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**HEALTH ORGANISATION**

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**REPORT**

OF THE

**SECOND LABORATORY CONFERENCE**

ON THE

**SERODIAGNOSIS OF SYPHILIS**

*held at Copenhagen, May 21st to June 4th, 1928*

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Series of League of Nations Publications

III. HEALTH ✓

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# Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis, held in Copenhagen from May 21st to June 4th, 1928.

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Since the first Serological Laboratory Conference held at Copenhagen from November 19th to December 3rd, 1923, several of the methods employed for the serodiagnosis of syphilis have improved considerably, and a number of new tests have been elaborated, especially of those depending on directly visible changes in mixtures of syphilitic serum and extract (Flocculation tests).<sup>1</sup> Requests from different Institutes and individuals particularly interested in these problems have therefore been addressed to the Health Organisation with a view to the establishment of a further comparative examination of the methods of serodiagnosis of syphilis. On the proposal of its President, the Health Committee, at its eleventh session in October 1927, approved the convocation of the Second Serological Laboratory Conference on the Serodiagnosis of Syphilis. This Conference was held from May 21st to June 4th, 1928, at the Danish State Serum Institute at Copenhagen. The following attended the Conference, either as experts invited by the Health Organisation or as delegates of Governments or Institutions:

### *Austria.*

Serodiagnost. Untersuchungsanstalt Allgemeines Krankenhaus, Vienna: Professor R. MÜLLER, Dr. R. BRANDT, Mme. S. BERZELLER.

### *Denmark.*

State Serum Institute, Copenhagen: Dr. Th. MADSEN, Dr. H. BOAS, Dr. J. R. MÖRCH, Dr. K. NORÉL, Mlle. OJGAARD.

### *Federated Malay States.*

Institute for Medical Research, Kuala-Lumpur: Dr. A. NEAVE  
KINGSBURY.

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<sup>1</sup> See resolutions.

*France.*

Faculty of Medicine, School of Serology, Paris: Dr. R.  
DEMANCHE;  
Pasteur Institute, Paris: Dr. E. DEBAINS;  
Prophylactic Institute, Paris: Dr. A. VERNES, M. R. BRICQ,  
Mlle. M. KERDAL.

*Germany.*

Reichsgesundheitsamt, Berlin: Professor H. DOLD;  
Institute "Robert Koch", Berlin: Professor R. OTTO, Dr. G.  
BLUMENTHAL;  
Serological Institute, Ambrock, Hagen, W.: Dr. E. MEINICKE,  
Mme. L. MEINICKE;  
Allgemeines Krankenhaus St. Georg, Hamburg, 5: Dr. E.  
JACOBSTHAL, Mlle. M. HAUSCHILDT;  
Institut für experimentelle Krebsforschung, Heidelberg:  
Professor H. SACHS, Dr. E. WITEBSKY.

*Great Britain.*

Ministry of Health, London: Dr. L. W. HARRISON, Dr. E. J.  
WYLER, Mr. E. TWEED.

*India.*

Medical College, Lucknow: Dr. J. G. MUKERJI.

*Italy.*

Hygiene Institute of University Royale, Naples: Professor  
Dante DE BLASI.

*Japan.*

Government Institute for Infectious Diseases: Professor  
M. NAGAYO, Dr. K. NOBECHI.

*Poland.*

State Institute of Hygiene, Warsaw: Professor L. HIRSZFELD,  
Dr. S. SIERAKOWSKI, Mlle. H. RABINOWICZ.

*Kingdom of the Serbs, Croats and Slovenes.*

Serological Service of the Central Hygiene Institute, Belgrade:  
Dr. D. PAVLOVITCH, Mme V. KITCHEVATZ.

*Sweden.*

Pathological Institute, Lund: Professor J. FORSSMAN.

*Turkey.*

State Institute of Hygiene, Angora: Dr. V. WASSAF.

*Union of Socialist Soviet Republics.*

State Institute of Experimental Medicine, Epidemiological  
Division, Leningrad: Professor D. ZABOLOTNY.

*United States of America.*

State Hygienic Laboratory, Lansing, and University of  
Michigan, Ann Arbor: Professor R. L. KAHN.

*Uruguay.*

Laboratory of the Prophylactic Institute, Montevideo:  
Dr. A. SCALTRITTI.

The following are the names of those invited to the Conference who were unable to accept the invitation:

*Argentine.*

Public Health Institute, Buenos Ayres: Professor Alfredo  
SORDELLI.

*Belgium.*

Pasteur Institute, Brussels: Dr. RENAUX.

*United States of America.*

Graduate School of Medicine of the University of  
Pennsylvania: Dr. J. A. KOLMER.

The participants were requested to acquaint themselves before the Conference began with the technique of the various methods which were to be compared during their work.

These methods were the following:

1. *The Bordet-Wassermann Reaction (B.-W.R.) represented by Several Modifications:*

- (a) *B.-W.R.* described by Medical Research Council Special Report, Series No. 14, Method No. 1, carried out by Dr. E. J. Wyler, assisted by Mr. E. Tweed.
- (b) *B.-W.R.* described by D. de Blasi, carried out by Professor Dante de Blasi.
- (c) *B.-W.R.* described by Calmette and Massol (modified by Prof. E. Debains, for the examination of non-heated sera), carried out by Prof. Debains.

- (d) *B.-W.R.* synoptic method described by Jacobsthal, carried out by Dr. E. Jacobsthal, assisted by Mlle. M. Hauschildt.
- (e) *B.-W.R.* original method, carried out by Dr. G. Blumenthal.
- (f) *B.-W.R.* original method, carried out by Dr. D. Pavlovitch, assisted by Mme. V. Kitchevatz.
- (g) *B.-W.R.* described by McIntosh and Fildes, carried out by Dr. S. Sierakowsky, assisted by Mlle. H. Rabinowicz.

## 2. Flocculation tests.

- (a) *Kahn reaction*, carried out by (1) Dr. H. Boas, assisted by Mlle. E. Oejgaard, (2) Professor R. L. Kahn.
- (b) *Meinickes Trübungsreaktion (Meinicke's Turbidity Test) (M.T.R.)*, carried out by Dr. E. Meinicke, assisted by Mme. L. Meinicke.
- (c) *Müllers Ballungsreaktion (Müller's Clotting Test) (M.B.R.)*, carried out by Professor R. Müller, assisted by Mme. S. Berczeller.
- (d) *Murata Reaction*, carried out by Dr. K. Nobechi.
- (e) *Sachs-Georgi (Cito- and Lentochol Reaction)*, carried out by Dr. E. Witebsky.
- (f) *Sigma Reaction modified*, carried out by Dr. K. Norél.
- (g) *Vernes Syphilimetric Reaction*, carried out by M. R. Bricq, assisted by Mlle. M. Kerdal.

A detailed description of the techniques of the various methods in question is given in Annex 2 of this report.

The samples of blood and spinal fluid were placed at the disposal of the Conference by a number of Danish hospitals and dispensaries, as well as by clinics in Berlin, Hamburg, London, Vienna and Paris (see Annex 3). As the result of the Conference depended entirely upon the sufficiency and variety of the material placed at its disposal, its cordial thanks are due to the chief physicians of the different clinics who so



generously offered their work and these samples for the use of the participants.

The origin of the samples tested is indicated in the Tables attached to this report (Annex 1) and where no indication is given the sample was collected at Copenhagen. The specimens received from abroad were despatched to the Copenhagen Institute by air mail, and were kept in ice until their distribution. Owing to the time taken in transit and that required for their preparation prior to distribution, the sera from abroad were from three to nine days old, whilst the Danish sera were quite fresh. Thus the material before the Conference consisted, as is usually the case in the routine work of large diagnostic centres, of both old and fresh sera.

The clinical diagnosis of the sera submitted for the examination of the Conference were not published until the participants had submitted their reports on the results obtained, the sera and spinal fluids being distributed without any indication as to their origin or the diagnosis. In the numerous cases in which the clinical diagnosis was uncertain, and where discrepancies occurred between the serological findings and the clinical diagnoses, the case was thoroughly examined by Dr. A. K. H. CORNING. In cases showing one or more positive sero-reactions, if no history of syphilis was available, a new sample of blood was withdrawn where possible and the serum retested. The Conference is greatly indebted to Dr. Corning for his valuable aid.

#### A. BLOOD SERUM TESTS.

During the Conference, 944 sera <sup>1</sup> were tested by various methods. Of these, 502 were derived from known cases of syphilis in all stages of the disease both treated and untreated, seven were derived from cases with no definite history of syphilis, but where there was nevertheless some evidence of syphilis. 435 sera were derived from patients suffering from various other diseases (tuberculosis, cancer, scarlet fever, gonorrhœa; pregnancy, etc.) but with no clinical signs of syphilis. It must be remembered, however, that, as experience

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<sup>1</sup> In addition to these, thirteen blood sera were examined by a limited number of participants after the actual close of the Conference (Sera Nos. 945-957).

shows, a certain number of the latter cases may nevertheless be syphilitic.

The detailed results of the tests carried out by the different methods are presented in *Table 1* of Annex 1, prepared on the basis of the daily reports furnished by each worker immediately after having read the results. In *Table 2* of Annex 1 are collected the detailed results of the tests which, with one or more of the methods, gave a positive reaction in cases without clinical signs of syphilis.

It should be noted that, in judging the results obtained, it was not considered the purpose of the Conference to recommend any special method. It must be remembered that the choice of a method should depend on various considerations especially with regard to sensitiveness and specificity, and also as to local conditions, time required, cost. It is hoped, however, that the tables referred to below, which contain indications with regard to sensitiveness and specificity, will facilitate the analysis of the results.

In summarising the results of the different tests, some difficulty arose from the seven sera mentioned above with uncertain diagnosis. These cases, although without any definite history of syphilis, showed strong evidence of being nevertheless syphilitic; but, as the clinical diagnosis of syphilis could not be established with certainty, it was thought best to count them neither as "syphilitic" nor as "non-syphilitic", but to classify them in a special group as "doubtful". The detailed results of the tests in these cases (performed both during and after the close of the Conference) are given in *Table (i)* with a brief clinical report. A summary of the results of the different methods in respect of the *total number* of cases examined (944) is presented in *Table (a)*. It will be noted that the cases are divided into three groups: syphilitic, doubtful and non-syphilitic.

The results of the different methods in question can only be compared in cases when the tests on sera were examined by *all* the workers. Unfortunately, a considerable number of sera were not tested by all of the participants and, in order to obtain a *direct* comparison, it would be necessary to reject a considerable part of the material available. The following procedure — that of *indirectly* comparing the results — was therefore adopted.

The results obtained by the majority of the participants (ten) who tested all or practically all the 944 sera, are compared directly, omitting the small number of sera (six cases of syphilis, six non-syphilitic) which were not tested by one or another of these workers. The tests of the other workers (five), who did not examine more than a limited number of sera, are then each compared individually with the corresponding sera tested by the principal group of workers.

The details of the tests in the twelve sera omitted from the comparison are given in *Table (k)*. It is obvious that this omission does not change the relative position of the methods with regard to their apparent value.

The results of the group of methods which are directly comparable is given in *Table (b)*.

The results of those methods which are compared separately in their relationship to the main group are given in *Tables (c)—(h)*.

## B. CEREBRO-SPINAL FLUIDS.<sup>1</sup>

The detailed results of the examination of cerebro-spinal fluids is presented in *Table 3* of Annex 1. As the number of samples received was relatively small (122) and owing to the fact that in several cases the quantity of fluid available was insufficient, a definite conclusion can hardly be drawn from the results obtained by the Conference, especially as, owing to the time taken in transit from abroad, a considerable proportion of the material was not suitable for examination. Tables have been drawn up on the basis of the daily reports of the investigators, but no summary of the results was made.

The following resolutions were adopted:

### RESOLUTIONS.

#### I.

The Conference,

Having considered the results of the blood-serum<sup>2</sup> tests for syphilis according to the methods under review:

<sup>1</sup> See also Appendix.

<sup>2</sup> The material available at this Conference has not been sufficient to permit of a conclusive comparison between the Bordet-Wassermann and the flocculation tests in the examination of cerebro-spinal fluid.

Notes that in the case of those which depend on directly-visible changes in mixtures of extract and serum, hereinafter referred to as flocculation<sup>1</sup> tests certain new tests have been elaborated and certain others have improved considerably since the last Conference in 1923;

And is of opinion that the best of them may be regarded as equal in value to the best of those which depend on fixation of complement (Bordet-Wassermann).

It desires nevertheless to emphasise the fact that, no less than the complement-fixation tests, these flocculation methods are, despite their apparent simplicity, extremely sensitive to the slightest differences in experimental conditions and subject to so many sources of error, in connection both with the execution of the test and the reading and interpretation of the results, that they must be placed only in the hands of specially trained serologists.

## II.

The Conference,

Being of opinion that some serological tests may have the advantage of greater sensitiveness without being absolutely specific, and *vice versa*, and that concordance of reaction to two or more tests has greater diagnostic value than has a single reaction:

Recommends that, in order to secure the most reliable information to the clinician, at least two different sero-diagnostic methods should be used<sup>2</sup>.

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<sup>1</sup> The term "flocculation" is employed here for convenience without prejudice to opinions as to whether any given reaction is one of flocculation, increase of turbidity, precipitation, or clotting. It is applied here to the following tests: Kahn, Meinicke (M.T.R. and M.M.R.), Müller (M.B.R.), Murata, Sachs-Georgi, Sigma and Vernes.

<sup>2</sup> The following, namely: Dr. de Blasi, Dr. Debains, Dr. Demanche, Dr. Dold, Dr. Harrison, Dr. Hirsfeld, Dr. Jacobsthal, Dr. Kingsbury, Dr. Meinicke, Dr. Muckerji, Dr. Müller, Dr. Nagayo, Dr. Otto, Dr. Pavlovitch, Dr. Sachs, Dr. Scaltritti and Dr. Zabolotny, considering:

(a) That, as theoretical considerations give reason to expect, some sera react to the Bordet-Wassermann but not to the flocculation tests and *vice versa*, and that the Bordet-Wassermann and the flocculation tests supplement each other;

(b) That strong confirmation of a weak or  $\pm$  flocculation test is afforded by a positive Bordet-Wassermann test, and *vice versa*,

Would for the present prefer that one of the methods should be a Bordet-Wassermann test.

### III.

The Conference,

Having in view the necessity for constantly readjusting serodiagnostic methods in order to obtain the highest degree of specificity :

Recommends that the serologist should check the accuracy of his tests by regular and very frequent reference to clinical data, in consultation with the clinician, whose assistance in supplying adequate information as to the history of syphilis and the clinical particulars of the case is of great value for the interpretation of the results.

### IV.

The Conference,

Having in view the fact that serological tests for syphilis are primarily for the purpose of assisting clinicians in diagnosis, in observing progress under treatment and in tests for cure;

And having in view also the fact that patients frequently pass from the care of one clinician to that of another, the serum of one patient being tested from time to time in different laboratories:

Is of opinion that a uniform method of notation of serological results bearing approximately the same clinical interpretations would be of great value to clinicians, and proposes the following general rules:

(1) That a negative reaction should be reported as “—” or “negative”;

(2) That a reaction which is just positive to a degree which in the hands of the serologist has been afforded practically only by sera from cases of syphilis (and of a few well-defined pathological conditions) should be reported as “+” or “positive”.

It is recommended, in this connection, that serologists should so adjust their tests that practically only sera from cases of syphilis afford reactions which they report as “+” or “positive” ;

(3) That a reaction which is neither negative nor positive as defined in (2) should be reported as “±”.

In making these recommendations, the Conference would remark that there is nothing in them which would prevent the serologist adding to his report any amplifying or explanatory note which may be considered desirable (*e.g.* signs expressing the strength of the reaction).

## V.

The Conference wishes to reiterate with particular emphasis:

(1) That, in spite of the increased sensitiveness which the various serodiagnostic methods have shown at the present Conference, serological results may, notwithstanding the presence of a syphilitic infection, be negative in certain cases;

(2) That a positive reaction in the absence of a clear history or of signs of syphilis should, if only to exclude all possibility of error, never be accepted until a test of at least one more specimen has afforded the same result;

(3) That, except in the case of a few well-defined pathological conditions, syphilis is indicated with a degree of probability which closely approaches certainty, when several tests performed according to different methods give a positive result.

The Conference would suggest that the gist of the remarks in sub-sections (1) to (3) might be printed on the backs of the reports on serum tests for syphilis which are rendered by serologists to clinicians.

## VI.

The Conference,

Having in view the special importance of serodiagnosis for the diagnosis, treatment and prevention of syphilis:

Desires to record its view that considerable misunderstanding would be avoided and reports on tests of sera would be greatly enhanced in value if clinicians would study closely the diagnostic and therapeutic implications of such reports.

## VII.

The Conference,

Considering that the work in common and the discussions among participants have contributed greatly to a fuller knowledge and better understanding of the subject;

Bearing in mind, however, that the methods for the serodiagnosis of syphilis are constantly improving and that they are of capital importance for public health, and in order to stamp out a social scourge:

Considers it extremely desirable that the Health Organisation of the League of Nations should keep this question on the programme and take steps to secure further comparisons of this kind in the future.

