,000 r.p.m. for 10 mins. to deposit the sedimentable constituents of the suspension on to the cover glass. The cover glass is removed, dried and stained in 0.5 per cent. basic fuchsin.)

**Fig. A.** Mouse lung suspension after preliminary purification by centrifugation, but before removal of tissue particles by flocculation with kieselguhr.

**Fig. B.** Mouse lung suspension after kieselguhr purification.



TABLE LIV

20.12.43				Read at 2 hours									Read at 4 hours								
<i>Rabbit sera</i>	<i>Suspension</i>	<i>Reaction</i>		20	40	80	160	320	640	1,280	2,560	5,120	20	40	80	160	320	640	1,280	2,560	5,120
ne .. ..	Murine ..	m/M <sup>1</sup> ..	..	S+	S+	S+	S+	S	tr	0	0	0	T-	T-	T-	T-	S	S-	tr	0	0
iv ML) ..	Cairo ..	c/M <sup>2</sup> ..	..	tr	tr	tr	0	0	0	0	0	0	S-	S-	S-	tr	0	0	0	0	0
.. ..	Murine ..	m/C <sup>3</sup> ..	..	tr	tr	tr	0	0	0	0	0	0	S	S	S	tr	0	0	0	0	0
v YS) ..	Cairo ..	c/C <sup>1</sup> ..	..	tr	S	S+	S+	S	tr	0	0	0	S+	T-	T-	T-	S	S-	0	0	0

168 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

*The Absorption of M<sup>1</sup> and C<sup>1</sup>*

The agglutinins M<sup>1</sup> and C<sup>1</sup> are those responsible for the agglutination of the homologous suspension (Table LV).

TABLE LV

Date	Guinea-pig sera	Absorbing suspensions	Agglutinating suspensions	Serum dilutions				
				40	80	160	320	640
18.11.43	Murine .. .. (146/157) .. ..  Absorbed at 1/10	—	Murine ..	T	T	S	0	0
		Murine (m) ..	.. ..	S	0	0	0	0
		Murine boiled 10' (m <sup>100</sup> ) ..	.. ..	T	T-	0	0	0
		Cairo (c) ..	.. ..	T	T	tr	0	0
		Cairo boiled 10' (c <sup>100</sup> ) ..	.. ..	T	T	tr	0	0
21.11.43	Cairo .. .. (F) .. ..  Absorbed at 1/20	.. ..	Cairo ..	T	T	T	tr	0
		Murine .. ..	.. ..	T	T	S+	0	0
		Murine boiled 10'	.. ..	T	S+	tr	0	0
		Cairo .. ..	.. ..	tr	0	0	0	0
		Cairo boiled 10'	.. ..	T-	S+	tr	0	0

Interpretation : M<sup>1</sup> is absorbed by m, but only weakly by m<sup>100</sup>, c or c<sup>100</sup>. Similarly C<sup>1</sup> is strongly absorbed by c, but only weakly by c<sup>100</sup>, m or m<sup>100</sup>.

*The Absorption of M<sup>2</sup> and C<sup>2</sup>*

The agglutinins M<sup>2</sup>, C<sup>2</sup> are those responsible for the agglutination of heterologous suspensions (Tables LVI and LVII).

TABLE LVI

Date	Rabbit sera	Absorbing suspension	Agglutinating suspension	Serum dilutions				
				40	80	160	320	640
1.12.43	Murine .. .. (127 iv ML) .. .. Absorbed at 1/20	—	Cairo ..	T	T	T-	S-	0
		Cairo .. ..	.. ..	0	0	0	0	0
		Cairo boiled 10'	.. ..	T	T	T	S	0
29.11.43	Cairo .. .. (30 iv YS) .. .. Absorbed at 1/20	—	Murine ..	T	T	T	S	0
		Murine .. ..	.. ..	0	0	0	0	0
		Murine boiled 10'	.. ..	T	T	S+	tr	0

Interpretation : M<sup>2</sup> is absorbed by c, but not significantly by c<sup>100</sup>. C<sup>2</sup> is absorbed by m, but not significantly by m<sup>100</sup>. The M<sup>2</sup>, C<sup>2</sup> antibodies prove extraordinarily easy to absorb.

It is apparent that antisera prepared against the murine strain will agglutinate the Cairo strain, though to a lower titre than they agglutinate the homologous strain. Similarly, Cairo antisera will agglutinate the murine strain.

It is not possible to infer that the two rickettsial strains possess two antigenic determinant groups in common, only differing in their quantitative distribution.



170 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE LVIX

Guinea-pig sera				Serum dilutions					
	Absorbing suspension	Agglutinating suspension		80	160	320	640	1,280	2,560
Cairo .. .. .	—	Cairo .. .. .		T	T	T	S	0	0
Absorbed at 1/40	*Cairo .. .. .	.. .. .		0	0	0	0	0	0
	*Murine .. .. .	.. .. .		T	T	T—	S	0	0
(a) Cairo absorbed at 1/40	†Murine .. .. .	Cairo .. .. .		T	T	T—	0	0	0
(β)a reabsorbed ..	†Murine .. .. .	.. .. .		T	T	S	0	0	0
(γ)β reabsorbed ..	†Murine .. .. .	.. .. .		T	T	S	0	0	0

\* Opacity to match a coli suspension of 1,000 × 10<sup>6</sup>/ml.

† Opacity to match a coli suspension of 2,000 × 10<sup>6</sup>/ml.

2. The surface of the murine strain behaves as a single antigenic determinant group m, but this gives rise to a complex of antibodies. A fraction—M<sup>1</sup>—can only agglutinate the homologous organism, whereas a fraction —M<sup>2</sup>—is sufficiently close to the c structure to agglutinate the Cairo strain as well.

Similarly, the Cairo antigen c gives rise to some antibodies (C<sup>2</sup>) which can react with the murine antigen. In other words, the antigenic receptors which give rise to M<sup>1</sup> (C<sup>1</sup>) antibodies also give rise to M<sup>2</sup> (C<sup>2</sup>) antibodies.

The first hypothesis can only be inferred when m and a (c and a) have been separated. On the other hand there is abundant evidence that a single antigen gives rise to antibodies which are not entirely uniform, some of the antibody molecules being "more avid" than others. We have, therefore, preferred to accept the second as our working hypothesis.

The single antigen of our hypothesis is, of course, made up of many different chemical groups arranged in a specific pattern, the arrangement of these groups giving rise to the specific configuration of the antigen looked at as a whole.

We imagine the antibodies of the Cairo antisera as constituting a heterogeneous group. Some of the antibody molecules (C<sup>1</sup>) in this group are highly specific and will react only with Cairo suspensions (c). Other antibody molecules (C<sup>2</sup>) are less specific and, as well as reacting with Cairo suspensions (c), will also react with murine suspensions (m).

We think the reason why the C<sup>2</sup> antibodies are absorbed so much better by m than by c is that c has a large range of antibodies (C<sup>1</sup>, C<sup>2</sup>) with which it can react; the relative absorbing dose for the C<sup>2</sup> component is therefore smaller than when m is used for the absorption, since m can only react with the C<sup>2</sup> component.

Thus, we picture the surface antigen of the murine strain as being different from, but not wholly unlike, the surface antigen of the Cairo strain.

*The M<sup>100</sup>, C<sup>100</sup> Antibodies*

If the agglutinating suspensions are boiled for five minutes, they behave differently. They react better with the heterologous sera, being agglutinated to a higher titre, but with the homologous serum they may be agglutinated to a lower titre, to a higher titre, or the agglutination may remain apparently unaltered, according to the particular serum employed (Table LX). These boiled suspensions are stable in 2.5 per cent. saline and they are not agglutinated by normal rabbit and guinea-pig in the lowest dilutions we have used (1/20).

TABLE LX

Serum*	Date	Agglutinating suspensions	Serum dilutions									Read at 18 hours
			20	40	80	160	320	640	1,280	2,560	5,120	
Rabbit sera	28.11.43	Murine unheated ..	T	T	T	T	T	T	S	0	0	m/M <sup>1</sup> Titre 1,280 m <sup>100</sup> /M <sup>100</sup> Titre 2,560
		Murine boiled 5' ..	T	T	T	T	T	T	T	S	0	
	21.1.44	Cairo unheated ..	S+	T-	T-	S+	S	tr	0	0	0	c/M <sup>2</sup> Titre 320 c <sup>100</sup> /M <sup>100</sup> Titre 1,280
		Cairo boiled 5' ..	T	T	T	T	T-	T-	S	tr	0	
Cairo .. (30 iv YS)	2.12.43	Murine unheated ..	T	T	T	T-	S	0	0	0	m/C <sup>2</sup> Titre 320 m <sup>100</sup> /C <sup>100</sup> Titre 2,560	
		Murine boiled 5' ..	T	T	T	T	T	T-	T-	S+		tr
2.12.43	Cairo unheated ..	T	T	T	T	T	T-	S+	tr	0	c/C <sup>2</sup> Titre 1,280 c <sup>100</sup> /C <sup>100</sup> Titre 2,560	
	Cairo boiled 5' ..	S+	S+	S+	T-	T-	T-	T-	S	0		
Guinea-pig sera	2.12.43	Murine unheated ..	T	T	T	T-	0	0				m/M <sup>1</sup> Titre 160 m <sup>100</sup> /M <sup>100</sup> Titre 80
		Murine boiled 5' ..	T	T	S+	tr	0	0				
	2.12.43	Cairo unheated ..	T-	S	0	0	0	0				c/M <sup>2</sup> Titre 40 c <sup>100</sup> /M <sup>100</sup> Titre 40
		Cairo boiled 5' ..	T-	T-	tr	0	0	0				
Cairo .. (F)	20.12.43	Murine unheated ..	T	S	tr	0	0	0				m/C <sup>2</sup> Titre 40 m <sup>100</sup> /C <sup>100</sup> Titre 160
		Murine boiled 5' ..	T	T	T	S	tr	0				
Tunis ..	27.12.43	Tunis unheated ..	T	T	T	T-	S	0				t/T <sup>1</sup> Titre 320 t <sup>100</sup> /T <sup>100</sup> Titre 160
		Tunis boiled 5' ..	T	T	T	S+	0	0				

\* It is emphasised that these antisera were *not* prepared by the injection of heated rickettsiae. They are the ordinary immune sera of convalescent animals.

## 172 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### *The Absorption of M<sup>100</sup>, C<sup>100</sup> Antibodies*

Agglutinin absorptions show that the antibody with which the boiled suspension reacts is distinct from either M<sup>1</sup> or M<sup>2</sup> (C<sup>1</sup>, C<sup>2</sup>) (Table LXI).

TABLE LXI

Date	Guinea-pig sera	Absorbing suspension	Agglutinating suspension	Serum dilutions					
				20	40	80	160	320	640
23.11.43	Murine .. (146/157) .. Abs. at 1/10	—	Murine boiled	T	T	tr	0	0	
		Murine ..	5' .. ..	T	S	0	0	0	
		Murine boiled 10'	.. ..	0	0	0	0	0	
26.12.43	Cairo .. .. (F) .. .. Abs. at 1/10	—	Cairo boiled	T—	T—	T—	S	0	0
		Cairo .. ..	5' .. ..	T—	T—	T—	S	0	0
		Cairo boiled. 10'	.. ..	S+	0	0	0	0	0
21.11.43	Cairo .. .. (F) .. .. Abs. at 1/20	—	Murine boiled		T	S+	tr	0	0
		Murine ..	5' .. ..		T—	S	tr	0	0
		Murine boiled 10'	.. ..		0	0	0	0	0
		Cairo .. ..	.. ..		T—	S	tr	0	0
		Cairo boiled 10'	.. ..		0	0	0	0	0

Interpretation : M<sup>100</sup> is absorbed by m<sup>100</sup>, but not significantly by m.

C<sup>100</sup> is absorbed by m<sup>100</sup> and c<sup>100</sup>, but not significantly by m or c.

Likewise it can be shown that M<sup>100</sup> is absorbed by c<sup>100</sup> but not by c.

It would seem as if the antibody M<sup>100</sup> was identical with or very similar to C<sup>100</sup>. It would also seem that, by boiling, it is possible to degrade the specific antigen m to a form which is identical with, or very similar to, the specific antigen c degraded in a like manner. We shall give our reasons later for avoiding the more familiar explanation that boiling destroys a heat labile surface antigen and "uncovers" a heat stable antigen.

Thus, we imagine the surface antigen of the Cairo strain as giving rise to a very heterogeneous group of antibodies, not only of the C<sup>1</sup>, C<sup>2</sup> variety, but also antibodies (C<sup>100</sup>) formed in response to degradation products of c. These C<sup>100</sup> antibodies will react with similar degradation products of m.

### COMPLEMENT-FIXATION

#### METHODS ;

#### *Antigen*

Castaneda (1936) obtained a murine antigen by using the peritoneal washings of infected rats whose resistance had been lowered by X-ray treatment. It was not possible to grow epidemic strains in this way, so that the problem remained a difficult one until the development of simple ways of cultivating epidemic rickettsiae.

Bengtson (1941) and Bengtson & Topping (1941) have described a complement-fixation test using rickettsial antigens prepared from yolk sacs. We have employed as antigen purified rickettsial suspensions prepared from the lungs of infected rats and mice. The antigen is the agglutinating suspension

previously described, standardised to match a *coli* suspension of  $300 \times 10^6$ /ml. These antigens are best used fresh; if they are allowed to stand for 3-4 weeks at  $+4^\circ\text{C}$ ., they become degraded.

The sera are inactivated at  $56^\circ\text{C}$ . for 20 minutes. When rabbit sera are being titrated, it is necessary first to absorb out the haemolysin for sheep erythrocytes which is often present. The removal is effected in this way: the inactivated serum is diluted one in ten with saline and to each 1.0 ml. of the diluted serum are added 0.2 ml. of packed, washed sheep erythrocytes; the suspension is left in the ice box for 15 minutes, after which the cells are centrifuged down.

The test is set up as follows: 0.5 ml. of two-fold serial dilutions (a) of the serum, 0.5 ml. of guinea-pig complement diluted to contain 3 M.H.D., 0.5 ml. of antigen standardised by opacity. The primary incubation is for one hour at  $37^\circ\text{C}$ . in a water bath. Then 1.0 ml. of sensitised sheep cells is added. This sensitised suspension is made by mixing, just before use, a 3 per cent. suspension of washed sheep erythrocytes with an equal volume of haemolysin diluted so that 0.5 ml. contains 3 M.H.D.

The test is read after a further incubation at  $37^\circ\text{C}$ . for half-an-hour. The following controls have always been included:—

- (1) Anti-complementary control of antigen.—0.5 ml. of saline, 0.5 ml. of antigen dilution as used in the test, 0.5 ml. of complement (3 M.H.D.)—one hour primary incubation—1.0 ml. of sensitised sheep cells.
- (2) Anti-complementary control of serum.—0.5 ml. of saline, 0.5 ml. of the lowest serum dilution used in the test, 0.5 ml. of complement (3 M.H.D.)—one hour primary incubation—1.0 ml. of sensitised sheep cells.
- (3) Haemolytic control of antigen.—Prepared in the same way as (1) except that 1.0 ml. of unsensitised sheep cells is added after the primary incubation. The unsensitised suspension is made by diluting a 3 per cent. suspension of sheep cells with an equal volume of saline.
- (4) Haemolytic control of serum.—Prepared in the same way as (2) except that 1.0 ml. of unsensitised sheep cells is added after the primary incubation.

In all the tests described below (1) and (2) have been completely haemolysed, and (3) or (4) have shown no trace of haemolysis at the time of reading the test. The antigens do not fix complement in the presence of normal rabbit or guinea-pig sera in the dilutions employed.

In reading the test the degree of haemolysis is expressed by one of five symbols—4, 3, 2, 1, 0. Four signifies no haemolysis, and 0 signifies complete haemolysis. The titre of the serum is expressed as the highest serum dilution (a) showing complete (4) or nearly complete (3) fixation.

#### *Absorptions*

Absorptions have been carried out in exactly the same way as has been described for the absorption of agglutinins.

#### *Sera*

The sera were those used for comparison of agglutinins.

### RESULTS

#### *Simple Complement Fixation Results*

With guinea-pig antiserum the antigens used are strictly specific. With rabbit antiserum, however, it is only possible to demonstrate specificity where the murine antigen is concerned, the Cairo antigen fixing a considerable amount of complement in the presence of murine antiserum (Table LXII).

TABLE LXII

	Serum	Antigen	Serum dilutions						Interpretation
			20	40	80	160	320	640	
Guinea-pig sera	Murine .. 10.7.43.	Murine .. Cairo ..	4 0	4 0	3 0	0 0	0 0	0 0	m/M <sup>1</sup> Titre 80 —
	Cairo .. (F) .. 27.10.43.	Murine .. Cairo ..	0 4	0 4	0 4	0 4	0 1	0 0	— c/C <sup>1</sup> Titre 160
Rabbit sera	Murine .. (127 iv ML*) 2.12.43	Murine .. Cairo ..	4 4	4 4	4 3	2 0	0 0	0 0	m/M <sup>1</sup> Titre 80 †c/M <sup>2</sup> Titre 80
	Cairo .. (30 iv YS) .. 2.12.43	Murine .. Cairo ..	2 4	0 4	0 4	0 4	0 2	0 0	— c/C <sup>1</sup> Titre 160

\* Sera from rabbits infected with yolk sac material behave in the same way.

† This interpretation was proved to be wrong by a subsequent absorption test, Table LXIV.

#### Absorption of M<sup>1</sup> and C<sup>1</sup>

The antibodies M<sup>1</sup> and C<sup>1</sup> are those responsible for the fixation with the homologous antigen (Table LXIII).

#### The Absorption of M<sup>2</sup> Antibody from Murine Rabbit Antisera

We had interpreted the complement fixation found with unboiled Cairo suspensions reacting with murine rabbit sera as being due to the c/M<sup>2</sup> reaction. This interpretation proved to be wrong (Table LXIV).

It is clear that c is not reacting with M<sup>2</sup> but with M<sup>100</sup>. We conclude that under the conditions of the test the reaction c/M<sup>2</sup> or m/C<sup>2</sup>, which has been described in the agglutination reaction, does not fix 3 M.H.D. of complement.

Why the unboiled Cairo suspension should react with a part of the M<sup>100</sup> antibodies in the complement fixation reaction is difficult to explain. The reaction is at a minimum when perfectly fresh antigen is used, but we have not succeeded in entirely obviating it. There would seem to be two possibilities: either the Cairo suspension, unlike the murine suspension, has been partially degraded by the purification process, or the surface antigen of the Cairo strain has a configuration such that it can react with certain elements among the M<sup>100</sup> fraction of murine antibodies.

This difference between the murine and Cairo suspensions is shown also by the fact that the reaction c/C<sup>1</sup> is somewhat reduced by absorption with c<sup>100</sup> and with m<sup>100</sup>, whereas the m/M<sup>1</sup> reaction is only significantly reduced by absorption with m.

#### The M<sup>100</sup>, C<sup>100</sup>. Antibodies

If the antigens are boiled, they, like the agglutinating suspensions, behave differently. They react better with the heterologous sera, but their degree of reaction with homologous sera varies. Boiling a rickettsial suspension makes it slightly more anti-complementary. Therefore, whether the titre with homologous sera goes up, stays the same, or goes down, depends upon:—

- (i) The level of the M<sup>100</sup> antibodies.
- (ii) The concentration of the antigen used.

The more concentrated the antigen, the more anti-complementary it becomes and thus the less effective complement is present in the system. By choosing a level of antigen which is still not anti-complementary after boiling, as judged



TABLE LXIII

		Absorbing suspension	Antigen	Serum dilutions						
				20	40	80	160	320	640	
Guinea-pig sera	Murine (146/157)	.. ..	Murine..	4	4	0	0	0	0	
	10.8.43	.. ..	.. ..	0	0	0	0	0	0	
	Abs. at 1/10.	.. ..	.. ..	4	4	0	0	0	0	
		Murine ..	.. ..	Murine..	4	2	0	0	0	0
		10.12.43	.. ..	.. ..	0	0	0	0	0	0
		Abs. at 1/10	.. ..	.. ..	4	1	0	0	0	0
		Cairo ..	.. ..	4	1	0	0	0	0	
		Cairo boiled 10'	.. ..	4	1	0	0	0	0	
	Tunis ..	.. ..	Cairo ..	4	4	4	0	0	0	
	24.12.43	.. ..	.. ..	4	4	3	0	0	0	
		Murine ..	.. ..	4	4	0	0	0	0	
		Murine boiled 10'	.. ..	4	4	0	0	0	0	
		Abs. at 1/20	.. ..	.. ..	4	0	0	0	0	0
			Cairo ..	.. ..	4	4	0	0	0	0
		Cairo boiled 10'	.. ..	4	4	0	0	0	0	
Rabbit sera	Murine (122 v YS)	.. ..	Murine..	4	4	1	0	0	0	
	28.11.43	.. ..	.. ..	0	0	0	0	0	0	
		Murine ..	.. ..	4	3	0	0	0	0	
		Murine boiled 10'	.. ..	4	4	0	0	0	0	
		Abs. at 1/20	.. ..	.. ..	4	4	0	0	0	0
			Cairo ..	.. ..	4	4	0	0	0	0
		Cairo boiled 10'	.. ..	4	4	0	0	0	0	
	Cairo (30 iv YS)	.. ..	Cairo ..		4	4	3	0	0	
	27.12.43	.. ..	.. ..		4	4	2	0	0	
		Murine ..	.. ..		4	4	0	0	0	
		Murine boiled 10'	.. ..		4	4	0	0	0	
		Abs. at 1/40	.. ..	.. ..		2	0	0	0	0
			Teheran ..	.. ..		4	3	0	0	0
		Teheran boiled 10'	.. ..		4	3	0	0	0	

Interpretation: M<sup>1</sup> is absorbed by m, but not significantly by m<sup>100</sup>, c or c<sup>100</sup>. C<sup>1</sup> is absorbed strongly by t (=c), less strongly by t<sup>100</sup> (=c<sup>100</sup>) and m<sup>100</sup>, and not significantly by m.

TABLE LXIV

28.11.43	Serum	Absorbing suspension	Antigen	Serum dilutions				
				40	80	160	320	640
Rabbit serum	Murine (122 v YS)	.. ..	Cairo ..	4	4	0	0	0
		.. ..	.. ..	4	4	0	0	0
		Murine ..	.. ..	0	0	0	0	0
		Murine boiled 10'	.. ..	4	4	0	0	0
		Abs. at 1/20	.. ..	.. ..	0	0	0	0
		Cairo ..	.. ..	4	4	0	0	0
		Cairo boiled 10'	.. ..	0	0	0	0	0

by the control, it is possible to demonstrate an apparent rise in titre on boiling the antigen even in those sera which have a smaller amount of M<sup>100</sup> (C<sup>100</sup>) antibodies than M<sup>1</sup> (C<sup>1</sup>) antibodies.

With heterologous sera, however, the titre always goes up on boiling the antigen, because there is always an increase in the number of antibodies with which the antigen can react, since the unboiled heterologous antigen does not react with the M<sup>1</sup> (C<sup>1</sup>) fraction (Table LXV). The degradation of the surface

## 176 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE LXV

	Serum	Antigen	Serum dilutions						Interpretation	
			20	40	80	160	320	640		
Rabbit sera	Murine .. (122 v YS) 28.11.43 ..	Murine ..	4	4	4	1	0	0	m/M <sup>1</sup> Titre 80	
		Murine boiled 10'.	4	4	4	4	4	4	m <sup>100</sup> /M <sup>100</sup> Titre >640	
		Cairo ..	4	4	0	0	0	0	c/M <sup>100</sup> Titre 40	
		Cairo boiled 10'.	4	4	4	4	4	4	c <sup>100</sup> /M <sup>100</sup> Titre >640	
	Cairo .. (30 vi YS) 29.11.43	Murine ..	1	0	0	0	0	0	—	
		Murine boiled 10'.	4	4	4	4	4	4	m <sup>100</sup> /C <sup>100</sup> Titre >640	
		Cairo ..	4	4	4	4	4	3	c/C <sup>1</sup> Titre 640	
		Cairo boiled 10'.	4	4	4	4	4	4	c <sup>100</sup> /C <sup>100</sup> Titre >640	
	Guinea-pig sera	Murine .. (146/157).. 21.12.43	Murine ..	4	4	0	0	0	0	m/M <sup>1</sup> Titre 40
			Murine boiled 10'.	4	0	0	0	0	0	m <sup>100</sup> /M <sup>100</sup> Titre 20
Cairo ..			0	0	0	0	0	0	—	
Cairo boiled 10'.			4	2	0	0	0	0	c <sup>100</sup> /M <sup>100</sup> Titre 20	
Cairo .. (F) .. 21.12.43		Murine ..	0	0	0	0	0	0	—	
		Murine boiled 10'.	4	4	4	0	0	0	m <sup>100</sup> /C <sup>100</sup> Titre 80	
		Cairo ..	4	4	4	0	0	0	c/C <sup>1</sup> Titre 80	
		Cairo boiled 10'.	4	4	2	0	0	0	c <sup>100</sup> /C <sup>100</sup> Titre 40	

antigen may be accomplished by methods other than boiling. Thus, when the kieselguhr method of purification was first used, the precipitation was found to be optimal at pH4. This procedure degraded the surface antigen.

#### *The Absorption of M<sup>100</sup>, C<sup>100</sup> Antibodies*

It seems that the antibody M<sup>100</sup> is identical with or very similar to the antibody C<sup>100</sup> (Table LXVI).

These antibody absorptions, using the complement-fixation technique, give a picture of the antigenic structure of rickettsiae which confirms what we have already deduced from consideration of agglutinin absorptions.

#### **THE "SOLUBLE" RICKETTSIAL ANTIGEN**

Elford & van den Ende (1944) found that, if mouse lung rickettsial suspensions were filtered through collodion membranes, no viable rickettsiae would pass through a membrane of average pore size 500  $\mu\mu$ . Such filtrates, under certain conditions, still contain a substance capable of specifically fixing complement in the presence of rickettsial immune sera. This soluble antigen (Plotz, unpublished observations; Topping & Sheer, 1944), has been used by Craigie, Watson, Clark & Malcomson (1946) as an antigen in the complement-fixation reaction, and it has, therefore, seemed of importance to determine how it behaves in comparison with the rickettsial suspensions which we have used as antigen.

#### *The Presence of a Soluble Rickettsial Antigen in some Yolk Sacs Heavily Infected with Rickettsiae*

Two yolk sacs (M 36, 37), heavily infected with the murine strain of typhus rickettsiae, were harvested. Judged by smears, the rickettsiae were very numerous. The yolk sacs were then treated separately. Each was emulsified in

ANTIGENIC STRUCTURE OF TYPHUS RICKETTSIAE 177

TABLE LXVI

	Serum	Absorbing suspension	Antigen	Serum dilutions					
				20	40	80	160	320	640
Guinea-pig sera	Murine ..	—	Murine boiled	4	4	4	2	0	0
	10.8.43 ..	Murine ..	10'	4	4	4	2	0	0
	Abs. at 1/10	Murine boiled 10'		0	0	0	0	0	0
—	Cairo ..	—	Murine boiled	4	4	4	4	4	0
	(F) ..		10' (m <sup>100</sup> )	4	4	4	4	4	0
	10.8.43 ..	—	Cairo, boiled	4	4	4	4	4	0
	Abs. at 1/10		10' (c <sup>100</sup> )	4	4	4	4	1	0
		Murine ..	m <sup>100</sup> ..	4	4	4	4	1	0
		Murine ..	c <sup>100</sup> ..	4	4	4	4	1	0
		Murine boiled 10'	m <sup>100</sup> ..	4	0	0	0	0	0
		Murine, boiled 10'	c <sup>100</sup> ..	4	0	0	0	0	0
		Cairo ..	m <sup>100</sup> ..	4	4	4	4	4	0
		Cairo ..	c <sup>100</sup> ..	4	4	4	4	4	0
		Cairo, boiled	m <sup>100</sup> ..	4	3	0	0	0	0
	Cairo, boiled	c <sup>100</sup> ..	4	3	0	0	0	0	
Rabbit sera	Murine ..	—	Murine, boiled	4	4	4	4	4	4
	(122 v YS)	Murine ..	10' ..	4	4	4	4	4	4
		Murine boiled 10'	.. ..	3	2	0	0	0	0
	28.11.43	Cairo ..	.. ..	4	4	4	4	4	4
	Abs. at 1/20	Cairo, boiled 10'	.. ..	4	3	1	0	0	0

Interpretation : M<sup>100</sup> is absorbed by m<sup>100</sup> and c<sup>100</sup>, but not significantly by m or c. C<sup>100</sup> is absorbed by c<sup>100</sup> and m<sup>100</sup>, but not significantly by c or m.

15 ml. of Hartley broth, and the emulsion was centrifuged for 30 minutes at 10,000 r.p.m. The mid-zone, avoiding the top fat, was removed and filtered through a 500-m $\mu$  membrane. These filtrates were then used undiluted as antigens in a complement fixation reaction (Table LXVII).

TABLE LXVII

Rabbit serum	Serum	Antigen	Serum dilutions					
8.12.43			20	40	80	160	320	640
	Murine ..	M 36 .. ..	4	4	3	0	0	0
	(127 iv ML) ..	M 37 .. ..	0	0	0	0	0	0
		Standard antigen* ..	4	4	4	3	0	0

\* Rat lung rickettsial suspension previously described.

Even in the yolk sac suspension in which it was possible to demonstrate a soluble antigen, this was a weak antigen despite the small volume of the suspending fluid.

*The Presence of a Soluble Rickettsial Antigen in Rickettsial Consolidated Mouse Lungs at Harvest.*

Twenty-two mouse lungs infected with the murine strain were harvested. Eleven lungs were put aside in the ice-box. The other 11 lungs were ground up in 11 ml. of Hartley broth containing 0.75 per cent. sodium citrate. The suspension was centrifuged at 10,000 r.p.m. for 20 minutes, and the supernatant was filtered through a filter made up of three collodion membranes of 1,000 m $\mu$ , 750 m $\mu$  and 500 m $\mu$  in that order from top to bottom.

178 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

This filtrate (antigen A) was stored overnight in the ice-box. Next day the 11 lungs which had been set aside were processed in an identical manner (antigen B). At 1/1 both were anti-complementary. Dilutions of the antigens were made in physiological saline (Table LXVIII).

TABLE LXVIII

Rabbit serum	Serum	Antigen	Antigen dilutions	Serum dilutions.					
				20	40	80	160	320	640
19.10.43	Murine (122 v YS) ..	A	1/2	4	3	2	1	0	0
1/4			1	0	0	0	0	0	
1/8			0	0	0	0	0	0	
		B	1/2	4	4	4	4	2	1
1/4			3	3	1	0	0	0	
1/8			1	0	0	0	0	0	
		Standard antigen 22.11.43	—	4	4	4	4	3	0

We think there is probably a weak soluble antigen in consolidated mouse lung at harvest, though for its demonstration it is necessary to use a very concentrated suspension. As might be expected, the storage of the mouse lungs at +4° C. overnight before processing increases the amount of soluble antigen.

*The Absence of a Soluble Antigen in a Recently Purified Mouse Lung Rickettsial Suspension*

A purified suspension of murine rickettsiae of opacity equal to a *coli* suspension of  $500 \times 10^6$ /ml., prepared two days previously from mouse lung by the kieselguhr technique and since then stored at +4° C., was divided into two parts:—

Part I was not further treated=antigen I.

Part II was centrifuged at 10,000 r.p.m. for 20 minutes. The water-clear supernatant was used as antigen II (Table LXIX).

TABLE LXIX

Guinea-pig serum	Serum	Antigen	Serum dilutions					
			20	40	80	160	320	640
20.2.43	Murine (146/157) ..	I	4	4	2	0	0	0
		II	0	0	0	0	0	0

*The Presence of a Soluble Antigen in a Purified Rat Lung Rickettsial Suspension after Storage*

A purified suspension of murine rickettsiae prepared from rat lung was diluted to match a *coli* suspension of  $1,000 \times 10^6$ /ml. and was stored for one month at +4° C. At the end of this time the suspension was centrifuged at 10,000 r.p.m. for 20 minutes, and the supernatant was mixed with an equal volume of Hartley broth and was filtered through a 500-m $\mu$  membrane.

TABLE LXX

Guinea-pig serum			Serum dilutions			
15.10.43	Serum	Antigen	20	40	80	160
	Murine .. .. (146/157)	Filtrate .. ..	4	4	4	1

*Boiling a Purified Rat Lung Rickettsial Suspension for 10 minutes does not lead to the Release of a Soluble Antigen into the Surrounding Medium.*

A purified suspension of murine rickettsiae prepared from rat lung was diluted in saline to match a coli suspension of  $500 \times 10^6$ /ml. It was centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant was discarded and the deposit was re-suspended in an equal volume of saline. The suspension was boiled for 10 minutes, and then centrifuged for 20 minutes at 10,000 r.p.m. The deposit was re-suspended in an equal volume of saline=antigen I. The supernatant was mixed with half volume of Hartley broth and was then filtered through a 500-m $\mu$  membrane. The filtrate=antigen II. (Table LXXI).

TABLE LXXI

20.10.43	Rabbit sera	Murine (127 iv ML)						Cairo (30 iv YS)					
	Antigen	20	40	80	160	320	640	20	40	80	160	320	640
	I	4	4	4	4	3	0	4	4	4	4	4	3
	II	0	0	0	0	0	0	0	0	0	0	0	0

*The Liberation of a Soluble Antigen from Rickettsial Suspensions shaken with Ethyl Ether*

Following Craigie (1945), ethyl ether has been widely used as a means of purifying rickettsial suspensions derived from yolk sacs. We have found that ether provokes the liberation of a soluble antigen from rickettsial suspensions (Tables LXXII and LXXIII).

*Yolk Sac suspensions*

A yolk sac heavily infected with an epidemic strain of typhus rickettsiae (Breinl) was harvested. Judged by smears, the rickettsiae were very numerous. The yolk sac was emulsified in 30 ml. of saline and the suspension was divided into two parts.

Part I was centrifuged at 10,000 r.p.m. for 15 minutes. The supernatant was filtered through a 500-m $\mu$  membrane. The filtrate=antigen I. The deposit was re-suspended in an equal volume of saline=antigen II.

TABLE LXXII

Guinea-pig serum			Serum dilutions					
25.9.43	Serum	Antigen	20	40	80	160	320	640
	Breinl ..	I	0	0	0	0	0	0
		II	4	4	4	0	0	0
		III	4	4	4	2	0	0

## 180 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Part II was shaken with an equal volume of ethyl ether. The emulsion was centrifuged lightly to facilitate the development of an ether-emulsion layer and an aqueous layer. The ether-emulsion layer was discarded and the aqueous layer was exposed to a partial vacuum to remove the dissolved ether. It was then centrifuged at 10,000 r.p.m. for 15 minutes and the supernatant was filtered through a 500-m $\mu$  membrane. The filtrate=antigen III. (Table LXXII).

Thus, shaking a rickettsial suspension with ethyl ether leads to the release of a soluble antigen.

### *Purified lung suspensions*

A kieselguhr purified suspension of murine rickettsiae, prepared the day before, was diluted to an opacity to match a *coli* suspension of  $1,000 \times 10^6$ /ml. It was divided into two parts.

Part I was centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant was mixed with an equal volume of Hartley broth and filtered through a 500-m $\mu$  membrane. The filtrate=antigen I.

Part II was shaken with 2/3 vol. of ethyl ether. The aqueous layer was removed, exposed to a partial vacuum and centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant was mixed with an equal volume of Hartley broth and filtered through a 500-m $\mu$  membrane. The filtrate=antigen II.

TABLE LXXIII

Rabbit serum	Serum	Antigen	Serum dilutions					
			20	40	80	160	320	640
18.10.43								
	Murine ..	I	0	0	0	0	0	0
	(122 v YS) ..	II	4	4	4	4	2	0

It is clear that a "soluble" rickettsial antigen exists naturally under certain conditions. It seems probable that a surface antigen has dissociated from the rickettsial bodies into the fluid menstruum in which the bodies are suspended. But shaking a rickettsial suspension with ethyl ether leads to a further release of soluble antigen, and it is this which has been widely used in the complement fixation test. It seems probable that the soluble antigen is derived from the surface antigen of the rickettsiae. Thus, Miles and Pirie (1939) found that shaking *Brucella melitensis* with chloroform brought the bulk of the SSS into solution. We have not, so far, succeeded in producing a soluble antigen preparation which will pass a 93-m $\mu$  membrane. This would suggest that the antigen is of the order of size of 30-40 m $\mu$ . Preliminary centrifugation experiments tend to confirm this (Elford & Fulton, unpublished observations).

### *The Serological Behaviour of the Soluble Rickettsial Antigen Released as a Result of Ether Treatment of Rickettsial Suspensions*

The main facts have been established by using filtrates which have passed through a 500-m $\mu$  membrane. In most of the experiments which follow, the ether-treated suspensions have been centrifuged at 10,000 r.p.m. for 20 minutes, and the supernatants have been used, without filtration, as a source of soluble antigen. If a rickettsial suspension is diluted to match the faint opalescence often found in such supernatants, and if this very weak suspension is used as antigen in the complement fixation test, there is no fixation. It follows, therefore, that the complement fixation observed with the unfiltered supernatants of ether-treated suspensions is not due to the few rickettsiae which are probably still present.

ANTIGENIC STRUCTURE OF TYPHUS RICKETTSIAE 181

A yolk sac vaccine of the murine type, prepared by us some months before, according to the latest *p. H7'* method of Craigie (unpublished), was dialysed against saline to remove the formalin. The dialysed vaccine was then divided into two parts:—

Part I was not further treated=antigen I.

Part II was centrifuged at 10,000 r.p.m. for 15 minutes.

The deposit was re-suspended in an equal volume of saline=antigen II. The supernatant was filtered through a 500-m $\mu$  membrane=antigen III.

TABLE LXXIV

Guinea-pig sera	Antigen	Murine (146/157)						Breinl					
		20	40	80	160	320	640	20	40	80	160	320	640
10.10.43	I	4	4	4	0	0	0	4	4	4	0	0	0
	II	4	4	3	0	0	0	0	0	0	0	0	0
	III	4	4	4	0	0	0	4	4	4	0	0	0

From this result we thought that, whereas the rickettsial bodies (m) were reacting with the antibody M<sup>1</sup> of the homologous serum, the soluble antigen mSA was reacting with the M<sup>100</sup> antibody of the homologous serum and the B<sup>100</sup> (Breinl serum) antibody of the heterologous serum. However, absorptions have shown that this is not so; for the mSA reacts with the M<sup>1</sup> antibody of its homologous guinea-pig serum (Table LXXV).

For antigen in this experiment we have used the supernatant of the vaccine described in the previous experiment. This supernatant was not, however, filtered (mSA).

TABLE LXXV

Guinea-pig sera	Serum	Absorbing suspensions	Antigen	Serum dilutions				
				20	40	80	160	320
15.10.43	Murine ..	—	mSA ..	4	4	3	0	0
	(146/157) ..	Murine .. ..	.. ..	4	3	0	0	0
	Abs. at 1/10 ..	Murine boiled 10'	.. ..	4	4	3	0	0
	Cairo ... ..	—	mSA ..	4	4	4	0	0
	(F) .. ..	Murine .. ..	.. ..	4	4	4	0	0
	Abs. at 1/10 ..	Murine boiled 10'	.. ..	4	0	0	0	0

Interpretation: The mSA is reacting with M<sup>1</sup> and C<sup>100</sup>. It would seem that the surface antigen has been partially degraded by removal by ether from the surface of rickettsiae.

The soluble antigens prepared from purified rat or mouse lung suspensions of rickettsiae behave in the same way. The suspensions, after being shaken with half a volume of ether, are centrifuged at 10,000 r.p.m. for 20 minutes. The supernatants are then used as antigens.

In Table LXXVI below, the murine soluble antigen is written mSA and the Cairo soluble antigen cSA.

## 182 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

TABLE LXXVI

Guinea-pig sera	Date	Serum	Absorbing suspension	Antigen	Serum dilutions					
					10	20	40	80	160	320
24.12.43	Murine .. (146/157) .. Abs. at 1/10	— Murine .. .. Murine boiled 10'	mSA .. ..	4	4	4	3	0	0	
				4	4	0	0	0	0	
				4	4	4	0	0	0	
24.12.43	Murine .. (146/157) .. Abs. at 1/10	— Murine .. .. Murine boiled 10'	cSA .. ..	4	3	0	0	0	0	
				4	0	0	0	0	0	
				0	0	0	0	0	0	
6.12.43	Cairo .. (F) .. .. Abs. at 1/10	— Murine .. .. Murine boiled 10'	mSA .. ..	—	4	4	1	0	0	
				—	4	4	0	0	0	
				—	0	0	0	0	0	

Interpretation : mSA is reacting with M<sup>1</sup> and C<sup>100</sup>, cSA is reacting with M<sup>100</sup>.

It may be that the antibody fractions with which the mSA antigen is reacting are not identical with the fractions with which m (the rickettsial bodies) reacts.

If such supernatants containing the soluble antigen are boiled for five minutes, they behave differently. Just as with the rickettsial suspensions, the m (c) antigen is converted into the m<sup>100</sup> (c<sup>100</sup>) form. In Tables LXXVII and LXXVIII the unboiled murine soluble antigen is written mSA and the boiled mSA<sup>100</sup>. Similarly, the Cairo soluble antigen is written cSA and cSA<sup>100</sup>.

TABLE LXXVII

Guinea-pig serum	Date	Serum	Antigen →	Absorbing suspensions	Serum dilutions											
					mSA						mSA <sup>100</sup>					
					20	40	80	160	320	640	20	40	80	160	320	640
15.12.43	Murine .. 146/157 Abs. 1/10	— Murine .. .. Murine boiled 10'	mSA .. ..	4	4	3	0	0	0	0	4	0	0	0	0	0
				4	0	0	0	0	0	3	0	0	0	0	0	
				4	4	1	0	0	0	0	0	0	0	0	0	0

Interpretation : mSA reacts with M<sup>1</sup>, mSA<sup>100</sup> reacts with M<sup>100</sup>.

TABLE LXXVIII

Guinea-pig sera	Date	Serum	Antigen →	Absorbing suspensions	Serum dilutions											
					cSA						cSA <sup>100</sup>					
					20	40	80	160	320	640	20	40	80	160	320	640
24.12.43	Tunis	— Murine .. .. Murine boiled 10'	cSA .. ..	4	4	4	0	0	0	0	4	4	1	0	0	0
				4	4	4	0	0	0	4	4	0	0	0	0	
				4	4	1	0	0	0	0	0	0	0	0	0	
27.12.43	Tunis	— Cairo .. .. Cairo boiled 10'	cSA .. ..	4	4	4	3	0	0	0	4	4	4	0	0	0
				4	4	0	0	0	0	4	4	3	0	0	0	
				4	4	3	0	0	0	4	0	0	0	0	0	

Interpretation : cSA reacts with C<sup>1</sup>, cSA<sup>100</sup> reacts with C<sup>100</sup>.



ANTIGENIC STRUCTURE OF TYPHUS RICKETTSIAE 183

These absorptions again emphasise our previous finding that, whereas the M<sup>1</sup> antibody is not significantly absorbed by m<sup>100</sup> or c<sup>100</sup>, the C<sup>1</sup> antibody (as measured by the reaction c/C<sup>1</sup> or cSA/C<sup>1</sup>) is absorbed to some extent by both m<sup>100</sup> and c<sup>100</sup>. Thus, from whatever cause, in the Cairo suspension the surface antigen is relatively degraded and is not so satisfactory for serological analyses as the murine suspension. Craigie, Watson, Clark and Malcomson (1946) have found that when epidemic antigens derived from yolk sac suspensions are used, the specific antibody, which we have named C<sup>1</sup>, is not sensibly absorbed by homologous rickettsiae heated at 65° C. for one hour. This would suggest that such yolk sac epidemic antigens are as specific as we have found our murine antigen to be. Considering the murine antigen which we have prepared from mouse lung and which is probably the equal of the antigens prepared from yolk sacs, there would seem to be advantages in using the whole organism as antigen in the complement-fixation reaction, rather than in using the soluble antigen. For the soluble antigen reacts with a group of antibodies in heterologous sera (X<sup>100</sup>) with which the whole organism does not. The behaviour of the influenza virus provides an analogy to these findings (Friedewald, 1943).

We have shown that with guinea-pig sera the murine soluble antigen (mSA) reacts with the M<sup>1</sup> antibody of its homologous serum and the C<sup>100</sup> antibody of an heterologous serum. When rabbit sera are used, the results are different. (Table LXXIX). The mSA behaves as if it had been totally degraded by the ether and it reacts with the M<sup>100</sup> antibodies of its homologous serum. Epidemic soluble antigens behave in the same way.

TABLE LXXIX

Rabbit sera	Serum	Antigen →	Serum dilutions									
			mSA					mSA <sup>100</sup>				
Date		Absorbing suspensions	40	80	160	320	640	40	80	160	320	640
4.12.43	Murine .. (122 v YS) .. Abs. at 1/20	—	4	4	4	0	0	4	4	4	0	0
		Murine ..	4	4	4	0	0	4	4	4	0	0
		Murine boiled 10'	1	0	0	0	0	0	0	0	0	0
		Antigen →	cSA									
		Absorbing suspensions	20	40	80	160	320					
6.1.44	Breinl .. (27 iv YS) .. Abs. at 1/20	—	4	4	4	2	0					
		Cairo ..	4	4	4	0	0					
		Cairo boiled 10'	4	3	0	0	0					

THE EFFECT OF ETHYL ETHER ON THE RICKETTSIAL SURFACE ANTIGEN

The effect of ethyl ether on the rickettsial bodies themselves is probably very small, but it is possible under certain conditions to demonstrate a slight degradation of the surface antigen. For such a demonstration Cairo suspensions are of no value, since they, even before ether treatment, react with a portion of the M<sup>100</sup> antibodies. The murine suspensions purified by the keiseluhr technique do not react with the C<sup>100</sup> antibodies, but after shaking with ether, such suspensions do show a measurable reaction with C<sup>100</sup> antibodies (Table LXXX).

## 184 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

A concentrated suspension of murine rickettsiae was diluted so that the opacity matched a *coli* suspension of  $500 \times 10^6$ /ml. This suspension was divided into two parts:—

Part I was untreated=antigen I.

Part II was shaken for two minutes with half a volume of ethyl ether. The aqueous layer was exposed to a partial vacuum. When all the dissolved ether had been removed, the suspension was centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant was discarded and the deposit was re-suspended in an equal volume of saline=antigen II.

TABLE LXXX

Rabbit serum	Serum	Antigen	Serum dilutions					
			20	40	80	160	320	640
21.12.43								
	Cairo ..	I	2	0	0	0	0	0
	(30 iv YS) ..	II	4	2	0	0	0	0

It can also be shown that an ether-treated murine suspension is capable of absorbing more of the T<sup>100</sup> antibodies from Tunis (epidemic rickettsiae) guinea-pig antisera than similar untreated murine suspensions (Table LXXXI).

Absorbing suspension I: Concentrated murine suspension diluted to match a *coli* suspension of  $2,000 \times 10^6$ /ml.

Absorbing suspension II: 1 ml. of this concentrated murine suspension was shaken with 3 ml. of ethyl ether. The suspension was centrifuged to deposit the rickettsiae and the supernatant was discarded. The deposit was then washed in 4 ml. of saline. Finally, the washed deposit was re-suspended in saline to match a *coli* suspension of  $2,000 \times 10^6$ /ml.

Absorbing suspension III: Concentrated murine suspension was diluted to match a *coli* suspension of  $1,000 \times 10^6$ /ml. It was then boiled for 10 minutes.

TABLE LXXXI

Guinea-pig serum	Serum	Absorbing suspension	Antigen	Serum dilutions				
				20	40	80	160	320
23.12.43								
	Tunis ..	—	mSA	4	4	0	0	0
		I		4	4	0	0	0
	Abs. 1/20	II		4	0	0	0	0
		III		0	0	0	0	0

Thus, the effect of ethyl ether on the surface antigens is probably very slightly to degrade them.

### THE ANTIBODIES TO *PROTEUS OX19*

It is well known that rabbits infected with typhus rickettsiae develop agglutinins in their sera which will agglutinate *Proteus OX19*. We have studied these antibodies to determine their relationship to the other rickettsial antibodies. In the tables that follow, the murine strain has been used for illustration, but the Cairo strain behaves in exactly the same way.

METHODS

For the titration of the *OX19* agglutinins we have used the Oxford standard suspension of *Proteus OX19*. This is an alcoholised suspension which is re-suspended in formol-saline. The agglutination test is conducted in the same way as the rickettsial agglutination test.

In most instances we have also used the Oxford standard suspension as an absorbing suspension. The concentrated suspension is centrifuged and the deposit re-suspended in an equal volume of saline. The opacity is approximately equal to a *coli* suspension of  $11,280 \times 10^6$ /ml. The sera are diluted directly in the absorbing suspension. The absorption is for one hour at 37° C., after which the organisms are removed by centrifugation.

In two instances, noted in the text, we have used instead a fresh suspension of *Proteus OX19*, prepared by suspending in saline the growth of a 24-hour agar slope and diluting to the required opacity.

The *Proteus OX19* rabbit immune serum (157) was prepared by injecting 2 ml. of the concentrated Oxford standard suspension of *Proteus OX19* intravenously, and bleeding after 14 days. The *OX19* agglutinin titre of this serum is 1/5,000.

*The Relationship of OX19 Antibody and M<sup>1</sup> and M<sup>2</sup>*

Tables LXXXII and LXXXIII show that the *OX19* antibody is distinct from M<sup>1</sup> and M<sup>2</sup>.

TABLE LXXXII

Rabbit serum	Serum	Agglutinating suspensions	Serum dilutions												
			Murine						OX19						
			160	320	640	1,280	2,560	5,120	160	320	640	1,280	2,560	5,120	
Murine (119 v YS) ..	—	—	T	T	S+	tr	0	0	0	T	T	T-	tr	0	0
Abs. 1/80 ..	*Murine ..	0	0	0	0	0	0	T	T	T	T	0	0	0	0
	†OX19 ..	T	T	S+	tr	0	0	0	0	0	0	0	0	0	0

\* Opacity to match a *coli* suspension of  $2,000 \times 10^6$ /ml.  
 † Oxford standard suspension.

TABLE LXXXIII

Rabbit serum	Serum	Agglutinating suspensions	Serum dilutions									
			Cairo					OX19				
			40	80	160	320	640	40	80	160	320	640
Murine (119 v YS) ..	—	—	T-	S+	S	S-	0	T	T	T	T-	S-
Abs. 1/20 ..	Cairo ..	0	0	0	0	0	0	T	T	T	T-	S-
	OX19* ..	T	T-	S	tr	0	0	0	0	0	0	0

\* Oxford standard suspension.

*The Relationship of the OX19 Antibody and the M<sup>100</sup> Antibodies*  
*Agglutination*

*Rickettsial antiserum*

Absorptions have shown that *Proteus OX19* removes the *OX19* antibody but does not affect M<sup>100</sup>, whereas boiled murine suspension (M<sup>100</sup>) removes both antibodies (Table LXXXIV).

TABLE LXXXIV

Rabbit sera		Agglutinating suspension →	Serum dilutions															
			Murine boiled 5 mins. (m <sup>100</sup> )								OX19 (Oxford Standard Suspension)							
Date	Serum	Absorbing suspension	40	80	160	320	640	1,280	2,560	5,120	40	80	160	320	640	1,280	2,560	5,120
18.12.43	Murine (119 v YS)	—	T	T	T	T	T	T-	S	0	T	T	T	T	S	0	0	0
		Murine boiled 10'	T	T	T-	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abs. at 1/20	OX19 (Oxford standards)	T	T	T	T	T	T-	tr	0	0	0	0	0	0	0	0	0
—	—	—	160	320	640	1,280	2,560	5,120	10,240	—	160	320	640	1,280	2,560	5,120	10,240	—
6.2.44	Murine (119 iii YS)	—	T	T	T	S+	0	0	0		T	T	T	S	0	0	0	—
		Murine boiled 10'	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
	—	*OX19 (fresh suspension)	T	T	T	S+	0	0	0		0	0	0	0	0	0	0	—
	Abs. at 1/80	*Ditto, boiled 10'	T	T	T	S+	0	0	0		S+	S	0	0	0	0	0	0

\* Opacity to match a coli suspension of 3,000 × 10<sup>6</sup>/ml.

*Rabbit Proteus OX19 antiserum*

Castaneda & Zia (1933) found that, if they heated their suspensions of murine rickettsiae at 75° C. for half-an-hour in the presence of formalin, the rickettsiae became agglutinable by rabbit *Proteus OX19* antisera. We have not been able to repeat this observation with our suspensions.

*Complement Fixation*

*Rickettsial antiserum*

Using the complement-fixation technique, it is confirmed that the M<sup>100</sup> antibody is absorbed by m<sup>100</sup>, but not by *Proteus OX19* (Table LXXXV).

TABLE LXXXV

Date	Serum	Absorbing suspension	Antigen	Serum dilutions					
				20	40	80	160	320	640
6.2.44.	Murine (119 iii YS)  Absorbed at 1/20	—	Murine boiled 10'	4	4	4	4	2	2
		Murine boiled 10'		1	0	0	0	0	0
		*OX19 (fresh suspension).		4	4	4	4	3	2
		†OX19 (fresh suspension).		4	4	4	3	2	2

\* Opacity to match *coli* suspension of 3,000 × 10<sup>6</sup>/ml.  
 † Opacity to match *coli* suspension of 21,000 × 10<sup>6</sup>/ml.

No complement fixation could be demonstrated when rabbit rickettsial antisera were used with *Proteus OX19* as antigen.

*Rabbit Proteus OX19 antiserum*

The boiled murine suspension fixes complement in the presence of *Proteus OX19* antiserum in low dilution, but the unboiled suspension does not (Table LXXXVI).

TABLE LXXXVI

Date	Serum	Antigen	Serum dilutions					
			20	40	80	160	320	640
6.2.44 ..	<i>Proteus OX19</i> (157) .. ..	*OX19 (fresh suspension).	4	4	4	4	4	2
		Murine .. ..	0	0	0	0	0	0
		Murine boiled 10'	4	2	0	0	0	0

\* Opacity to match a *coli* suspension of 1,000 × 10<sup>6</sup>/ml.

There would seem to be two possibilities :—

- (i) The *OX19* antibody is formed in response to an entirely distinct antigen which is not situated on the surface of the rickettsia, but is uncovered by boiling.
- (ii) The *OX19* antibody is formed in response to a degraded form of m—the specific surface antigen of the rickettsiae. The *OX19* antibodies would then represent a very non-specific fraction of the M<sup>100</sup> antibodies, just as the M<sup>8</sup> fraction represents a relatively non-specific fraction of the M antibodies.

The results of the immunisation of rabbits with the rickettsial soluble antigen have led us to believe that the latter is the true explanation.

### THE IMMUNISATION OF RABBITS WITH VARIOUS RICKETTSIAL ANTIGENS

If the *OX19* antibodies are a fraction of the  $M^{100}$  antibodies, then it should be possible to demonstrate that the soluble antigen, either undegraded or degraded, gives rise to *OX19* agglutinins.

We have also immunised rabbits with unboiled and boiled rickettsiae to show that no  $M^1/M^2$  antibodies are provoked by the boiled rickettsiae.

#### Preparation of the Antigen

(a) Twenty ml. of purified murine suspension prepared from mouse lung were diluted with saline to match a *coli* suspension of  $500 \times 10^6$ /ml. This diluted suspension was divided into two parts:—

Part I was not further treated=antigen m.

Part II was boiled for 30 minutes=antigen  $m^{100}$ .

(b) Twenty-five ml. of purified murine suspension prepared from rat lung were diluted with saline to match a *coli* suspension of  $1,000 \times 10^6$ /ml. The diluted suspension was then shaken with 12.5 ml. of ether. The ether layer was discarded and the aqueous layer was drawn off. The dissolved ether was removed by bubbling warm air through, after which the aqueous layer was centrifuged at 10,000 r.p.m. for 20 minutes. The deposit was discarded, and the supernatant was mixed with half a volume of Hartley broth and filtered through a 500  $m\mu$  membrane. The filtrate was divided into two parts:—

Part I was not further treated=antigen mSA.

Part II was boiled for 30 minutes=antigen  $mSA^{100}$ .

#### Immunisation of the Rabbits

Four rabbits were immunised—one with each of the antigens m,  $m^{100}$ , mSA and  $mSA^{100}$ . The scheme of immunisation is shown below.

Day →	1	5	9	13
	1st bleed then 1 ml. of antigen i.v.	1 ml. of antigen i.v.	1 ml. of antigen i.v.	2nd bleed

#### The Agglutinin Response

TABLE LXXXVII

Antigen used for immunisation	Agglutinin titre of serum after immunisation			
	$M^1$	$M^2$	$M^{100}$	<i>OX19</i>
m .. ..	5,120	1,280	10,240	320
$m^{100}$ .. ..	20	<20	10,240	1,280
mSA .. ..	320	80	2,560	1,280
$mSA^{100}$ .. ..	<20	<20	1,280	320

It has been our experience that the *OX19* antibodies produced by individual rabbits in response to the injection of rickettsiae show a wide quantitative variation; differences in the titres, therefore, should not be interpreted as meaning a difference in the antigen. On the other hand, the titres of the  $M^1$ ,  $M^2$  and  $M^{100}$  antibodies are more uniform and differences have more significance.