## VIII.

The Conference,

Being of opinion that the special value of its work lies in the fact that the authors have themselves been able to compare, on the same test material, the results of their own methods with those of others, and contemplating the possibility of ultimately securing uniformity in the serodiagnosis of syphilis;

Holds it to be desirable that the Danish State Serum Institute, acting as the central laboratory of the Health Organisation of the League of Nations, should, in continuation of the work of the Conference,

Undertake the distribution of a series of serum samples for comparative tests in the different laboratories;

Itself test, at request, any other serum samples which it may receive, or distribute them for purpose of comparative re-testing;

And undertake to arrange in the same way an exchange of extracts, thus initiating a co-ordination of work which might be further developed in due course.

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Tableau a).

SÉRUM SANGUIN.

Résumé des résultats obtenus par l'examen du total des cas  
(944 cas)<sup>1</sup>.

Réaction Reaction	Effectuée par Carried out by	Cas syphilitiques (502 cas) Syphilitic cases (502 cases)					
		Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & ++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done
B.-W. R.	De Blasi . . . . .	461	130	72	259	0	41
»	Debains . . . . .	315	167	9	135	4	187
»	Harrison-Wyler . . . . .	502	210	78	214	0	0
»	Jacobsthal . . . . .	502	265	65	172	0	0
»	Otto-Blumenthal . . . . .	502	260	65	176	0	1
»	Pavlovitch . . . . .	501	220	56	225	1	0
»	Sierakowski . . . . .	502	195	57	250	0	0
Kahn R.	Boas . . . . .	502	294	27	181	0	0
»	Kahn . . . . .	499	305	33	161	0	3
M.T.R.	Meinicke . . . . .	502	246	38	218	0	0
M.B.R.	Müller . . . . .	499	317	45	137	0	3
Murata R.	Nagayo-Nobechi . . . . .	497	255	67	155	20	5
S.G.R. lento.	Sachs-Witebsky . . . . .	497	208	23	253	13	5
» cito.	Sachs-Witebsky . . . . .	496	254	21	209	13	6
Sigma R.	Noréi . . . . .	502	257	76	169	0	0
Vernes R.	Vernes-Bricq . . . . .	453	174	62	198	19	49

<sup>1</sup> Y compris les sérums réexaminés.

<sup>2</sup> Cas sans historique défini de syphilis, mais qui sont néanmoins suspects; voir tableau i) (détails sérologiques) et rapports cliniques.



Table (a).

BLOOD SERUM

Summary of results obtained with the total amount of cases examined (944 cases)<sup>1</sup>.

Cas douteux (7 cas) Doubtful cases <sup>2</sup> (7 cases)						Cas non syphilitiques (435 cas) Non-syphilitic cases (435 cases)					
Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & ++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done	Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & ++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done
5	0	2	3	0	2	397	13	34	350	0	38
4	2	0	2	0	3	249	26	4	213	6	186
7	3	2	2	0	0	435	0	12	423	0	—
7	3	2	2	0	0	435	29	36	369	1	—
7	3	3	1	0	0	435	24	40	371	0	—
7	3	1	3	0	0	434	6	25	403	0	1
7	3	2	2	0	0	435	0	13	422	0	—
7	6	0	1	0	0	435	3	6	426	0	—
7	6	0	1	0	0	434	0	5	429	0	1
7	7	0	0	0	0	435	9	13	413	0	—
7	6	0	1	0	0	432	1	10	421	0	3
7	4	1	2	0	0	432	2	36	372	22	3
7	4	0	2	1	0	431	0	1	430	1	4
7	4	1	1	1	0	427	0	1	425	1	8
7	4	2	1	0	0	434	6	35	393	0	1
5	2	1	2	0	2	369	2	36	316	15	66

<sup>1</sup> Including re-tested sera.

<sup>2</sup> Cases with no definite history of syphilis but with some evidence of being syphilitic; see table (f) (serological details) and clinical reports.

Tableau b).

SÉRUMS SANGUINS.

Comparaison des résultats obtenus  
au moyen des différentes méthodes.

Table (b).

BLOOD SERA.

Comparison of results obtained by  
means of the different methods.

Méthode Method	Exécutée par Carried out by	Nombre de + et de ++ parmi les cas diagnostiqués cliniquement comme: Number of + and ++ in cases with the clinical diagnosis:		
		Syphilis (496 cas) (496 cases)	Douteux Doubtful (7 cas) (7 cases)	Sans syphilis Non-syphilitic (429 cas) (429 cases)
Bordet-Wassermann	De Blasi . . . . .	Voir tableau c) See table (c)		
Bordet-Wassermann (Calmette-Massol)	Debains . . . . .	Voir tableau d) See table (d)		
Bordet-Wassermann (Méthode 1) (Method 1) "Medical Research Council Report Series No. 14"	Harrison-Wyler . .	208	3	0
Bordet-Wassermann (Méthode synoptique) (synoptic method)	Jacobsthal . . . . .	261	3	28
Bordet-Wassermann (originale) (original)	Otto-Blumenthal .	256	3	24
Bordet-Wassermann (originale) (original)	Pavlovitch . . . . .	218	3	6
Bordet-Wassermann (McIntosh-Fildes)	Sierakowski . . . . .	192	3	0
Kahn	Boas . . . . .	289	6	3
Kahn	Kahn . . . . .	303	6	0
Meinicke (Réaction d'opaci- fication) (Turbidity test)	Meinicke . . . . .	241	7	9
Müller (Réaction de conglomé- ration) (Clotting test)	Müller . . . . .	314	6	1
Murata	Nagayo-Nobechi . .	Voir tableau e) See table (e)		
Sachs-Georgi (Lentochol)	Sachs-Witebsky . .	Voir tableau f) See table (f)		
Sachs-Georgi (Citochol)	Sachs-Witebsky . .	Voir tableau g) See table (g)		
Sigma	Norél . . . . .	253	4	6
Vernes	Vernes . . . . .	Voir tableau h) See table (h)		

Tableau c).

Table (c).

Le professeur DE BLASI (B.-W.R.)  
a examiné:

Professor DE BLASI (B.-W.R.)  
examined:

455 sérums provenant de cas syphil.  
5 » » » douteux.  
391 » » » non syphil.

455 sera from syphilitic cases.  
5 „ „ „ doubtful „  
391 „ „ „ non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par le professeur de Blasi, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Professor de Blasi, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	De Blasi . . . . .	129	0	13
B.-W.R.	Harrison-Wyler . . . . .	184	1	0
B.-W.R.	Jacobsthal . . . . .	241	2	28
B.-W.R.	Otto-Blumenthal . . . . .	233	1	23
B.-W.R.	Pavlovitch . . . . .	201	2	6
B.-W.R.	Sierakowski . . . . .	173	2	0
Kahn R.	Boas . . . . .	263	4	3
Kahn R.	Kahn . . . . .	279	4	0
M.T.R.	Meinicke . . . . .	219	5	9
M.B.R.	Müller . . . . .	289	4	1
Sigma R.	Norél . . . . .	234	4	6

Tableau d).

Le D<sup>r</sup> DEBAINS (B.-W.R.)  
a examiné:

311 sérums provenant de cas syphil.  
4 " " " douteux.  
244 " " " non syphil.

Table (d).

DR. DEBAINS (B.-W.R.)  
examined:

311 sera from syphilitic cases.  
4 ,, ,, doubtful ,,  
244 ,, ,, non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par le Dr Debains, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Dr. Debains, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	Debains . . . . .	164	2	26
B.-W.R.	Harrison-Wyler . . . . .	126	1	0
B.-W.R.	Jacobsthal . . . . .	172	1	13
B.-W.R.	Otto-Blumenthal . . . . .	162	1	12
B.-W.R.	Pavlovitch . . . . .	148	2	6
B.-W.R.	Sierakowski . . . . .	118	2	0
Kahn R.	Boas . . . . .	184	3	1
Kahn R.	Kahn . . . . .	202	3	0
M.T.R.	Meinicke . . . . .	156	4	7
M.B.R.	Müller . . . . .	207	3	1
Sigma R.	Norél . . . . .	171	3	5

Tableau e).

Table (e).

Les Drs NAGAYO et NOBECHI (Murata R.) ont examiné:

Drs. NAGAYO and NOBECHI (Murata R.) examined:

491 sérums provenant de cas syphil.  
 7 » » » douteux.  
 426 » » » non syphil.

491 sera from syphilitic cases.  
 7 „ „ doubtful „  
 426 „ „ non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par les Drs Nagayo-Nobechi, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Drs. Nagayo and Nobechi, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphilitic cases + & ++
B.-W.R.	Harrison-Wyler . . . . .	205	3	0
B.-W.R.	Jacobsthal . . . . .	259	3	28
B.-W.R.	Otto-Blumenthal . . . . .	254	3	24
B.-W.R.	Pavlovitch . . . . .	216	3	6
B.-W.R.	Sierakowski . . . . .	190	3	0
Kahn R.	Boas . . . . .	286	6	3
Kahn R.	Kahn . . . . .	299	6	0
M.T.R.	Meinicke . . . . .	241	7	9
M.B.R.	Müller . . . . .	310	6	1
Murata R.	Nagayo-Nobechi . . . . .	251	7	2
Sigma R.	Norél . . . . .	251	4	6

**Tableau f).**

**Table (f).**

Les Drs SACHS et WITEBSKY (S.G.R. Lentocho) ont examiné:

Drs. SACHS and WITEBSKY (S.G.R. Lentocho) examined:

491 sérums provenant de cas syphil.  
 7 » » » douteux.  
 425 » » » non syphil.

491 sera from syphilitic cases.  
 7 „ „ „ doubtful „  
 425 „ „ „ non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par les Drs Sachs et Witebsky, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Drs. Sachs and Witebsky, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	Harrison-Wyler . . . . .	206	3	0
B.-W.R.	Jacobsthal . . . . .	258	3	27
B.-W.R.	Otto-Blumenthal . . . . .	253	3	24
B.-W.R.	Pavlovitch . . . . .	216	3	5
B.-W.R.	Sierakowski . . . . .	190	3	0
Kahn R.	Boas . . . . .	285	6	3
Kahn R.	Kahn . . . . .	299	6	0
M.T.R.	Meinicke . . . . .	239	7	9
M.B.R.	Müller . . . . .	311	6	1
S.G.R. Lento.	Sachs-Witebsky . . . . .	206	4	0
Sigma R.	Norél . . . . .	250	4	6

Tableau g).

Table (g).

Les D<sup>rs</sup> SACHS et WITEBSKY (S.G.R. Citochol) ont examiné:

Drs. SACHS and WITEBSKY (S.G.R. Citochol) examined:

490 sérums provenant de cas syphil.  
 7 » » » douteux.  
 421 » » » non syphil.

490 sera from syphilitic cases.  
 7 „ „ doubtful „  
 421 „ „ non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par les Drs Sachs et Witebsky, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Drs. Sachs and Witebsky, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphilitic cases + & ++
B.-W.R.	Harrison-Wyler . . . . .	205	3	0
B.-W.R.	Jacobsthal . . . . .	257	3	27
B.-W.R.	Otto-Blumenthal . . . . .	252	3	23
B.-W.R.	Pavlovitch . . . . .	215	3	5
B.-W.R.	Sierakowski . . . . .	189	3	0
Kahn R.	Boas . . . . .	284	6	3
Kahn R.	Kahn . . . . .	298	6	0
M.T.R.	Meinicke . . . . .	238	7	8
M.B.R.	Müller . . . . .	310	6	1
S.G.R. Cito.	Sachs-Witebsky . . . . .	250	7	0
Sigma R.	Norél . . . . .	249	4	6

Tableau h).

Table (h).

Le professeur VERNES et M. BRICQ  
(méthode syphilométrique)  
ont examiné:

Professor VERNES and M. BRICQ  
(Syphilimetric method)  
examined:

448 sérums provenant de cas syphil.  
5 " " " douteux.  
365 " " " non syphil.

448 sera from syphilitic cases.  
5 " " doubtful "  
365 " " non-syphilitic cases.

*Ce tableau montre les résultats obtenus dans ces cas par le professeur Vernes et M. Bricq, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.*

*This table shows the results obtained in these cases by Professor Vernes and M. Bricq, compared with the corresponding results obtained by the other methods.*

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphili- litic cases. + & ++
B.-W.R.	Harrison-Wyler . . .	190	3	0
B.-W.R.	Jacobsthal . . . . .	234	2	22
B.-W.R.	Otto-Blumenthal . . .	231	3	17
B.-W.R.	Pavlovitch . . . . .	198	2	5
B.-W.R.	Sierakowski . . . . .	172	2	0
Kahn R.	Boas . . . . .	264	4	3
Kahn R.	Kahn . . . . .	277	4	0
M.T.R.	Meinicke . . . . .	225	5	5
M.B.R.	Müller . . . . .	287	4	1
Sigma R.	Norél . . . . .	234	4	6
Vernes R.	Vernes-Bricq . . . . .	171	2	2



**Tableau i)**

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**Table (i)**

Tableau i)

RÉACTIONS SÉROLOGIQUES DANS LES CAS CLASSÉS COMME DOUTEUX:

Date	Sérum No.	B.-W. R.						
		De Blasi	Debains	Harrison-Wyler	Jacobsthal	Otto-Blumenthal	Pavlovitch	Sternkowsky
21.V 14.VI 14.VII	60 <sup>1</sup> 60 60	0	0	++	+	++	++	+
21.V 1.VI 14.VI	80 <sup>1</sup> 80 (838) 80 (838)	0 -	0 0	+	± ++	++ ±	- ±	± ±
25.V	418	±	++	++	++	++	++	++
29.V 2.VI	490 <sup>1</sup> 490(953)	- 0	- 0	- ±	- 0	± +	- 0	- 0
30.V 14.VII	657 <sup>1</sup> 657	±	-	-	-	-	-	-
31.V 2.VI 15.VI	709 <sup>1</sup> 709(952) 709(952)	-	++	± ±	±	±	++	+

<sup>1</sup> Les Sérums ont été examinés plusieurs fois (voir dates). — These sera have been tested several times (see dates).

<sup>2</sup> M. T. R. effectués par le D<sup>r</sup> Boas. — M. T. R. tested by Dr. Boas.

HISTORIQUE CLINIQUE DES CAS CLASSÉS COMME DOUTEUX.

N° 60.

Femme M., née en 1910. Diagnostic: tuberculose pulmonaire, urétrite gonococcique. Tuberculose floride bilatérale. Température 38,5° C. Crachats + T.B.C. Sécrétion urétrale + pour gonocoque. Pas de signes cliniques de syphilis. Décédée le 14.IX.1928. Autopsie: pas de lésions syphilitiques.

N° 80.

Homme L., né en 1887. Diagnostic tuberculose pulmonaire avec bronchiectasie. En traitement par la sanocrysine. Temp. 38,5° C. Nie la syphilis; pas de signes cliniques de cette maladie.

N° 418.

Homme P., né en 1903. Diagnostic: chancre mou. Pas d'anamnèse syphilitique. Recherche des spirochètes dans le chancre négative. Malade en observation à la clinique, en attendant des manifestations de syphilis. Réaction Bordet-Wassermann + à la clinique (Berlin).

N° 490.

Homme O. E., né en 1903. Diagnostic: tuberculose pulmonaire. En 1921: chancre pénien trouvé à l'hôpital Frederiksberg. Frottis répétés avec résultats négatifs. Réaction Bordet-Wassermann faiblement positive. En 1922: réaction B.-W. négative; pas de cicatrices pénienne du chancre; pas d'exanthème.

N° 657.

Femme K., née en 1861. Diagnostic clinique: « cancer rectal ? ». Diagnostic autopsique: cholé lithiase, cholécystite chronique et aigue. Extrait de l'observation: A la clinique R.B.-W. négative pour le sang; liquide céphalo-rachidien non examiné. Réaction à la luétine (+); pupilles normales. Réflexes achilléens et patellaires normaux; Babinski positif à droite, négatif à gauche. Réaction d'Oppenheim positive à droite, négative à gauche. Pas d'ataxie.

A la peau du tronc: lésions eozématiformes, serpigineuses, avec rebord croûteux. Ces lésions, d'apparence syphilitique au début, cédèrent à un traitement local cutané, mais des nodules indurés restèrent perceptibles sous la peau.

N° 709.

Femme L., née en 1895. Diagnostic: pleurésie exsudative gauche. Tuberculose laryngée? Infiltration marquée des deux cordes vocales, sans bacilles tuberculeux. Pas de syphilis dans l'anamnèse, ni de signes cliniques de cette maladie. (La lésion laryngée est-elle syphilitique?). Réaction d'Herxheimer après injection réactivante de 0 gr. 15 de salvarsan.