As was to be expected, the boiled antigens gave rise only to the  $M^{100}$  and the *OX19* antibodies, whereas the unboiled antigens provoked  $M^1$  and  $M^2$  antibodies in addition. There is further evidence here that the soluble antigen, even before boiling, is partly degraded, as the specific agglutinins produced in response to the antigen mSA are of relatively low titre.

We believe that the *OX19* agglutinins are a fraction of the  $M^{100}$  antibodies; that is to say, they are produced in response to a degraded form of the specific rickettsial antigen.

It is often maintained that the rickettsiae contain a minor antigen which is identical with a minor antigen in *Proteus OX19*, and this is held to explain the cross reactions observed. But in our opinion the evidence does not support this view. Bruce White's observations (1936) serve, in the absence of a demonstration of the postulated alkali labile factor, only to show that the *OX19* SSS will react with the *Proteus* antisera and with typhus antisera, but that after treatment with alkali the SSS is so modified that it will now only react with the antibodies in the typhus antisera.

We specifically state our disbelief in the existence of an identical common antigen.

One must suppose that the surface antigen of *Proteus OX19* has a structure which is very similar to the structure of the degraded rickettsial antigen.

The fact that the serum from a guinea-pig convalescent from rickettsial infection does not agglutinate *Proteus OX19*, is explained simply by saying that the grade of antibodies this animal produces in response to degraded forms of the rickettsial surface antigen is not of such a type that they can react with the similar, but not identical, surface antigen of the *Proteus OX19*.

It is known, in other instances, that such differences in the grades of antibodies produced by different species of animal can occur. Thus, in the serological analysis of *Neisseria*, antisera produced in rabbits are of little value owing to the cross agglutininations which occur, whereas antisera produced in chickens are very specific (Phair *et al.*, 1943).

## DISCUSSION

When a murine antigen reacts with a Cairo antiserum, complement is not fixed, but if the murine antigen is boiled, there is specific complement-fixation. One explanation of this phenomenon might be that a heat-labile surface antigen had been destroyed by the boiling, uncovering a heat-stable antigen. What actually happens when a rickettsial suspension is shaken with ethyl ether is obscure. But it seems very likely that whatever is dislodged from the rickettsial bodies into the suspending fluid was itself occupying a position on the surface of the rickettsia.

This soluble antigen behaves serologically in many ways as do the rickettsiae. It seems, in fact, that the soluble antigen may be a fragment of the surface antigen. If this is so, it is difficult to visualise boiling as uncovering another antigenic substance, since the surface antigen itself behaves, when boiled, in the same way as the postulated uncovered antigen.

The picture of the antigenic structure which we have drawn is a possible synthesis of the experimental results. The murine strain has a certain surface antigen m, which on injection into animals gives rise to a wide range of antibodies. Some of these antibodies are very specific ( $M^1$ ) and will react only with their exact homologue; others ( $M^2$ ) are less specific and will react with a wider range of structural types, such as the slightly different surface antigen of the Cairo strain.

In addition to this series, there is another group of antibodies ( $M^{100}$ ) which are formed in response to degraded forms of m. *In vitro* this degradation may be achieved by boiling the antigen—how it occurs in the body is unknown.

## 190 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

These  $M^{100}$  antibodies will react with similar degraded forms of the Cairo antigen, and a fraction of them will also react with *Proteus OX19*. There may be other antibodies in the sera for the demonstration of which we have not, as yet, suitable antigens. Antisera made with the Cairo strain will contain a large number of antibodies indistinguishable from antibodies in the murine antisera, but a fraction will be truly strain specific.

Craigie, Watson, Clark and Malcomson (1946) conclude that the murine and epidemic strains contain the same heat-stable antigen, but that in addition each possesses a specific heat-labile antigen. Our conception of the complexity of the rickettsial antibodies is, of course, entirely opposed to the application, in the present instance at any rate, of the mosaic theory of antigenic structure, whereby different fractions of the antibody complex are interpreted as having been formed in response to clearly defined separate antigenic receptors.

The specific antigen is made up of different chemical groups arranged in a specific pattern, and in this sense the antigen is a mosaic. But the mosaic theory of antigen structure envisages macroscopic areas on the surface of an organism consisting of complex substances with different properties, and which are potentially separable by chemical or physical methods.

The complexity of the antibodies formed is likely to be very great in those cases, such as the present, where the antigens are extremely labile substances. Not only do the antibodies differ because of the varying specificity of the antibodies produced against a particular antigen, but there are a large number of antigens of slightly different structure formed because of the ease with which the original antigen is degraded.

In the psittacosis-lymphogranuloma group, where the organisms are known to be exceptionally labile, the serological distinction of the members of the group is very difficult, because in most laboratory animals the antisera produced have a complex array of antibodies, and, in addition, all antigens, when prepared for *in vitro* study, are to a variable degree degraded. When they are used as undegraded living suspensions, as in the toxin-antitoxin titrations, the different members of the group may be sharply differentiated (Rake & Hamre, 1944).

For the serological distinction of the epidemic and murine strains of rickettsiae, it is essential to confine attention to that fraction of antibodies, in each immune serum, which is highly specific. And to do this, it is necessary to use undegraded antigens.

In our opinion the differences between the murine and epidemic strains are sufficient to justify according each specific status.

### SUMMARY

1. We have compared the antigenic structure of a murine strain of typhus rickettsiae with that of a strain of epidemic typhus rickettsiae.

2. The comparison has been made by the agglutination and complement-fixation techniques, using rabbit and guinea-pig antisera, and rickettsial suspensions prepared from the lungs of infected rats and mice.

3. The murine surface antigen gives rise to a heterogeneous group of antibodies, some of which can react with the surface antigen of the epidemic strains. Similarly, the epidemic surface antigen gives rise to antibodies, some of which can react with the murine surface antigen.

4. Boiling a rickettsial suspension so alters the surface antigen that it acquires a new serological specificity. Such degraded antigens have lost their strain specificity.



5. There is present in rabbit and guinea-pig rickettsial antisera an antibody which reacts with rickettsiae degraded by boiling. This antibody is entirely distinct from the antibody which reacts with the undegraded rickettsiae.

6. The OX19 antibody of rabbit rickettsial antisera is entirely distinct from the antibodies to the undegraded surface antigen of the rickettsiae, but it is a fraction of the group of antibodies which reacts with the boiled rickettsiae.

7. Shaking a rickettsial suspension with ethyl ether provokes the release into the surrounding fluid of a soluble antigen. This soluble antigen behaves as if it were a fragment of the surface antigen, though there is evidence that it is partially degraded.

8. We conclude that the surface antigen of the murine strain is similar to, but not identical with, the surface antigen of the epidemic strain.

Whereas murine antisera contain many antibodies which are not distinguishable from antibodies in epidemic antisera, yet there remains a strain specific fraction in each group of rickettsial antibodies.

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## APPENDIX A

## ILLUSTRATIVE CASE REPORTS.

The summarised case reports of 116 patients considered in the sections of this monograph dealing with the North African and Naples typhus epidemics have been deposited in the Library of the National Institute for Medical Research, Hampstead, London, N.W.3, where they are available for consultation by interested workers, on application to the Librarian.

Summarised reports of 13 representative cases are reproduced here to illustrate the respective features of severe, moderate and mild typhus, treated with or without chemotherapy. The cases have been chosen to illustrate clinical and pathological points of interest.

## Controls.

Naples 45	..	..	Mild case; recovered.
Naples 23	..	..	Moderately severe case; recovered.
Algiers M2	..	..	Moderately severe case; recovered.
Naples 43	..	..	Grave case; died.
Naples 66	..	..	Grave case; recovered.
Naples 77	..	..	Grave case; died.

## Treated with V186.

Naples 42	..	..	Mild case; recovered.
Naples 32	..	..	Moderately severe case; recovered.
Algiers EK13	..	..	Grave case; recovered.
Naples 62	..	..	Grave case; died.
Naples 60	..	..	Grave case; died.

## Treated with Penicillin.

Naples 93	..	..	Grave case; died.
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## Doubtful Typhus Treated with V186.

Naples 58	..	..	Grave case; died.
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A key to the abbreviations used throughout the tables showing the results of laboratory examinations is given below. The normal values determined by the methods used, in eight hospital employees, are shown in Appendix Table I.

## KEY TO TABLES

## Agglutination.

<i>Proteus</i>	..	..	Highest final dilution of serum showing visible agglutination with "O" suspensions of <i>Proteus X19</i> , <i>X2</i> , and <i>XK</i> .
+	..	..	Approximately equals "standard" agglutination.
<i>M.</i>	..	..	Murine typhus rickettsiae.
<i>C.</i>	..	..	Epidemic (Cairo strain) typhus rickettsiae.
<i>T.A.B.</i>	..	..	"O" suspensions of <i>Bact. typhosum</i> , <i>paratyphosum A</i> , and <i>paratyphosum B</i> .
<i>M.A.</i>	..	..	<i>Brucella melitensis</i> and <i>abortus</i> suspensions.

## Blood.

<i>Hgl.</i>	..	..	Haemoglobin, "Sahli" corrected (100 per cent.=116.6 on the Sahli scale).
<i>Hcrt.</i>	..	..	Approximate haematocrit determined in Wintrobe tubes. Not corrected for oxalate shrinkage.
<i>ESR.</i>	..	..	Erythrocyte sedimentation rate in mm. per hour.
<i>Urea</i>	..	..	Blood urea in mg. per 100 c.c. whole blood.
<i>Cl.</i>	..	..	Chloride as mg. per 100 c.c. plasma.
<i>Rbc.</i>	..	..	Red cell count in millions per c.mm.
<i>Wbc.</i>	..	..	White cell count per c.mm.
<i>P.</i>	..	..	Polymorphonuclear neutrophils ( <i>S.</i> segmented nuclei, <i>U.</i> unsegmented nuclei).
<i>L.</i>	..	..	Lymphocytes.
<i>M.</i>	..	..	Monocytes.
<i>E.</i>	..	..	Eosinophils.
<i>B.</i>	..	..	Basophils.
<i>B.M.</i>	..	..	Basophilic mononuclear cells (see text).
<i>S.G.</i>	..	..	Specific gravity determined by the copper sulphate method.
<i>Bl.</i>	..	..	Whole blood.
<i>Pl.</i>	..	..	Plasma.
<i>CO<sub>2</sub></i>	..	..	Total plasma CO <sub>2</sub> determined by Conway's method.

APPENDIX TABLE I  
NORMALS

	<i>Rbc.</i>	<i>Wbc.</i>	<i>Hgl.</i>	<i>Differential</i>	<i>Urea</i>	<i>S.G. Blood/Plasma</i>	<i>CO<sub>2</sub></i>	<i>Cl.</i>	<i>X19.</i>
Vi ♀	4.14	9700	90	P.S.5529 L.2910, U.291 M.679, E.291	27	1057, 1028	67.8	355	+50
Lo ♀	4.40	11950	83	—	39	1058, 1028	69.6	368.5	+50
Pel ♂	4.97	5600	83	P.S.2016 L.1344 U.1568 M.560, E.56, B.56	32	1058, 1028	68.9	374.2	+++50
De ♂	4.68	7250	81	P.S.3191 L.1812 U.1812 M.362, E.72	30	1062, 1028	65.8	362.1	-50
Pet ♂	4.73	6700	83	P.S.1755 L.2161 U.2092 M.607, E.67, B.67	39	1059, 1028	70.0	376.3	—
La ♀	4.26	5750	77	P.S.2357 L.978 U.2242 M.57, E.115	31	1057, 1028	73.1	369.2	++50
Te ♂	5.18	8550	76	P.S.4446 L.3676 U.171 E.85	24	1058, 1026	66.3	367.1	+50
Ir ♀	4.67	8700	—	P.S.4350 L.3828 U.174 M.261, B.87	36	1057, 1028	—	—	—

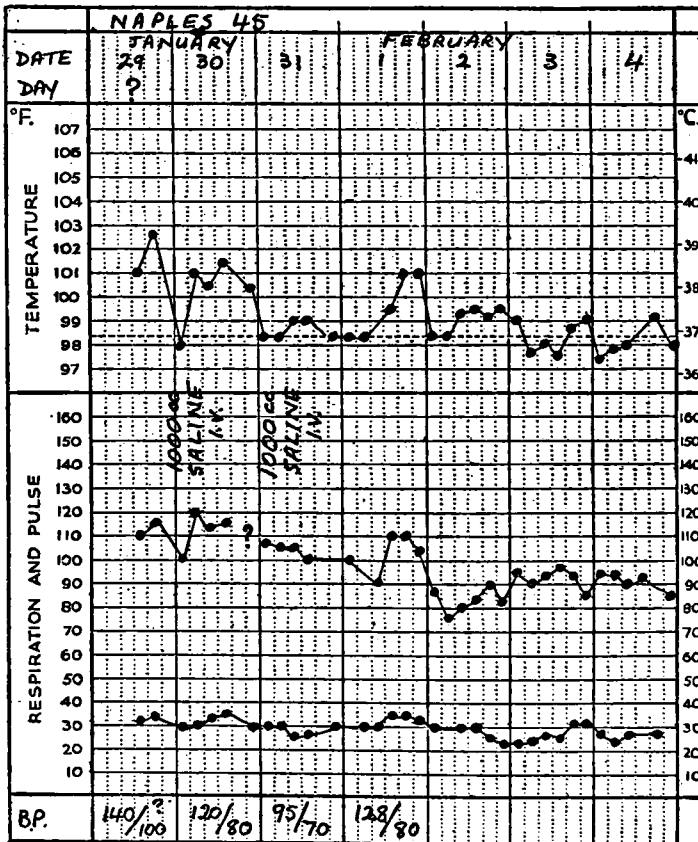
194: CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Control

Mild case.  
Recovered.

NAPLES 45.

Female, aet. 43, married, admitted on the 29th January, after an illness of indefinite duration, the details of which could not be remembered clearly. She had felt feverish and cold for at least seven days and possibly 10, and had a slight headache. The chief complaint was of difficulty in breathing, owing to a tight feeling in the chest and throat, and of soreness on swallowing. On admission, she appeared moderately ill, and dehydrated; her temperature was 101° F., her pulse 108, and respirations 30. She had difficulty in speaking, owing to extreme dryness of the tongue and buccal mucosa. She appeared clear mentally, although apprehensive. There was a faint macular rash on the trunk, and suffusion of the eyes, with a haemorrhage into the left sclera. The heart was enlarged, with apex beat 11 cm. from the mid-line in the 5th intercostal space. The first heart sound was loud; the pulse was apparently irregular, because of variation in the blood pressure with individual beats. Systolic pressure varied between 140 and 150, and diastolic between 100 and 110. The lungs appeared normal. There were irregular twitchings of the face and arms. The urine was normal. The spleen was not palpable. She was given glucose saline intravenously by slow drip, and after receiving 1,000 c.c. she appeared improved and was less restless. On the 4th day after admission she appeared amazingly better, was bright mentally and could breathe more easily, but occasional twitching of the face was still seen. Convalescence was uneventful. Fever had on no occasion exceeded 103° F., and the temperature reached normal on the 3rd day, was elevated for two readings on the 4th day, after which it remained normal.



APPENDIX TABLE II

Index No. : NAPLES 45

Control

Date	Day	Agglutinations					Blood							Animal inoculations				
		Proteus	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.			Rbc.	Wbc.	Differential	
												Bt.	Pl.					
30/1		X19 : ++800 X2 : +200 XK : +50	++180	++640	T:-25 A:-25 B:-25	M:-25 A:-25	++	31			71			3.63	10700	P.S.5778 U.2247 M.749 B.M.107	L.1819	None.
6/2		X19 : ++++320	++320	++1280			++	25			72			3.67	8850	P.S.2457 U.877 M.292, B.58	L.2164	
30/1		Blood culture : sterile.																

## 196 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### Control

*Moderately severe case.  
Recovered.*

#### NAPLES 23.

Male, aet 17, admitted on the 15th January, on the 5th day of illness which began suddenly with chilliness and pain in the back and neck. He became constipated and could not eat, coughed for three days before admission and had headache on coughing. His brother became ill at the same time, and was admitted on the same day (Naples 22). He was a very flushed, mildly ill, well-nourished boy. Temperature 104·8° F., pulse 112, respirations 24. He was mentally clear, and co-operative. There was suffusion of both eyes, and the conjunctiva of the left eye was injected. Heart was acting vigorously, pulse of good volume and B.P. 120/74. The spleen was felt one finger's breadth below the left costal margin. There was a pink macular rash, profuse on the limbs and sparse on the trunk. Twenty-four hours later, petechiae between the macules were found on both sides of the back, the left upper arm and both legs. The urine contained a light cloud of albumin but no red cells nor casts. Illness increased, and on the 7th day he was more prostrated, and complained of pain in the throat, particularly on swallowing. The neck was painful on movement and slightly rigid. The fauces appeared reddened and dry. The heart sounds, though clear, were fainter, and the B.P. 86/46. During the next three days he became more severely ill, with increasing drowsiness and periods of delirium, difficulty in swallowing and in articulation; and on the 10th day he appeared dazed and confused, with slurred, incoherent speech. He was unable to swallow, and had to be fed through a nasal tube. On the 11th day a sudden fall of temperature occurred, unaccompanied by a change in pulse rate. The fall was succeeded by a rigor and a rise of temperature to its previous level. The urine still contained a light cloud of albumin and a few hyaline and granular casts. On the next day he had improved remarkably, and was responsive, though deaf and with speech still slightly slurring. His temperature was normal on the following day. The fever had lasted for 13 days from the day of onset. Although cough and slight expectoration had occurred from time to time, he never showed abnormal physical signs in the chest. The output of urine was reduced during the febrile period, but on the 14th day urinary output was approximately equal to the day's fluid intake.

APPENDIX TABLE III

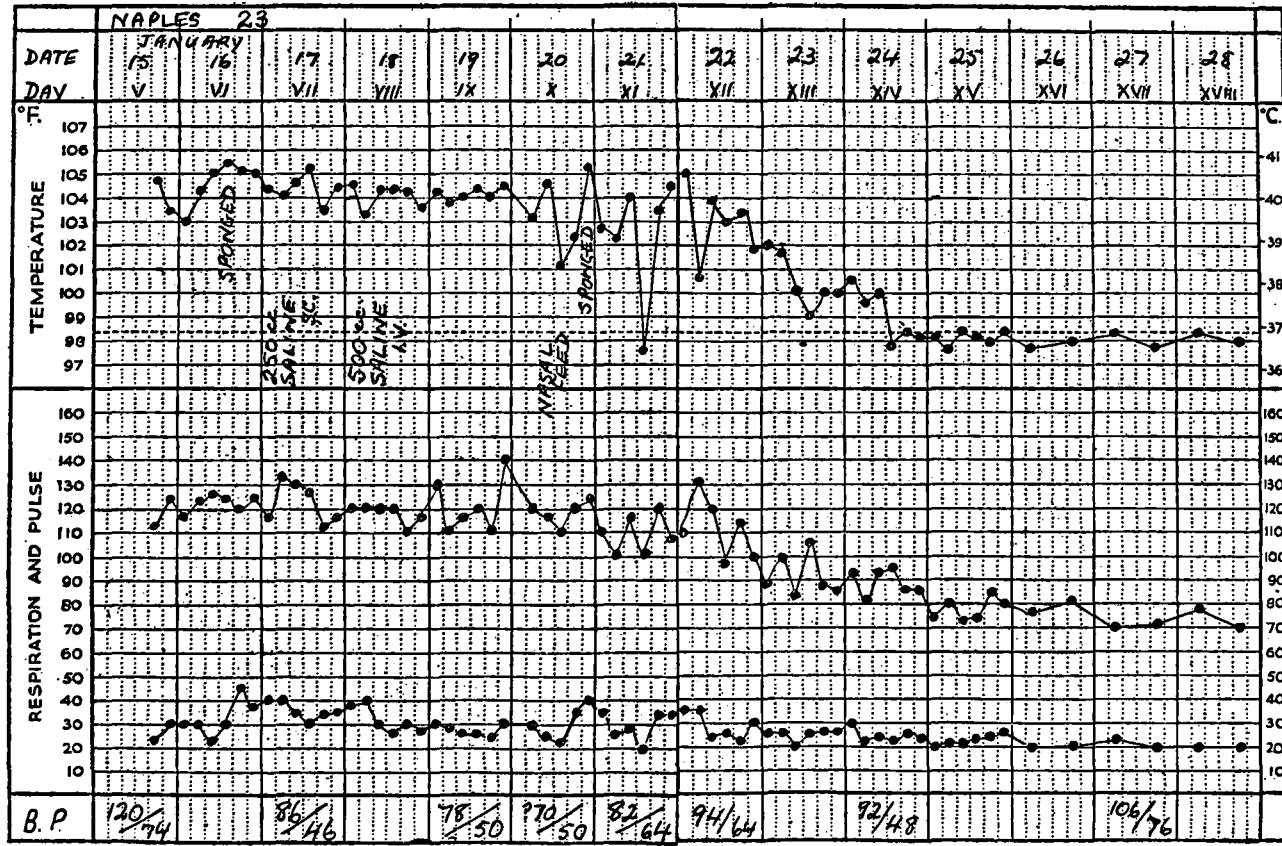
Index No. : NAPLES 23.

Control

Date	Day	Agglutinations						Blood										Animal inoculations	
		Proteus	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.		Rbc.	Wbc.	Differential			
											Bl.	Pl.							
16.1	6	X19 : +++400 X2 : +50 XK : +100	+++40	++160	T.-25 A.-26 B.-25	M.-25 A.-25	+	36							3-41	5550	P.S.1382 L.1110 U.2608 M.444, B.M.55	Two guinea pigs: whole blood intraperitoneally on 16.1.44. Brain of 6th passage intranasally to mice. Mice: 1st passage: few "inclusions." Third passage: gross pulmonary consolidation with ++ rickettsiae in smears. Strain also isolated from whole blood by a member of the U.S.A. Typhus Commission.	
20.1	10		+++80	++640											3-41	4200	P.S.2100 L.672 U.1092 M.252, B.M.84		
24.1	14	X19 : +1600 X2 : +100 XK : +100	+++160	++2560				47							3-63	5650	P.S.2655 L.1808 U.565 M.821		
30.1	20	X19 : +3200 X2 : +++50 XK : +++200	+++160	++2650			++	30							3-64	2750	P.S.880 L.1485 U.165 M.192, B.27		
4.2	25						++	25							3-56	5000			
16.1		Blood culture: sterile.																	

APPENDICES

197





**Control****ALGIERS M2.***Moderately severe case.  
Recovered.*

Male, aet. 59, docker, European (French), admitted on the 14th October, on the 8th day of illness which began with malaise, headache and fever. He did not shiver. Illness developed gradually—with aching pains in the back and limbs, cough, general tiredness, anorexia and constipation. Past history included several attacks of haematuria and pains in the loins, reputed to be due to renal calculi. On admission, temperature was 104° F., and he was considered to be gravely ill. Treatment with intravenous hypertonic saline (20 per cent.) 60 c.c. daily, with subcutaneous glucose saline (500 c.c.) daily, and with suprarenal cortical extract parenterally was given. He was first seen on 22nd October—the 16th day of illness—when he was a prostrated, seriously ill man, drowsy and slightly confused but able to give a history of his illness. A fading macular and purpuric rash was present on the trunk and limbs, including the thenar and hypothenar eminences. The eyes were very injected, and there was a large haemorrhage into the bulbar conjunctiva of the right eye. The tongue was dry but clean; the chest showed weak breath sounds and many râles at the extreme bases. The heart sounds were feeble, and the pulse was poor in volume; the radial arteries were tortuous, and the B.P. was 120/70. The liver was enlarged to one and one-half fingers' breadth below the costal margin; the spleen was not felt; the urine contained no albumin but a few red cells. The temperature fell to normal by the 19th day of disease, and he slowly became better. Oedema appeared around the ankles on the 17th day, and increased so as to involve the forearms, scrotum and legs. He was not dyspnoeic but much prostrated. The liver was still enlarged. Convalescence was complicated by slight evening rises in temperature to 100° F. or over, and he had some excess of white blood cells in the urine at this time. This infection was treated with hexamine. The total duration of fever, not including that due to the urinary tract infection, was considered to be 18 days.

The pathological findings were believed to indicate that this case was one of murine typhus, though no animal inoculations had been carried out and therefore the final proof of isolation of the causative rickettsiae was not obtained.

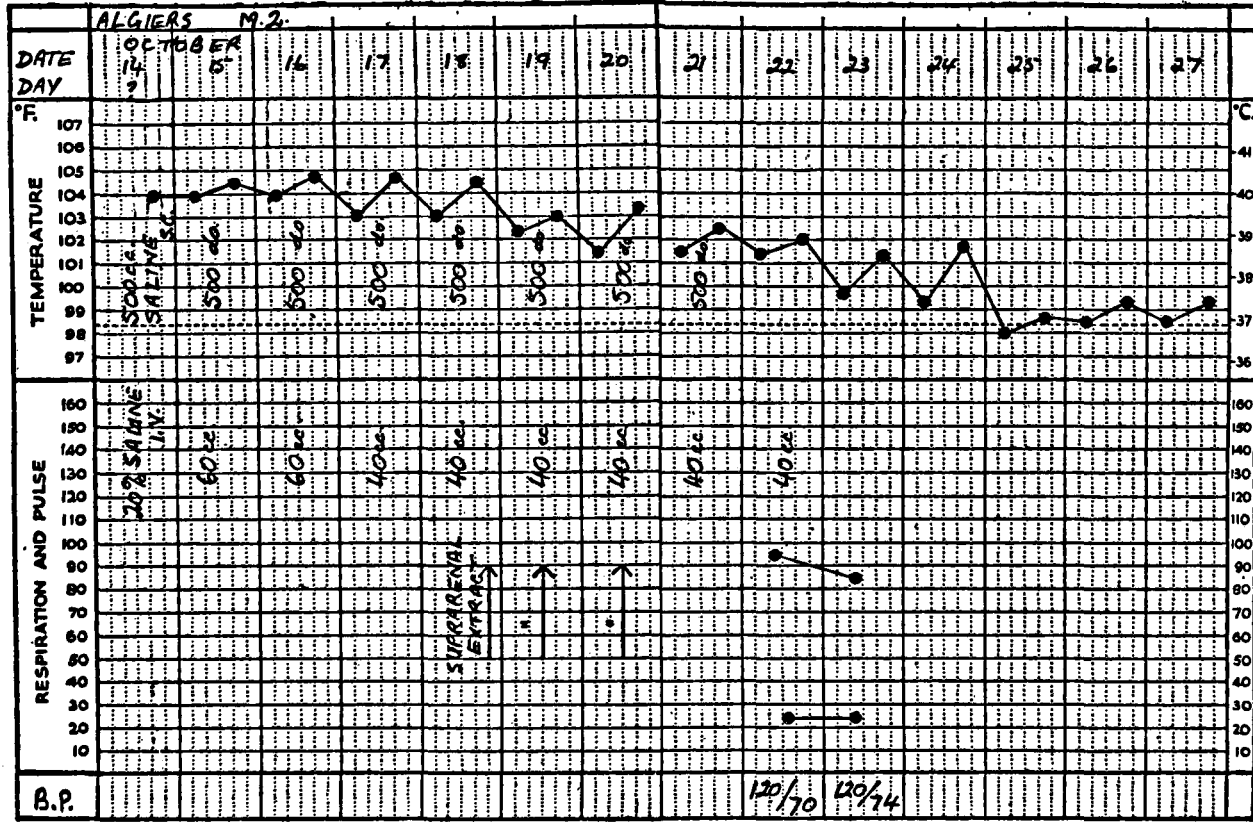
APPENDIX TABLE IV

Index No. : ALGIERS M2

Control

Date	Day of disease	Agglutinations					Blood							Animal inoculations	
		Proteus	M.	C.	T.A.B.	M.A.	Hgl.	Hcrit.	ESR.	Urea	Cl.	Rbc.	Wbc.		Differential
22.10.43	16	±12800	-3200	+++400	T.-25 A.-25 B.-25	—	57	27.5	16.5	30	337.2	2.83	7600	P S.1900 L.532 U.4788 M.152, B.152, B.M.38	None.
29.10.43	23*	±12800	tr+6400	+1600	—	—	55	28	6	20	—	2.93	5400	—	
10.11.43	35	—	—	—	—	—	57	31	17	25	364.9	3.2	6700	P S.2378 L.2278 U.1240 M.670, E.101, B.33	
16.11.43	41	±6400	±12800	+3200	—	—	72	—	—	—	—	3.56	8350	P S.2505 L.3173 U.1461 M.835, E.250, B.84, B.M.41	

\* Examination of urine showed very many pus cells.



## 202 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### Control

*Grave case.  
Died.*

#### NAPLES 43.

Male, aet. 52, fruiterer, was admitted on the 29th January, on the 4th day of illness which developed gradually with giddiness, headache, shivering and pains in the legs. He felt feverish and lost his appetite. On admission, temperature was 101° rising to 104.2° F., pulse 128, and respirations 30. He did not appear ill, was bright and cheerful, though shivering constantly. A faint macular rash was visible over the shoulders and trunk; the eyes were watery, the tongue slightly furred, and the heart was apparently normal. Although the volume of the pulse appeared to be low, the B.P. was 100/76. The lungs were clear; the spleen was just palpable on inspiration. The neck was not stiff. The urine contained a light cloud of albumin and very occasional red cells. The temperature remained between 103° and 105° F. during the next day, and by the 6th day he was very ill, prostrated and tremulous, with slight rigidity of the limbs. There was anuria at this time (see below). He was responsive, though somewhat deaf. A tinge of icterus was present in the conjunctivae. There were râles at both lung bases and rhonchi anteriorly. B.P. was 74/60 and heart sounds were faint. Spleen was enlarged to one finger's breadth below the costal margin. The rash was still present and, in addition, a few doubtful petechiae were seen in the slightly oedematous folds of skin on the back. Tendon reflexes were exaggerated in the limbs, but plantar responses were flexor. On the 7th day he was drowsy with laboured respirations, muscles of the arms were twitching and the B.P. was 65/45. During that day disorientation and a muttering delirium developed. On the 8th day he was breathing stertorously and became comatose, and he died during the afternoon. The output of urine had never been at normal levels, and no urine could be obtained on the 6th day, even by catheter. In addition to nasal feeds and fluids by mouth in small quantity, he was given intravenous glucose saline daily in 500 c.c. quantities or more. Excretion of urine recommenced on the 7th day, some ten ounces of urine being passed on the day preceding death. Fluid balance and biochemical findings are shown in Fig. 23, p. 110 above.

### *Post-mortem Findings*

#### *Naples 43.*

*(Died 3.30 p.m., 2nd February, 1944. P.M., 5.30 p.m., 2nd February, 1944.)*

The body was that of a rather poorly nourished adult male of small stature. Rigor mortis was absent and the body was still warm at the time of autopsy. A few very tiny haemorrhages were present in the palpebral conjunctivae.

#### *Rash.*

Not visible at autopsy. (The autopsy was performed in the evening with the aid of torchlight only.)

#### *Respiratory.*

The larynx was normal. Some congestion was present in the lower part of the trachea, which contained a small amount of very tenacious muco-pus. A few delicate adhesions were present over the apex of the right lung. No free fluid was present in the pleural cavities. The lungs were normal in texture, and there was no evidence of oedema. Anthracotic pigmentation of the lungs and bronchial lymph nodes was well marked. No bronchiolitis was seen at autopsy.

#### *Cardiovascular.*

The pericardium was normal. The heart was of approximately normal size. The right auricle and ventricle were greatly distended, but the left ventricle was firmly contracted. A large milk spot was present over the base of the left ventricle. The myocardium was pale in the deeper layers, especially on the posterior aspect of the right ventricle. The pallor had an indefinite patchy distribution. The valves and coronary arteries were normal. The aorta showed fatty streaking and slight early atheroma.

#### *Gastro-intestinal.*

The oesophagus, stomach and intestines were normal. There was moderate discrete enlargement of the mesenteric lymph glands. The spleen was two or three times its normal size (wt. 340 gm.). It was soft in consistency. The capsule was thickened and wrinkled, with dense adhesions over a very hard nodular scar near the upper pole. On the cut surface the splenic pulp was soft, dark red in colour and bulging slightly, but not easily scraped away on the knife. Malpighian bodies and trabeculae were obscured.

The liver was normal, apart from a slight degree of acute passive congestion.

#### *Genito-urinary.*

The kidneys were normal in size and texture (wt. 240 gm.). The capsule stripped with ease, leaving a smooth surface with no obvious abnormality. On the cut surface the radial markings were prominent and the demarcation between cortex and medulla pronounced. The apices of the pyramids were pale.

Ureters and bladder were normal. The bladder contained about 100 c.c. of clear urine. *Supravrenals* were normal.

*Brain.*

Slight oedema of the meninges was seen. The pia-arachnoid over the superior and lateral aspects of the frontal and parietal lobes was more than usually opaque, and with small nodular thickenings along the sulci. The pia-arachnoid in this area was almost gelatinous in appearance, and the dura lightly adherent to it. Superficial vessels were slightly engorged. No other naked eye abnormality could be seen in the brain.

Specimen of urine taken at autopsy showed clear straw colour; albumin—trace, sugar—nil. Microscopically—oxalate crystals only.

Tissues were kept for section in 10 per cent. formol-saline and in Zenker's fixative.

*Histology**Naples 43.**Conjunctivae.*

The submucous layer contained many large mononuclears and plasma cells, with a tendency to accumulate into fairly compact masses round the blood vessels. Some of the vessels showed swelling of the endothelium, and small thrombi. Marked congestion was present, but there were very few haemorrhages.

*Respiratory.*

The lungs showed congestion and some vesicular emphysema only.

*Cardiovascular.*

The heart muscle showed little swelling of the interstitial connective tissue, but muscle fibres were separated by densely packed linear collections of mononuclear cells. The muscle fibres showed irregularity in intensity of staining, but very little degenerative change. In some sections, cellular exudate was closely related to blood vessels in which the endothelial layer was swollen. Small areas of old-standing fibrosis were present.

The aorta showed the usual round cell infiltration round the vasa vasorum, involving small areas in the outer layer of the media. Early atheromatous changes were present in the intima.

*Gastro-intestinal.*

The stomach showed no histological abnormality. The intestines were not examined.

The *liver* showed mild congestion, and striking cellular infiltration of the portal tracts. There was a slight increase in the connective tissue of the portal tracts.

The *spleen* showed marked congestion and hyperplasia of the pulp. Malpighian bodies were small and the trabeculae obscured.

The *suprarenals* showed congestion of the subcapsular zones, and a little focal degenerative change in the parenchyma. Few nodular accumulations of lymphocytes were present.

*Genito-urinary.*

A moderate degree of congestion was present in the kidneys, most marked in the medulla, where also fairly numerous haemorrhages into interstitial tissues and tubules were seen. Foci of cellular infiltration occurred throughout the kidneys.

The glomeruli were slightly enlarged but without cellular increase. Endothelial cells were only slightly swollen, and a few pyknotic nuclei were present in some of the glomeruli. Aniline blue staining demonstrated a patency of all the capillaries, some of which were congested or simply distended. Extravasated blood or coagulated albuminous material was present in some of the capsular spaces. Slight focal thickening of the basement membrane was frequent, but there was no intra-capillary fibril formation. A few sclerosed glomeruli were surrounded by areas of fibrosis.

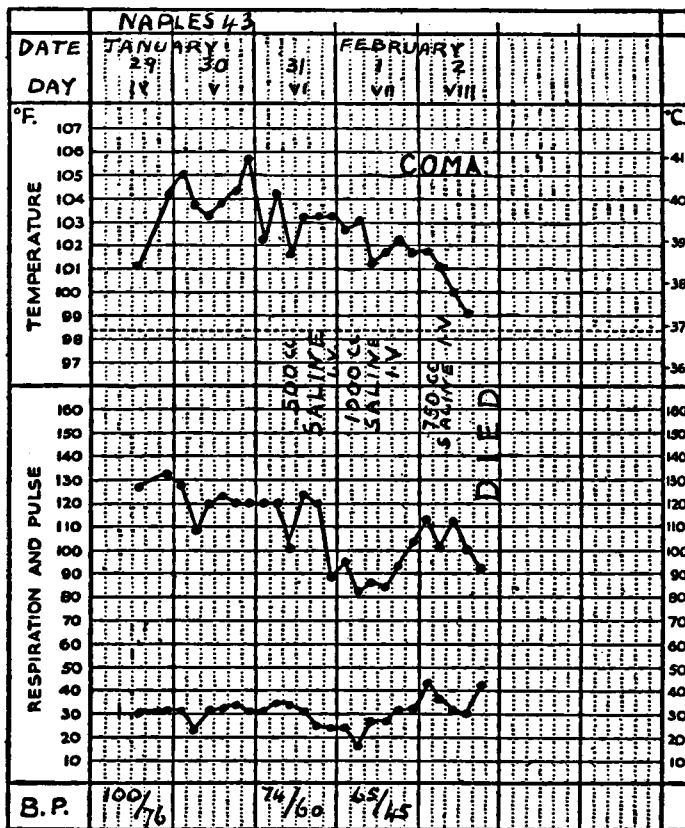
The proximal and distal convoluted tubules near the kidney surface were slightly dilated. In the deeper layers the tubular epithelium showed evidence of cloudy swelling and occasional small foci of necrosis. Albuminous material and a few hyaline casts were found in the tubules and in the medulla.

Some of the medium-sized arteries in the junctional zone showed moderate focal intimal thickening.

*Brain.*

Very few nodular lesions were seen, although at least four sections from each of the usual sites were examined. No such lesions were seen in the medulla, midbrain or basal ganglia, and only a few in the pons, cerebellum and cerebral cortex. Vascular lesions, on the other hand, were more prominent than usual. There were many small haemorrhages—some apparently into foci of necrotic brain, others into the Virchow-Robin space, and several vessels were encountered in which there appeared to be endothelial thickening or even destructive changes in the vessel walls, with superimposed thrombi. The pia-arachnoid over the occipital cortex was thickened and sclerotic. In other areas the meninges showed diffuse mononuclear cell infiltration.





## Control

NAPLES 66.

*Grave case.  
Recovered.*

Female, aet 55, married, admitted on the 11th February, on the 8th day of illness which began with shivering. She developed persistent headache, photophobia, pains in the back and limbs, a non-productive cough and constipation. She vomited a number of times but did not lose her appetite. Temperature on admission was 100.2° F., rising to 103° F., pulse 96, rising to 120, respirations 20. She appeared weak, was mentally clear, and showed neither cyanosis nor dyspnoea. A fine haemorrhagic rash was sparsely scattered over the trunk and limbs. Eyes were injected and the lower lids engorged. The tongue was dry and brown. No abnormality was found on auscultation of the chest. The heart's action appeared to be normal; the pulse was of good volume and the B.P. 75/60. The spleen was enlarged to one finger's breadth below the costal margin. Reflexes were normal. The urine contained a light cloud of albumin without any abnormal formed elements. She became drowsy and prostrated, with remittent fever, exceptionally low-blood pressure (55/45); and she passed little urine although taking fluids well. On the 10th day she seemed weak and for a time had periodic breathing. The amount of urine excreted increased on this day, and she became incontinent. During the next two days, the temperature fell; she developed abdominal distension, and retention of urine; and her respiration became grunting, though abnormal signs were not found in the lungs. Large volumes of urine were obtained by catheter daily. Urine samples contained only traces of albumin and few casts. On the 14th day the temperature again rose, and pyrexia continued irregularly for two further days; a bedsore appeared over the sacrum, she was incontinent of faeces and urine, and her general condition deteriorated. Large amounts of glucose saline were now given intravenously by slow drip, and her general condition and blood pressure improved a little, though the bedsore extended. She was afebrile on and after the 16th day and was excreting large volumes of urine, but on the 19th day she passed thick dirty brown urine, containing mucus, many red cells, pus, epithelial cells and bacteria, and had pain in the suprapubic region. Hexamine, 1 gm. three times a day, was given for two days; and then sulphathiazole 1 gm. six-hourly for two days, followed by 1½ gm. six hourly for a further seven days. She received in all a total of 43 gm. of sulphathiazole. The urinary infection slowly cleared, and the bedsore healed. She was very weak and fainted once during convalescence, but was quite well by the 33rd day of illness. The total duration of fever was 15 days. There were several days after the termination of fever when the daily output of urine equalled the daily intake of fluids. Fluid balance and biochemical findings are recorded in Fig. 12, p. 104, above.



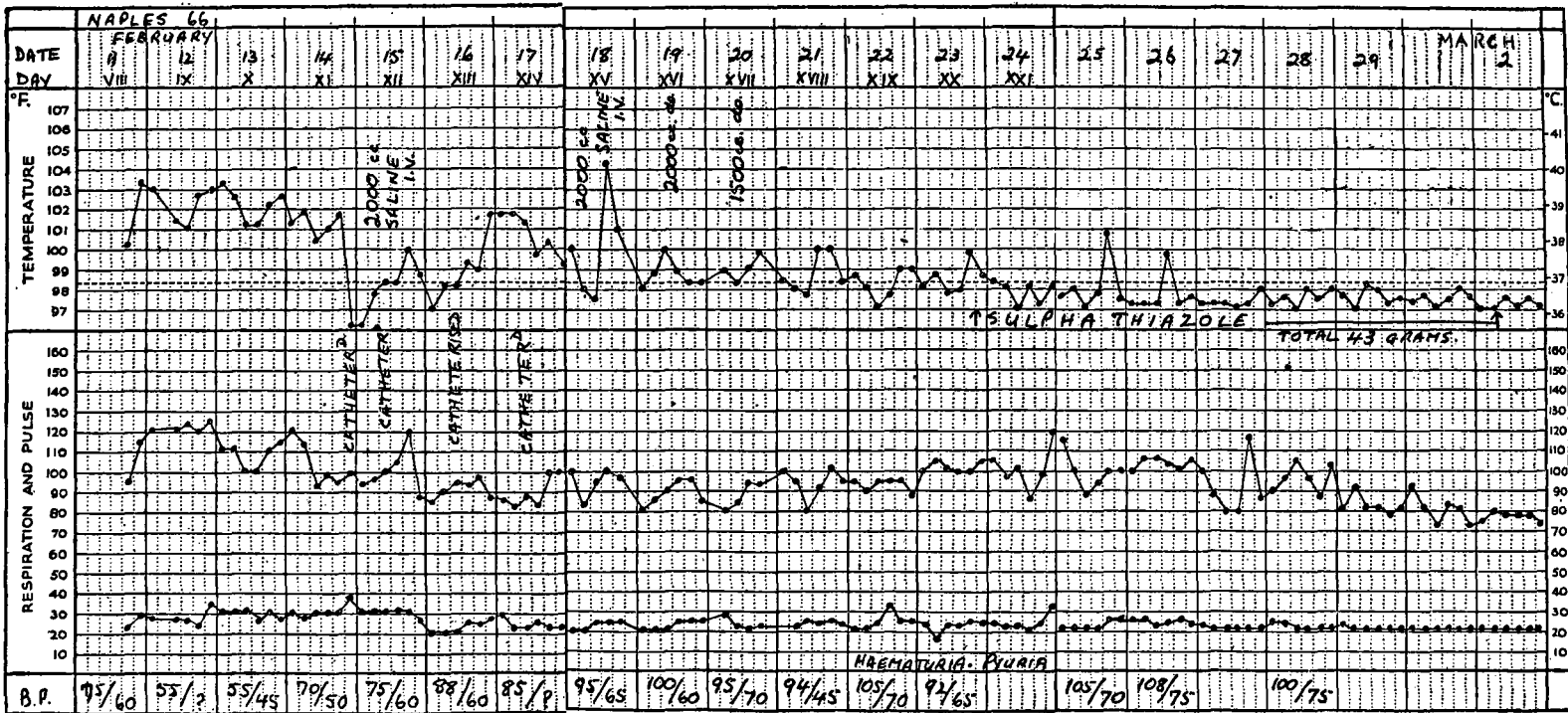
APPENDIX TABLE VI

Index No. : NAPLES 66

Control

Date	Day	Agglutinations						Blood								Animal inoculations	
		Proteus	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.		Rbc.	Wbc.		Differential
												Bl.	Pl.				
12.2	10	X19: +++400 X2: ++200 XK: +200	-20	++160	T.-25 A.-25 B.-25	M.-25 A.-25	++	83	280.5	62.4	72			3-32	8750	P.S.2450 U.4200 L.1225 M.175, E.O, B.O B.M.700	None.
18.2	11						62	275.5									
14.2	12						149	269.1				1048	1023				
15.2	13	X19: ++640	+80	+2560			164	281.2				1050	1023				
16.2	14						108	294.7				1047	1021.5				
18.2	16	X19: +800 X2: +400 XK: ++80	+160	++5120			++	104	312.1	58.6	60	1048	1025	3-20	8800		
21.2	19							86	353.6	63.7		1046	1024				
24.2	22							37	361.4	60.8		1045	1025				
27.2	25	X19: +200 X2: +100 XK: +80	+++40	+++840			-										
29.2	27							86	340.8	68.4		1045					

APPENDICES



## Control

*Grave case.  
Died.*

## NAPLES 77.

Male, aet. 20, shoemaker, admitted on the 19th February on the 9th day of an illness which began suddenly with headache, and pains in the body and legs. He shivered and became feverish and felt steadily worse each day. He complained also of dizziness and photophobia. A cough and deafness developed shortly before admission. He vomited once and had epistaxis once. He slept poorly. Temperature on admission was 104° F., pulse 116, respirations 28. He was prostrated, slightly cyanosed but mentally clear. There were blotchy macules over the arms and trunk. The eyes were heavily injected, the tongue moist and clean. Lymph glands in the axillae and epitrochlear regions were palpable. A few rhonchi were heard on auscultation of the chest. The heart was normal in size, the sounds distinct but diminished in intensity. The pulse was regular, rapid and soft, and the systolic B.P. was 92; the diastolic pressure could not be recorded. The abdomen, reflexes, and urine were normal. For the next four days high fever and tachycardia continued; he became drowsy and more gravely ill, though remaining rational. The rash became more evident but was not haemorrhagic; râles became audible in the chest, and there was persistent cough. The blood pressure fell to 65/30 on the 13th day, and the urine output on the day prior to this was 700 c.c., although fluid intake was 2,800 c.c. There was mild creasing of the skin. During the morning of the 14th day, the temperature fell sharply to 98.4° F. and then rose again to 104° F.; the pulse fell to 86 and then rose to 160. He became somewhat collapsed. The heart had an irregular apical impulse, and a third heart sound of questionable origin was audible; the B.P. was 76/40. He became very restless and orthopnoeic, and by the evening the apical cardiac impulse was in the anterior axillary line. Fine tracheal râles and asthmatic rhonchi could be heard in the lungs. Morphine, gr.  $\frac{1}{4}$  to  $\frac{1}{2}$ , was given twice daily to control the restlessness. He was, however, found out of bed trying to urinate on the 15th day, when his condition had improved only slightly. He was, therefore, catheterised regularly. The heart was still dilated, and the sounds, which were easily heard, had a well-marked presystolic gallop rhythm. There was tenderness over the right costal margin, but the liver edge could not be felt. Slight oedema over the sacrum developed during the day, and the lungs appeared to become increasingly oedematous. During the next two days, but for a diminution in the fever, the condition remained unaltered, and orthopnoea continued. The pulse volume was poor and the systolic pressure variable. Some oedema of the feet appeared on the 17th day. On the 19th day his condition was bad, cyanosis was marked, respirations were laboured and jerky, the cardiac dullness extended 4 cm. to the right of the sternum and the left border of the heart was 9 cm. from the midline. The liver edge could not be palpated, but oedema of the feet and sacrum persisted. He died that evening, the 19th day of the disease. The urinary output was well maintained from the 14th day onwards, and the urine was normal on the 16th and 17th days. "Mersalyl," 1 c.c., was given 24 hours before death, but there was no resultant diuresis.

*Post-mortem Findings*

## Naples 77.

(Died 10.20 p.m., 28th February, 1944. P.M. 10 a.m., 29th February, 1944.)

The body was that of a well-developed young adult male. Rigor mortis was present in all limbs. Moderate hypostatic congestion was present. There was a superficial pressure sore about 2 in. in diameter over the left side of the sacrum. Pitting oedema of the feet extended up to the ankles.

*Rash.*

A few purplish lesions were seen on the anterior aspect of the trunk. No haemorrhages were present in the conjunctivae.

*Respiratory.*

A few petechiae were present in the mucous membrane of the larynx, and the vocal cords were congested. A little congestion of the trachea was seen and the lumen contained a small amount of mucopurulent secretion. The pleural cavities were normal.

The posterior parts of the lungs were of rubbery consistency and the pleural surface over these areas had a misty ground-glass appearance. There were a few nodules of consolidation about the size of walnuts in the anterior portions of the lungs, especially the right. On section the posterior aspects of the lungs showed marked congestion and some oedema. There was a faint granularity in appearance, and the consistency was firmer than could be accounted for simply by congestion and oedema. Mucopus exuded from the bronchioles.

## 210 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### *Cardiovascular.*

The heart, both on the right and left sides, was enormously dilated; its weight was 360 gm. It extended from a point about half-an-inch to the right of the sternum to beyond the left nipple. The pericardium was normal and contained approximately the normal amount of fluid. The heart muscle was very flabby and pale. The interventricular septum and the papillary muscles, showed a well-marked characteristic thrush breast appearance of fatty degeneration, and a few irregular purplish streaks. The endocardium as a whole was pale. The valves and coronary arteries were normal. The aorta showed fatty streaking in the abdominal part only.

### *Gastro-intestinal.*

The oesophagus was normal. The stomach contained a number of roundworms; the mucous membrane on the posterior surface, especially around the lesser curvature, showed numerous superficial haemorrhages and one or two definite erosions. The intestines were normal. A fairly large number of lymph glands enlarged to the size of walnuts, discrete and firm rubbery in consistency, were found in the mesentery.

The *spleen* weighed 195 gm., was firm in consistency and showed a few fibrinous flecks of capsular thickening. The cut surface was firm and revealed some hyperplasia of the Malpighian bodies. The pulp was a little congested and the trabeculae obscured.

The *pancreas* was normal.

The *liver* was normal in size and pale in colour. Shining through the capsule were patches in which the haemorrhagic congested zones contrasted sharply with the pale remainder of the organ. On the cut surface the liver was pale, due to fatty change, but with the central zones of the lobules congested.

The gall bladder and bile ducts were normal.

### *Genito-urinary.*

The kidneys weighed 355 gm. The capsules stripped easily, leaving a smooth shiny outer surface studded with discrete petechial haemorrhages. The petechiae were larger and more discrete than had been seen in previous autopsies. The cut surface of the right kidney was pale and the glomeruli were prominent; the medulla, especially in the junctional zone, was congested, and the radial markings more prominent than usual. The left kidney was rather more congested even in the cortex.

A few petechiae were present in the renal pelvises.

The ureters were normal. The bladder contained about 8 oz. of clear urine. The mucous membrane of the trigone was congested. Slightly milky fluid exuded from the posterior urethra.

*Testes* showed no gross abnormality.

### *Brain.*

Venous engorgement of the meninges was pronounced, but there was no oedema. Definite petechiae were seen in the peduncles and in the white matter of the cerebrum.

Tissues were kept for section in 10 per cent formol-saline and in Bouin's and Zenker's fixatives.

## *Histology*

### *Naples 77.*

#### *Respiratory.*

The lungs showed very extensive congestion and oedema, with many large mononuclear phagocytic cells (heart-failure cells) in the alveolar spaces. One medium-sized vessel was partially occluded by a mural thrombus. Some of the sections showed bronchopneumonic patches at various stages—the exudate being predominantly mononuclear in some areas and fibrino-purulent in others.

#### *Cardiovascular.*

The heart muscle showed little swelling of the interstitial connective tissue and very scanty cellular infiltration. Degenerative changes, on the other hand, were more extensive. In addition to small foci of degeneration and necrosis involving fragments of single fibres, there was a large area, visible to the naked eye, in the papillary muscles which had the appearance of Zenker's hyaline necrosis. This may have been ischaemic in origin. Small areas of fatty degeneration, especially in the inner layers, were present.

The aorta showed accumulations of lymphocytes round the vasa vasorum in the adventitia, and there was slight fragmentation of the elastic lamina of the media.

#### *Gastro-intestinal.*

The stomach showed marked congestion of the submucosa and the tips of the mucosal folds. There was focal infiltration of mononuclear cells, and a number of superficial erosions were present.

The intestines were not examined.

The *liver* showed extensive fatty degeneration and haemorrhagic focal necrosis of the central zones of the liver lobules. Slight lymphocytic infiltration of the portal tracts was present.

The *spleen* was congested, and the pulp cells showed an increase in mononuclear cells. There was a thin layer of haemorrhagic exudate on the surface of the spleen.

The *pancreas* showed no histological abnormality.

The *suprarenals* showed small focal areas of degeneration in the cortical parenchyma. Haemorrhages were not seen.

*Lymph glands*: The sinusoids were dilated and loosely packed with large pale mononuclear phagocytic cells. Germinal centres were small.

#### *Genito-urinary.*

The medulla of the kidneys was moderately, and the cortex slightly congested. Occasional small haemorrhages into the tissue spaces and tubules occurred throughout the organs. Foci of cellular infiltration were scanty.

The glomeruli were enlarged, a few almost filling Bowman's capsule. Abundant coagulated albuminous material was present in the capsular spaces. The enlargement was due to generalised rounded patency with engorgement of capillary loops. Erythrocytes were occasionally found in the capsular space, and in some instances actual rupture of a glomerular capillary was seen. The basement membrane of the tufts was not thickened, and some of the endothelial cells showed slight swelling.

Near the surface of the kidney the proximal convoluted tubules were slightly dilated; more deeply, swollen epithelial cells partially obliterated the lumina. All the tubules showed cloudy swelling to a varying degree, and occasionally small groups of epithelial cells were necrotic. The distal convoluted tubules and ascending limbs of Henle's loop were dilated, but showed less evidence of degeneration than the proximal part of the nephron. Coagulated albuminous material was present in the medullary zone.

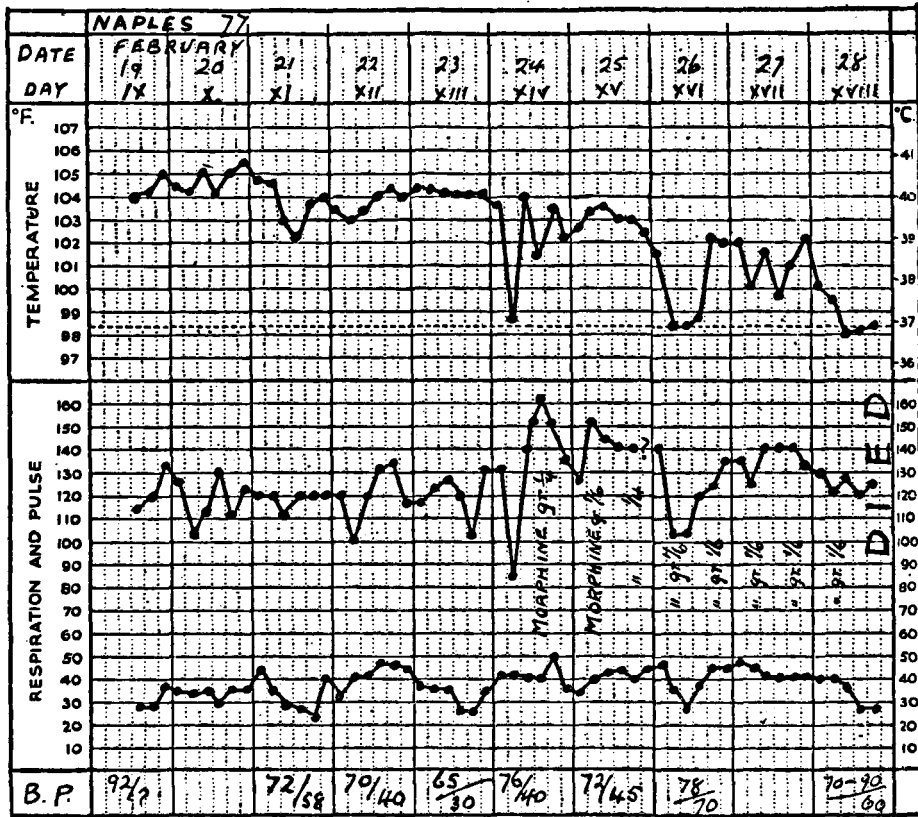
A few of the arterioles showed slight hyaline thickening of the intima.