Table (i)

## SEROLOGICAL REACTIONS IN CASES REFERRED TO AS DOUBTFUL

Kahn R.		M. T. R.	H. B. R.	Hurata R.	S. G. R.	Sigma R.	Vernes R.	B.-W. R. Stalona Serum Institut
Eons	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs- Witelsky	Nordl	Vernes- Brieg	Eons
++ ++ ++	++	++	++	++	++	+	+	++ ++ ++
++ ++ ++	++ ++	++ ++ +++ <sup>2</sup>	++ ++	++ -	++ E.Fl.	± + +	± 0	+ + ++
++	++	++	++	++	++	+	+	++
- 0	- -	++ +	- 0	- 0	-± 0	- -	- 0	
++ ++	++	+ +++ <sup>2</sup>	+	±	-	± ++	-	0 ++
++ ++ ++	++ ±	++ ++ +++ <sup>2</sup>	++	++	+	+ + +	0	++ ++ ++

## CLINICAL HISTORY OF CASES REFERRED TO AS "DOUBTFUL".

- No. 60. Female M., born in 1910. — Diagnosis: pulmonary tuberculosis, gonococcal urethritis. Active bilateral tuberculosis. — Temperature 38.5° C. Sputum + tuberculosis. — Urethral secretion + for gonococci. No signs of clinical syphilis. Died on 14.IX.1928. — Post-mortem: no syphilitic lesions.
- No. 80. Male I., born in 1887. — Diagnosis: pulmonary tuberculosis with bronchiectasis. Under treatment with Sanocrysin. — Temperature 38.5° C. Denies syphilis; no clinical signs of this illness.
- No. 418. Male P., born in 1903. — Diagnosis: soft chancre. No patient's history of syphilis. Examination of the chancre for spirochetes: negative. Patient under observation in a clinic for manifestation of syphilis. Reaction Bordet-Wassermann done at the clinic (Berlin): positive.
- No. 490. Male O.E., born in 1903. — Diagnosis: pulmonary tuberculosis. In 1921: penile chancre found at the hospital Frederiksberg. Repeated slides with negative results. Bordet-Wassermann reaction feebly positive. In 1922: B.-W. reaction: negative; no penile cicatrices of the chancre; no rash.
- No. 657. Female K., born in 1861. — Clinical Diagnosis "rectal cancer" ? P. M. diagnosis: cholelithiasis, cholecystitis chronic and acute. Extract from the note: At the clinic: B.-W. reaction for blood: negative. Cerebro-spinal fluid not examined. Reaction to lucline: positive; pupils normal. Patella and ankle reflexes normal. Babinski sign, positive on the right side, negative on the left. Oppenheim reaction: positive on the right side, negative on the left. No ataxia. On the skin of the trunk: serpiginous eczematous lesions surrounded with crusts. These lesions of a syphilitic appearance at the beginning, yielded to local skin treatment, but hard nodules remained perceptible under the skin.
- No. 709. Female L., born in 1895. — Diagnosis: Pleurisy with exsudation, on the left side. Tuberculous laryngitis? Marked infiltration of the two vocal cords. No tubercle bacilli. No history of syphilis and no clinical signs of this illness. (Was the laryngeal lesion syphilitic?) Reaction of Herxheimer after an activating injection of 0.15 gr. of salvarsan.

Tableau k).

Table (k).

CAS EXCLUS DE LA COMPARAISON (voir page 11).

CASES EXCLUDED FROM THE COMPARISON (see page 11).

		B.-W. R.							Kahn R.		M.T.R.	M.B.R.	Murata R.	S.'G.R.	Sigma R.	Vernes	
Date	No.	De Blasi	Debains	Harrison-Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Bons	Kahn	Meinicke	Müller	Nagayo-Nobechi	Sachs-Witebsky	Nordé	Vernes-Brieg	Diagnosis
<i>1. Syphilis (6 cases)</i>																	
	193	-	++	-	-	+	±	-	±	-	0	±	E.Fl.	-	+	Lues latens	
	308	-	++	+	+	++	++	++	++	++	0	+	+ / ++	+	-	D. p.	
	535	-	±	±	+	±	±	±	++	++	0	+	- / +	+2½	±	Lues III	
	827	++	++	++	++	++	++	++	++	0	++	++	- / +	++	++	D. p.	
	840	-	0	±	±	+	-	+	++	0	+	+	± / +	±	0	Lues latens	
	871	-	0	±	-	±	±	±	++	0	++	++	++	+2½	+7	D. p.	
<i>2. Controls (6 cases)</i>																	
	377						0				0	?-c <sub>2</sub>				Gon.	
	379						0					±				Gon.	
	447									0				±	0	Gon.	
	484													0		Pleurit. Tub	
	536					±					0					Cancer uteri	
	860		0		±	±					0				0	Tub.	

## Annex I.

### TABLES SHOWING THE RESULTS OF THE COMPARISON OF VARIOUS SEROLOGICAL METHODS.

*The hieroglyphics used in the tables:*

# = strong positive =  $> 3.9$   $\Sigma$  units in 22 hours =  $> 10$   
Vernes units.

+ = positive = 1.5—3.9  $\Sigma$  units in 22 hours = 6-10  
Vernes units.

$\pm$  = neither negative nor positive = 1.0—1.4  $\Sigma$  units in  
22 hours = 1-5 Vernes units.

— = negative =  $< 1.0$   $\Sigma$  units in 22 hours.

? = doubtful.

0 = test not done.

C = Chylosis, H = Haemolysis, E.Fl. = Spontaneous  
flocculation.

The  $\bullet^t$  in the tables under "Diagnosis" indicates that  
syphilitic cases had been treated.

$\square$  = positive reaction in cases not diagnosed clinically  
as "Syphilis".

N. V. D. = no venereal disease.

N. H. Syph. = no history of syphilis.

$\theta$  = Naught.

A ref. = to be repeated.

*Method used by Dr. E. Debains*

Record of intensity of reactions.

(A = minimal dose of complement).

Dose of complement determined by the serum examined:

A<sub>1</sub> = Very weak

A<sub>2</sub> = Weak

A<sub>3</sub> = Medium

A<sub>4</sub> = Rather strong

A<sub>5</sub> = Strong

$> A_5$  = Very strong.

*Method used by Sachs-Witebsky.*

If two hieroglyphics are indicated, the first refers to the  
Lentochol, the second to the Citocholreaction.

**Tableau 1.**  
**RÉSULTATS OBTENUS PAR LA COMPARAISON DES DIVERSES**  
**MÉTHODES SÉROLOGIQUES.**

Date	No.	B.-W. R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.			
		De Blasi	Debrains	Harrison Wyler	Jacobsthal	Olto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meincke	Müller	Nagayo Nobuchi	Sachs Witelsky	Norel	Vernes Briquet		
21/5	1	0	0	—	—	—	—	—	—	—	—	—	—	± 1.3	—	θ		
	2	0	0	—	—	—	—	—	—	—	—	—	—	± 1.3	—	θ		
	3	—	0	—	—	—	—	—	—	—	—	—	—	—	à ref.	—		
	4	0	0	—	±	+	—	±	—	—	—	±	—	+	3.0	—	θ	
	5	0	0	#	+	#	—	+	#	#	#	#	#	#	10.0	#	13	
	6	0	0	#	+	#	#	+	#	#	#	#	#	#	+	1.6	#	14
	7	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	8	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	9	0	0	—	—	—	#	—	—	—	—	—	—	—	—	—	θ	
	10	0	0	—	—	+	—	—	—	—	±	—	—	—	—	±	4	
*	11	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	12	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	13	0	0	#	#	#	#	#	#	#	#	#	#	± 1.3	#	64		
	14	0	0	#	+	#	±	+	#	#	#	#	+	± 1.1	+	10		
	15	0	0	—	±	—	—	—	—	±	±	±	—	± 1.1	±	4		
	16	0	0	+	±	±	—	±	#	#	#	#	+	± 1.1	—	θ		
	17	0	0	—	±	—	—	—	—	—	—	—	#	—	—	θ		
	18	0	0	#	±	#	#	—	±	±	—	—	—	± 1.3	±	4		
	19	0	0	—	—	±	—	—	—	—	—	±	—	—	—	θ		
	20	0	0	+	—	—	—	+	#	#	±	+	+	± 1.6	+	6		
*	21	0	0	#	±	#	#	+	#	#	+	#	#	+	3.0	+	6	
	22	0	0	—	—	—	—	—	—	—	—	—	—	—	—	θ		
	23	0	0	—	—	—	—	—	—	—	—	—	—	—	—	θ		
	24	0	0	#	+	#	#	+	#	#	#	#	#	#	5.0	+	7	
	25	0	0	±	—	#	—	—	#	+	+	±	+	± 2.5	—	θ		
	26	0	0	—	—	±	—	—	—	—	—	—	—	—	—	±	4	
	27	0	0	#	+	#	#	+	#	+	#	+	0	± 2.5	+	8		
	28	0	0	#	+	#	#	#	#	#	#	#	0	# 5.0	#	20		
	29	0	0	—	—	—	—	—	—	—	—	—	0	—	—	θ		
	30	0	0	—	—	—	—	—	—	—	—	—	0	—	—	θ		
*	31	0	0	#	#	#	#	#	#	#	#	+	#	0	# 25.0	#	40	
	32	—	0	—	—	—	—	±	#	+	±	±	#	± 2.5	+	6		
	33	0	0	—	—	—	—	—	—	—	—	—	—	± 1.1	—	θ		
	34	0	0	—	—	—	—	—	—	—	—	—	—	—	—	θ		
	35	0	0	—	—	—	—	—	—	—	—	—	0	—	—	θ		

\* Examiné le 22/5. — Testé on 22/5.

Table (1)

RESULTS OF THE COMPARISON OF VARIOUS SEROLOGICAL METHODS.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. P.	Tbc.	Grav.	Tumor	Diagn. Aliae		
								•		Gon. Condylom	1	
										Lupus vulg.	2	
										Rheumatismus	3	
			• t					•		Condylom.	= n° 822	
			• t							Lues cerebrospin.	4	
											5	
										Aneurism. aortae	6	
								•		Cancer recti	7	
								•		Cancer ventr.	8	
			• t							Puerperium	9	
								•		Bronchit. chr.	= n° 869	
			• t								10	
			• t					•		Neuralgia	11	
			• t								12	
			• t					•			13	
			• t					•			14	
			• t					•			15	
			• t					• ?			16	
					• t			•		Lues cerebrospin.	17	
			• t					•		Lues cerebrospin.	18	
			• t							Lues cerebrospin.	19	
										Neuritis	20	
			• t							Apoplexia cerebr.	21	
											22	
			• t								23	
											24	
			• t								25	
										Gon.	26	
			• t								27	
			• t								28	
										Scarlatina	29	
									•		30	
			• t							Balanitis.	31	
											32	
									•	Cancer uteri	33	
										Pleuro-pneumonia	34	
										Ischias	35	

Tableau 1. — Table 1.

Date	No.	B.-W. R.						Kahn R.		H. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wylor	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinike	Müller	Nagayo Nobechi	Sachs Witebsky	Nord
21/5	36	0	0	#	#	#	#	+	#	#	#	#	#	+ 1.6	# 48
	37	0	0	-	-	-	-	-	-	-	-	-	-	+ 1.3	- 6
	38	0	0	-	+	-	-	-	-	-	-	-	-	-	- 6
	39	0	0	-	-	-	-	-	-	-	-	-	-	+ 1.1	- 6
	40	0	0	-	-	-	-	-	-	-	-	-	0	-	- 6
	*	41	0	0	#	#	#	#	#	#	#	#	#	# 15.0	# 98
	*	42	-	0	0	0	0	0	0	0	0	0	0	-	+ 4
	*	43	0	0	+	+	-	-	+	+	+	+	+	+ 1.3	+ 7
	*	44	#	0	#	#	#	#	#	#	#	#	#	+ 20.0	# 65
	*	45	-	0	-	-	-	-	-	-	-	-	-	-	+ 3
*	46	-	0	-	-	-	-	-	-	-	-	-	-	+ 3	
*	47	0	0	-	-	-	-	-	-	-	-	-	-	- 6	
*	48	0	0	-	-	-	-	-	-	-	-	-	-	+ 4	
*	49	0	0	-	-	-	-	-	-	-	-	-	-	+ ref.	
*	50	-	0	#	+	#	-	+	#	-	#	-	+ 1.3	+ 5	
*	51	-	0	-	-	-	-	-	-	-	-	-	-	+ 3	
*	52	-	0	-	-	-	-	-	-	-	-	-	-	+ 3	
*	53	-	0	-	+	-	-	-	-	-	-	-	+ 1.3	+ 9	
*	54	0	0	-	-	0	-	-	-	-	-	-	+ 1.3	- 6	
*	55	0	0	-	-	+	-	-	-	-	-	-	-	- 6	
*	56	0	0	#	#	#	#	+	#	+	#	+	+ 2.0	# 12	
*	57	0	0	-	-	-	-	-	-	-	-	-	-	- 6	
*	58	0	0	-	-	-	-	-	-	-	-	-	-	- 6	
*	59	0	0	-	-	-	-	-	-	-	-	-	-	- 6	
*	60	0	0	#	+	#	#	+	#	#	#	#	+ 2.0	+ 7	
*	61	0	0	#	+	#	#	#	#	#	#	#	+ 2.5	# 23	
*	62	0	0	+	-	-	-	-	-	-	-	+	-	- 6	
*	63	0	0	+	-	-	-	-	-	-	-	+	-	- 6	
*	64	0	0	+	+	+	-	-	-	-	-	?	-E.FI.	- 6	
*	65	0	0	-	-	-	-	-	+	-	-	?	-E.FI.	- 6	
*	66	0	0	-	-	-	-	-	-	-	-	-	-	+ 4	
*	67	0	0	-	-	-	-	-	-	-	-	-	-	- 6	
*	68	-	0	#	#	#	#	#	#	#	#	#	# 35.0	# 55	
*	69	0	0	-	+	-	-	-	-	-	-	-	-	- 6	
*	70	0	0	-	+	-	+	+	+	#	#	-	+ 1.3	+ 7	
				-	-	-	-	-	-	-	-	-	-	+ 4	

\* Examiné le 22/5. — Tested on 22/5.



Tableau 1. — Table 1.

Diagnosis										Observations	No.
Syphilis						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor		
			• t					•		Insuff. cordis	36
										Pyelitis	37
										Scarlatina	38
			• t							Scarlatina	39
											40
		•								Aortit. luica	41
			• t								42
					• t						43
			• t								44
			• t								45
			• t								46
			• t								47
			• t								48
			• t								49
			• t								50
										Lupus vulg.	51
										Lymphom colli	52
			• t							Malaria tertian.	53
											54
										Scarlatina	55
				• t							56
										Scarlatina	57
			• t							Scarlatina	58
											59
							•			Gon.	N. H. Syphilis 60
		• t								Hemiplegia sin.	61
			• t				•				62
			• t					•			63
											64
										Dermatitis	65
										Scarlatina	66
•											67
		•					•				68
									•	Lues cerebrospin.	69
										Melanosarcom	70

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. H. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Venus R.
		De Blas	Debains	Harrison Wylor	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witebsky	Norel	Vernes Bricef
21/5	71	0	0	+	-	-	-	+	-	-	-	-	-	-	-	0
	72	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	73	0	0	-	-	-	-	-	-	-	-	-	-	-	-	4
	74	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	75	0	0	-	-	-	-	-	-	-	-	-	-	-	-	4
	76	0	0	-	-	+	-	-	-	-	-	-	-	-	-	0
	77	0	0	-	-	-	+	-	-	-	-	-	-	-	+	0
	78	0	0	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	79	-	0	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	80	0	0	+	+	+	-	+	+	+	+	+	+	+	+	5
*	81	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	82	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0
	83	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	84	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	85	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	86	0	0	-	-	-	-	-	-	-	-	-	-	-	+	0
	87	0	0	+	+	+	+	+	+	+	+	+	+	+	+	4
	88	0	0	+	+	+	+	+	+	+	+	+	+	+	+	6
	89	0	0	+	+	+	+	+	+	+	+	+	+	+	+	10
	90	0	0	-	-	-	-	-	-	-	-	-	-	-	-	4
22/5	91	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	92	0	0	+	+	-	+	+	+	+	+	+	+	+	+	0
	93	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0
	94	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	95	-	0	-	-	-	-	-	-	-	-	-	-	-	+	3
	96	-	0	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	97	-	0	-	-	+	+	+	+	+	+	+	+	+	+	0
	98	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	99	-	0	-	-	-	-	-	-	-	-	-	-	-	+	0
	100	-	0	-	-	-	-	-	-	+	+	+	+	+	+	0
101	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0	
102	-	0	-	-	+	+	+	+	+	+	+	+	+	+	0	
103	0	0	+	+	+	+	+	+	+	+	+	+	+	+	3	
104	-	0	-	-	+	+	+	+	+	+	+	+	+	+	9	
105	+	0	+	+	+	+	+	+	+	+	+	+	+	+	à ref.	
														+	162	

\* Examiné le 22/5. — Tested on 22/5.



Tableau 1. — Table 1.