#### *Brain.*

A moderate number of nodular lesions was seen. Some of these were compact aggregations of 6 to 8 or more cells. In others the cells were more diffuse and there was evidence of necrosis in the centres of the nodules. Fairly large pale cells, as well as smaller ones with dense pyknotic nuclei, were present. Occasionally, polymorphonuclears were present in the lesions. An occasional lesion was encountered in which haemorrhage was the predominant feature. Sometimes the haemorrhage and cell infiltration occupied a perivascular situation. The lesions were more numerous in the brain stem (medulla, pons and midbrain) than elsewhere, and the sites of election appeared to be the grey matter and the subependymal layers.

Congestion and slight round cell infiltration of the meninges were present.





214 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

Treated with V186

NAPLES 42.

Mild case.  
Recovered.

Female, aet 36, married, with three children, admitted on the 27th January, on the 4th day of an illness which was detected because her sisters' families had both developed typhus. Although feeling well, she was afraid that she also would get the disease, and she took her temperature on the night of the 23rd January and found it to be 38° C. (100·4° F.). She felt well next day, but took to bed on the 25th on account of menstruation. On the day before admission, she again took her temperature, and found it to be 39° C. (102·2° F.); and on this day she shivered, had a slight cough and pain in the chest. She could not sleep during this period, because she was worried and afraid. Temperature was 103·4° F. on admission, pulse 120 and respirations 26. She did not appear ill, though she was anxious and alert. A few indefinite macules were seen on the back, where there were also scars and pigmented areas from acne and scabies. Eyes were glistening, tongue moist with slight fur. A few rhonchi were heard at the lung bases; the heart was rapid but not otherwise abnormal, and B.P. was 124/70. The abdomen and reflexes were normal. The urine contained a slight trace of albumin, but no casts. Treatment with V186 was begun at once. It was administered orally at two-hourly intervals. She remained febrile for three days, with remittent fever and irregular tachycardia, but did not complain of any discomfort. She took fluids well, but the urinary output during the first two days was low in relation to the fluid intake. After the second day, good urinary excretion was maintained. She was convalescent on the 8th day, the duration of fever having been seven days from the onset. She received 116·5 gm. of V186 in seven days. Sodium bicarbonate in 4-gm. doses was given occasionally with the V186, on the 6th and 7th days.

Dosage of V186

Date	Quantity (grams)	Interval	Daily total (grams)	Grand total (grams)
27th January, 1800 hours .. ..	4	Two-hourly	} 8·5	} 116·5
2000 hours .. ..	3	Two-hourly		
2200 hours .. ..	1½	Two-hourly		
28th January .. ..	1½	Two-hourly	18	
29th January .. ..	2	Two-hourly	24	
30th January .. ..	2	Two-hourly	24	
31st January .. ..	2	Two-hourly	24	
1st February .. ..	2	Four-hourly	12	
2nd February .. ..	2	Four-hourly	6	



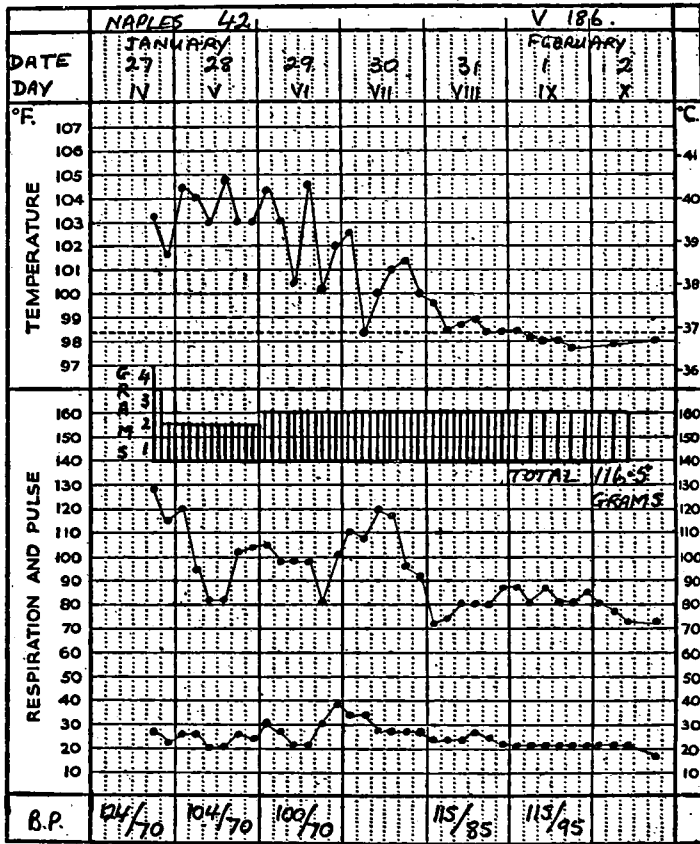
APPENDIX TABLE VIII

Index No. : NAPLES 42

Treated with V186

Date	Day	Agglutinations						Blood							Animal inoculations			
		Proteus	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.		Rbc.		Wbc.	Differential	
												Bl.	PL					
28.1	8	X19: ++1600 X2: +100 KK: +50	-20	+++160	T.-25 A.-25 B.-25	M.-25 A.-25	++	40				74			4-21	9250	P S.5272 L.1665 U.1480	Two guinea-pigs: whole blood intraperitoneally, 28.1.44. Brain of seventh passage intranasally to mice. Mice: first passage: few "inclusions" in lung smears. Fifth passage: moderate numbers (+) of "inclusions" and rickettsiae in smears. No scrotal reactions were seen in the guinea-pigs.
21.2	10	X19: ++800 X2: +100 KK: +100	+++640	+2560				53				76			3-49	10900	P S.6431 L.1853 U.1306 M.891, B.218, E.M.109	
8.2	16	X19: +800 X2: +++50 KK: +++50	+++640				+	41				82			4-15	11650	P S.8176 L.2214 U.2097 M.815, E.116, B.232	

216 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS



## Treated with V186

Moderately severe case.  
Recovered.

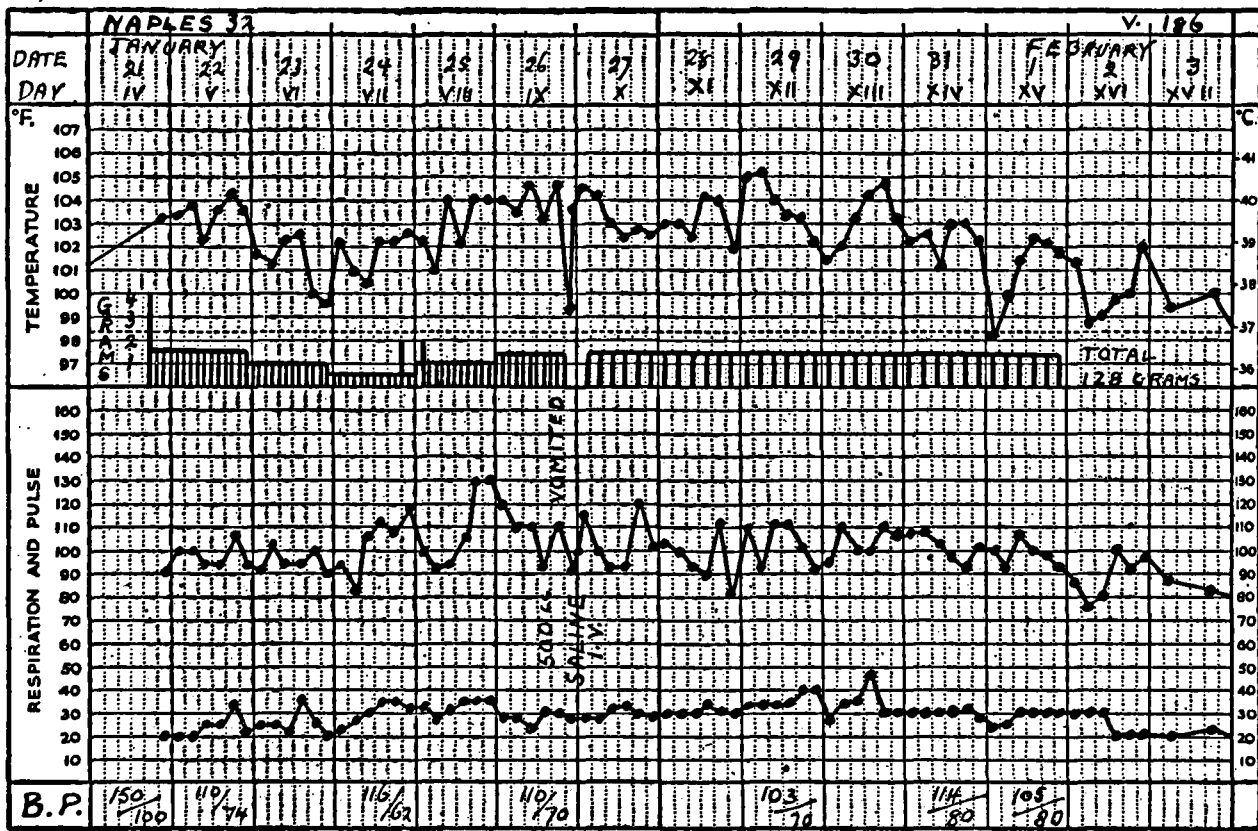
## NAPLES 32.

Female, aet. 20, admitted on the 21st January, on the 4th day of illness which began suddenly with intense headache and shivering. Her temperature rose to 104° F., she had pains in the back and legs, lost her appetite and was constipated. On admission, her temperature was 103.2° F., her pulse 92, and respirations 20. She was an adipose, alert, intelligent girl, who did not look ill and was mentally clear. There were about 12 discrete petechial haemorrhages on the abdomen and chest, and no macules were visible. The eyes were clear, tongue rather dry and furred. The chest was normal, and the spleen not palpable. The heart sounds were normal, the pulse good in volume, the B.P. 150/100. Urine was normal, of specific gravity 1027. There was no neck rigidity. V186 was given by the mouth every two hours. The patient was co-operative, and the drug, as well as adequate amounts of fluid, was taken well. On the 5th day, there was transient blotchy erythema on the trunk but no fresh haemorrhages; the spleen was palpable two fingers' breadth below the costal margin; headache was severe. The temperature fell gradually on the following day, and the dosage of the drug was reduced. From midday on the 7th day the temperature rose steadily; it reached a height of 104.6° F. on the 9th day, and on the evening of this day she appeared very ill, with severe headache, restlessness and mild delirium. A sharp remission of fever to 99.4° F. was accompanied by vomiting and signs of collapse, but improvement followed administration of intravenous fluid. The dosage of V186 had been steadily increased in the two days preceding this event, to 1½ gm. two-hourly; the drug was withheld for four hours during collapse, but treatment was then recommenced with four-hourly dosage. On the 12th day, the temperature reached 105° F. for the first time, and she still had severe headache. A small petechial haemorrhage was seen in the conjunctiva of the left eye, but no other haemorrhages were found. Fever was remittent for the next four days, and she became apyrexial on the 17th day of illness. The clinical condition varied considerably during the last few days of pyrexia, with periods of intense headache, depression and reluctance to drink; but no further vomiting or apparent collapse occurred. She made a rapid recovery as soon as the pyrexia terminated. She received a total of 128 gm. of V186 during a period of 11 days. At times the output of urine was low, falling on one day to one-seventh of the daily fluid intake; but she did not develop albuminuria, and only a few granular casts were found in the urine on the 14th day. The blood pressure never fell to a very low level. The blood urea remained within normal limits throughout the illness. Diuresis had not occurred up to the 15th day, when measurement was discontinued. The vomiting and collapse on the 9th day, following as they did a period of intensive dosage with V186, were considered probably to be due to a toxic effect of the drug.

## Dosage of V186

Date	Quantity (grams)	Interval	Daily total (grams)	Grand total (grams)
21st January .. .. .	4	Two-hourly	7	128
22nd January .. .. .	1½	Two-hourly		
23rd January .. .. .	1½	Two-hourly		
24th January .. .. .	1	Two-hourly		
	↓		7.5	
	(Extra dose 1½ g. at 20.00 hrs.)			
25th January .. .. .	1	Two-hourly	13	
	(Extra dose 1 g. at 02.00 hrs.)			
26th January until 20.00 hours	1½	Two-hourly	16.5	
27th January .. .. .	1½	Four-hourly	9	
28th January .. .. .	1½	Four-hourly	9	
29th January .. .. .	1½	Four-hourly	9	
30th January .. .. .	1½	Four-hourly	9	
31st January .. .. .	1½	Four-hourly	9	
1st February .. .. .	1½	Four-hourly	9	





## Treated with V186

## ALGIERS EK13.

*Grave case.  
Recovered.*

Male, aet 21, waiter, Arab, admitted on the 14th October, on the 7th day of illness which began suddenly with headache and shivering. He slept poorly, developed aching pains in the shoulders, knees, feet and back, and had a severe frontal headache. He lost his appetite, had some pain in the epigastrium and was constipated. Vomiting occurred each day from the onset. There was cough, with pain in the region of the larynx on coughing. On admission, the temperature was 103·8° F., rising to 104° F., pulse 104, and respirations 30. He was mentally clear, but appeared anxious, slightly prostrated and dyspnoeic. The face was flushed and slightly bloated. A rose-pink macular eruption was present on trunk and limbs. Eyes were slightly injected, tongue was dry but covered with white fur; glands were felt in the axillae and epitrochlear regions; the chest had generalised rhonchi, and impaired percussion over the left base, where the scar of an old knife-wound was located. Heart sounds could not be heard, owing to the rhonchi; pulse was fair in volume, and B.P. 108/64. The liver was enlarged to one finger's breadth below the costal margin; the spleen was similarly enlarged. Reflexes were slightly diminished in the legs. The urine had a specific gravity of 1020, contained no albumin but a few hyaline casts. Treatment with V186 was instituted on the 15th October, with rather bigger doses than in previous cases, and was continued four-hourly until the morning of the 19th October, by which time he had received 86 gm. Fever, somewhat remittent in type, continued; he became more prostrated and ill; the tongue was very dry, and protruded with difficulty; and although the rash had faded on the trunk, a large haemorrhage appeared in the bulbar conjunctiva of the left eye. He was more dyspnoeic, with deep, slow respirations. On the evening of the 11th day, he vomited after the V186, and continued to vomit each time he was given the drug. The V186 was stopped on the 12th day, when the temperature fell sharply. He continued to vomit throughout the day, and glucose saline was given both subcutaneously and intravenously, together with 20 per cent. (hypertonic) saline intravenously in 20 c.c. volume. The urine output on this day fell to zero, and, on catheterisation, only a few drops of urine, with a heavy cloud of albumin and many granular and epithelial casts, was obtained. For the next six days he remained desperately ill, could take no fluid by mouth without vomiting, and began to have liquid stools, which on the 17th and 18th days showed melaena. The blood urea had now reached very high values (600 mg. per 100 c.c.), but the urinary output slowly rose, and it reached 2000 c.c. per diem from the 16th day onwards. The urine still contained much albumin and many casts. Fluid was given by rectal, subcutaneous and intravenous routes, and administration of hypertonic saline intravenously was repeated. In spite of this, he appeared very dehydrated; the tongue was dry, the voice had diminished to a whisper, and he had become much wasted, with a sacral bed sore. Improvement set in on the 19th day, though he continued to vomit for a further four days. On the 23rd day, he again developed fever and the right parotid gland was acutely swollen and tender. The fever lasted for three days, the parotitis slowly subsided and he made an uninterrupted recovery. The urine output continued to be 2000 c.c. daily, but the albumin slowly declined in amount and casts were not present after the 21st day. The retinal and radial arteries of this patient were thought to be a little thickened for his age, and it is therefore possible that there was some contributory cause for the uraemia, apart from the recent circumstances. The blood pressure did not rise excessively at any time. The duration of fever, excluding that due to the parotitis, was 12 days, and fever terminated four days after the commencement of chemotherapy. Dosage of V186 is summarised in Table IV (p. 27).

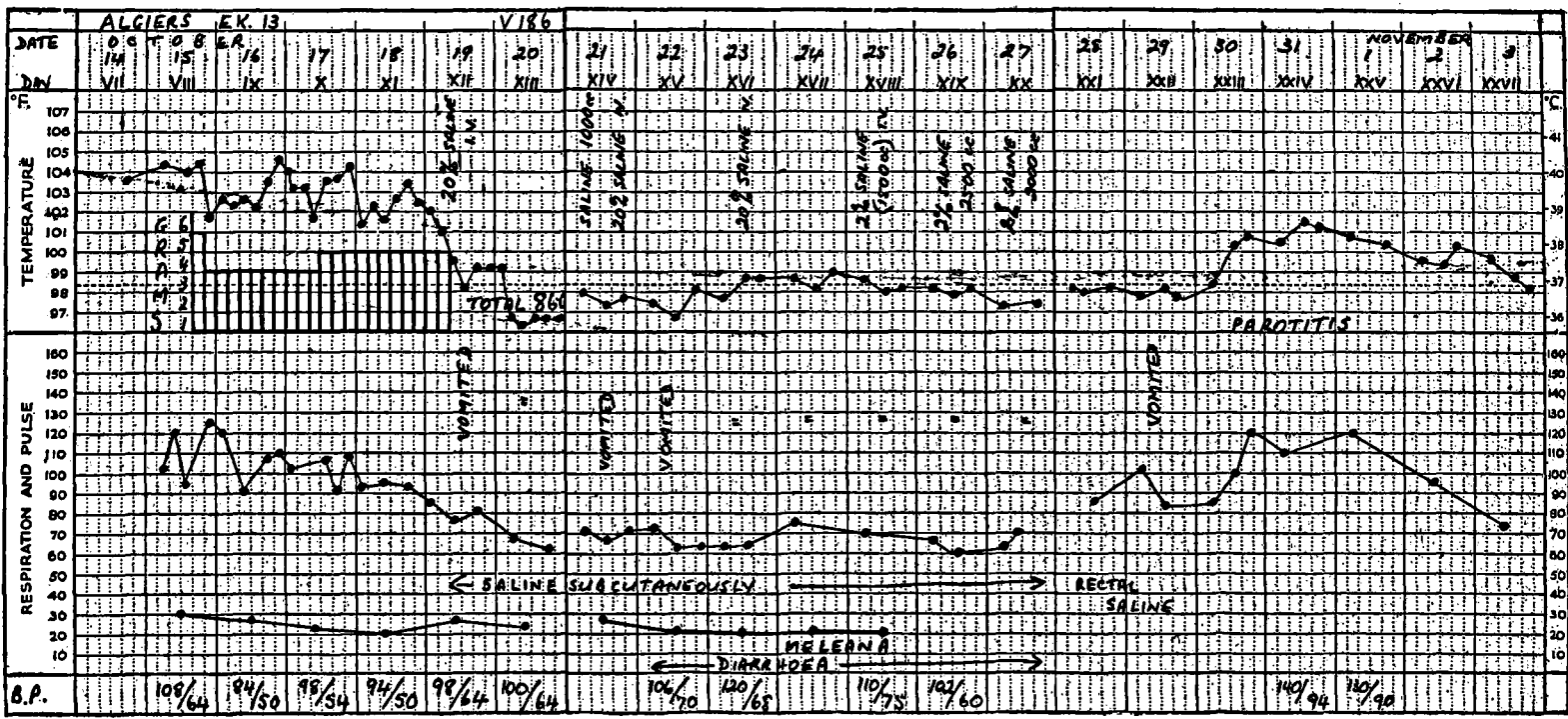
APPENDIX TABLE X

Index No. : ALGIERS EK 13

Treated with V186

Date	Day	Agglutinations					Blood								Animal inoculations
		Proteus	M.	C.	T.A.B.	M.A.	Hgt.	Hcrit.	ESR.	Urea.	Cl.	Rbc.	Wbc.	Differential	
18.10.43	8	X19 : +200 X2 : tr200	-25		T.-25 A. tr25 B.-25	M.-25	67	45	<1	40	306.7	4.99	6900	PS. 1449 L.586 U.4450 M.345, B.M.69	Two guinea-pigs inoculated with whole blood of 15.10.43. One developed mild fever and agglutinins for epidemic rickettsiae in the blood. Further passage however failed. Mice : inoculated I.N. with sternal marrow 15.10.43. Small focal lesions and inclusion bodies in smears after third passage. Further adaptation failed.
17.10.43	10	X19 : +++400	tr+100	±800			76	33	<	50	328.6	4.90	5400		
19.10.43	12						69	32	<1	220+	317.4	4.81	14650	PS. 2564 L.2784 U.7546 M.366, E.73 B.146, B.M.1171	
20.20.43	13						62					4.98	21900	PS. 5694 L.1423 U.12150 M.1533, E.219 B.109, B.M.656,	
21.10.43	14						73	30	<1	±500		4.51	21000		
23.10.43	16	X:19 ±3200	±3200	> ++6400				28	<1	460	358.6				
25.10.43	18						63	30	<1	±800		4.58	10250	PS. 5228 L.717 U.4895 M.350, B.M.51	
26.10.43	19							29	<1	±800					
28.10.43	21						69	29	<1	±880		4.63	12250	PS. 4815 L.649 U.4392 M.311, B.85	
1.11.43	25						53	26	<1	140		3.44	34900	PS. 12220 L.1920 U.19200 M.1046, E.174 B.174, M ye. 174	

APPENDICES





## Treated with V186

NAPLES 60.

*Grave case.  
Died.*

Female, aet. 24, admitted on the 7th February in a delirious state. Relatives stated that she had been ill for six days. Temperature on admission was 100° F., pulse 144, and respirations 20. She was a well-built woman, confused mentally, with a flushed face and a most profuse macular and haemorrhagic rash on the trunk and limbs, and around the left eye; in addition to haemorrhage into the macules, there were discrete petechial haemorrhages. She was slightly cyanosed and dyspnoeic. The eyes were injected and the eyelids showed many fine haemorrhages. The tongue was dry and brown, the voice was husky. Lymph glands in both axillae were palpable. Musical rhonchi were heard on auscultation of the lungs. The heart sounds were faint, the pulse volume poor and the B.P. 95/65. The abdomen was tender in both lower quadrants. The spleen was enlarged to one finger's breadth below the costal margin. Reflexes were normal and there was no stiffness of the neck. She was incontinent of urine. V186 was given four-hourly. On the 8th February she was agitated, restless and irritable, complaining of generalised pains; she was given paraldehyde and "Luminal." The tongue was dry; and an area of blueish discoloration of the skin, 3 inches in diameter, was present on the left buttock. Intravenous glucose saline was begun at mid-day, and continued at the rate of 1250 c.c. per diem until death. On the 9th February she was more co-operative, though restless. The rash was even more profuse, haemorrhages being seen in the nail-beds but not in the fundi. The face was still flushed and cyanosis persisted. Only a few fine râles could be heard in the chest. Treatment with V186 was given two-hourly on this day. On the 10th she was worse and became much more dyspnoeic and hoarse. The heart sounds remained good, however, and the B.P. was 95/65. On the 11th February she could not speak, the flush had become cyanotic, the respirations laboured and rapid (46), and cyanosis of the toes was commencing. The heart was irregular, the cardiac impulse being of varying intensity and the systolic blood pressure varying with each beat; the pressure was recorded as 90/50. Moist râles were increasing at both lung bases. She died in the afternoon, with an extreme livid cyanosis. She was incontinent of urine throughout, but the output did not seem to be notably diminished. A total dosage of 65.5 gm. of V186 was given during four days. Sodium bicarbonate was given with each dose of V186 in the last 24 hours of therapy.

*Dosage of V186*

<i>Date</i>	<i>Quantity (grams)</i>	<i>Interval</i>	<i>Daily total (grams)</i>	<i>Grand total (grams)</i>
7th February, 20.00 hours .. ..	4	Four-hourly	4	} 65.5
8th February .. .. ..	3	Four-hourly	18	
9th February .. .. ..	1½	Two-hourly	18	
10th February .. .. ..	{ 1½	Two-hourly	} 19.5	
	{ 3	Four-hourly		
11th February .. .. ..	3	Four-hourly	6	

*Post-mortem Findings**Naples 60.**(Died 3 p.m. 11th February, 1944. P.M. 3.45 p.m. 11th February, 1944)*

The body was that of a well-developed adult female. Rigor mortis was absent and the body still warm. There was purplish discoloration of the skin over several toes, and over small areas on the dorsum and heel of the right foot.

*Rash.*

A very profuse purplish haemorrhagic eruption was present over the trunk and limbs. Many haemorrhages were seen in the palpebral conjunctivae.

*Respiratory.*

The larynx was normal. The trachea showed slight inflammatory congestion only. The pleural cavities showed no abnormality. There was hypostatic congestion of both lung bases. In the right lung there was a large area of collapse in the lower lobe, and one

## 224 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

nodule of bronchopneumonia, the size of a walnut, in the lateral part of the middle lobe. The left lung showed, in addition to congestion, some oedema of the lower lobe and one small septic infarct.

### *Cardio-vascular.*

Pericardial sac normal. The heart weighed 230 gm. It showed tremendous right-sided dilatation. The heart muscle showed some pallor near the endocardial surface, and in the sub-endocardial areas there were several indefinite purplish streaks of infiltration. This appearance was most striking on the interventricular septum. The valves and coronary arteries were normal. The aorta showed a few fatty streaks in the abdominal part.

### *Gastro-intestinal.*

The oesophageal mucous membrane was dry, and covered with a coating very much like that seen on the tongue in severe typhus. In the stomach, the mucosa near the fundus showed two large areas 1-2 in. in diameter, with a black haemorrhagic appearance and evidence of "erosion." The rest of the gastric mucous membrane showed many tiny pin-point haemorrhages. The intestines were normal. There was no mesenteric glandular enlargement.

The *spleen* was enlarged (weight 280 gm.). It was of firm rubbery consistency and a reddish brown colour. The capsule was smooth. The cut surface was smooth, with Malpighian bodies quite prominent; the pulp somewhat pale and the trabeculae obscured.

The *liver* was approximately normal in size and appearance.

The *pancreas, gall bladder, bile ducts* and *suprarenals* were normal.

### *Genito-urinary.*

The kidneys were slightly enlarged. Each kidney weighed 195 gm. They were firm in consistency, and the capsule stripped easily, leaving a smooth glistening rather pale outer surface. On the cut surface cortex and medulla were clearly demarcated. The glomeruli were prominent and the radial markings normal. The cortex was pale, with some very small yellowish areas of tubular degeneration.

The ureters, bladder, uterus and adnexa were normal.

### *Brain.*

The meninges were congested and slightly oedematous. No other naked-eye abnormality of the brain could be seen.

Tissues were kept for section in 10 per cent. formol-saline and in Zenker's fixative.

## *Histology*

### *Naples 60.*

#### *The conjunctivae.*

There was a diffuse accumulation of cells in the sub-epithelial layer—masses of mononuclear cells, and smaller numbers of polymorphs and plasma cells. Congestion and interstitial haemorrhages were also present. In the deeper layers mononuclear cell infiltration was more sharply limited around blood vessels. These frequently showed swelling and proliferation of the endothelium, and occasionally disorganisation of the vessel walls and partial occlusion of the lumina.

#### *Respiratory.*

The lungs contained areas of bronchopneumonic consolidation, the alveolar septae being thickened by congestion and mononuclear cell infiltration, and the alveolar exudate, consisting predominantly of mononuclear cells, showing active phagocytosis of polymorphs. The bronchi seen showed acute inflammatory exudate throughout the walls, and the epithelial linings were completely desquamated into the lumen. Other areas of the lung showed different stages of bronchopneumonic oedema and emphysema. One medium-sized bronchus showed a mononuclear cell infiltration in its walls, while the lumen contained a polymorphonuclear inflammatory exudate. Many areas of bronchopneumonia were sharply localised to the immediate periphery of small and medium-sized bronchi.

#### *Cardio-vascular.*

The heart muscle showed great irregularity in the intensity of staining. Many fibres, in addition to alteration in depth of staining, showed slight irregular swelling and fragmentation. Interstitial infiltration of large mononuclears and plasma cells, often concentrated round vessels, was moderate in severity. Interstitial haemorrhages were frequent. Endothelial swelling was noticeable in some of the vessels.

Histological changes of the usual type were present in the aorta.

*Gastro-intestinal.*

The stomach showed mononuclear cell infiltration of the superficial layers of the mucous membrane, together with congestion and petechial haemorrhages. Nodular accumulations of mononuclears also occurred in deeper layers of the mucosa.

The intestines were not examined.

*Liver.*—There was slight fatty degeneration of the parenchyma. The portal tracts showed slight lymphocytic infiltration.

*Spleen.*—There was moderate congestion of the pulp and an increase in mononuclears, some of which showed erythrophagocytosis. Polymorphs were scanty. Malpighian bodies were not enlarged.

The *pancreas* showed diffuse cystic dilatation of the ducts, and a few nodular perivascular accumulations of large mononuclears.