Date	No.	B. W. R.						Kahn R.		M. T. R.	M. S. R.	Muras R.	S. G. R.	Sigma R.	Venes R.			
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witschky	Norel	Vernes Bricq		
22/5	106	-	0	-	+	±	-	-	±	+	-	±	-	+	1.6	-	θ	
	107	-	0	±	+	+	+	±	±	±	-	-	-	+	1.6	+	7	
	108	-	0	±	-	+	-	-	+	±	+	±	E. Fl.	+	2½	à ref.		
	109	-	0	-	+	±	-	-	+	±	-	±	-	-	-	±	5	
	110	-	0	-	-	-	-	-	-	-	-	-	±	-	-	±	4	
	111	-	0	+	+	±	-	+	±	±	±	±	+	±	5	+	10	
	112	-	0	-	±	+	-	-	±	+	±	±	-	-	+	3	±	4
	113	-	0	-	-	-	-	-	+	-	-	±	?-C <sub>2</sub>	-	±	1.3	à ref.	
	114	-	0	-	-	-	-	-	-	-	-	-	?-C <sub>2</sub>	E. Fl.	-	-	à ref.	
	115	-	0	±	±	±	±	-	±	+	-	±	±	-	+	2	+	11
	116	-	0	-	-	+	-	±	±	±	±	±	-	+	+	1.6	±	4
	117	-	0	-	-	-	-	-	-	+	-	+	-	-	±	-	±	3
	118	±	0	±	±	±	±	+	±	±	±	±	+	+	+	2	±	28
	119	-	0	+	-	+	-	±	±	±	±	±	+	±	±	1.3	+	8
	120	-	0	-	-	-	-	-	-	-	-	-	-	-	±	1.3	±	3
	121	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	±	4
	122	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	±	θ
	123	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	124	-	0	±	±	±	-	-	-	-	-	±	-	-	-	-	-	θ
	125	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	à ref.	
	126	-	0	±	±	±	-	-	±	+	±	±	±	0	+	1.6	-	θ
	127	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
128	-	0	-	-	-	-	-	-	-	±	-	-	-	-	-	à ref.		
129	±	0	±	-	±	±	±	±	±	±	±	±	±	±	2	±	20	
130	-	0	±	-	±	±	±	±	±	±	±	±	±	±	5	-	θ	
131	-	0	-	-	-	-	-	±	+	-	+	±	-	-	-	-	θ	
132	-	0	±	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
133	-	0	±	±	±	-	+	±	±	±	±	±	±	±	5	±	12	
134	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
135	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
136	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
137	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
138	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
139	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
140	-	0	±	+	±	±	+	±	±	±	±	+	+	±	5	à ref.	à ref.	





Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latus	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
•			• <sup>t</sup>									141
			• <sup>t</sup>									142
			• <sup>t</sup>							Eczema manus		143
			• <sup>t</sup>							Psoriasis		144
			• <sup>t</sup>									145
			• <sup>t</sup>									146
			• <sup>t</sup>									147
			• <sup>t</sup>							Psoriasis		148
			• <sup>t</sup>							Psoriasis		149
			• <sup>t</sup>							Psoriasis		150
			• <sup>t</sup>							Scabies	Berlin	151
			• <sup>t</sup>							Ulcus cruris		152
			• <sup>t</sup>									153
			• <sup>t</sup>							Gon.		154
			• <sup>t</sup>							Psoriasis		155
			• <sup>t</sup>									156
			• <sup>t</sup>							Gon.		157
			• <sup>t</sup>									158
			• <sup>t</sup>							Gon.		159
			• <sup>t</sup>							Gon.		160
			• <sup>t</sup>							Mb. cordis		161
			• <sup>t</sup>						•?	Ulcus ventr.		162
			• <sup>t</sup>						•?	Cancer coli ?		163
			• <sup>t</sup>							Dyspepsia		164
			• <sup>t</sup>									165
			• <sup>t</sup>									166
			• <sup>t</sup>							Ulcus ad frenul.	= n° 798	167
			• <sup>t</sup>							Lymphoma		168
			• <sup>t</sup>							Lupus vulg.		169
			• <sup>t</sup>							Lupus vulg.		170
			• <sup>t</sup>									
			• <sup>t</sup>						•	Nephritis		172
			• <sup>t</sup>						•			173
			• <sup>t</sup>						•			174
			• <sup>t</sup>						•			175

Tableau 1. — Table 1.

Date	No.	B.-W. R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witelsky	Norel
23/5	176	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	177	—	—	—	+	—	—	—	—	+	—	—	—	—	0
	178	—	—	—	—	—	—	—	—	—	—	—	—	+2 <sup>1</sup> / <sub>2</sub>	0
	179	—	+	+	+	+	+	+	+	+	+	+	+	± 1.1	5
	180	+	+	+	+	+	+	+	+	+	+	+	+	± 25	56
	181	—	—	—	—	—	—	—	—	—	—	—	E. Fl.	—	à ref.
	182	—	—	—	+	—	—	—	—	—	—	+	—	—	0
	183	±	+	+	+	+	+	+	+	+	+	+	+	± 10	6
	184	—	+	+	+	+	+	+	+	+	+	+	+	± 5	0
	185	—	—	—	+	—	—	±	±	—	—	±	0	—	0
	186	—	±	—	+	±	+	+	+	+	+	+	+	± 5	± 3
	187	—	+	+	+	+	+	+	+	+	+	+	+	± 5	± 29
	188	—	à ref.	+	+	+	+	+	+	+	+	+	+	± 1.6	± 8
	189	+	+	+	+	+	+	+	+	+	+	+	+	± 10	à ref.
	190	+	+	+	+	+	+	+	+	+	+	+	+	± 35	± 130
	191	+	—	+	+	+	+	+	+	+	+	+	+	± 25	± 153
	192	—	—	—	—	±	—	—	—	—	+	—	—	± 1.6	0
	193	—	+	—	—	±	—	±	—	—	0	?	E. Fl.	—	± 6
	194	—	±	—	—	—	—	—	—	—	—	±	—	—	0
	195	—	à ref.	—	—	—	—	+	—	+	±	+	±	+2 <sup>1</sup> / <sub>2</sub>	0
	196	—	—	—	—	—	—	—	—	—	±	—	—	—	0
	197	+	+	+	+	+	+	+	+	+	+	+	+	± 25	± 132
	198	—	—	±	—	—	±	±	±	±	±	±	—	—	0
	199	—	—	±	+	+	+	+	+	+	+	+	+	± 1.6	à ref.
	200	—	—	+	+	+	+	+	+	+	+	+	+	± 3	± 8
	201	—	—	—	—	—	—	—	—	—	±	—	—	± 2	0
	202	—	+	—	—	±	—	—	—	—	±	—	+	± 1.6	0
	203	—	—	—	—	—	—	±	+	±	±	—	—	± 1.3	0
	204	—	—	±	—	±	±	±	+	—	±	±	—	± 1.6	0
	205	—	—	—	—	—	—	—	—	—	±	—	—	—	± 3
	206	+	+	+	+	+	+	±	+	+	+	+	+	± 5	± 45
	207	—	—	—	—	±	±	±	±	—	±	—	+	± 1.3	± 6
	208	—	—	±	+	±	±	±	±	±	±	—	+	± 2½	± 8
	209	—	±	±	±	±	±	±	±	±	±	—	+	± 3	± 4
	210	—	+	+	+	+	+	+	+	±	±	+	+	± 1.6	± 7

<sup>1</sup> Précipitation non spécifique. — Non specific precipitation.







Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae			
• t		• t									Atheroscleros. cer.	} Wien	211
• t					• t								212
•													213
• t													214
•													215
											Gon.		216
													217
		• t	• t								Hemipares. sin.		218
		• t	• t										219
		• t	• t										220
		• t											221
											Lupus vulg.		222
											Ulcera cruris		223
											Lupus vulg.		224
			•								Gon.		225
	•												226
• t													227
			• t										228
			• t										229
											Lupus vulg.	= n° 884	230
											Lupus vulg.		231
											Lupus vulg.		232
											Ulcus molle	= n° 898	233
			• t										234
			•										235
			• t										236
			• t										237
			• t								Haemorrhag. cerebri	N. H. Syph.	238
			• t										239
											Pneumonia	N. H. Syph.	240
		•											241
	•												242
			• t										243
			• t										244
							•						245

Tableau 1. — Table 1.

Date	No.	B.-W. R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.	
		De Blas	Debains	Harrison Wyley	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecchi	Sachs Witebsky	Norel	Vernes Bricq
24/5	246	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	247	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	248	-	+	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	250	-	+	-	-	-	-	-	-	-	-	-	-	-	-	θ
	251	±	à ref.	-	-	-	-	-	-	±	-	-	-	-	-	θ
	252	-	-	-	-	±	±	-	-	-	±	-	-	-	-	θ
	253	-	+	±	±	±	±	±	±	±	±	±	±	±	5.0	0
	254	±	-	-	-	-	±	-	-	±	-	-	-	-	-	θ
	255	±	± A <sub>5</sub>	±	±	±	±	±	±	±	±	±	±	±	3	± 15
*	256	±	-	-	-	±	-	±	±	-	±	±	-	±	1.3	± θ
	257	±	± A <sub>4</sub>	±	±	±	±	±	±	±	±	±	±	±	1.6	± 28
	258	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	259	-	0	-	-	-	-	-	-	-	-	±	-	-	-	θ
	260	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	261	±	0	+	+	±	+	±	±	±	±	±	±	±	10	± 12
	262	±	0	+	+	±	±	±	±	±	±	±	±	±	1.3	± 3
	263	±	0	±	±	±	±	±	±	±	±	±	±	±	10	± 15
	264	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	265	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
*	266	±	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	267	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	268	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ
	269	-	0	-	-	±	-	-	-	-	-	-	-	-	-	θ
	270	-	0	±	±	±	±	±	±	±	±	±	±	±	2½	± 7
	271	-	0	-	-	-	±	-	-	-	-	±	-	-	-	θ
	272	±	0	±	±	±	±	±	±	±	±	±	±	±	15	± 6
	273	±	0	-	-	-	-	-	-	-	-	-	-	-	-	± 3
	274	±	0	-	-	±	-	-	-	-	-	±	-	±	1.6	à ref.
	275	-	-	-	-	±	-	-	-	-	-	-	-	-	-	θ
*	276	±	0	±	±	±	±	±	±	±	±	±	±	±	10	± 8
	277	±	0	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	278	±	0	±	±	±	-	±	±	±	±	±	±	±	1.3	à ref.
	279	±	0	-	-	-	-	-	-	-	-	-	-	-	2½	± θ
	280	-	0	-	-	-	-	-	-	-	-	-	-	-	-	θ

\* Examiné le 20/5. \*\* Drs Boas et Norel ont changé leurs antigènes.  
 \* Tested on 20/5. \*\* Drs. Boas and Norel changed antigen.

Tableau 1. — Table 1.

Diagnosis										Observations	No.
Syphilis					Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Crav.	Tumor	Diagn. Aliae	
							•			Sang. cord. umbilic.	246
			• t					•			247
			• t					•			248
			• t					•			249
			• t					•			250
			• t					•		Ulcus molle	251
			• t					•			252
			• t					•			253
			• t					•			254
			• t					•			255
			• t				• t				256
			• t					•		Malaria tract.	257
			• t					•			258
			• t					•			259
			• t					•			260
			• t					•			261
			• t					•			262
			• t					•			263
			• t					•			264
			• t					•			265
			• t					•			266
			• t					•			267
			• t					•		Dementia senil. Encephalit. epid.	268
			• t			• t					269
			• t					•		Malaria tract.	270
			• t					•		Dementia alcohol.	271
			• t			• t					272
			• t			• t				Malaria tract.	273
		• t						•		Lues cerebri Schizophrenic	274
								•			275
						• t				Malaria tract.	276
		• t						•		Lues cerebrospin.	277
						• t				Malaria tract.	278
						• t				N. H. Syph.	279
								•		Gon.	280



Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tubes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae			
			e t							Ulcus cruris	Berlin	281	
			e t										282
			e t										283
			e t										284
			e t										285
										Dementia praecox	N. H. Syph.	286	
							•		•				287
													288
			e t										289
			e t										290
			e t									291	
			e t									292	
										Encephal. epid.	= n° 820	293	
										Eczema faciei			294
										Leucocytosis spin.			295
										Hypertroph. prostat.	N. H. Syph.	296	
			e t							Gon.			297
			e t										298
			e t									299	
			e t									300	
												301	
										Gon. Condylom.	= n° 821	302	
										Alopecia areata			303
							e t						304
							e t					305	
										Dementia paranoid.	Malaria tract.	306	
										Dementia praecox			307
							e t						308
										Dementia senil.		309	
			e t									310	
										Dementia praecox.	N. H. Syph.	311	
										Epilepsia			312
										Dementia praecox			313
										Schizophrenia			314
										Schizophrenia			315







Tableau 1. — Table 1.

Date	No.	B.-W.R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.		
		De Blasi	Debains	Harrison Wyley	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Bons	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witebsky	Norel	Vernes Ericq	
25/5	351	##	## A <sub>5</sub>	##	##	##	##	##	##	##	##	##	##	## 100	## 80		
	352	##	## A <sub>5</sub>	##	##	##	##	##	##	##	##	##	##	## 125	## 94		
	353	±	-	-	-	-	-	-	-	-	-	?	-C <sub>2</sub>	-	à ref.		
	354	+	-	-	-	-	-	-	-	-	-	?	+C <sub>2</sub>	-	à ref.		
	355	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
	356	##	## A <sub>5</sub>	##	##	##	##	##	##	##	##	##	##	##	## 15	## 58	
	357	##	## A <sub>5</sub>	##	##	##	##	##	##	##	##	##	##	##	## 10	à ref.	
	358	-	-	-	±	-	-	-	±	±	-	-	-	±	1.3	0	
	359	±	Anti c.	-	-	-	-	-	-	-	-	-	-	-	-	0	
	360	-	-	-	-	-	±	-	-	-	-	-	-	-	-	0	
361	±	-	-	-	-	-	-	-	-	-	-	±	-	-	0		
362	±	-	-	+	+	-	-	##	##	±	+	±	-	±	2½	± 3	
363	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
364	±	Anti c.	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
365	-	## A <sub>4</sub>	-	+	-	-	-	-	-	-	-	-	-	-	-	0	
366	-	## A <sub>3</sub>	-	+	+	-	-	±	##	##	##	+	+	± 2½	± 3		
367	±	-	-	±	-	-	-	-	±	±	±	-	-	-	-	0	
368	-	-	-	±	+	-	-	-	±	±	±	±	±	± 2½	-	0	
369	-	Anti c.	-	-	-	-	-	-	-	-	-	-	±	-	± 2½	-	0
370	-	## A <sub>3</sub>	-	-	-	-	-	-	-	##	-	-	-	-	-	-	0
371	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
372	-	-	-	-	-	-	-	##	##	+	##	##	##	± 2½	± 11		
373	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
374	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
375	±	## A <sub>3</sub>	±	±	±	+	-	##	##	+	##	+	+	± 2½	± 7		
376	+	## A <sub>3</sub>	±	±	-	±	+	-	-	-	##	+	+	± 1.3	-	0	
377	-	-	-	±	±	-	±	±	-	-	0	?	-C <sub>2</sub>	-	-	0	
378	-	-	-	±	±	±	-	±	-	-	-	?	-C <sub>2</sub>	-	-	0	
379	-	-	-	-	-	0	-	-	-	-	-	-	-	-	à ref.		
380	±	-	-	-	±	+	-	-	+	##	##	±	-	## 5	± 3		
381	±	-	-	±	±	±	±	-	-	-	-	-	-	-	-	0	
382	-	-	-	±	±	±	±	-	+	+	##	+	-	± 1.6	± 3		
383	-	## A <sub>4</sub>	±	±	##	-	-	-	+	##	##	+	-	± 1.3	± 4		
384	±	-	-	-	-	-	-	-	##	##	##	+	+	## 5	-	0	
385	±	-	-	-	-	+	-	-	±	±	±	-	-	± 1.3	-	0	
														± 1.6	± 3		

\* Examiné le 20/6. — Tested on 20/6.





Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	The.	Grav.	Tumor	Diagn. Aliae			
										Gon.	N. H. Syph.	386	
										Gon.		387	
										Gon.		388	
										N.V.D.	N. H. Syph.	389	
										Gon.		390	
										Gon.	N. H. Syph.	391	
										N.V.D.		392	
			• t									393	
										Gon.		394	
										Gon.		395	
										Gon.		396	
	• t											397	
												398	
			• t									399	
										Gon.	London	400	
										Gon.		401	
										Ulcus ven.		402	
• t												403	
												404	
										Gon.		405	
										Gon.		406	
										N.V.D.		407	
										Gon.		408	
										Gon.		409	
										Gon.		410	
										Gon.		411	
												412	
										Gon.		413	
	• t											414	
										Malaria tertian.	Hamburg	415	
												416	
											London	417	
										Ulcus molle		Berlin	418
											London	B.-W. R. † in clinic	419
										Gon.		420	

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Verne R.
		De Biasi	Debeins	Harrison Weyer	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witebsky	Norel	Verne Ericq
26/5	421	-	$\pm A_2$	-	-	$\pm$	-	-	-	-	-	-	-	-	-	$\theta$
	422	$\pm$	-	-	-	-	-	-	-	-	-	-	-	-	-	$\pm$ 4
	423	-	$\pm A_3$	$\pm$	$\pm$	$\pm$	-	-	-	-	-	-	-	-	-	$\theta$
	424	-	AMH c.	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	425	-	-	-	$\pm$	-	-	-	-	-	-	-	$\pm C_1$	-	-	$\theta$
	426	-	$\pm A_3$	-	-	-	-	-	-	$\pm$	-	-	-	-	$\pm$ 1.3	$\pm$ 3
	427	-	-	-	-	$\pm$	$\pm$	-	-	-	-	-	$\pm$	-	-	$\theta$
	428	-	$\pm A_3$	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	429	$\pm$	$\pm A_3$	$\pm$	-	$\pm$	$\pm$	$\pm$	-	$\pm$	-	$\pm$	-	-	$\pm$ 1.3	$\theta$
	430	-	$\pm A_4$	$\pm$	$\pm$	$\pm$	$\pm$	-	-	$\pm$	-	-	-	-	-	$\pm$ 3
	431	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	432	$\pm$	-	-	-	-	-	-	-	-	-	-	-	-	-	à ref.
	433	$\pm$	-	-	-	$\pm$	-	-	$\pm$	$\pm$	-	$\pm$	-	-	$\pm$ 1.3	$\theta$
	434	-	-	-	-	-	-	-	-	-	-	-	$\pm$	-	-	$\theta$
	435	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	436	-	-	-	-	$\pm$	-	-	-	-	-	-	-	-	-	$\pm$ 3
	437	$\pm$	-	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	438	$\pm$	-	-	$\pm$	$\pm$	-	-	-	-	-	-	-	-	-	$\theta$
	439	-	-	-	-	-	-	-	-	-	-	-	$\pm C_1$	-	-	$\theta$
	440	-	-	-	-	-	-	-	-	-	-	-	$\pm C_1$	-	-	$\theta$
	441	-	-	-	$\pm$	$\pm$	$\pm$	-	-	$\pm$	-	$\pm$	-	-	-	$\theta$
	442	-	-	-	$\pm$	$\pm$	-	-	-	$\pm$	-	$\pm$	-	-	$\pm$	$\theta$
	443	-	-	-	-	$\pm$	$\pm$	-	-	-	-	-	-	-	-	$\theta$
	444	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	445	-	-	-	-	-	-	-	-	-	-	-	-	-	$\pm$ 1.3	$\theta$
	446	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$\theta$
	447	-	-	-	-	-	-	-	-	0	-	-	$? - C_2$	-	-	$\theta$
	448	-	-	-	-	$\pm$	$\pm$	$\pm$	-	-	-	-	-	-	$\pm$ 1.3	0
	449	$\pm$	-	-	-	$\pm$	$\pm$	-	-	-	-	-	-	-	-	$\theta$
	450	-	-	-	-	-	$\pm$	-	-	-	-	-	-	-	-	0
451	-	-	-	-	-	-	-	-	-	-	-	$\pm C_1$	-	-	$\theta$	
452	-	$\pm$	-	-	-	-	$\pm$	-	-	-	-	-	-	-	$\theta$	
453	-	$\pm A_6$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	100	$\pm$ 81	
454	-	-	-	-	-	$\pm$	-	-	$\pm$	$\pm$	$\pm$	$? + C_2$	-	$\pm$ 1.3	$\pm$ 3	
455	-	-	-	-	-	-	-	-	-	-	-	$- C_1$	-	-	$\theta$	

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphills							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
			• t							Gon.	N. H. Syph.	421
										Gon.		422
										Gon.	N. H. Syph.	423
										Gon.		424
										Gon.		425
			• t									426
										Gon.		427
										Gon.	N. H. Syph.	428
			• t									429
										Gon.	= n <sup>o</sup> 957	430
										Gon.		431
										Gon.		432
					• t							433
										Gon.		434
										Gon.	London	435
										Ulcus molle		436
										Gon.		437
										Gon.		438
										Gon.		439
• t												440
												441
			• t									442
			• t									443
										Gon.		444
										Gon.		445
										Gon.		446
										Gon.		447
										Gon.		448
										Gon.	N. H. Syph.	449
	• t											450
												451
	•									Dement. senil.		452
			• t									453
			• t									454
										Psoriasis		455

Tableau 1. — Table 1.

Date	No.	B. W. R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.				
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq			
26/5	456	+	# A <sub>3</sub>	#	#	#	#	#	#	#	#	#	#	#	10	#	13		
	457	-	# A <sub>3</sub>	#	#	#	#	#	#	#	#	#	#	#	5	#	4		
	458	±	# A <sub>6</sub>	#	#	#	#	#	#	#	#	#	#	#	25	#	13		
	459	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	460	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	461	-	-	-	-	-	+	-	-	-	-	-	? - C <sub>2</sub>	-	-	-	±	3	
	462	±	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0	
	463	±	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0	
	464	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0	
	465	-	# A <sub>4</sub>	-	-	±	+	-	-	-	-	-	-	-	-	-	±	3	
	466	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	467	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	468	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	0	
	469	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	470	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	29/5	471	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		472	-	+ A <sub>2</sub>	±	+	+	-	-	#	#	#	+	? + C <sub>2</sub>	#	+ 2½	+	6	
473		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
474		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
475		±	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	0	
476		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	5	
477		+	-	-	-	+	±	±	-	-	-	-	-	-	-	-	-	0	
478		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	4	
479		-	-	±	+	+	+	-	#	#	+	#	±	#	+ 2½	-	-	0	
480		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
481		-	-	-	-	±	-	-	#	#	-	+	±	-	#	-	-	0	
482		-	0	-	-	-	-	-	-	-	-	-	±	-	-	-	-	0	
483		-	# A <sub>3</sub>	+	+	#	+	#	#	#	#	#	#	#	#	5	+	10	
484		-	-	-	-	+	-	±	±	#	#	-	±	-	#	0	-	0	
485		-	-	-	-	-	±	±	#	#	-	+	±	-	#	± 1.3	-	0	
486		-	# A <sub>3</sub>	-	±	#	-	-	#	#	#	+	+	-	#	#	5	-	0
487		-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	0
488	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
489	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
490	-	-	-	-	±	-	-	-	-	+	-	-	±	1.1	-	-	0		



Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. ali (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
					• t							456
			• t								Lues cerebrospin.	457
							•					458
											Neurasthenia	459
												460
			• t									461
								•			Lupus vulg.	= n° 947 462
								•				= n° 949 463
								•				= n° 948 464
								•				= n° 946 465
											Lupus vulg.	466
												467
								•			Lupus vulg.	468
												469
							•					470
											Mb. cordis	471
			• t									472
											Lupus vulg.	473
											Lymphom. colli	474
												475
								•				476
											Eclampsia	N. H. Syph. 477
											Sang. cord. umbilic.	478
			• t									479
				• t								480
												481
											Encephal. luica	481
											Lupus vulg.	482
											Hemiplegia dextr.	483
					• t						Pleurit. tub.	484
							•					485
												486
												487
												488
												489
												= n° 953 490

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meincke	Müller	Nagayo Nobuchi	Sachs Witbsky	Norel	Vernes Bricq
28/5	491	-	-	-	-	-	-	-	-	-	-	+	-	-	-	0
	492	-	-	-	±	-	-	±	-	-	-	-	-	-	-	0
	493	+	# A <sub>4</sub>	#	#	#	#	#	#	#	#	#	#	# 10	#	57
	494	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	495	-	-	-	-	-	-	-	-	-	-	±	-	-	-	0
	496	+	# A <sub>3</sub>	#	#	#	#	#	#	#	#	#	#	# 15	#	70
	497	-	# A <sub>3</sub>	#	#	#	#	±	#	#	#	#	#	± 1.3	±	10
	498	±	# A <sub>3</sub>	#	#	#	#	#	#	#	#	#	#	# 10	#	31
	499	-	-	-	±	-	-	-	-	-	-	±	-	-	-	0
	500	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0
	501	±	-	-	-	±	-	±	-	-	-	-	-	-	-	0
	502	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	# 20	#	57
	503	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	# 50	#	104
	504	±	# A <sub>3</sub>	+	±	-	±	±	#	±	#	±	#	± 1.3	±	6
	505	±	# A <sub>3</sub>	+	±	#	#	#	#	#	#	#	#	# 10	+	9
	506	-	-	-	±	-	±	-	#	#	-	+	±	± 1.3	-	0
	507	-	-	-	±	-	±	-	#	#	-	±	-	-	±	3
	508	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	509	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	510	-	-	-	±	-	-	-	-	-	-	-	-	-	-	0
	511	-	-	±	±	#	-	+	#	#	-	+	±	±	±	3
	512	-	0	#	#	#	+	#	#	#	-	#	±	± 1.6	±	4
	513	-	# A <sub>3</sub>	+	-	±	-	±	#	#	-	#	-	± 2½	±	4
	514	±	# A <sub>3</sub>	+	-	#	#	#	#	#	+	±	#	± 1.3	±	3
	515	-	-	-	-	±	-	-	-	-	-	-	#	# 20	-	0
	516	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	517	-	# A <sub>4</sub>	-	-	+	-	-	-	-	-	-	-	-	-	0
	518	-	-	#	#	#	±	+	#	#	-	-	±	-	-	0
	519	-	-	#	#	#	#	#	#	#	+	#	±	± 1.6	±	4
	520	-	0	±	±	-	-	-	-	-	-	±	#	± 2½	#	15
	521	-	-	-	-	-	-	-	-	-	-	±	-	-	-	0
	522	-	-	-	±	+	-	-	-	-	-	-	-	-	-	0
	523	-	0	-	-	-	-	±	±	-	-	-	-	-	-	0
	524	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	525	#	# A <sub>5</sub>	#	#	#	#	#	#	+	#	#	#	# 25	#	17

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. ali (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Gray.	Tumor	Diagn. Aliae		
										Neurasthenia, Rosac.	491	
			⊙ t					⊙			492	
											493	
											494	
											495	
		⊙ t									496	
			⊙								497	
			⊙ t								498	
										Psoriasis	499	
								●			= n° 945	
								●			500	
			⊙ t							N. H. Syph.	501	
	● t										502	
	● t										503	
			● t								504	
			● t								505	
	● t										506	
			● t					●			507	
			● t					●			508	
			● t								509	
			● t								510	
			● t								511	
			● t								512	
			● t								513	
			● t								514	
			● t								515	
			● t								516	
			● t								517	
			● t								518	
			● t								519	
			● t								520	
			● t								521	
			● t								522	
			● t								523	
								●			524	
						● t					525	

Tableau 1. — Tab. 1.

Date	No.	B. - W. R.						Kahn R.		M. T. R.	M. B. R.	Murats R.	S. G. R.	Sigma R.	Vernes R.		
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witelsky	Norel	Vernes Bricq	
29/5	526	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	125	+	96
	527	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	528	-	-	-	+	+	-	-	+	+	+	+	+	+	1.3	+	31
	529	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	5	+	21
	530	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	5	+	7
	531	-	-	-	-	+	-	-	+	+	+	+	+	+	1.3	+	4
	532	-	+	A <sub>3</sub>	+	+	+	+	+	-	+	+	-	+	2½	+	5
	533	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	534	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	3
	535	-	+	+	+	+	+	+	+	+	0	+	-	+	2½	+	4
	536	-	-	-	-	+	-	-	-	-	0	-	-	-	-	-	0
	537	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	538	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	3
	539	-	Anti compl.	-	-	-	-	-	-	-	-	-	-	-	-	+	4
	540	+	+	A <sub>2</sub>	-	+	+	-	-	-	+	+	+	+	1.3	+	4
	541	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	3
	542	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	543	+	-	-	+	+	+	+	+	+	+	+	+	+	5	à ref.	
	544	+	+	A <sub>3</sub>	+	+	+	+	+	+	+	+	+	+	10	+	20
	545	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	25	+	54
	546	-	+	A <sub>3</sub>	-	+	+	-	-	-	-	-	-	-	-	-	0
	547	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	25	+	81
	548	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	549	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	550	+	+	A <sub>5</sub>	+	+	+	+	+	+	+	+	+	+	2½	+	9
	551	+	+	A <sub>2</sub>	-	-	+	-	-	-	-	-	-	-	-	+	4
	552	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0
	553	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	554	-	-	-	+	+	+	+	+	+	+	+	+	+	10	+	15
	555	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0
	556	+	+	A <sub>2</sub>	+	+	+	+	+	+	+	+	+	+	10	à ref.	
	557	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0
	558	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0
	559	-	0	-	+	+	+	+	+	+	+	+	+	+	5	+	13
	560	-	-	-	+	+	+	-	-	-	-	-	-	-	1.6	+	3

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Thc.	Grav.	Tumor	Diagn. Aliae		
	•									Ulcus molle		526
			•									527
			•									528
			•									529
			•	•								530
			•									531
			•									532
			•									533
							•			Lues cerebrospin.	= n° 19	534
		•									= „ 21	535
								•		Cancer uteri	= „ 72	536
								•		Cancer ventr.	= „ 95	537
								•			= „ 96	538
								•		Eclampsia	= „ 121	539
			•					•			= „ 126	540
								•			= „ 127	541
								•		Cancer ventr.	= „ 137	542
			•								= „ 229	543
			•								= „ 235	544
			•								= „ 236	545
			•								= „ 237	546
			•								= „ 241	547
			•					•			= „ 248	548
			•								= „ 249	549
						•					= „ 272	550
			•							Dementia praecox	= „ 287	551
			•								= „ 299	552
			•							Gon.	= „ 302	553
			•									554
			•									555
			•									556
		•								Aneurisma aortæ		557
		•								Hemiplegia sin.		558
												559
												560

\* Les mêmes sérums réexaminés. — The same sera re-tested.

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murasu	S. G. R.	Signa R.	Vernis R.		
		De Blas	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Siernkowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witelsky	Norel	Vernis Bricq		
29/5	561	—	—	+	+	+	±	+	+	+	+	+	+	+	5	+	38	
	562	—	—	—	—	—	—	—	—	—	—	±	—	—	—	±	3	
	563	±	0	—	—	+	—	—	—	+	—	—	—	—	—	—	0	
	564	±	± A <sub>3</sub>	±	—	+	—	—	—	±	—	—	—	—	—	—	0	
	565	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	566	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	567	±	—	±	—	—	—	±	—	—	—	—	—	—	—	—	0	
	568	±	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	±	±	±	20	±	18
	569	±	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	±	±	±	20	±	26
	570	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
30/5	571	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	572	—	—	—	—	—	—	—	+	—	+	—	—	+	±	1.3	0	
	573	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	574	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	575	—	—	—	—	—	—	—	—	—	—	—	—	—	±	1.3	0	
	576	±	—	+	+	±	±	±	±	±	±	±	±	±	±	10	±	15
	577	±	—	±	±	±	±	±	±	±	±	±	±	±	±	10	±	34
	578	—	± A <sub>2</sub>	—	—	±	—	—	±	±	±	±	±	±	±	1.3	—	0
	579	±	± A <sub>6</sub>	±	±	±	±	±	±	±	±	±	±	±	±	15	±	50
	580	—	—	—	—	—	±	—	±	—	—	+	—	—	±	1.3	—	0
30/5	581	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	582	—	± A <sub>3</sub>	±	+	±	±	±	±	±	±	±	±	±	±	5	±	4
	583	—	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	E. Fl.	±	±	5	—	0
	584	—	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	E. Fl.	±	2½	±	4	
	585	+	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	±	±	±	20	±	14
	586	—	± A <sub>3</sub>	±	—	±	+	±	±	±	±	±	±	±	+	±	±	17
	587	—	—	±	±	±	±	—	±	±	±	±	±	±	+	±	±	0
	588	—	±	±	±	±	±	—	±	±	±	±	±	±	+	±	±	0
	589	—	—	±	±	±	±	—	±	±	±	±	±	E. Fl.	+	2½	±	4
	590	—	± A <sub>2</sub>	±	±	±	±	±	±	±	±	±	±	±	+	±	±	9
30/5	591	+	± A <sub>3</sub>	±	±	±	±	±	±	±	±	±	±	±	±	±	±	0
	592	±	± A <sub>6</sub>	±	±	±	±	±	±	±	±	±	±	±	±	5	±	26
	593	—	—	—	—	—	—	—	—	—	—	—	±	±	±	±	±	16
	594	—	± A <sub>4</sub>	±	+	±	+	±	±	±	±	±	±	±	±	1.3	—	0
	595	—	± A <sub>2</sub>	—	+	±	—	+	±	±	±	±	±	±	±	2	—	0

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D.p.	Tbc.	Gray.	Tumor	Diagn. Aliae		
		⊙ t								Observatio		561
			⊙ t									562
			⊙ t									563
			⊙ t									564
			⊙ t									565
			⊙ t									566
				● ?								567
			⊙ t									568
			⊙ t									569
			⊙ t									570
			⊙ t				●			Pleuritis exsud. sin.		571
							●					572
							●					573
							●					574
									●			575
												576
			⊙ t			● t						577
			⊙ t									578
			⊙ t									579
		⊙ t										580
			⊙ t									581
						● t						582
			● t									583
			● t									584
		⊙ t										585
						● t						586
		⊙ t										587
						● t						588
		⊙ t								Lues cerebri		589
			● t									590
		●										591
		● t								Aortitis luica		592
												593
				● t								594
					●							595







Tableau 1. — Table 1.

Date	No.	B. - W. R.							Kahn R.		M. T. R.	G. S. R.	Murasz	S. G. R.	Sigma R.	Vernas R.			
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinteke	Müller	Nagayo Nobechi	Sachs Witelsky	Notei	Vernas Brucq			
30/5	631	+	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	#	25	#	79		
	632	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
	633	±	-	-	-	-	-	-	-	-	-	-	-	-	± 1.3	-	0		
	634	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	#	#	25	#	76	
	635	-	# A <sub>3</sub>	±	-	±	±	-	#	+	#	#	+	±	±	1.3	-	0	
	636	-	# A <sub>5</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	637	-	-	±	±	±	-	±	#	#	+	#	+	#	#	5	+	7	
	638	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	#	#	25	#	68	
	639	-	-	±	±	-	-	-	#	#	±	#	+	-	±	± 1.3	-	0	
	640	±	-	±	±	±	-	-	-	-	-	±	-	-	-	0.9	-	0	
	641	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	0	
	642	-	+ A <sub>2</sub>	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0	
	643	#	+ A <sub>2</sub>	#	#	#	#	±	#	#	#	#	#	#	#	10	+	7	
	644	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	645	#	# A <sub>3</sub>	#	-	#	#	+	#	#	#	#	#	+	#	+	3	#	13
	646	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	647	-	-	-	+	-	-	-	#	#	-	#	+	-	-	+	1.6	0	
	648	±	# A <sub>2</sub>	±	+	-	#	±	#	#	-	#	+	±	+	± 1.6	±	3	
	649	-	# A <sub>1</sub>	-	-	-	±	-	#	+	-	+	±	-	-	± 1.3	-	0	
	650	-	# A <sub>3</sub>	#	#	#	±	±	#	#	+	#	±	-	+	± 1.3	-	0	
	651	-	# A <sub>4</sub>	±	+	-	±	-	#	#	-	#	-	-	-	± 1.3	-	0	
	652	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	653	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	654	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	655	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	656	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
	657	±	-	-	-	-	-	-	#	#	+	+	±	-	-	± 1.3	-	0	
	658	-	# A <sub>3</sub>	-	-	-	-	-	-	-	-	-	±	-	-	-	-	0	
	659	#	# A <sub>6</sub>	#	#	#	#	+	#	-	#	+	+	+	#	10	+	7	
	660	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	#	#	100	#	139	
661	#	# A <sub>5</sub>	#	#	#	#	#	#	±	#	#	#	#	#	100	#	110		
662	-	-	-	-	-	-	-	-	-	-	-	#	#	#	100	#	110		
663	-	# A <sub>3</sub>	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0		
664	+	# A <sub>5</sub>	#	#	#	#	#	#	#	#	±	-	-	-	-	-	0		
665	-	-	-	-	-	-	-	-	-	-	-	#	#	#	10	#	75		

Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis						Mb. alii (non-syphilit.)							
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Gray.	Tumor	Diagn. Aliae			
			•							Gon.	Berlin	631	
			•										632
			•										633
			•										634
			•										635
			• t										636
			• t										637
			• t										638
			• t										639
			• t										640
			• t								Wien	641	
			• t									642	
			• t									643	
			• t									644	
			•									645	
			• t							Hemiplegia sin.		646	
	• t		• t								Malaria tract.	647	
			• t									648	
			• t									649	
			• t	• t								650	
			•							Paralysis agitans		651	
			•							Angina pectoris		652	
							•				N. H. Syph.	653	
							•					654	
											Hamburg	655	
										Mb. Hodgkin		656	
									•	Cancer recti	1900-15 Malaria	657	
										Dementia praecox		N. H. Syph.	658
			• t							Exhibitionismus		659	
		• t										660	
			• t							Imbecillitas		661	
			• t							Psychos. man. depr.		662	
			• t									663	
						• t						664	
										Paranoia		665	



Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliac		
						• t				Paranoia	Malaria tract.	666
						• t						667
			•									668
			•									669
												670
								•		Degen. psychopath. Dementia praecox Hemiplegia Trauma capitis Encephal. luica	N. H. Syph.	671
												672
			•									673
												674
												675
			•							Lupus vulg. Lupus erythem.		676
												677
			• t				•				= n° 951	678
										Lues cerebrospin.		679
			• t									680
										Schizophrenia Sang. cord. umbilic		681
												682
			• t									683
												684
			• t									685
										Scabies		686
			• t									687
												688
												689
										Dementia praecox	Malaria tract. N. H. Syph.	690
												691
										Dementia praecox		692
										Dementia praecox		693
												694
			• t									695
												696
									•			697
									•			698
												699
										Dementia praecox	N. H. Syph.	700

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murat R.	S. G. R.	Sigma R.	Vernes R.	
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meimicke	Müller	Nagayo Nohechi	Sachs Wilebsky	Norel	Vernes Bricq	
30/5	701	—	# A <sub>3</sub>	±	+	—	#	—	+	—	+	±	—	± 1.3	—	θ	
	702	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	703	#	# A <sub>5</sub>	#	#	#	#	—	#	+	#	#	#	# 10	#	71	
	704	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
31/5	705	—	+ A <sub>2</sub>	±	#	±	±	+	#	#	+	#	#	± 1.3	—	0	
	706	—	# A <sub>5</sub>	—	—	#	±	—	—	#	—	—	—	—	—	θ	
	707	—	—	—	+	—	±	—	—	—	—	—	—	—	—	θ	
	708	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	# 25	#	75	
	709	—	# A <sub>4</sub>	±	±	±	#	+	#	#	#	#	+	+	3	0	
	710	—	—	—	±	—	±	—	—	—	—	—	—	—	—	θ	
	711	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	712	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	713	—	# A <sub>5</sub>	—	#	±	±	—	+	+	±	—	—	—	±	3	
	714	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	715	—	—	—	±	—	±	—	—	—	—	—	—	—	—	θ	
	716	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	717	—	# A <sub>5</sub>	—	—	±	—	—	—	—	—	—	—	—	—	θ	
	718	—	—	—	—	—	—	—	—	—	—	?-C <sub>5</sub>	—	—	—	θ	
	719	—	—	+	#	±	±	+	#	#	±	+	—	#	± 1.3	—	θ
	720	—	# A <sub>4</sub>	—	—	±	—	—	—	—	—	—	—	± 1.3	—	θ	
	721	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	722	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	723	+	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	# 10	#	14	
	724	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	725	±	# A <sub>5</sub>	±	#	—	#	—	#	#	—	#	—	+	± 1.3	—	θ
	726	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	#	#	# 5	—	0	
	727	—	±	—	—	—	—	—	—	±	—	—	—	—	—	0	
	728	±	—	—	+	+	—	—	±	—	—	—	—	—	—	θ	
	729	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	730	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ	
	731	—	# A <sub>4</sub>	+	#	#	±	±	#	#	#	#	#	# 5	#	15	
	732	—	—	—	+	—	—	—	—	—	—	—	—	—	—	θ	
	733	—	—	—	±	—	—	—	—	—	—	—	—	—	—	θ	
	734	+	# A <sub>5</sub>	±	#	+	±	—	—	—	—	—	—	—	—	θ	
	735	—	# A <sub>4</sub>	—	+	#	±	±	±	—	±	±	±	± 2½	—	0	
														± 1.3	—	0	













Tableau 1. — Table 1.

Date	No.	B.-W. R.						Kahn R.		M. T. R.	M. B. R.	Muria R.	S. G. R.	Sigma R.	Venus R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witebsky	Norel
31/5	806	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	807	-	0	-	-	-	-	-	-	-	-	-	-	-	θ
	808	-	0	-	-	-	-	-	-	-	-	-	-	-	θ
	809	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	810	±	0	-	-	±	-	-	-	-	-	±	-	-	0
	811	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	812	±	0	#	#	#	#	+	#	#	#	#	#	# 10	0
	813	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	814	-	± A <sub>3</sub>	-	-	±	±	-	-	±	-	-	-	0	0
	815	-	-	-	-	-	-	-	-	-	-	-	-	0	θ
	816	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	817	-	0	-	-	-	-	-	-	-	-	-	-	-	θ
	818	-	± A <sub>3</sub>	-	-	-	-	-	-	-	-	-	-	0	θ
	819	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	820	±	± A <sub>5</sub>	-	-	-	±	-	-	-	-	-	-	-	0
	821	-	-	-	±	-	-	-	-	-	-	-	-	-	θ
	822	±	-	-	±	±	-	-	-	-	-	-	-	-	θ
	823	-	± A <sub>5</sub>	±	±	#	±	-	±	-	-	-	-	-	θ
	824	±	0	#	#	#	#	±	#	#	#	#	#	# 50	# 72
	825	#	# A <sub>5</sub>	#	#	#	#	#	#	#	#	±	#	# 50	# 59
826	-	± A <sub>2</sub>	±	±	#	±	-	#	#	±	±	±	# 2½	-	
827	#	# A <sub>4</sub>	#	#	#	#	#	#	0	#	#	#	# 25	# 39	
828	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
829	-	± A <sub>3</sub>	-	-	-	-	-	-	-	-	-	-	-	0	
830	±	0	-	+	+	-	-	-	-	-	-	-	-	0	
831	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
832	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
833	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
834	#	0	#	#	#	#	#	-	#	-	+	#	± 2½	± 6	
835	±	0	-	±	-	±	-	-	±	-	-	±	± 1.3	0	
836	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
1/6	837	-	0	-	-	-	-	-	-	-	-	-	-	0	
	838	-	0	±	±	±	±	±	±	±	±	-	-	θ	
	839	-	0	-	±	-	-	-	-	-	-	E. Fl.	± 2½	0	
	840	-	0	±	#	+	-	+	#	0	+	+	±	± 1.3	0

\* Température de l'incubateur: 33° C. — Temperature of incubator: 33° C.

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alit (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	G. a. v.	Tumor	Diagn. Aliae		
			• t				•			Pleuritis exsud.	806	
							•			Pleuritis exsud.	807	
			• t								808	
			• t								809	
											810	
			• t					•			811	
								•			812	
								•			813	
								•		N. H. Syph.	814	
								•			815	
								•			816	
								•			817	
								•		N. H. Syph.	818	
								•			819	
								•		Angina Vincenti Eczema faciei	cf. n° 294; N. H. Syph. 820	
								•		Gon. Condylom.	cf. n° 302; N. H. Syph. 821	
			• t								cf. n° 4 822	
			• t								823	
			• t								824	
											825	
							• t				826	
							• t				827	
										Dementia praecox Encephalit. epid. Lues cerebrospin.	N. H. Syph. 828	
			• t								829	
											830	
										Dementia praecox Paranoia Depressio ment.	831	
											832	
											833	
			• t				•				Malaria tract. 834	
											835	
							•			Pleurit. exsud. sin.	cf. n° 79; N. H. Syph. 836	
							•			Pleurit. exsud.	cf. n° 178; N. H. Syph. 837	
							•				cf. n° 80; N. H. Syph. 838	
										Encephalit. epid. Paranoia	N. H. Syph. 839	
			•								840	

Tableau 1. — Table 1.

Date	No.	B. W. R.						Kahn R.		M. T. R.	M. S. R.	Murata R.	S. G. R.	Sigma R.	Vernos R.		
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witbsky	Norel	Vernos Briceq	
$\frac{1}{6}$	841	-	0	-	+	-	-	-	-	-	-	-	-	-	0		
	842	-	0	-	+	+	-	-	-	-	+	-	-	-	-	0	
	843	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0	
	844	-	0	-	+	-	-	-	-	-	-	-	-	-	0		
	845	-	0	-	+	-	-	-	-	+	-	-	-	-	-	0	
	846	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0	
	847	-	0	+	+	+	+	+	+	-	+	+	+	-	0		
	848	-	0	-	-	-	-	-	-	-	-	-	-	-	0		
	849	+	0	+	+	+	+	+	+	+	+	+	+	+	100	0	
	850	+	0	+	+	+	+	+	+	+	+	+	+	+	1.3	-	0
	851	-	0	-	+	-	-	-	-	-	-	-	-	-	-	-	0
	852	-	0	-	+	+	+	-	+	-	-	-	-	-	-	-	0
	853	-	0	-	-	-	-	-	-	-	-	-	-	-	0	-	0
	854	-	0	+	+	+	+	+	+	-	+	-	+	-	+	4	0
	855	-	0	+	+	+	+	+	+	+	+	?+C <sub>2</sub>	-	+	5	-	0
	856	+	0	+	+	+	+	+	+	+	+	+	+	+	2½	0	
	857	+	0	+	+	+	+	+	+	+	+	+	+	+	20	0	
	858	-	0	-	-	-	-	-	-	-	-	-	-	-	0	0	
	859	-	0	-	+	-	-	-	-	-	-	-	-	-	0	0	
	860	-	0	-	+	+	-	-	-	-	0	-	-	-	0	0	
	861	-	0	-	-	-	-	-	-	-	-	-	-	-	0	0	
	862	-	0	-	-	+	-	-	-	-	-	-	-	-	0	0	
	863	-	0	-	+	+	-	-	-	-	+	-	-	-	0	0	
	864	-	0	-	+	+	-	-	-	-	+	-	-	-	-	0	
	865	-	0	-	-	-	-	-	-	-	+	?	-	+	1.3	0	0
	866	-	0	-	-	-	-	-	-	-	-	-	-	-	0	0	
	867	+	0	-	+	+	+	-	-	-	-	-	-	-	0	0	
	868	-	0	+	+	+	-	-	-	-	-	-	-	-	-	0	
	869	-	0	-	+	+	-	-	-	-	-	-	-	-	0	0	
	870	-	0	-	+	+	-	-	-	-	+	-	-	-	0	0	
	871	-	0	+	-	-	-	-	-	0	+	+	+	+	2½	+	7
	872	-	0	-	+	+	+	+	+	+	+	+	+	+	+	+	0
	873	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0
	874	-	0	-	-	-	-	-	-	+	+	+	+	+	5	+	85
	875	-	0	-	-	-	-	-	-	+	-	-	-	-	-	-	0

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphills							Mb. alii (non-syphillit.)					
I	II	III	Latens	Cong.	Tabes	D. P.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						• t				Dementia praecox	N. H. Syph.	841
										Dementia praecox	} <b>Hamburg</b>	842
										Kachexia sen.		843
										Dementia praecox		844
										Dementia praecox		845
						• t				Schizophrenia		846
										Schizophrenia		847
						• t				Kachexia sen.	<b>Hamburg</b>	848
						• t				Kachexia sen.		849
						• t				Kachexia sen.		850
										Dementia praecox	N. H. Syph.	851
						• t				Dementia praecox		852
						• t				Dementia praecox		853
						• t				Dementia praecox		854
						• t				Dementia praecox		855
		• t								Dementia praecox		856
						• t				Dementia praecox		857
										Dementia alcohol.		858
							•			Dementia alcohol.	N. H. Syph.	859
							•			Dementia alcohol.	N. H. Syph.	860
										Gon.	<b>Berlin</b>	861
										Gon.	cf. n° 192; N.H. Syph.	862
										Gon.	cf. n° 193;	863
									•	Gon.	cf. n° 195; N.H. Syph.	864
										Degenerat. psych.		865
										Encephalopath. art.		866
									•	Encephalopath. art.	= n° 955	867
									•	Epilepsia	N. H. Syph.	868
										Bronch. acut.	cf. n° 10; N. H. Syph.	869
										Dementia praecox	N. H. Syph.	870
						• t				Dementia praecox		871
						• t				Dementia praecox	N. H. Syph.	872
										Dementia praecox		873
									• ?	Melaena	N. H. Syph.	874
										Observatio		875

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Muras.	S. O. R.	Sigma R.	Varies R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobuchi	Sachs Witebsky	Norel	Verres Bricq
1/6	876	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	877	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	878	#	0	#	#	#	#	#	#	#	#	#	#	#	5	74
	879	-	0	-	-	+	-	-	-	-	-	-	-	-	-	0
	880	-	0	-	#	+	-	-	-	-	-	-	-	-	-	0
	881	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	882	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	883	-	0	-	+	-	-	-	-	-	-	-	-	-	-	0
	884	-	0	-	#	+	-	-	-	-	-	-	-	-	-	0
	885	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	886	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	887	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	888	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	889	-	0	-	-	-	-	-	-	-	-	-	-	-	0	0
	890	-	0	+	+	+	-	-	-	-	-	-	-	-	-	0
	891	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	892	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	893	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	894	#	0	#	#	#	#	#	#	#	#	#	#	#	50	100
	895	+	0	#	#	#	#	+	#	#	#	#	#	#	10	21
	896	#	0	#	#	#	#	#	#	#	#	#	#	#	100	147
	897	+	0	#	#	#	#	#	#	#	#	#	#	#	10	11
	898	+	0	-	#	-	-	-	-	-	-	+	+	-	-	0
	899	+	0	-	-	-	-	-	-	-	+	-	-	-	-	0
	900	-	0	-	-	-	-	-	+	#	-	+	-	+	1.3	0
	901	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	902	+	0	-	-	-	+	-	-	-	-	-	-	-	-	0
	903	+	0	-	#	-	-	-	-	-	-	-	-	-	-	0
	904	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
	905	#	0	#	#	#	#	#	#	#	#	#	#	#	75	0
	906	+	0	#	+	#	+	+	-	-	+	+	-	-	-	0
	907	-	0	-	-	-	+	-	-	-	+	+	-	-	-	0
	908	+	0	#	#	#	#	#	#	+	#	+	-	-	-	0
	909	#	0	#	#	#	#	#	#	#	#	#	+	+	1.3	0
	910	-	0	+	+	+	+	+	#	#	#	+	+	+	5	31
				+	+	+	+	+	#	#	#	+	+	+		3



Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliac		
			•					•				876
								•		Eclampsia		877
								•				878
								•				879
								•		Kachexia sen.	<b>Hamburg</b>	880
								•			N. H. Syph.	881
								•				882
										Acne vulg. faciei	N. H. Syph.	883
										Lupus vulg.	cf. n°230; N. H. Syph.	884
										Observatio		885
										Lupus vulg.		886
										Lupus vulg.		887
				• t								888
										Bronchitis		889
										Observatio		890
										Observatio		891
			• t									892
			•					•				893
			• t									894
			• t									895
			• t									896
			• t									897
			•							Ulcus molle	cf. n°233; N. H. Syph.	898
												899
										Ulcus molle		900
			• t									901
			• t							Morbilli	= n° 956	902
			• t							Scabies		903
			• t									904
						•						905
						• t						906
						• t				Schizophrenia		907
						•						908
						•						909
						• t					Malaria tract.	910

Tableau 1. — Table 1.