*Suprarenals.*—A few irregular accumulations of cells (lymphocytes and mononuclears) were present both in the cortex and the medulla. In the cortex they were mainly subcapsular and perivascular in distribution. The glands were congested and small foci of glandular degeneration were present.

*Genito-urinary.*

The cortex was moderately congested, the medulla slightly more so. Foci of cellular infiltration were abundant throughout.

The glomeruli were moderately enlarged and many almost entirely filled the cavity of Bowman's capsule. In some tufts, endothelial nuclei were enlarged; in others, they were flattened and deeply staining. No definite increase in cellularity of the glomeruli could be made out, but the epithelium lining Bowman's capsule in some of the glomeruli showed an increase in number and prominence of the nuclei. Albuminous material occurred in the capsular space. The capillary loops were patent: some were markedly distended and stuffed with erythrocytes; others, though showing varying degrees of distension, were empty. The basement membrane of the tufts appeared normal, and there was no formation of intra-capillary fibrils. A few glomeruli were sclerotic.

In parts of the section there was complete necrosis of proximal convoluted tubules, with desquamation of the epithelium; in other parts the change was limited to intense cloudy swelling with focal necrosis. The distal tubules were less severely affected, cloudy swelling being the main feature. Numerous casts, consisting of cellular debris, hyaline material or masses of erythrocytes, were present in the tubules. The tubules in the functional zone and medulla were dilated.

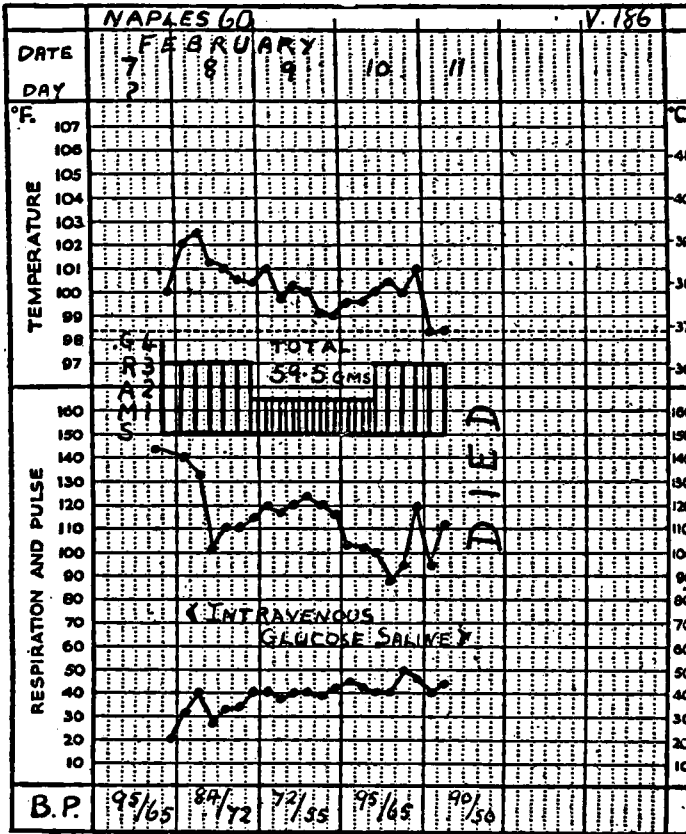
In the deeper parts of the cortex there was oedema of the interstitial tissue.

The blood vessels showed no apparent abnormality. Many capillaries seemed to contain an unusually large number of mononuclear cells.

*Brain.*

Small numbers of nodular lesions were encountered in the medulla, pons and midbrain only. Very few perivascular foci or haemorrhages were encountered, and lesions of the vessel walls were not seen. A moderate degree of mononuclear infiltration of the meninges was present.





## Treated with V186

Grave case.

NAPLES 62.

Died.

Female, aet. 24, admitted on the 8th February, on the 6th day of illness. She first took to her bed on account of grief over her sister's death from typhus. Two days later, she was feverish, with headache, pains in the back and thighs, vomiting each day but with no loss of appetite. She was constipated. Her nose bled on one occasion two days before admission. Temperature on admission was 103.6° F., pulse 100, respirations 20. She was a mentally clear, rather pale but well-built woman, with slight cyanosis but without dyspnoea. No definite rash was seen, but the eyes were very injected and there were haemorrhages in the lower eyelids. The tongue was moist and pink. The superficial lymph glands were not enlarged. The lungs appeared normal. The heart sounds were faint, but the rhythm normal; the pulse was small in volume, and the B.P. 80/65. No abnormality was found in the abdomen. Reflexes were normal. The urine had a specific gravity of 1022 and contained a trace of albumin and a very occasional granular cast. She was given V186 two-hourly for 20 hours. At the end of this time, she vomited the tablets. In spite of repeated efforts, the drug could not be retained and the administration was therefore stopped. On this, the 7th day of illness, there was a faint lilac cyanosis of the cheeks. She remained drowsy until evening, when she became restless and got out of bed. On the 8th day, she continued to vomit at intervals, was restless and passed no urine. Intravenous glucose saline had been given continuously since the previous evening, and was continued until the evening of the 12th day, when she seemed better, comfortable and more talkative. A petechial eruption appeared on the right forearm on the 10th day. On the same day she became afebrile. She was incontinent of urine on the 9th and 10th days, when apparently only small amounts were passed. On the 11th day, 300 c.c. were obtained by catheterisation. This specimen had a specific gravity of 1002; contained a light cloud of albumin and a few leucocytes; no casts or R.B.C. were present. Improvement was, however, short-lived; she vomited, and thrombosis of the cephalic vein in the left arm developed. The blood urea was found to be very high, although urine was now being excreted in amounts of one to two litres daily; the specific gravity of the urine remained low, and it contained leucocytes but at this time no albumin nor casts. Intravenous saline was again given by continuous slow drip from the 12th day onwards, but the blood urea continued to climb. On the 14th day, fever recommenced, diarrhoea set in, and her condition steadily grew worse. High remittent fever with rigors was present on the 15th and 16th days, and on the latter day she twice had haematemesis. Plasma was infused instead of saline during the afternoon, and later Group O blood was given, but the pulse and respiration rate rose steadily and she died on the evening of the 16th day. During the last three days of life, she passed 2000, 1600 and 100 c.c. of pale urine containing a heavy cloud of albumin. The specific gravity had risen to 1018 and 1020, and rare leucocytes and granular casts were present. She had received in all 20.5 gm. of V186 during 20 hours, but had vomited the last three doses. She received small doses (1½ gm. four times daily) of sulphathiazole for 12 hours shortly before death. Evidence of a terminal bacteraemia (coliform) was obtained by blood culture. Fluid balance and biochemical findings are shown in Fig. 19, p. 108 above.

## Dosage of V186

Date	Time	Quantity (grams)	Interval	Total (grams)
9th February.	Midnight	4	Two-hourly	} 20.5
	0200 hours	3	Two-hourly	
	0400 hours	1½	Two-hourly	
	0600 hours	1½	Two-hourly	
	0800 hours	1½	Two-hourly	
	1000 hours	1½	Two-hourly	
	1200 hours	1½	Two-hourly	
	1400 hours	1½	Two-hourly	
	1600 hours	1½	(Vomited immediately)	
	1630 hours	1½	(Vomited immediately)	
	2000 hours	1½	(Vomited immediately)	

*Post-mortem Findings*

*Naples 62.*

(Died 10.45 p.m., 20th February, 1944. P.M., 11 a.m., 21st February, 1944.)

The body was that of a well-nourished adult female. Post-mortem lividity was slight. The skin over the right median cubital vein had been incised for intravenous therapy. There was no local sepsis.

*Rash.*

No rash was visible, and there were no conjunctival haemorrhages.

*Respiratory.*

The trachea showed slight congestion only, and contained no exudate. A few petechial haemorrhages were present over the epiglottis. Each pleural cavity contained about 2 ounces of clear straw-coloured fluid. A few pleural haemorrhages were present along the interlobar septa. The posterior parts of the lungs showed congestion and slight oedema, and anteriorly there was a slight degree of vesicular emphysema. There was no naked eye evidence of bronchiolitis.

*Cardio-vascular.*

There were numerous bright purple petechial haemorrhages on the posterior part of the base of the left ventricle and the adjacent part of the parietal pericardium. The pericardial sac contained a normal amount of fluid. The heart weighed 280 gm., and the right side was grossly distended. The myocardium, especially near the endocardial surface, was pale, and the endocardial surfaces were streaked with irregular purplish lines. The valves, auricles and coronary arteries were normal. The aorta showed a moderate degree of atheroma in the abdominal portion only.

*Gastro-intestinal.*

Oesophagus normal. The stomach was distended and contained a small amount of undigested food. Areas of the mucous membrane along the lesser curvature showed petechial haemorrhages, varying from 0.5 to 2 mm. in diameter. Intestines were normal. A few firm fleshy lymph glands, the size of peas, were present in the mesentery.

*Spleen.*

Enlarged (weight 370 gm.). The capsule appeared thickened, and was studded with a few fibrinous translucent nodules. It was of firm rubbery consistency and deep purple in colour. The cut surface did not bulge; the pulp was deep purplish red, and swollen to obscure the trabeculae.

*Liver.*

Very dark in colour, with rounded edges. A great deal of blood escaped from the veins after removal, leaving the organ paler and with slight mottling of passive congestion. On the cut surface, the lobular pattern was distinct, and there was some early fatty change. In addition, especially in the subcapsular zone, there were pin-point areas of bright yellow colour. The largest of these yellow necrotic foci were about 1 mm. in diameter.

*Gall bladder* and bile ducts normal.

*Pancreas* was normal.

*Suprarenal* medullae were deeply congested.

*Genito-urinary.*

The kidneys were very markedly enlarged. The left kidney weighed 320, and the right 240 gm.; they were of firm, rubbery consistency. The capsule stripped with ease, leaving a smooth surface not grossly abnormal except for distention of the capillaries. The cut surface was slightly bulging. The swelling affected both the cortex and the medulla. The radial markings of the cortex were prominent and in parts distorted. The appearance suggested marked interstitial infiltration. Although the kidneys were pale, there was no naked eye evidence of gross tubular degeneration. The junctional zone was intensely congested. In the medulla the striations were prominent and broad. Pallor, which increased in intensity towards the apices of the pyramids, was striking. There was delicate incrustation at the apices of the pyramids, which gave a scraping sensation to the knife. The renal pelves were studded with very numerous petechiae. The ureters were normal. The bladder was contracted and empty. It showed no abnormality. Uterus and adnexa were normal.

*Brain.*

Apart from very marked pallor, it showed no naked eye evidence of abnormality

Tissues were kept for section of 10 per cent. formol-saline, and in Bouin's and Zenker's fixatives.

At autopsy: blood culture was sterile; spleen culture yielded a Gram-negative coliform organism.

*Histology**Naples 62.**Respiratory.*

The lungs showed patches of oedema, congestion, emphysema and bronchopneumonia.

*Cardio-vascular.*

The heart showed diffuse interstitial infiltration of mononuclear cells, and widening of the interstitial spaces. A few more definite nodular collections of large mononuclears, with a perivascular distribution, were seen.

Aorta: only very few tiny cell accumulations were seen round the vasa vasorum.

*Gastro-intestinal.*

The stomach and intestines were not examined.

*Liver.*—There was marked engorgement of the sinusoids, and early fatty degeneration in the central zones of the liver lobules. Occasional lobules showed necrosis of the central liver cells.

*Spleen.*—The pulp was markedly congested and contained many mononuclears as well as polymorphonuclear cells. The trabeculae and malpighian bodies were obscured. The capsule was thickened, and covered with a thin layer of fibrinous exudate with many polymorphs.

*Supravernals.* There was lake-like engorgement of the blood vessels, especially under the capsule, but no other histological abnormality.

*Genito-urinary.*

Congestion was marked throughout the kidneys, and haemorrhages were present in the medulla. Focal cellular infiltrations were scanty.

Many glomeruli were enlarged, and the capillary loops were usually patent and markedly rounded, although they were not all distended with blood. The endothelial cells were often enlarged and resembled the covering epithelium of the tuft so closely that they could be distinguished only in specially stained (aniline blue) sections. In a few tufts, foci of increased cellularity were present, sometimes involving as much as one-half of the glomerulus, and showing the features of a typical intra-capillary glomerulitis, including formation of intra-capillary fibrils. In others, a slight diffuse increase in cellularity, and a tendency to lobulation of the glomerulus, were observed. The basement membrane of the tufts, except in the areas of focal intra-capillary glomerulitis, showed no demonstrable abnormality but gave the impression of being stiffer than normal. The basement membrane of Bowman's capsule, on the other hand, was often thickened. Associated with the thickening of the basement membrane of Bowman's capsule, there often appeared to be an increase in the number of capsular cells, all of which remained, however, in the usual single layer.

The medulla had a normal architecture except for oedema and haemorrhages, but the cortex and boundary zone showed considerable alteration, due to regenerative, degenerative and atrophic changes in the tubules, and considerable diffuse increase in the interstitial tissue. In some parts, groups of convoluted tubule cells showing faintly-staining vacuolated cytoplasm or cloudy swelling occurred; in others, the tubules were either quite necrotic or were undergoing atrophy; while in yet other areas, recently regenerated tubules containing granular debris were found—all these various types of lesion or appearance being separated by an increased amount of oedematous connective tissue. The non-specialised tubules were dilated and contained casts of all kinds.

Interstitial tissue was generally increased, but connective tissue cells, though swollen, were not abundant, the bulk being made up to some extent by oedema. Mononuclear and polymorphonuclear leucocytes were scattered irregularly throughout.

No degenerative changes were found in the arteries at the boundary zone nor in the interlobular arteries. Some of the smaller arterioles showed hyaline thickening of the intima.

*Brain.*

Very few nodular lesions were encountered. In 10 sections of the brain stem, only two lesions were seen—one a characteristic nodular lesion of neuroglia cells and polymorphs in the medulla, the other a small perivascular lesion. One lesion of a rather diffuse type was seen in the basal ganglia, and a single nodule in two sections of cerebellum. No vascular lesions were seen, and mononuclear infiltration of the meninges was slight.



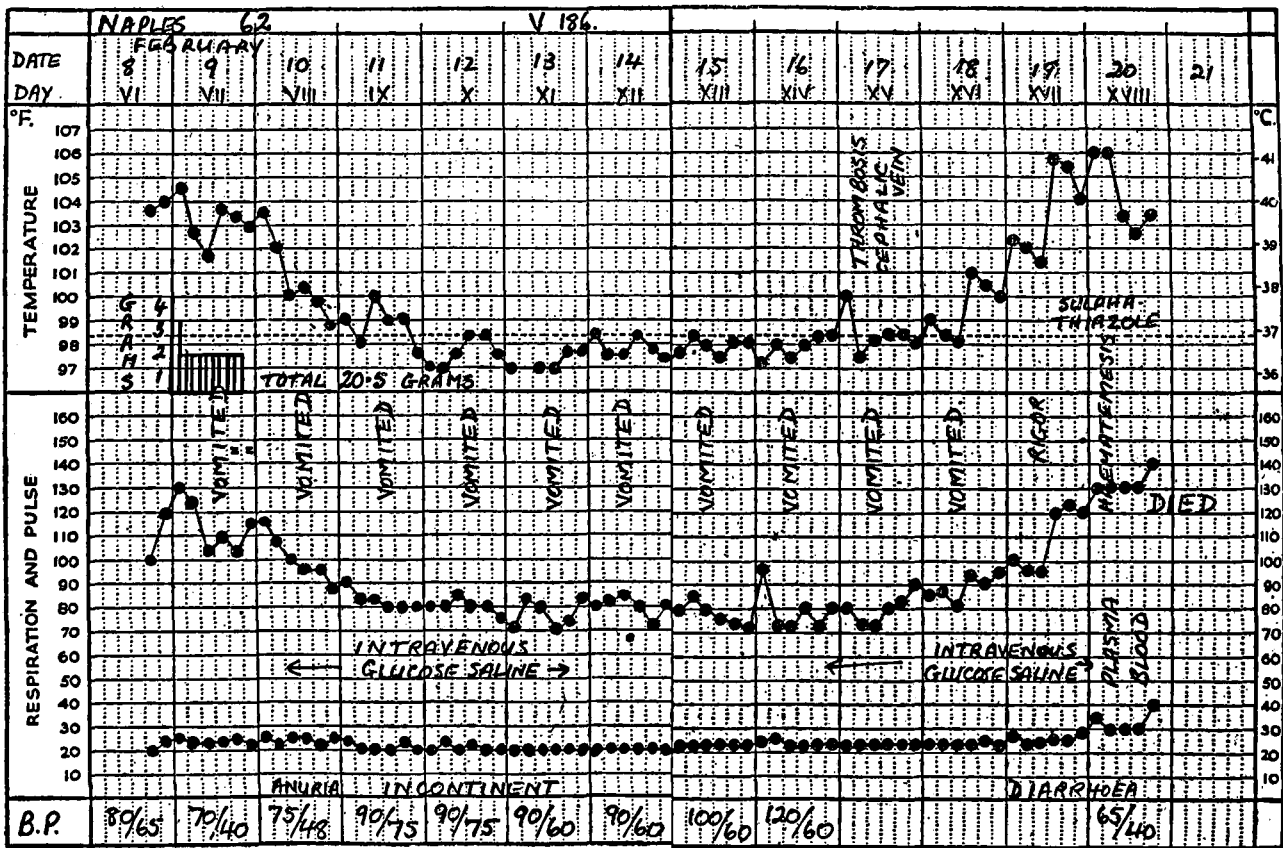
APPENDIX TABLE XII

Index No.: NAPLES 62

Treated with V186

Date	Day	Agglutinations					Blood									Animal inoculations	
		Protous	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.		Rbc.	Wbc.		Differential
												Bl.	Pl.				
9.2	7	X19: ++++800 X2: +200 KK: +200	-20	+++20	T.-25 A.-25 B.+25	M.-25 A.-25	++	76		46.6	73			3.31	9800	P.S.608 L.950 U.2052 M.190	None.
14.2	12							293	308.4		64	1047	1027				
16.2	14	X19: ++++3200 X2: +++400 KK: +400	+++80	+++80			++	348			67	1051	1027	3.07	5800	P.S.1798 L.10944 U.2494 M.406, B.58	
18.2	16							480	292.3	57.4	52	1048	1030	2.58	4950		
20.2	18	X19: ++++10240	+++40	+1280				420					1023				
12.2	Blood	culture: sterile.															
19.2	Blood	culture: growth of motile gram + coliform; negative bacilli; A.G. in glucose. A. in lactose.															
21.2	Hear	t blood: at post-mortem; spleen: at post-mortem: sterile; same organism as grown from blood 19/2.															

APPENDICES



## Treated with Penicillin.

*Grave case.  
Died.*

## NAPLES 93.

Female, aet. 24, admitted on the 6th March after an illness of about seven or eight days' duration, with shivering, fever, headache, generalised aches and pains and constipation. She had been incontinent of urine and faeces for three days. Temperature on admission was 102.3° F., rising to 104° F., pulse 160, respirations 28. She was semi-comatose and did not reply to questions, but lay quietly with eyes open. There was no response to pin-pricks on the limbs. There was slight cyanosis of the lips and cheeks. She was not dyspnoeic. There was a macular rash of cyanotic hue on the trunk, with subcuticular mottling over the shoulders. The eyes were very injected and showed a few tiny haemorrhages into the lower lids. The tongue was moist; the chest was full of bubbling râles. The heart sounds were faint, the pulse was very soft and rapid, and the B.P. 120/80. The abdomen and reflexes were normal. She was incontinent of urine. Treatment with penicillin—20,000 units intramuscularly every three hours—was instituted and continued for four days, by the end of which time she had received 720,000 units. Penicillin treatment was then discontinued, because of the lack of response.

On the day after admission (7th March), she began to have a remittent fever, averaging 103° F., with pulse at times over 180 and respirations rising to 50. On this day she was a little more responsive, and resented nasal feeding, which had been given since admission. There were gross twitching movements of the wrist, and tremors of the lips and facial muscles. There was a large purplish-pink indurated area, 4 in. by 5 in., over the right buttock. Many of the macules were haemorrhagic, and petechiae were present on the backs of the hands and feet. The neck was a little stiff. By nightfall, there was rigidity of the limbs, and twitching movements were prominent. The lower lobe of the right lung appeared consolidated, but there was no cough. On the 8th March, rash, fever, tachycardia and tachypnoea continued. She was still not responsive. The lower parts of both lungs now appeared consolidated. In spite of the extreme tachycardia, the B.P. was 127/90. On the 9th, the cyanosis of the face had increased. On the 10th, she responded slightly to questions, the pulse rate was a little slower, but the lung signs remained the same. Fever continued, but became increasingly remittent; cyanosis and dyspnoea increased, and the rash persisted. She remained slightly responsive, and at times alert and watchful, until her death on the 16th March, 11 days after admission. The muscle twitching was a striking feature. At times it appeared to be tetaniform. It was not, however, relieved by intravenous calcium gluconate. She developed some oedema of the back several days before death, but congestion of the liver did not occur. The total duration of illness was unknown, but it must have been at least 18 days. The signs of consolidation at the lung bases persisted throughout the illness. She was incontinent throughout, but the amount of urine passed did not at any time suggest oliguria. Purulent sputum, coughed up two days before she died, suggested a condition of lung abscess. Cultivation of the sputum yielded a growth of *Proteus*, but direct films indicated the presence of Gram-positive cocci and fusiform bacilli as well.

*Post-mortem Findings**Naples 93.*

(Died 5.45 a.m., 16th March, 1944. P.M., 10.45 a.m., 16th March, 1944.)

The body was that of a fairly obese adult female. Rigor mortis was present in the limbs, and hypostatic congestion was slight. Two areas, about 2 in. in diameter, of purple, discoloured skin were present over the medial aspects of the feet. A large superficial bed sore was present over the sacrum.

*Rash.*

None visible. No conjunctival haemorrhages were seen.

*Respiratory.*

The larynx was congested, and there was a slightly "moth-eaten" appearance of the mucous membrane over the vocal cords. The tracheal mucosa was congested, and the lumen contained abundant mucopurulent secretion. The pleura over the right lung was covered with a thin irregular layer of fibrin, due to an early pleurisy extending from an abscess in the upper lobe. The upper and middle lobes were of soft rubbery consistency in the posterior parts, due mainly to oedema and some bronchopneumonia. More anteriorly, there was vesicular emphysema only. The posterior two-thirds of the lower lobe were of deep purple colour, and showed numerous petechias on the pleural surface. In consistency,

## 234 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

the lower lobe was firm ; and scattered throughout it were hard nodules, the size of peas. On section, there was definite bronchopneumonia, and, between the pneumonic patches very extensive congestion and oedema. The upper lobe contained, near the anterior border, an abscess cavity covered only by the pleura. The walls were of necrotic, dark foul-smelling lung ; surrounding the abscess were a few bronchopneumonic patches. In the right lung, there was consolidation of the posterior two-thirds, and of the anterior tip of the upper lobe. The consolidated lung was deep purple in colour, and the pleura over it showed many petechiae. On the cut surface, there was evidence of a confluent bronchopneumonia and much oedema. The appearance of both lower lobes was that of bronchopneumonia in massively-collapsed lung.

### *Cardio-vascular.*

The pericardium contained many petechial haemorrhages in a small area round the base of the large vessels behind the heart. The amount of pericardial fluid was normal. The heart was of approximately normal size ; its weight 230 gm. The right side was moderately distended. The myocardium was pale, and on the endocardial surface could be seen irregular faint purplish streaks. The endocardium was very pale. The haemorrhagic appearance could be seen in the auricles as well as the ventricles, but it was most marked in the papillary muscles and the trabeculae. Valves and coronary arteries were normal.

The aorta showed slight fatty streaking only in the arch and abdominal portions.

### *Gastro-intestinal.*

Oesophagus normal. Stomach normal. Intestines normal. The mesentery contained a few slightly enlarged lymph glands. The spleen weighed 180 gm. ; it was soft in consistency, with a deep purple outer surface. On the cut surface the pulp was slightly swollen and soft. The Malpighian bodies were prominent but not very large. The trabeculae were not obscured.

The *liver* was soft and swollen, with rounded edges ; the outer surface was smooth ; the cut surface bulged slightly. Both the outer and cut surfaces showed extensive fatty change, and areas with the nutmeg appearance of passive venous congestion.

The *pancreas* was hard but showed no other abnormality.

*Gall bladder* and bile ducts were normal.

*Suprarenals* normal.

### *Genito-urinary.*

The kidneys were of approximately normal size ; their weight 320 gm. The capsule stripped with a little difficulty, tearing away the somewhat softened cortex. The outer surface was pale. On the cut surface the cortex was pale, and the glomeruli prominent. There were a few irregular linear points of congestion. The junctional zone was congested, and the radial markings of the medulla were prominent. Some of the medullary vascular markings were abnormally broad.

*Ureters* normal.

*Bladder mucosa* showed some irregular haemorrhagic foci.

*Uterus* and *adnexa* normal.

### *Brain.*

Apart from a large number of abnormally prominent small vessels in the substances of the brain and brain stem, there was no gross abnormality.

Tissues were kept for section in 10 per cent. formol-saline, and in Bouin's fixative.

## *Histology*

### *Naples 93.*

#### *Respiratory.*

The lungs showed fairly extensive bronchopneumonia, which, in parts, was very early, consisting of congestion and infiltration with mononuclear cells. In some areas of the lung there was acute suppurative inflammation, very sharply limited to small bronchi. Other areas showed extension of the bronchial lesions to alveoli in the immediate vicinity, resulting in sharply defined nodules of bronchopneumonia.

#### *Cardio-vascular.*

The myocardium showed marked swelling of interstitial spaces but very little cellular infiltration ; there was some early proliferation of fibroblasts. Myocardial degeneration, on the other hand, was more prominent—as evidenced both by irregularity in staining and by the presence of small areas of necrosis involving fragments of single fibres only.

The aorta showed a few very tiny perivascular accumulations of lymphocytes in the adventitia.

*Genito-urinary.*

Haemolysis was marked in histological material from this case, and congestion was definitely present only in the boundary zone and superficial part of the medulla. Foci of cellular infiltration were scanty.

The glomeruli were swollen, so as to fill the capsular spaces, the capillary loops being generally patent and frequently widely distended. Cellularity was not increased, the endothelial nuclei not enlarged, and no necrotic areas were present. The basement membrane of some of the loops appeared swollen, but there was no formation of intracapillary fibrils.

The epithelium of the tubules, particularly the proximal convoluted portions, showed cloudy swelling.

The blood vessels appeared normal.

*Gastro-intestinal.*

The intestines were not examined. The *spleen* was markedly congested; the pulp cells were predominantly mononuclear, and Malpighian bodies were partially obscured. In the *liver*, there was some increased fibrosis of the portal tracts, and infiltration by small round cells. There was marked congestion of the liver sinusoids, but Kupffer cells were not prominent.

The *pancreas* showed fairly widespread degenerative changes of the parenchyma, with here and there persistence only of the stroma. Some areas showed diffuse interlobular fibrosis. The islets were not grossly abnormal.

*Lymph Glands.*—There was great dilatation of the sinusoids, with reticular-cell hyperplasia. Nearly all the cells filling the sinusoids were mononuclear in type. The germinal centres were not hyperplastic.

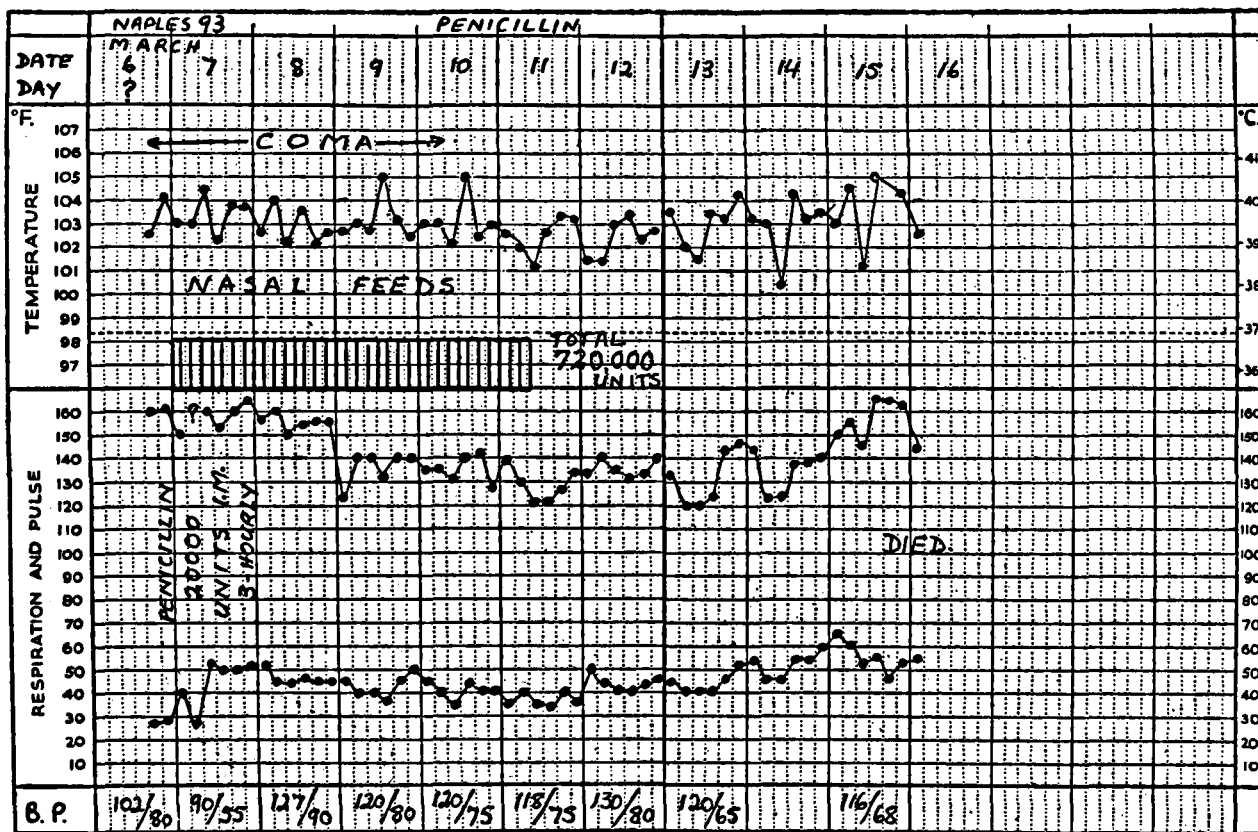
*Suprarenals.*—A few small compact areas of lymphocytic infiltration were present throughout the glands. In relation to these were many small foci of granular degeneration, mostly in the outer zones of the cortex.

*Conjunctiva.*—There was a diffuse accumulation of lymphocytes and plasma cells in the deeper layers of the epithelium and the subepithelial layers. Accumulations of these cells were present round small blood vessels. In many of the smaller vessels the endothelium was swollen, sometimes sufficiently to obliterate the lumen. No interstitial haemorrhages were seen.

*Brain.*

The number of focal lesions was small, and they were found almost exclusively in the brain stem, in the region of the olivary nuclei and substantia nigra. The basal ganglia showed an apparent increase in cellular elements but no definite focal lesions, and no such lesions were found in the cerebellum. Most of the lesions present were compact and consisted of the usual cell types. Small areas of softening were seen in perivascular areas in the medulla. A few perivascular haemorrhages, and one small thrombosed vessel, were seen in the pons and mid-brain. One small area of perivascular cellular reaction was seen in the cortex.





## Doubtful Typhus Treated with V186

Grave case.  
Died.

NAPLES 58

*(This case was rejected from the research series as there was insufficient evidence for a certain diagnosis of typhus.)*

Female, aet 40, married; admitted on the 6th February after an illness stated to have lasted only five days. It was subsequently found that she had been ill for an unknown period before this, with "pneumonia". Four days before admission, she had headache and pains in the legs. She vomited during two nights before admission. Pains were chiefly in the knee, ankle and wrist joints. She complained of a cough and felt shivery. Temperature on admission was 100.8° F., pulse 112, respirations 22. She did not appear seriously ill or distressed, and was mentally clear. There were numerous fine purpuric spots on the trunk, legs and arms, and around the neck in collar-fashion. No macules were visible. Her eyes were clear, and her tongue clean. Superficial lymph glands were not palpable. Râles and rhonchi were heard over the posterior aspect of the right lung. The heart appeared normal, the pulse volume was good, and the B.P. 104/76. The abdomen was normal. Reflexes were normal. The urine contained a trace of albumin with a few granular casts. V186 was administered two-hourly. On the 7th February, the temperature had risen to 104° F., with a pulse rate of 96 and respirations 20. She seemed iller, though continuing to co-operate and taking fluids well. On the 8th, the fever continued, the rash remained, but she did not look ill. The urine contained a moderate cloud of albumin, and granular and hyaline casts. Temperature fell to below 100° F. She became irrational during the afternoon and vomited after the 6.0 p.m. dose of V186. The dose was repeated together with sodium bicarbonate, but she again vomited. At 9.30 p.m. she again vomited, and appeared collapsed, with feeble pulse and a normal temperature. The pulse rate was 60, and the B.P. could not be recorded. She remained seriously ill all night, with a further episode of vomiting. Pulse remained feeble and slow. On the 10th February she still seemed collapsed and ill, with temperature of 98.4° F., an impalpable pulse and respirations of 24. She complained of abdominal pain. The B.P. could not be recorded. Intravenous glucose saline was begun, and continued throughout the day. Later, reconstituted plasma was given. She did not rally, became very noisy and restless, and died at midnight with a subnormal temperature. She had received 59.5 gm. of V186 in three days when the first attack of vomiting occurred, and after this no further V186 was administered. Urine output dropped to 500 c.c. on the day before her death, and she took only 900 c.c. of fluid on this day and vomited several times. She did not pass urine during the day of her death, and the bladder was empty at autopsy.

## Dosage of V186

Date	Quantity (grams)	Interval	Daily total (grams)	Grand total (grams)
6th February, 1800 hours	4	Two-hourly	8.5	59.5
2000 hours	3	Two-hourly		
2200 hours	1½	Two-hourly		
7th February	1½	Two-hourly	18	
8th February	1½	Two-hourly	18	
9th February	1½	Two-hourly	15	

## Post-mortem Findings

Naples 58.

*(Died, 00.15 a.m., 11th February, 1944. P.M., 11.45 a.m., 11th February, 1944.)*

The body was that of a moderately well-developed adult female. Rigor mortis was present in arms and legs. Post-mortem lividity was slight. There was old-standing scarring of both upper eyelids, with loss of eyelashes. There was an incision over the medium cubital vein on the left side.

## Rash.

Very sharply defined bright purpuric spots were present in a broad zone over the upper part of the trunk, shoulders and chest.



*Respiratory.*

Larynx appeared normal. The trachea showed very slight inflammatory congestion, and contained a little mucopurulent secretion. Each pleural cavity contained about half-a-pint of clear straw-coloured fluid (a sample was taken for urea estimation). Pleural adhesions were present on both sides. These were easily broken down on the right side over the lateral aspect of the lung, exposing a soft oedematous gelatinous covering, about half-an-inch thick, over the chest wall. On the left side, the adhesions were strong over the diaphragmatic surface of the lung. The outer surfaces of the lungs were covered with a layer of very gelatinous fibrinous material. The interlobar septae on both sides were obliterated by the same gelatinous adhesions. The lower lobes of both lungs were rubbery in consistency. On the cut surface, they were oedematous and presented a pale solid appearance, in which a fine whitish alveolar pattern was distinguishable. The appearance and texture suggested diffuse fibrosis as would occur in an organising pneumonia. The upper lobes were oedematous posteriorly, and the anterior portions showed some compensatory vesicular emphysema.

*Cardio-vascular.*

The pericardium contained about 20 c.c. of clear straw-coloured fluid. On the parietal pericardium in the region of the ascending aorta were a number of pearly translucent nodules, about 1 mm. in diameter. The heart was of approximately normal size (weight, 300 gm.). The myocardium showed slight pallor of the papillary muscles, but was not otherwise abnormal. The valves and coronary arteries were normal. The aorta showed slight fatty streaking only.

*Gastro-intestinal.*

Oesophagus normal. The stomach was contracted and normal. Intestines normal—no petechial haemorrhages.

The *spleen* was not enlarged (weight, 135 gm.). A few slight adhesions bound it to the diaphragm. The cut surface was pale, the pulp shrunken and the trabeculae prominent.

The *liver* was acutely congested, very dark in colour and with rounded edges. It was firm in consistency, and the cut surface had the mottled appearance of passive congestion.

*Pancreas* and *suprarenals* were normal.

*Genito-urinary.*

The kidneys appeared moderately swollen; the left weighed 160 gm., the right 135 gm. The capsule stripped easily, leaving a smooth pale surface on which the vascular markings were prominent. On the cut surface, there was some pallor of the cortex, which was approximately normal in width. There were a number of irregular pale-yellowish areas in the cortex, consistent with the presence of tubular degeneration. The glomeruli were very prominent and reddish. Demarcation of the medula was clear, and the radial markings were prominent and not distorted.

The renal pelves and ureters were normal.

The bladder was contracted, and contained only about 1 c.c. of milky urine. Uterus and adnexa were normal.

*Brain.*

Vascular congestion of the meninges was prominent. No other abnormalities were noted, and no petechiae were found.

Tissues were kept for section in 10 per cent. formol-saline, and in Zenker's fixative.

*Histology**Naples 58.**Respiratory.*

The appearance of the lung tissue varied in different sections. A thick layer of organised exudate covered the consolidated lobes. The lungs themselves, on the other hand, showed little evidence of organisation. The alveoli were filled with exudate, which, in parts, was granular and completely non-cellular. In some areas, there was a fibrin network separated from alveolar septae by healthy-looking polymorphonuclears. The fibrin mass, in other parts, appeared more hyaline and contained a few fibroblasts. The appearance suggested a partially-unresolved lobar pneumonia.

*Cardio-vascular.*

There was some fatty degeneration of the heart muscle, but no cell infiltration.

The liver showed congestion limited to the central zones of the lobules.

## 240 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### Genito-urinary.

Congestion was slight in the renal cortex and medulla, and foci of cellular infiltration were scanty.

The glomeruli were enlarged, and some of them swollen to fill the capsular space. Their cellularity was not increased, and many of the endothelial cell nuclei appeared shrunken and pyknotic. In many tufts the capillary loops were dilated and packed with erythrocytes or granular debris; in an occasional loop, fibrin was present. The basement membrane appeared normal. The epithelial lining of the tufts was normal, but in occasional glomeruli the capsular epithelium was partly desquamated. A few sclerosed glomeruli, surrounded by areas of fibrosis, were seen.

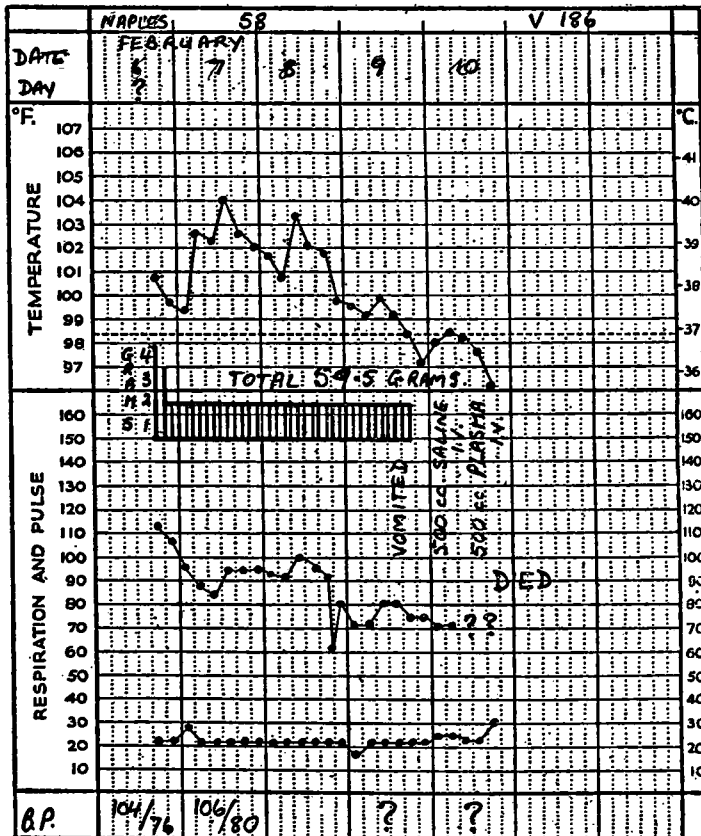
The specialised epithelium of the cortex showed gross abnormality. In some parts, the epithelial cells were deeply eosinophilic and swollen, with coarsely granular cytoplasm and nuclei either pyknotic or in a state of karyorrhexis; others showed areas of intense hyaline droplet degeneration interspersed with zones in which the cell cytoplasm was swollen and vacuolated. The proximal and distal convoluted tubules appeared almost equally affected, and the ascending limb of Henle's loop rather less so. Casts were abundant, some obviously consisting of epithelial cell debris, others hyaline, and many of the collecting tubules in the medulla contained erythrocytes.

There was no general increase in interstitial tissue, but in the deeper layers of the cortex and medulla interstitial oedema was present.

Some of the larger branches of the arcuate arteries showed patchy intimal thickening, and in a few of the smaller arterioles, hyaline thickening of the intima was present.

### Brain.

Characteristic nodular lesions were seen in fairly large numbers in the brain stem, cerebellum and cerebral cortex.



APPENDIX TABLE XIV

Index No. : NAPLES 58

Doubtful Typhus treated with V186

Date	Day	Agglutinations						Blood								Animal inoculations	
		Proteus	M.	C.	T.A.B.	M.A.	Slide	Urea	Cl.	CO <sub>2</sub>	Hgl.	S.G.		Rbc.	Wbc.		Differential
												Bl.	Pl.				
7.2	?	X19 : -50 X2 : ++++50 XK : ++++25	-20	-20	T.-25 A.-25 B.-25	M.-25 A.-25	-	45		52.3	87.5			3.82	16800	P.S.10956 L.1328 U.2988 M.1328	Guinea-pigs inoculated with whole blood intraperitoneally. No fever. Passage negative.
8.2		X19 : -50 X2 : -50 XK : -50	-20	-20	T.-25 A.-25 B.-25	M.-25 A.-25	-		342.9	46.2							
10.2							-	64		19.7				4.08			
11.2	p.m.	X19 : -50 X2 : -50 XK : -50	-20	-20	T.-25 A.-25 B.-25	M.-25 A.-25		201									
8.2	Blood	culture : sterile.															
11.2	Lung	culture at post-mortem : growth of pneumococci (not typed).															

APPENDICES

## APPENDIX B

## LIST OF DRUGS TESTED FOR CHEMOTHERAPEUTIC ACTIVITY AGAINST EXPERIMENTAL TYPHUS INFECTION IN MICE

The drugs in the appended lists have been tested for activity against typhus by the methods described in the Introduction (see also Andrewes, King, van den Ende and Walker, 1944). The majority of drugs, particularly those related to the active substances, were made by our colleagues—Dr. H. King, F.R.S., and Dr. J. Walker. An account of the chemical side of their work has been published elsewhere (Andrewes, King and Walker, 1946a).

A number of compounds were selected from lists supplied by the Therapeutic Research Corporation of Great Britain and by Imperial Chemical (Pharmaceuticals) Ltd., and a few came from individual sources.

## I.—SUBSTANCES SHOWING ACTIVITY

	A*	B*
	mg.	mg.
V147 <i>p</i> -Sulphonamidobenzamidine hydrochloride .. .. .	24	4
V186 <i>p</i> -Sulphonamidobenzamidoxime hydrochloride .. .. .	24	2
V207 <i>p</i> -Sulphonamidobenzamidoxime (free base of V186) .. .. .	16	4
V192 <i>p</i> -Sulphonmethylamidobenzamidine hydrochloride .. .. .	16	8
V262 <i>p</i> -Sulphonhydroxylamidobenzamidoxime base .. .. .	16	4
V279 <i>p</i> -Sulphonamido- <i>m</i> -tolamidoxime hydrochloride .. .. .	16	>8
V276 <i>p</i> -Sulphonamido- <i>m</i> -tolamidine hydrochloride .. .. .	8	>4
V231 <i>p</i> -Sulphonamidobenzamidoxime methyl ether hydrochloride .. .. .	16	8
V232 <i>p</i> -Sulphonamidobenzamidoxime ethyl ether hydrochloride .. .. .	8	4
V280 NN'-Dipropoxybenzamidine- <i>p</i> -sulphonamide .. .. .	16	8
V281 NN'-Dimethoxybenzamidine- <i>p</i> -sulphonamide .. .. .	8	4
V283 NN'-Diethoxybenzamidine- <i>p</i> -sulphonamide .. .. .	8	>8
V238 <i>p</i> -Sulphonamidobenzamidoxime ureide .. .. .	8	2

All the hydrochlorides in the above substances were readily soluble in water; the free bases were very sparingly so and were suspended in 5 per cent. gum-acacia in saline.

As will be seen, none of the related substances has as favourable a ratio between toxicity and activity as have V147 and V186. The bearing of all our findings on the relation between chemical constitution and chemotherapeutic activity against rickettsiae is discussed in a separate paper (Andrewes, King and Walker, 1946a).

## II.—SUBSTANCES SHOWING NO ACTIVITY

The figures in brackets give the maximum dose in milligrammes tolerated by a 15 g. mouse. The figures should be taken as only approximate. Injections were intraperitoneal except where the letters "s.c." indicate subcutaneous injections.

## Long-chain compounds—

V5 Stearamidine hydrochloride .. .. .	(0.4)
V8 Stilbamidine dihydrochloride .. .. .	(0.4)
V13 Propamidine dihydrochloride .. .. .	(0.4)
V12 Pentamidine dihydrochloride .. .. .	(0.4)
V9 Diphenylmethane-4 : 4'-diamidine dihydrochloride .. .. .	(0.4)
V11 Naphthalene-2 : 7-diamidine dihydrochloride .. .. .	(0.4)
V3 Hexadecylguanidine hydrochloride .. .. .	(0.15)
V1 Dodecanediguandine dihydrochloride .. .. .	(0.022)
V2 Hexadecanediguandine dihydrochloride .. .. .	(0.02)
V10 Diphenylmethane-4 : 4'-diguandine dinitrate .. .. .	(0.4)
V82 O-Nonylhydroxylamine hydrochloride .. .. .	(0.4)
V83 O-Nonylhydroxyguanidine nitrate .. .. .	(0.4)
V4 Dodecanedi-4sothiurea dihydrobromide .. .. .	(0.4)
V75 Cetylpyridinium bromide .. .. .	(0.03)

A\* = Max. tolerated dose for 15 g. mouse.

B\* = Minimal effective (wholly suppressive) dose given twice daily.

*Dyes—*

V14	2 : 7-Diaminoacridine dihydrochloride		..	..	..	..	..	..	(8)
V16	2 : 8-Diaminoacridine dihydrochloride		..	..	..	..	..	..	(0.06)
V17	Acridavine	..	..	..	..	..	..	..	(0.06)
V18	Rivanol	..	..	..	..	..	..	..	(0.35)
V19	Mepacrine (Atebrin)	..	..	..	..	..	..	..	(1.8)
V346	Congo Red		..	..	..	..	..	..	—
V37	Trypan Red	} Congo Red Type	..	..	..	..	..	..	(0.32)
V32	Trypan Blue		..	..	..	..	..	..	(0.16)
V29	Bayer 205		..	..	..	..	..	..	(1.5)
*V24	Methylene Blue		..	..	..	..	..	..	(0.95)
*V25	Selenium Methylene Blue		..	..	..	..	..	..	(2.5) s.c.
V52	Toluidine Blue		..	..	..	..	..	..	(1)
V345	Thionin	} Thiazine type	..	..	..	..	..	..	—
V373	Methylene Green B		..	..	..	..	..	..	(1) s.c.
V375	New Methylene Blue NSS		..	..	..	..	..	..	(1) s.c.
V45	Brilliant Cresyl Blue		..	..	..	..	..	..	(2)
V46	Nile Blue		..	..	..	..	..	..	(1)
V85	Nile Blue R		..	..	..	..	..	..	(1)
V86	Meldola Blue	} Phenoxazines	..	..	..	..	..	..	(0.1)
V87	Capri Blue GON		..	..	..	..	..	..	(0.22)
V88	Cresyl Fast Violet 2B		..	..	..	..	..	..	(0.5)
V47	Neutral Red		..	..	..	..	..	..	(1)
V49	Safranin	} Phenazines	..	..	..	..	..	..	(0.2)

*Alkaloids—*

V20	Quinine hydrochloride	..	..	..	..	..	..	..	(1.25)
V21	Cinchonidine hydrochloride	..	..	..	..	..	..	..	(2.25)
V22	Optochin hydrochloride	..	..	..	..	..	..	..	(1.4)
V93	Apoquinine dihydrochloride	..	..	..	..	..	..	..	(1.9)
V319	isoEmetine hydrochloride	..	..	..	..	..	..	..	(2)

*Arsenicals—*

V54	Sodium methylarsonate	..	..	..	..	..	..	..	(4)
V55	Sodium cacodylate	..	..	..	..	..	..	..	(5)
V61	Tryparsamide	..	..	..	..	..	..	..	(15)
V94	Allylarsonic acid	..	..	..	..	..	..	..	(2)
V299	$\gamma$ -n-Butylaminopropylarsonic acid hydrochloride	..	..	..	..	..	..	..	(4)
V310	$\gamma$ -cycloHexylaminopropylarsonic acid hydrochloride	..	..	..	..	..	..	..	(2)
V318	$\gamma$ -Benzylaminopropylarsonic acid hydrochloride	..	..	..	..	..	..	..	(0.5)

*Aromatic Amidoximes without Sulphur—*

V173	Benzamidoxime hydrochloride	..	..	..	..	..	..	..	(2)
V182	p-Chlorobenzamidoxime hydrochloride	..	..	..	..	..	..	..	(1)
V179	p-Bromobenzamidoxime hydrochloride	..	..	..	..	..	..	..	(1)
V183	3 : 4-Piperonylamidoxime hydrochloride	..	..	..	..	..	..	..	(2)

*Aromatic Amidines (without sulphur)—*

V158	Benzamidine hydrochloride	..	..	..	..	..	..	..	(16)
V162	p-Chlorobenzamidine hydrochloride	..	..	..	..	..	..	..	(0.55)
V161	m-Chlorobenzamidine hydrochloride	..	..	..	..	..	..	..	(0.075)
V174	p-Bromobenzamidine hydrochloride	..	..	..	..	..	..	..	(2)
V171	p-Tolamidine hydrochloride	..	..	..	..	..	..	..	(1)
V150	p-Aminobenzamidine hydrochloride	..	..	..	..	..	..	..	(1.5)
V170	m-Aminobenzamidine hydrochloride	..	..	..	..	..	..	..	(2)
V164	p-Nitrobenzamidine hydrochloride	..	..	..	..	..	..	..	(0.05)
V163	m-Nitrobenzamidine hydrochloride	..	..	..	..	..	..	..	(0.25)
V169	p-Hydroxybenzamidine hydrochloride	..	..	..	..	..	..	..	(1.7)
V172	p-Methoxybenzamidine hydrochloride	..	..	..	..	..	..	..	(2)
V290	p-Methoxybenz- $\beta$ -diethylaminoethylamidine dihydrochloride	..	..	..	..	..	..	..	(4)
V185	p-Ethoxybenzamidine hydrochloride	..	..	..	..	..	..	..	(2)
V180	3 : 4-Piperonylamidine hydrochloride	..	..	..	..	..	..	..	(2)
V190	p-Carbamylbenzamidine hydrochloride	..	..	..	..	..	..	..	(8)

\* These substances were ineffective when tested by our routine technique.

Toluidine blue has, however, been shown by Peterson (1944) to have a therapeutic effect when given in the food to mice infected with murine typhus. We confirmed this result and found methylene blue equally effective, when fed in sufficient quantity. Some activity was found in a few other closely related dyes (Andrewes, King and Walker, 1946b).

## 244 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### *Aromatic Amidines (without sulphur)—continued*

V159	Phenylacetamide hydrochloride	..	..	..	..	..	..	(4)
V168	p-Nitrophenylacetamide hydrochloride	..	..	..	..	..	..	(0·5)
V160	m-Phenylbutylamide hydrochloride	..	..	..	..	..	..	(0·05)
V166	N-2-Pyridylbenzamide hydrochloride	..	..	..	..	..	..	(0·17)
V165	N-2-Pyridyl-p-chlorobenzamide hydrochloride	..	..	..	..	..	..	(0·25)
V149	N-2-Pyridyl-m-nitrobenzamide hydrochloride	..	..	..	..	..	..	(0·5)
V167	N-2-Pyridylphenylacetamide hydrochloride	..	..	..	..	..	..	(0·15)
V261	N-2-Thiazolyl-p-nitrobenzamide	..	..	..	..	..	..	(8)

### *Aromatic Guanidines—*

V156	Phenylguanidine nitrate	..	..	..	..	..	..	—
V314	p-Chlorophenylguanidine nitrate	..	..	..	..	..	..	(2)
V315	p-Bromophenylguanidine nitrate	..	..	..	..	..	..	(1)
V304	m-Tolylguanidine sulphate	..	..	..	..	..	..	(2)
V302	o-Tolylguanidine nitrate	..	..	..	..	..	..	(1)
V184	p-Tolylguanidine nitrate	..	..	..	..	..	..	(1)
V324	o-Xyllylguanidine nitrate	..	..	..	..	..	..	(0·5)
V317	m-Xyllylguanidine nitrate	..	..	..	..	..	..	(1)
V322	p-Xyllylguanidine nitrate	..	..	..	..	..	..	(1)
V321	p-Ethylguanidine nitrate	..	..	..	..	..	..	(0·5)
V316	o-Anisylguanidine nitrate	..	..	..	..	..	..	(2)
V313	p-Anisylguanidine nitrate	..	..	..	..	..	..	(2)
V303	p-Phenethylguanidine nitrate	..	..	..	..	..	..	(1)
V312	Benzylguanidine nitrate	..	..	..	..	..	..	(1)
V300	p-Acetophenylguanidine hydrochloride	..	..	..	..	..	..	(1)

### *Biguanides—*

V157	Phenylbiguanide hydrochloride	..	..	..	..	..	..	(2)
V326	p-Chlorophenylbiguanide hydrochloride	..	..	..	..	..	..	(2)
V327	p-Bromophenylbiguanide hydrochloride	..	..	..	..	..	..	(2)
V320	p-Tolylbiguanide hydrochloride	..	..	..	..	..	..	(2)
V333	m-Tolylbiguanide hydrochloride	..	..	..	..	..	..	(1)
V329	o-Tolylbiguanide hydrochloride	..	..	..	..	..	..	(1)
V325	o-Xyllylbiguanide hydrochloride	..	..	..	..	..	..	(2)
V334	m-Xyllylbiguanide hydrochloride	..	..	..	..	..	..	(0·5)
V328	p-Xyllylbiguanide hydrochloride	..	..	..	..	..	..	(0·5)
V323	p-Ethylphenylbiguanide hydrochloride	..	..	..	..	..	..	(1)
V330	o-Anisylbiguanide hydrochloride	..	..	..	..	..	..	(1)
V331	p-Anisylbiguanide hydrochloride	..	..	..	..	..	..	(1)
V332	p-Phenethylbiguanide hydrochloride	..	..	..	..	..	..	(0·5)
V336	p-Acetophenylbiguanide hydrochloride	..	..	..	..	..	..	(2)

### *Sulphanilamide and Related Substances—*

V100	Sulphanilamide	..	..	..	..	..	..	(7·5)
V144	p-Aminobenzenesulphonacetamide	..	..	..	..	..	..	(7·5)
V251	N <sup>1</sup> -3 : 4-Dimethylbenzoylsulphanilamide	..	..	..	..	..	..	(8)
V273	N <sup>1</sup> -3-Methoxy-4-hydroxybenzylsulphanilamide	..	..	..	..	..	..	(4)
V473	Uleron	..	..	..	..	..	..	(7·5)
V177	p-Aminobenzenesulphonylbenzamide	..	..	..	..	..	..	(4)
V178	iso-p-Aminobenzenesulphonylbenzamide	..	..	..	..	..	..	(8)
V127	2-p-Aminobenzenesulphonamido-4-ethyl-6-methylpyrimidine	..	..	..	..	..	..	(15)
V102	Sulphapyridine	..	..	..	..	..	..	(0·75)
V101	Sulphathiazole	..	..	..	..	..	..	(0·75)
V128	2-p-Aminobenzenesulphonamido-4-amyl-6-methylpyrimidine	..	..	..	..	..	..	(15)
V129	2-p-Aminobenzenesulphonamido-5 : 6-benzpyrimidine	..	..	..	..	..	..	(7·5)
V130	2-p-Aminobenzenesulphonamido-4-chloro-6-methylpyrimidine	..	..	..	..	..	..	(7·5)
V131	2-p-Aminobenzenesulphonamido-4-hydroxy-6-methylpyrimidine	..	..	..	..	..	..	(15)
V148	N-Phenylmercuri-N-acetylsulphanilamide	..	..	..	..	..	..	(7·5)
V257	N <sup>4</sup> -Dimethylsulphanilamide	..	..	..	..	..	..	(8)
V244	N <sup>4</sup> -Dimethylsulphanilamide methochloride	..	..	..	..	..	..	(2)
V189	N <sup>4</sup> -Guanylsulphanilamide hydrochloride	..	..	..	..	..	..	(4)
V286	NN'-Diphenylguanidine-4 : 4'-disulphonamide	..	..	..	..	..	..	(8)
V267	N <sup>1</sup> -Propylsulphanilamide	..	..	..	..	..	..	(8)
V259	2-Aminopyridine-5-sulphonamide	..	..	..	..	..	..	(8)
V289	Sulphanilyldiethylguanidine	..	..	..	..	..	..	(4)
V291	p-Aminobenzenesulphon-3 : 5-dichloroanilide	..	..	..	..	..	..	(4)
V292	p-Aminobenzenesulphon-3 : 5-dibromoanilide	..	..	..	..	..	..	(4)
V293	p-Aminobenzenesulphon-p'-guanylanilide	..	..	..	..	..	..	(4)
V288	6-Sulphonamidoquinoline	..	..	..	..	..	..	(1)
		..	..	..	..	..	..	(4)