Date	No.	B. - W. R.						Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernas R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witelsky	Norel
1/6	911	±	—	—	±	±	—	—	—	—	—	—	—	—	0
	912	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	913	+	—	#	#	#	#	#	#	#	#	#	± #	+ 2½	# 15
	914	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	915	#	—	#	#	#	#	#	#	#	#	#	#	+ 3	# 37
	916	±	—	—	±	—	—	—	—	—	—	—	—	—	0
	917	+	—	±	#	#	±	#	—	—	—	—	E.F.I.	—	0
	918	±	—	—	+	—	—	—	—	—	—	—	—	—	0
	919	—	—	—	—	±	—	—	—	—	—	—	—	—	0
	920	—	—	—	—	—	—	—	—	—	—	—	—	±	0
	921	+	—	#	#	#	#	+	#	#	#	#	#	# 10	0
	922	—	—	—	+	±	—	—	—	—	±	±	—	—	0
	923	—	—	—	—	—	—	—	—	—	+	—	—	—	0
	924	—	—	—	+	±	—	—	—	—	—	—	—	—	0
	925	±	—	±	+	+	+	±	#	±	—	+	—	± 1.3	0
	926	—	—	—	—	—	—	#	#	—	#	+	—	± 1.3	0
	927	+	—	#	#	#	#	#	#	#	#	#	±	# 5	0
	928	—	—	—	—	—	—	#	#	#	#	#	±	—	0
	929	—	—	—	—	—	—	#	#	±	#	+	—	—	0
	930	—	—	—	—	±	—	—	+	—	+	+	—	±	0
	931	—	—	—	—	—	—	—	+	—	+	+	?	±C <sub>2</sub>	0
	932	—	—	—	—	—	—	—	+	—	+	+	—	—	0
	933	±	—	#	#	+	#	+	#	+	#	±	—	± 1.3	0
	934	—	—	#	#	#	#	#	#	#	#	#	#	—	# 60
	935	#	—	#	#	#	#	#	#	#	#	#	#	# 5	0
	936	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	937	—	—	—	—	—	—	#	+	+	+	+	—	—	± 3
	938	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	939	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	940	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	941	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	942	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	943	—	—	—	—	—	—	—	—	±	—	—	—	—	0
	944	#	—	#	#	#	#	#	#	—	—	—	—	—	0
2/6	945	—	—	—	—	—	—	—	—	—	—	—	#	# 50	# 91

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis						Mb. alli (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc	Grav.	Tumor	Diagn. Aliae		
			• t					•		Ulcus plantae		911
			•									912
										Psychos. man. depr.		913
		• t										914
												915
			• t							Malaria tropic.	<b>Hamburg</b>	916
			• t									917
			• t									918
		•								Hemipleg. sin.	cf. n° 201	919
										Observatio		920
					• t						Malaria tract.	921
			• t									922
			• t		• t							923
			• t									924
			• t									925
			• t								<b>Wien</b>	926
• t			• t									927
			• t									928
			• t									929
			• t									930
					• t							931
			• t	• t								932
			• t									933
			• t									934
		• t										935
		•								Uraemia	<b>Hamburg</b>	936
												937
								•				938
								•			N. H. Syph.	939
								•				940
								•				941
								•				942
										Malaria tropic.	N. H. Syph	943
			•?							Malaria tropic.	<b>Hamburg</b>	944
								•				945
											cf. n° 500; N. H. Syph.	945



Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
								•			cf. n° 465; N. H. Syph.	946
								•			cf. n° 462; » »	947
								•			cf. n° 464; » »	948
										Lupus vulg. Obs.	cf. n° 463; » »	949
												950
							•				cf. n° 678; » »	951
							•			Pleurit. exsud. sin.	cf. n° 709; » »	952
							•				cf. n° 490; Ulc. pen. 1921	953
									• ?	Melaena	cf. n° 874; N. H. Syph.	954
									•		cf. n° 687; » »	955
										Morbilli Gon.	cf. n° 902; » »	956
											cf. n° 430; » »	957

Tableau 2.

RÉACTIONS POSITIVES DANS LES CAS QUI N'ONT PAS ÉTÉ  
POSITIVE REACTIONS IN CASES NOT

Les sérums qui ont été ré-  
Sera which have been re-

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blas	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witebsky	Norel	Vernes Bricq
21/5	10	0	0	-	-	+	-	-	-	+	-	-	-	-	-	0
1/6	869	-	0	-	+	+	-	-	-	+	-	-	-	-	-	0
22/5	53	-	0	-	±	-	-	-	-	-	-	-	-	± 1.3	+	9
22/5	79	-	0	-	-	-	-	-	-	-	-	-	-	+	1.6	à ref.
31/5	836	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
22/5	148	-	0	-	-	-	-	-	+	±	-	±	-	± 1.3	±	4
23/5	167	-	-	-	-	-	-	±	-	-	-	-	-	+	1.6	0
31/5	798	-	0	-	+	+	-	-	-	-	-	-	-	-	-	0
22/5	173	-	+	-	±	±	-	±	-	-	-	-	-	± 1.3	-	0
23/5	178	-	-	-	-	-	-	-	-	-	-	-	-	+	2½	0
1/6	837	-	0	-	-	-	-	-	-	-	-	-	-	-	-	0
23/5	185	-	-	-	+	-	-	±	±	-	-	±	0	-	-	0
23/5	192	-	-	-	-	±	-	-	-	-	+	±	-	+	1.6	0
1/6	862	-	0	-	-	+	±	-	-	-	±	-	-	-	-	0
23/5	195	-	à ref.	-	-	-	-	-	±	+	±	+	±	+	2½	0
1/6	864	-	0	-	-	-	-	±	±	±	±	±	-	± 1.3	-	0
24/5	230	-	+	-	±	±	-	-	-	±	-	-	-	-	-	0

Table 2.

**DIAGNOSTIQUÉS CLINIQUEMENT COMME « SYPHILIS ».**  
**DIAGNOSED CLINICALLY AS SYPHILIS.**

examinés sont groupés ensemble.  
 re-tested are grouped together.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
								•		Bronchit. acuta	10 869	
										Malaria tertian.	53	
							•			Pleurit. exsud.	79 836	
										Psoriasis	148	
										Ulcus ad fren.	Ulcus curatum spontan. $\frac{1}{6}$ 167 798	
								•			173	
							•			Pleurit. exsud.	178 837	
							•?			Pneumonia	185	
										Gon.	192 862	
									•	C. maxil. sup.	195 864	
										Lupus vulg.	230	









Tableau 2. — Table 2.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. P.	Tbc.	Grav.	Tumor	Diagn. Aliae		
										Gon.	421	
										Gon.	423	
										Gon.	428	
										Gon.	430	
										Gon.	957	
										Gon.	449	
								•			462	
										Lupus vulg.	947	
											463	
											949	
								•			464	
											948	
								•			465	
											946	
								•		Eclampsia	477	
								•			500	
											945	
								•			501	
										Hemiplegia sin.	559	
										Ulcus molle	612	
										Gon.	615	
										Cholecystitis	626	
										Gon.	627	
							•				654	



Tableau 2. — Table 2.

Diagnosis											Observations	No.
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D.p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
										Dementia praecox		658
								•		Degen. psychopath.	2/3, WR —, Kahn —, Σ —, M.T.R. —	671
							•					678
												951
										Dementia praecox	2/3, Sigma —, WR —, M.T.R. —	690
										Dementia praecox	Malaria tr.	700
								•				706
								•				707
								•				717
										Gon.		744
										Gon.		754
										Gon.		756
										Lupus vulg.		786
							•					796
								•				814
								•				818
										Encephalit. epid.	2/3, WR —, Kahn —, Σ —, M.T.R. —	829
										Encephalit. epid.		839
										Dementia praecox		841
										Dementia praecox		851
							•					859



Tableau 2. — Table 2.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
							•					860
								•				867
												955
										Epilepsia ?	<sup>19/4</sup> WR —, Kahn —, Σ —, M.T.R. —	868
										Dementia praecox		870
										Dementia praecox	<sup>19/4</sup> WR —, Kahn —, Σ —, M.T.R. —	872
									• ?	Melaena		874
												954
										Kachexia senil.		880
										Acne vulg. faciei		883
												902
										Morbilli		956
									•			939
										Malaria trop.		943





Tableau 2. — Table 2.

Diagnosis											Observations	No.
Syphills							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
							•					860
								•				867
												955
										Epilepsia ?	1/4 WR —, Kahn —, Σ —, M.T.R. —	868
										Dementia praecox		870
										Dementia praecox	1/4 WR —, Kahn —, Σ —, M.T.R. —	872
									• ?	Melaena		874
												954
										Kachexia senil.		880
										Acne vulg. faciei		883
										Morbilli		902
												956
								•				939
										Malaria trop.		943

Tableau 3.

LIQUIDES CÉPHALO-  
CEREBRO-SPINAL

Date	No.	B.-W. R.			Kahn R.	M. T. R.	M. B. R.	S. G. R.	Sigma R.	Vernes R.
		Harrison Wyley	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	Norel	Vernes Bricq
23/5	1	##	##	##	##	##	##	##	+ 3	## 126 <sup>1</sup>
	2	++	+	##	+	-	##	-	+ 1.6	## 48
	3	++	-	-	##	-	+	-	+ 1.6	## 30
	4	##	##	##	-	##	##	+	+ 2½	- 0 <sup>1</sup>
	5	##	##	##	+	++	##	E. Fl.	+ 2½	## 147 <sup>1</sup>
	6	##	+	##	+	-	##	-	+ 2½	## 12
	7	-	-	-	-	-	-	-	+ 1.3	+ 8
	8	-	-	-	-	-	-	E. Fl.	-	+ 7
	9	##	##	##	##	##	##	E. Fl.	+ 3	## 141
	10	##	##	##	##	+	##	E. Fl.	+ 2½	± 1
	11	-	-	-	-	-	-	-	- 0.9	+ 7
	12	##	-	-	-	-	-	-	+ 1.3	## 129 <sup>1</sup>
	13	-	-	-	-	-	-	-	-	+ 11 <sup>1</sup>
	14	-	-	-	-	-	-	-	-	+ 8 <sup>1</sup>
	15	-	-	-	-	-	-	-	-	+ 7
16	-	0	0	0	0	0	0	-	## 114 <sup>1</sup>	
17	++	-	-	-	-	-	-	+ 2½	## 36 <sup>1</sup>	
18	##	-	-	-	-	-	-	-	± 5	
19	##	-	-	-	-	-	-	+ 1.6	## 119	
20	0	-	-	-	±	-	-	-	± 2	
21	-	-	-	-	-	-	-	-	- 0	
22	-	-	-	-	-	-	-	-	## 119	
23	-	-	-	-	-	-	-	-	+ 9	
24	-	-	-	-	-	-	-	-	± 4	
25	-	-	-	-	-	-	-	-	+ 6	
26	-	0	0	0	0	0	0	-	-	
27	-	-	-	-	-	-	-	-	+ 8	
28	0	-	-	-	-	-	-	-	+ 9	
29	-	-	-	-	-	-	-	-	+ 7	
30	-	-	-	-	-	-	-	-	± 5	
									+ 8	

<sup>1</sup> Liquores contamin. decompos. <sup>2</sup> Examinés le 29/5. — Tested on 29/5.







Tableau 3. — Table 3.

Date	No.	B.-W. R.			M. R.	M. T. R.	M. B. R.	S. G. R.	Sigma R.	Vernes R.	
		Harrison Wyler	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	el N	Vernes Eriq	
25/5	66	+	-	±	-	-	#	-	- 0.9	± 3	
	67	-	-	-	-	-	0	E. Fl.	-	± 1	
	68	+	+	#	#	#	#	+	- 0.9	# 114	
	69	-	-	-	-	±	-	-	- 0.9	- 0	
	70	+	-	-	±	±	+	-	- 0.9	± 2	
	71	#	#	#	#	#	#	#	+ 2½	# 77	
	72	#	#	#	#	#	#	#	- 0.9	# 126	
	73	#	#	#	#	#	#	#	# 5	# 136	
	74	+	+	#	±	-	±	E. Fl.	-	- 0	
	75	-	-	-	0	-	-	E. Fl.	-	0	
	76	-	-	-	-	-	-	-	-	-	± 2
	77	-	-	-	-	-	±	-	-	-	0
	78	+	-	-	±	-	#	±	-	-	+ 7
	79	-	-	-	-	-	-	-	-	-	- 0
	80	#	#	#	±	+	#	±	+ 1.6	-	± 5
	81	#	#	#	#	#	0	+	+ 2½	-	# 70
	82	#	#	#	#	#	#	#	# 5	-	# 132
	83	#	#	#	#	#	#	#	+ 2½	-	# 127
	84	#	+	#	#	#	#	#	+ 2½	-	# 125
	85	#	#	#	#	#	+	#	-	-	0
	29/5	86	#	0	0	0	±	#	0	+ 2½	# 114 <sup>1</sup>
		87	+	—	—	—	-	±	0	-	+ 12 <sup>1</sup>
		88	+	—	—	—	#	#	-	+ 1.6	# 141 <sup>1</sup>
		89	-	—	—	—	-	-	-	-	± 3 <sup>1</sup>
		90	-	—	—	—	-	± <sup>1</sup>	-	-	+ 10 <sup>1</sup>
91		-	—	—	—	—	-	-	-	± 5 <sup>1</sup>	
92		+	-	-	#	-	#	-	-	# 49 <sup>1</sup>	
93		#	0	0	#	-	#	-	-	# 134 <sup>1</sup>	
94		#	—	—	0	#	#	0	+ 2½	# 96 <sup>1</sup>	
95		#	—	—	—	#	#	—	+ 2½	# 124 <sup>1</sup>	
96		+	—	—	—	#	#	—	-	-	
97		+	—	—	—	-	+	—	# 5	# 119 <sup>1</sup>	
98		-	-	-	-	-	-	-	-	- 0 <sup>1</sup>	
99		+	+	—	#	±	-	-	-	- 0	
100		#	#	#	#	#	#	-	+ 1.6	# 104	

<sup>1</sup> Examinés avec une quantité de liquide insuffisante (1,2 et 0,8 cm<sup>3</sup> au lieu de 1,60 cm<sup>3</sup>). — Tested with too small quantity of fluid (1.2 and 0.8 cc. instead of 1.60 cc.).

<sup>2</sup> Examinés le 30/5. — Tested on 30/5.

Tableau 3. — Table 3.

Diagnosis										Observations	No.	
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor			Diagn. Aliae
						• t						66
						• t						67
						• t						68
						• t						69
						• t						70
						• t						71
						• t						72
						• t						73
						• t						74
						• t						75
										Dementia alcohol.		
			•							Mb. Alzheimer.		76
										Lues cerebri		77
						• t					Malaria tract. 1926	78
						• t					» » 1925	79
						• t					» » 1925	80
										<b>Berlin</b>		
						•						81
						•						82
						•						83
						•						84
						•						85
						• t						86
						• t						87
						• t	• t					88
						• t						89
						• t						90
										Lues cerebri		91
						• t					<b>Wien</b>	92
						•						93
						• t						94
						• t						95
						• t					Malaria tract.	96
						• t						97
						• t						98
						• t						99
						• t						100

Tableau 3. — Table 3.

Date	No.	B.-W. R.			Kahn R.	M. T. R.	M. B. R.	S. G. R.	Signe R.	Vernes R.		
		Harrison Wyler	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	Norel	Vornes Bricq		
29/5	101	+	+	+	+	+	+	+	5	+	119	
	102	+	+	+	+	-	-	0	-	-	0	
	103	+	+	+	+	+	+	+	+ 1.6	+	123	
	104	-	-	-	-	-	-	-	-	-	0	
	105	+	+	+	+	+	+	+	+ 1.6	+	137	
	106	-	-	-	-	-	-	-	-	-	0	
	107	-	-	-	-	-	-	-	-	-	0	
	108	+	+	+	+	+	+	+	+ 0.9	+	146	
	109	+	+	+	+	-	+	-	+ 2	+	136	
	110	+	+	+	+	+	+	+	+	15	+	136
	111	+	+	+	+	-	+	-	-	-	0	
	112	-	-	-	+	-	+	-	-	-	0	
	113	+	+	+	+	+	+	+	+	+ 1.6	+	113
	114	+	+	+	+	+	+	+	+	+ 2½	+	133
	115	+	+	+	+	-	+	-	+	+ 0.9	+	65
30/5	116	-	-	-	-	-	-	-	-	-	+ 5 <sup>1</sup>	
	117	-	-	-	-	+	-	-	-	-	0	
	118	+	+	+	+	+	+	+	+ 2½	+	124	
	119	+	+	+	+	-	+	-	+ 0.9	+	34	
	120	+	+	+	+	+	+	-	+ 2½	+	116	
	121	-	-	-	-	-	-	-	-	-	0	
	122	+	+	+	+	+	+	+	+ 2½	+	112	

<sup>1</sup> Examinés avec une quantité de liquide insuffisante (1,2 et 0,8 cm<sup>3</sup> au lieu de 1,60 cm<sup>3</sup>). — Tested with too small quantity of fluid (1.2 and 0.8 cc. instead of 1.60 cc.).