*Marfanil and analogues—*

V256	p-Sulphonamidobenzylamine hydrochloride (marfanil)	..	..	..	(8)
V255	p-Sulphonamidobenzyltrimethylammonium chloride	..	..	..	(4)
V335	p-Methylsulphonylbenzylamine hydrochloride	..	..	..	(8)

*Diaminodiphenyl sulphone and related substances—*

V140	4 : 4'-Diaminodiphenyl sulphone	..	..	..	..	(15)
V151	Sodium 4 : 4'-bis- $\gamma$ -phenyl-n-propylaminodiphenyl sulphone tetrasulphonate	..	..	..	..	(40)
V134	Monosuccinyl-4 : 4'-diaminodiphenyl sulphone	..	..	..	..	(15)
V132	$\beta\beta'$ -Di-p-aminophenylsulphonylethyl ether	..	..	..	..	(15)
V133	p-Aminophenylsulphonylacetamide hydrochloride	..	..	..	..	(15)
V282	Sodium sulphanilylacetate	..	..	..	..	(8)
V146	5 : 5'-Diamino-2 : 2'-dipyridyl sulphone	..	..	..	..	(7.5)

*p-Sulphonamidobenzamide and related substances—*

V193	p-Sulphonbutylamidobenzamide hydrochloride	..	..	..	..	(1)
V204	p-Sulphondimethylamidobenzamide hydrochloride	..	..	..	..	(4)
V200	p-Sulphondiethylamidobenzamide hydrochloride	..	..	..	..	(1)
V206	p-Sulphondiethanolamidobenzamide hydrochloride	..	..	..	..	(8)
V196	p-Sulphonphenylamidobenzamide hydrochloride	..	..	..	..	(1)
V202	p-Sulphonpiperidobenzamide hydrochloride	..	..	..	..	(1)
V201	p-Sulphonmorpholidobenzamide hydrochloride	..	..	..	..	(1)
V195	p-Sulphon-2-pyridylamidobenzamide hydrochloride	..	..	..	..	(4)
V216	2'-p-Guanylbenzenesulphonamidothiazole hydrochloride	..	..	..	..	(2)
V197	2'-p-Guanylbenzenesulphonamido-4'-methylthiazole hydrochloride	..	..	..	..	(4)
V218	2'-p-Guanylbenzenesulphonamido-4' : 6'-dimethylpyrimidine hydrochloride	..	..	..	..	(1)
V243	p-Guanylbenzenesulphonamidoacetamide dihydrochloride	..	..	..	..	(4)
V277	p-Sulphon-O-methylhydroxylamidobenzamide hydrochloride	..	..	..	..	(4)
V284	p-Sulphon-O-ethylhydroxylamidobenzamide hydrochloride	..	..	..	..	(4)
V268	p-Sulphon-O-propylhydroxylamidobenzamide hydrochloride	..	..	..	..	(1)
V265	p-Sulphon-O-butylhydroxylamidobenzamide hydrochloride	..	..	..	..	(1)
V226	p-Sulphon-p'-sulphamylphenylamidobenzamide nitrate	..	..	..	..	(4)
V227	p-Sulphon-p'-dimethylsulphamylphenylamidobenzamide hydrochloride	..	..	..	..	(4)
V228	p-Sulphon-p'-guanylphenylamidobenzamide dihydrochloride	..	..	..	..	(4)
V221	p-Guanylbenzenesulphonylguanidine hydrochloride	..	..	..	..	(8)
V210	p-Sulphonamidobenz-N-methylamide hydrochloride	..	..	..	..	(8)
V294	p-Sulphonamidobenz-N-butylamide hydrochloride	..	..	..	..	(4)
V212	p-Sulphonamidobenz-NN'-dimethylamide hydrochloride	..	..	..	..	(2)
V214	p-Sulphondimethylamidobenz-NN'-dimethylamide hydrochloride	..	..	..	..	(4)
V230	p-Sulphonamidobenzamidrazone hydrochloride	..	..	..	..	(8)
V250	2'-p-Sulphonamidophenyl-4' : 6'-dimethylpyrimidine	..	..	..	..	(8)
V222	$\omega$ -p-Sulphonamidotolamide hydrochloride	..	..	..	..	(4)
V223	p-Sulphonamidophenylacetamide hydrochloride	..	..	..	..	(8)
V247	l-Sulphonamidobenanthamide hydrochloride	..	..	..	..	(8)
V175	m-Sulphonamidobenzamide hydrochloride	..	..	..	..	(8)

*p-Sulphonamidobenzamidoxime and related substances—*

V198	p-Sulphondiethylamidobenzamidoxime hydrochloride	..	..	..	..	(1)
V252	p-Sulphondiethanolamidobenzamidoxime hydrochloride	..	..	..	..	(16)
V203	p-Sulphonpiperidobenzamidoxime hydrochloride	..	..	..	..	(1)
V199	p-Sulphonmorpholidobenzamidoxime hydrochloride	..	..	..	..	(8)
V278	p-Sulphon-O-methylhydroxylamidobenzamidoxime hydrochloride	..	..	..	..	(4)
V270	p-Sulphon-O-ethylhydroxylamidobenzamidoxime hydrochloride	..	..	..	..	(2)
V269	p-Sulphon-O-propylhydroxylamidobenzamidoxime hydrochloride	..	..	..	..	(1)
V266	p-Sulphon-O-butylhydroxylamidobenzamidoxime hydrochloride	..	..	..	..	(2)
V217	2'-p-Hydroxyguanylbenzenesulphonamidothiazole hydrochloride	..	..	..	..	(1)
V253	p-Sulphon-p'-dimethylsulphamylphenylamidobenzamidoxime	..	..	..	..	(8)
V263	p-Sulphonamidobenzamidoxime O-propyl ether hydrochloride	..	..	..	..	(2)
V233	p-Sulphonamidobenzamidoxime O-butyl ether hydrochloride	..	..	..	..	(1)

*Amidines and Amidoximes containing Sulphone groups—*

V181	4 : 4'-Diguanyldiphenyl sulphone dihydrochloride	..	..	..	..	(0.47)
V187	p-Methylsulphonylbenzamide hydrochloride	..	..	..	..	(8)
V297	p-Propylsulphonylbenzamide hydrochloride	..	..	..	..	(4)
V338	p-Phenylsulphonylbenzamide hydrochloride	..	..	..	..	(1)
V339	p-Benzylsulphonylbenzamide hydrochloride	..	..	..	..	(1)
V205	p-2' : 5'-Dihydroxyphenylsulphonylbenzamide hydrochloride	..	..	..	..	(1)
V341	p-Guanylphenylsulphonylacetamide dihydrochloride	..	..	..	..	(2)
V191	p-Methylsulphonylbenzamidoxime hydrochloride	..	..	..	..	(8)

## 24:6 CHEMOTHERAPEUTIC AND OTHER STUDIES OF TYPHUS

### Miscellaneous Sulphonamides and related compounds—

V258 Benzenesulphonamide .. .. .	(8)
V254 p-Cyanobenzenesulphonamide .. .. .	(1)
V241 p-Sulphonamidobenzamide .. .. .	(8)
V136 Pyridine-3-sulphonic acid .. .. .	(2)
V135 2-Thiophenesulphonamide .. .. .	(0.5)
V351 p-Guanylbenzenesulphinic acid .. .. .	(8)
V145 2-p-Hydroxybenzenesulphonamidopyridine .. .. .	(7.5)
V239 p-Sulphonamidobenzhydroxamic acid .. .. .	(4)

### Antibiotics—

V155 Tyrothricin .. .. .	(0.05)
*V123 Penicillin .. .. .	—

### Pyrimidines—

V308 2-Diethylaminoisoamylamino-4 : 6-dimethylpyrimidine .. .. .	(2)
V271 2'-(6-Bromo- $\beta$ -naphthylamino)-4'-methyl-6'-diethylaminoethylaminopyrimidine .. .. .	(1)
V272 2'-4-Chlorophenylguanido-4'-methyl-6'-diethylaminoethylaminopyrimidine .. .. .	(0.5)

### Miscellaneous Compounds—

V56 Urotropine .. .. .	(0.075)
V76 Hexylresorcinol .. .. .	(0.6)
V67 Sodium $\beta$ -naphthaquinonesulphonate .. .. .	(1.5)
V139 9 : 10-Phenanthraquinone .. .. .	(15)
V97 Solganal B .. .. .	(3.3)
V98 Myochrysin .. .. .	(0.1)
V141 Vanadyl tartrate .. .. .	(0.5)
V208 p-Aminophenol .. .. .	—
†V209 p-Aminobenzoic acid .. .. .	(20)
V264 Thiourea .. .. .	(25)
V142 Thionicotinamide .. .. .	—
V260 2-Aminopyridine sulphate .. .. .	(0.25)
V344 Thiodiphenylamine .. .. .	—
V137 2-Amino-6-carboxybenzthiazole .. .. .	(15)
V138 9-Amylfluorene-9-carboxylic acid .. .. .	(2)
V307 Iodoacetamide .. .. .	(0.25)
V301 Tetrahydrogeranylamine hydrochloride .. .. .	(1)
V306 $\alpha$ -Naphthylacetic acid .. .. .	(4)
V296 2-Anilino-4-chloro-6-diethylaminoethylamino-1 : 3 : 5-triazine .. .. .	(1)
V295 4-Diethylaminoisoamylaminocinnoline .. .. .	(4)
V309 2-p-Chloroanilino-4-diethylaminoethylaminoquinoline .. .. .	(0.5)

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\* Penicillin has been shown by Moragues, Pinkerton and Greiff (1944) to have some therapeutic activity in murine typhus infection of mice. They used much higher doses than were available to us when our tests were carried out.

† p-Aminobenzoic acid was ineffective when tested by our routine method. We could, however, in confirmation of Snyder, Maier and Anderson (quoted by Yeomans *et al.*, 1944), find a little activity when the drug was given by mouth to typhus-infected mice.



## PLATE II

- Fig. A. Blood vessel in conjunctiva showing thrombosis with partial occlusion of the lumen, and perivascular accumulation of leucocytes. Hæmalum and eosin. × 450.
- Fig. B. Conjunctival blood vessel with swollen endothelium and perivascular infiltration. Hæmalum and eosin. × 450.
- Fig. C. Conjunctival blood vessel showing displacement of swollen endothelial cells into the lumen. Hæmalum and eosin. × 450.
- Fig. D. Bronchiole containing cellular exudate, and with surrounding localised infiltration of mononuclear cells. Hæmalum and eosin. × 67.

PLATE II

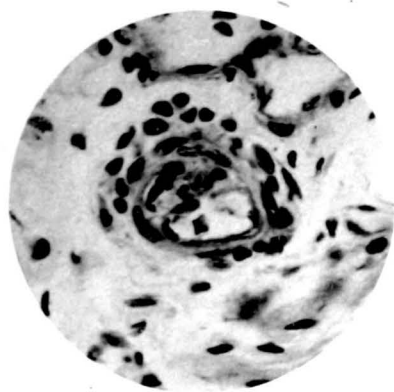


FIG. A

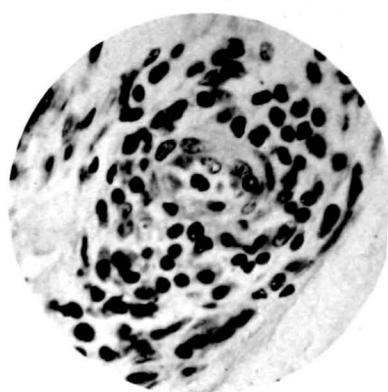


FIG. B

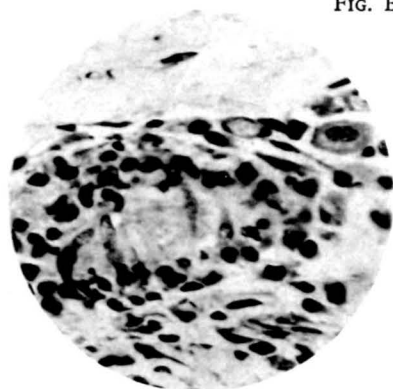


FIG. C

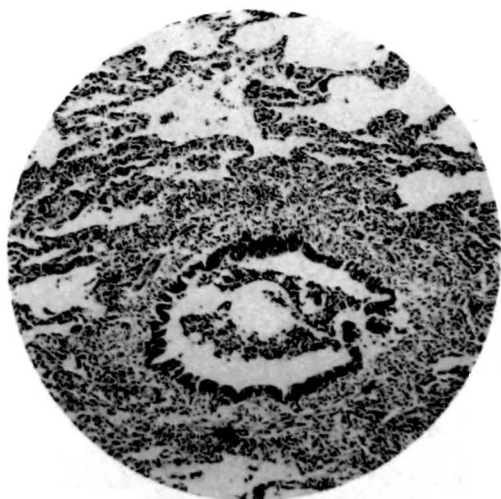


FIG. D

### PLATE III

- Fig. A. Myocardium showing diffuse interstitial infiltration and degenerative changes in the muscle fibres. Hæmalum and eosin.  $\times 67$ .
- Fig. B. High-power view of part of Fig. A, showing the characteristic mononuclear cells in and around a small blood vessel. Hæmalum and eosin.  $\times 375$ .
- Fig. C. Myocardium of patient who died with cardiac failure—showing extensive hyaline necrosis and relatively scanty interstitial cellular reaction. Hæmalum and eosin.  $\times 190$ .
- Fig. D. Focal lesion in the olivary nucleus. Hæmalum and eosin.  $\times 67$ .
- Fig. E. Focal lesion in cerebellum from a patient in whom serological tests for typhus remained persistently negative. Hæmalum and eosin.  $\times 67$ .
- Fig. F. Hæmorrhage into perivascular space round a small vessel in the brain. Hæmalum and eosin.  $\times 67$ .
- Fig. G. Petechial hæmorrhage in the mid-brain. Hæmalum and eosin.  $\times 67$ .

PLATE III

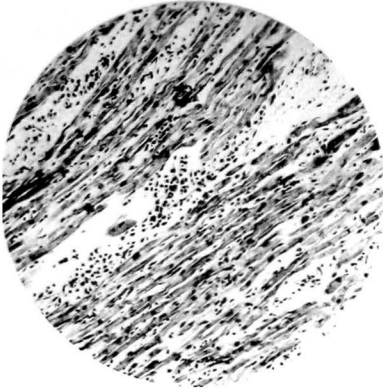


FIG. A

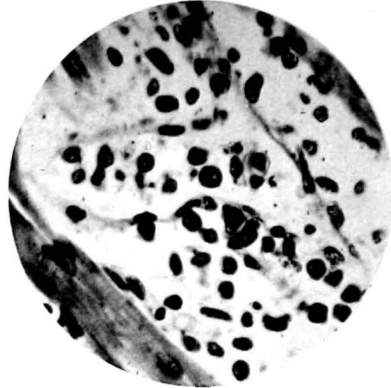


FIG. B



FIG. C

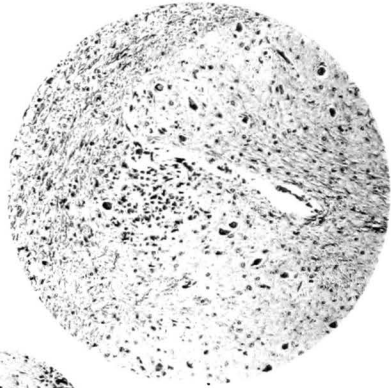


FIG. D

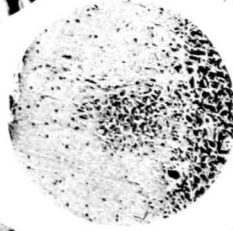


FIG. E

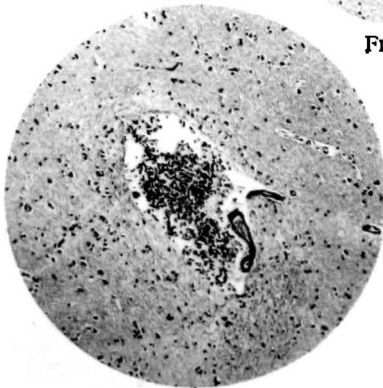


FIG. F

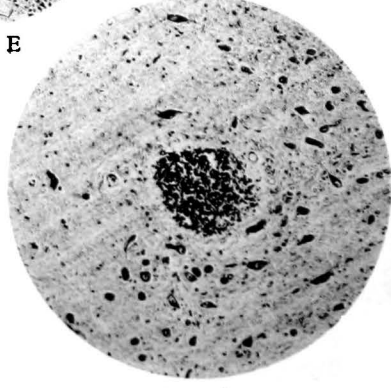


FIG. G

#### PLATE IV

Section of kidney from case Naples 62. Fairly abundant, oedematous, newly-formed connective tissue separates the tubules, many of which are degenerate or necrotic. Others show varying degrees of epithelial regeneration. Casts are abundant. Acid fuchsin, aniline blue, orange G.  $\times 67$ .

PLATE IV



#### PLATE V

- Fig. A. Section of kidney from case Naples 62. The enlarged glomerulus shows focal intra-capillary glomerulitis with marked increase of endothelial cells occluding the capillary lumina. Hæmalum and eosin.  $\times 230$ .
- Fig. B. Section of kidney from case Naples 62. The glomerulus shows focal development of fine intra-capillary fibrils. Aniline blue.  $\times 315$ .
- Fig. C. Section of kidney from case Naples 61, showing small arteriole occluded by thrombus. Adjacent tubules have disappeared, and the area is infiltrated by mononuclear cells. Hæmalum and eosin.  $\times 230$ .

PLATE V

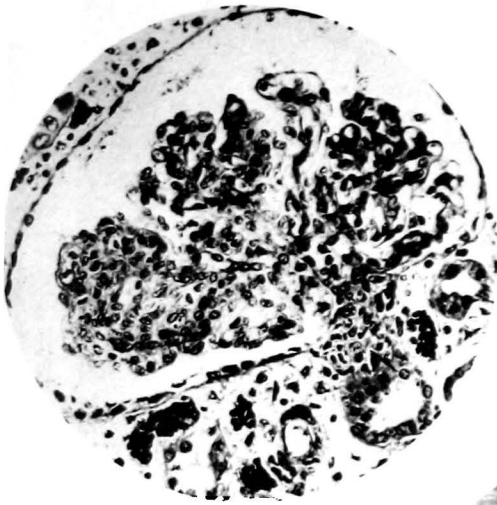


FIG. A



FIG. B

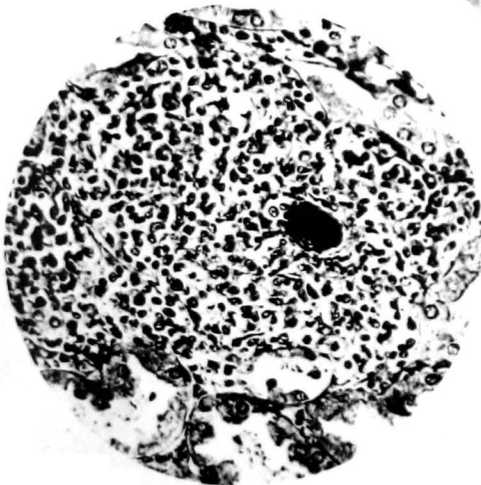


FIG. C



#### PLATE VI

- Fig. A. Section of kidney from case Naples 55, showing moderate enlargement of glomerulus and slight general increase in cellularity. Hæmalum and eosin.  $\times 230$ .
- Fig. B. Section of kidney from case Naples 55, showing general patency of glomerular capillaries without thickening of connective tissue membrane. Aniline blue.  $\times 315$ .
- Fig. C. Section of kidney from case Naples 77. This patient had signs of cardiac failure of congestive type. The glomerular capillaries are very congested and show extreme rounded patency. Aniline blue.  $\times 315$ .

PLATE VI

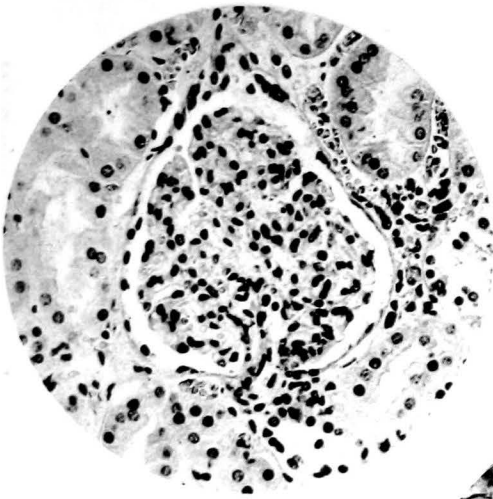


FIG. A



FIG. B

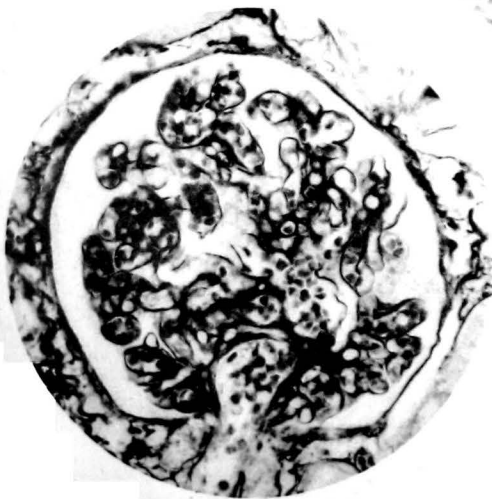


FIG. C

#### PLATE VII

- Fig. A. Section of kidney from case Naples 86. The glomerulus shows focal congestion and focal increased cellularity. Hæmalum and eosin.  $\times$  230.
- Fig. B. Section of kidney from case Naples 54. A small area of necrosis of capillary loops is seen close to the attachment of the tuft, with pyknosis and karyorrhexis of the nuclei. Hæmalum and eosin.  $\times$  230.

PLATE VII

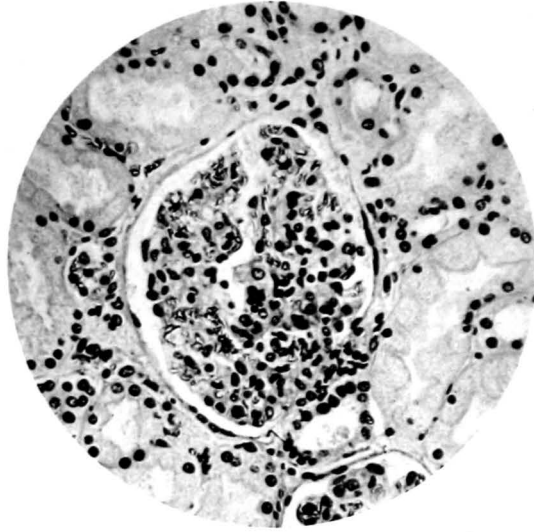


FIG. A

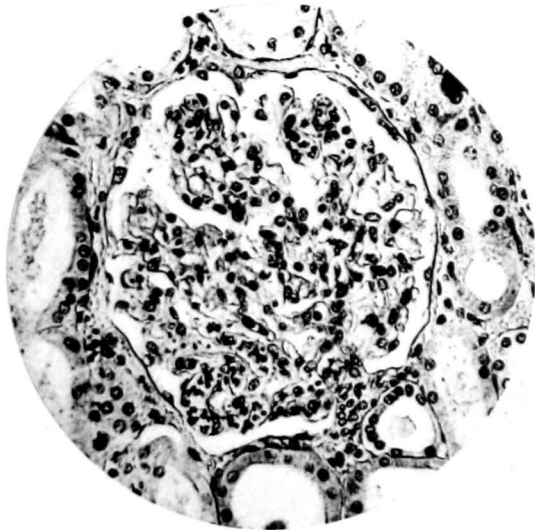


FIG. B

PLATE VIII

- Fig. A. Section of kidney from case Naples 43, showing hæmorrhage into the glomerular space and erythrocytes in the related convoluted tubules. Hæmalum and eosin.  $\times 120$ .
- Fig. B. Section of kidney from case Naples 54, showing interstitial periglomerular accumulation of mononuclear cells with slight focal increase in endothelial cellularity of the tufts. Hæmalum and eosin.  $\times 115$ .

PLATE VIII

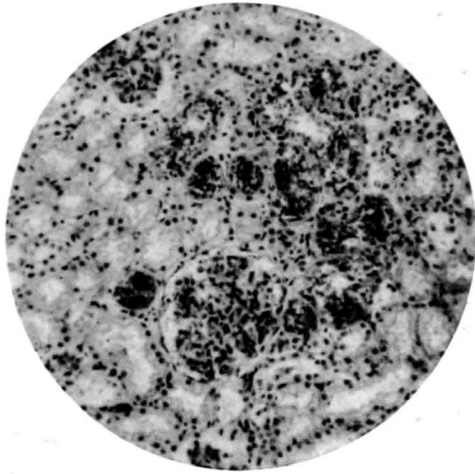


FIG. A

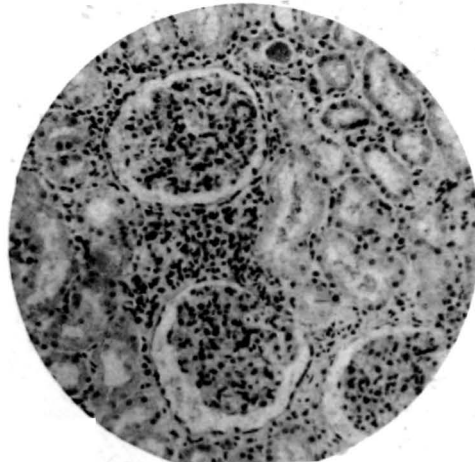


FIG. B

PLATE IX

- Fig. A. Section of renal cortex from case Naples 61, showing cloudy swelling of the cells of the convoluted tubules. Acid fuchsin, aniline blue, orange G.  $\times 67$ .
- Fig. B. Section of renal cortex from case Naples 60, treated with V186. An area of total necrosis of convoluted tubules is seen close to the glomerulus. Other tubules show varying degrees of cloudy swelling with desquamation and cast formation. Acid fuchsin, aniline blue, orange G.  $\times 67$ .

PLATE IX

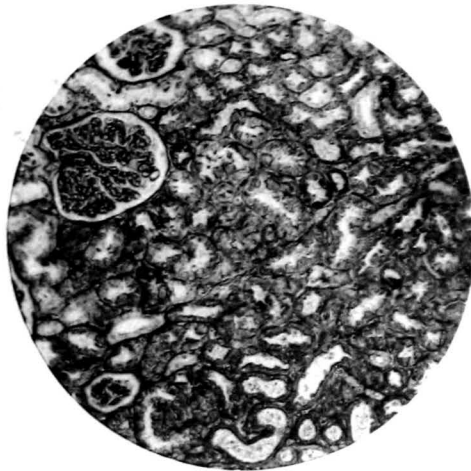


FIG. A

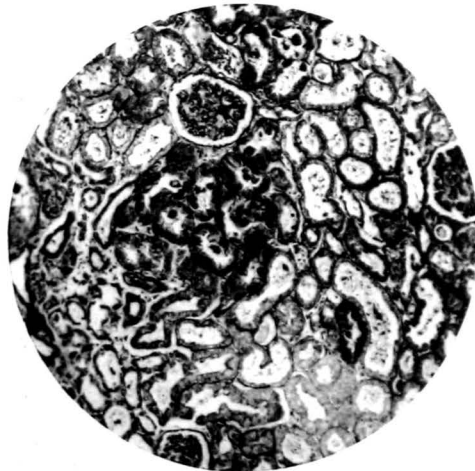


FIG. B



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