Tableau 3. — Table 3.

Diagnosis											Observations	No.
Syphills							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						• t				Encephalit. epid.	101	
						• t					102	
						• t				Dementia alcohol.	103	
						• t					104	
						• t					105	
										Encephalit. epid.	106	
										Schizophrenia	107	
						• t					108	
						• t					109	
						• t					110	
						• t					111	
						• t					112	
						• t					113	
		•				• t				Lues cerebrospin.	114	
						• t					115	
										Dementia praecox	116	
										Dementia praecox	117	
						• t					118	
						• t					119	
						• t					120	
										Psychos. man. depr.	121	
						• t					122	

## OBSERVATIONS ON THE RESULTS OBTAINED.

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PROFESSOR D. DE BLASI.

*Concerning the Results on May 30th.*

Not having been able, as is usually the case, to make a second test after a convenient interval, I have given the results of the first test, immediately after having taken the tubes from the thermostat. This must be borne in mind in view of the fact that some of the results, though strongly positive during the first test, can be due to a simple delay of hemolysis.

DR. E. DEBAINS.

Like all unheated-serum methods, our technique requires fresh sera, *i. e.*, sera taken twenty-four or forty-eight hours before examination. These are the normal conditions of work in the dispensaries, institutes and hospitals in the Paris area.

The Conference was working simultaneously on very fresh sera and sera of varying ages, up to nine days in certain cases (sera Nos. 554-569). We were obliged to increase the customary doses of alexin required for super-activation, and there was consequently an excess of alexin for the fresh sera and a shortage for the older sera; in the case of the latter, it was often difficult to read the results. These anomalies led in a number of cases to discrepancies as regards both sensitivity and specificity; this is what we found when we compared the results we obtained before the Conference with those obtained in the course of its work with sera from patients personally known to us which were sent to Copenhagen by aerial post on our instructions. This instance clearly illustrates our observations.

As nobody but ourselves uses a fresh-serum method, we were at a disadvantage, and our results are not strictly comparable with those of our colleagues. In spite of this unsatisfactory position, an examination of the results obtained will show the future importance of a comparison between our method, applied in normal conditions, and, in particular, the flocculation tests, in the detection of hereditary syphilis and latent or unrecognised syphilis in general medicine and surgery.

OBSERVATIONS BY DR. E. JACOBSTHAL.

The Serum Conference at Copenhagen yielded a surprising result, since it cannot be denied that the testing of the serum — not of the fluid — by means of complement-fixation gave less constant and less reliable results than the precipitation methods. It must, however, be pointed out that a research worker experimenting in a laboratory to which he is not accustomed is far less favourably situated than he would be in his own laboratory. Thus, at Hamburg for instance, the complement is never used only a few hours after collection, but is allowed to stand about twenty-four hours until it has acquired a certain consistency, while the sheep's blood is used in a far less concentrated form. Further, according to the unanimous opinion of the research workers who employed complement-fixing methods, the reagents supplied were, for some

unknown reason, less satisfactory than usual on certain days, especially the last days. If one considers, for example, the results of Jacobsthal's experiments, one finds that, while on the last three days of the experiments a large number of non-specific results were suddenly obtained, there were practically no such results during the first days of the Conference. (A confusion, no longer explicable now, must have arisen in the case of serum 185, which is a distinct exception to this rule and which gave a ++ result in Jacobsthal's experiments with all, even the weakest reactions.) The above consideration, moreover, shows that the precipitation methods are, as it were, less sensitive as far as changes of the local conditions are concerned, which must be considered a material advantage. It is only very seldom that we (Jacobsthal) obtain non-specific results in our own laboratory. The extract I used in Copenhagen appeared, for reasons unknown to me, to have reacted far more strongly than usual. At home such an occurrence is always counteracted by the clinic, and above all by the parallel use of precipitation methods. This, however, was not allowed at Copenhagen.

It must be pointed out in particular that, on the last day of the experiments, June 1st, 16 non-specific positive reactions were suddenly obtained. If that day's results were disregarded — in practice the results of such a "day of non-specific positivity" would always have been automatically eliminated — the Jacobsthal methodology would appear in a more favourable light, especially in view of its great sensitiveness in the indisputable cases of syphilis.

PROFESSOR R. MÜLLER.

I should like to make the following observations concerning our own M.B.R. results (clotting test). In non-syphilitic cases we obtained 7 doubtful = ± reactions and one + reaction. Of these 8 reactions, 3 were repeated. One of the 3 cases was the + case which gave a ± result on retesting. The results of the other 2 ± cases were unchanged when retested. We think that the results of this retest should be reckoned among the successes of the M.B.R., as it points to the constancy of the reaction, and the value of ± tests in cases of syphilis can be determined accordingly, since they may probably be construed as genuine and not merely accidental ± reactions. Only the one non-syphilitic + case became a ± case, which after all should also be counted as a success. Instead of this, in the final results, these 3 ± retest reactions are classed separately as non-specific results, and by this classification, in the clotting reaction, the results for non-syphilitic cases are given as one + and 10 ± reactions, instead of one + and 7 ±. This point might also be taken into account in the case of the other persons concerned in these researches.

DR. K. NOREL.

The extract for the Sigma reaction used during the first two days of the Conference gave a flocculation of quite a different type from the usual; it was therefore abandoned and a fresh preparation was used for the rest of the tests. It might be of interest to draw attention to the fact that five out of six non-specific weak positives (1.6 to 2 1/2 units per cc.) obtained during the Conference were found with precisely this non-typical reacting extract.

PROFESSOR OTTO<sup>1</sup>.

In checking the original report of Dr. Blumenthal, I discovered that, on June 30th, the serological diagnoses reported by him were erroneous in regard to a certain number of sera, because the reading

<sup>1</sup> See also Annex 2.

on that day differed from his usual procedure. The results of the following tests should therefore read:

Serum	584	+	instead of	±
„	586	+	„	„ ±
„	587	+	„	„ ±
„	589	+	„	„ ±
„	594	+	„	„ ±
„	595	+	„	„ ±
„	598	±	„	„ —
„	600	±	„	„ —
„	604	±	„	„ —
„	635	+	„	„ ±

PROFESSOR SACHS.

Professor Sachs desires to put forward the following considerations in connection with the critical estimate of the value of the material collected at Copenhagen.

The essential factors in the utility of any process of serological diagnosis for syphilis are sensitivity and specificity. In an estimate of the diagnostic value of a test, specificity must carry the greater weight. With the material in question, it is difficult to judge of the specificity, because all the tests under consideration — even those practised separately by individual methods — which yielded, by anamnesis, indications of latent syphilis, were taken as characteristic for syphilis. It is impossible to decide *a priori* whether in such cases the reactions are specific or non-specific. Accordingly, it must be remembered that the results in reference to the specificity factor are somewhat less satisfactory than would appear from the recapitulatory tables. In addition, a complete estimate of the value of the material must take account of the ± reactions, because experience shows that on repetition ± reactions sometimes give a +, and, conversely, + reactions may give a ±. For this reason, ± reactions in control cases should always be regarded as borderline reactions, which are equally capable of turning out as non-specific reactions in certain circumstances. The actual factor of sensitivity and specificity would then be found by adding the + and ± reactions together.

PROFESSOR A. VERNES.

The Serological Conference held by the League of Nations at Copenhagen in 1928 with the object of improving the general conditions necessary for the campaign against syphilis offered an excellent opportunity for representatives of sixteen different countries to meet and compare, by conducting experiments on about a thousand sera, the methods which they regarded as giving the best results. All this work, for which the preparations were carried out in a manner that does credit to the ability of its organisers, was conducted in an atmosphere of extreme courtesy and with a universal anxiety to achieve some progress.

In this spirit, the Institut prophylactique was obliged, in order to assure its co-operation in the Conference's work, to abandon

all its usual principles and all its methods for the time being; and we feel bound to give an explanation here.

1. In the days when it was almost impossible to detect syphilis except by skin or mucous-membrane lesions, these lesions were described as primary, secondary or tertiary according to their appearance.

In that distant period, it was not suspected that syphilis might take a different course.

We now know that manifestations of the secondary type (mucous patches) may be observed in the most advanced stages of the disease, and conversely manifestations of the tertiary (gummata) may appear even in the first stages.

Further, we know that the immense majority of syphilitic cases present no apparent symptoms, but that the expression "latent syphilis" itself merely conceals localisations which could only be established by very careful medical exploration: mediastinum, kidneys, liver, heart, ductless glands, Erb's paraplegia, etc., and, above all, that syphilitic meningitis which produces not the slightest disturbance of the reflexes until it has attacked the nerve centres and ducts enveloped by the meninges and which, if not promptly checked, ends in tabes and various forms of encephalitis.

All this shows the necessity for close contact between serology and clinical practice, so that serology, based primarily on the firm foundation of careful medical observation, may in due course furnish the doctor with accurate results.

2. It was through sustained clinical observation that we were led nearly twenty years ago to the fundamental conclusion that serology would be condemned to remain a rudimentary science if it could only produce + or - results, while in clinical practice and in all the other laboratory sciences *fluctuations* are observed and noted.

By making a protracted graphical study of these fluctuations in the blood and cerebro-spinal fluid of the same subjects, who were kept under constant clinical observation, we succeeded, by repeated improvements of our technique, in recording these fluctuations on a scale ranging from 0 to 150 and upwards, all personal influence on the part of the operator being eliminated.

At the Conference, however, in order that our results might be comparable with the others, we were asked to express them by the symbols +, ± and -, which we thought we had buried for ever when we established our first scale in 1910.

After some consideration, we agreed to make this temporary concession, as we saw no serious objection to it.

For one thing, no one smiles nowadays, as they did when I was beginning to demonstrate that "accurate serology throws light on the treatment of syphilis", and when only perhaps two foreign writers, Boas and Blaschko, shared my view. Also it was obviously useful that those who think only in terms of -, ±, +, or even ++ and further multiples of +, should be shown that a method of measurement mechanically regulated to throw light upon the treatment throws light also upon the diagnosis.

It must be also remembered that many sera were not in a sufficiently preserved state for our reaction.

Alteration of the serum due to age can produce a slight flocculation in the non-syphilitic sera, and can sensibly diminish the flocculation in cases of syphilis. A number of the results shown in the

tables must be set aside for this reason<sup>1</sup>, because it is apparent that the comparisons must bear on the modifications of the serum resulting from syphilis, and not on those which result from putrefaction.

Our method of measurement takes particularly into account the control of treatment, which gives it, in consequence, a fineness of diagnosis, on condition, I must repeat, that the sera are in a state of preservation such as we have in Paris. They should be used fresh, or have not been in transit more than three or four days, and all the usual precautions should, of course, be taken as regards temperature and asepsis.

Cerebro-spinal fluids should, however, be always examined on the same day as they are taken in the same way as for the leucocytosis test.

#### OBSERVATIONS UPON GUINEA-PIG COMPLEMENT BY DR. E. J. WYLER

For the Bordet-Wassermann tests carried out at this Conference by the Harrison method, complement was prepared as at home, the serum being taken off the clot four hours after the animal has been killed. The titres of the complements obtained in Copenhagen have for the most part been low as compared with those generally obtained at home, using the same hæmolytic amboceptor and the same strength of corpuscule suspension as ascertained by hæmoglobin measurement (Bigger's method). The titre of complement at home rarely falls below 1/60. The titres of complement (three guinea-pigs pooled in equal amount) obtained at this Conference were as follows:

May 21st: About 1/40.

May 22nd: do.

May 23rd: 1/30. The same result was obtained:

- (1) With two further samples of blood corpuscles;
- (2) With a different amboceptor;
- (3) With double the usual sensitisation.

May 24th: 1/55.

May 25th: 1/30. When the three component complements were separately titrated, two of them gave no lysis at 1/30, while the remaining serum gave a higher titre than 1/40 (no higher dilution than 1/40 was tried).

<sup>1</sup> Especially for the most obviously bad sera (chylous sera, granular sera, whether accompanied or not by the phenomenon of "silky waves" (*ondes soyeuses*), which we endeavoured to improve so far as possible by complications of technique (prolonged centrifugation, more-frequent test reading in the course of the experiment). Some of the numbers are given below:

3	114	190	254	329	393	472	599	729
4	121	191	255	332	394	476	600	737
6	123	192	256	333	401	479	601	738
9	128	193	264	334	402	485	602	751
15	138	194	265	335	403	490	623	807
24	139	195	266	336	404	491	625	813
32	140	196	274	339	412	511	632	839
49	143	199	277	346	413	513	634	855
53	146	203	280	347	415	518	639	876
63	150	215	281	349	417	520	641	892
64	151	216	282	353	430	543	648	893
65	153	218	283	354	432	548	651	897
73	171	220	284	357	438	556	667	899
78	173	226	285	361	439	578	668	902
79	174	228	286	362	446	582	677	903
84	179	229	294	373	450	583	683	924
96	183	233	295	376	453	584	688	925
104	184	244	296	377	455	588	693	929
108	187	245	306	378	459	595	694	937
109	188	248	317	386	461	596	717	941
113	189	253	327	392	470	597		

May 26th: 1/45. The three component complements, when separately titrated, gave about the same titre.

May 29th: 1/45. Ditto.

May 30th: 1/75. This complement was composed of two sera pooled in equal amounts. A third serum gave a slightly lower titre. The complement used on this day by the other delegates (consisting of the serum of numerous guinea-pigs) was also titrated by me and gave 1/40.

Three further complements of single guinea-pigs gave respectively  $>1/60$ ,  $>1/60$  and  $1/40$ .

May 31st: 1/70. This was the same complement as that used on the previous day, which had been kept frozen overnight. The mixed complement used by the other delegates gave 1/40.

June 1st: 1/70. This was the same complement as that used on the previous day (and also on May 30th) which had been kept frozen overnight.

In the technique under consideration, the complement is titrated in presence of excess of amboceptor. Low complement titres tend to decrease the sensitiveness of the test. Thus a serum tested at the conclusion of the Conference Tests with three different complements giving titres of 1/40, 1/70 and 1/70 respectively (the other ingredients of the test being, of course, the same) gave + with the weak complement and ++ with the other two. With seven other weakly reacting sera there was generally a tendency for a slightly increased degree of lysis with the weaker complement.

These low complement titres obtained at Copenhagen are of interest. Four of my guinea-pigs were despatched by aeroplane from London, two of these were used in the comparative tests indicated in the preceding paragraph. These were the two animals which yielded a titre of 1/70.<sup>1</sup> My animals are fed exclusively on oats and cabbage; the Institute guinea-pigs on barley, oats, beetroot and hay. Is it possible that this is connected with the differences of complement titre? I am not aware of any work in this connection except that of Griffith and Scott (Ministry of Health, London), who found that, when guinea-pigs are fed exclusively on green food, they get very thin, but their complement titre is not affected.

Certainly the animals used at the Serum Institute are often small. For my own work, I make it a rule to use only well-grown male animals (fed as stated) weighing usually about 700 grams. They are always weighed before use and if they have lost weight are rejected. They are never used for inoculation purposes.

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<sup>1</sup> The sera of the remaining two guinea-pigs were titrated a few days later and gave respectively 1/80 and 1/90.

Appendix

NOTE ON THE PREPARATORY WORK CARRIED OUT AT THE  
PROPHYLACTIC INSTITUTE (PARIS) FOR THE COPENHAGEN  
CONFERENCE, PRESENTED BY DR. VERNES, DURING  
THE CONFERENCE.

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Gentlemen,

I wish to bring to your notice the work that the Paris Prophylactic Institute has undertaken for the Copenhagen Conference. We have investigated certain tests for flocculation, comparing them with our own reagent, which, as you know, has remained absolutely unchanged since March 1917. The method we now follow, after successive improvements in our optical material, has not been changed since 1921. The work involved in the studies for this Conference has been considerable.

We received the material for the Sachs reaction on January 12th and have carried out 2,320 tests up to date.

The material for the Meinicke reaction was received on January 12th and 2,320 tests were made.

We received the material for the Sigma reaction, as modified by Dr. Madsen, on March 14th, and have carried out 580 tests.

The material for the Kahn reaction reached us on March 24th and has been employed for 640 tests.

Thus, including our current work, we have carried out 25,205 sero-reactions in our laboratory during this period, in addition to 4,796 serological tests for tuberculosis.

At the request of the President of the Health Committee, Dr. Madsen, and the Director of the Health Section, Dr. Rajchman, 128 specimens of cerebro-spinal fluid were sent by air mail from Paris to Copenhagen.

These tests were carried out in the laboratories of the Paris Prophylactic Institute (36, rue d'Assas) with, I assure you, just as much meticulous care as if we had performed them ourselves. The interests of the patient have been paramount.

So, in addition to the notation which had been suggested to us, we have added the photometric notation for the two reactions which lend themselves to it, namely, the Sachs and Meinicke tests:

When measured with the photometer (this is employed nowadays for all kinds of ultra-fine measurements in serological work: urea, cholesterolin, calcium, phosphates, magnesium, glucose, uric acid, albumin, etc.), both these tests, particularly the Sachs reaction, have much in common with our own, in that they tend themselves to a graphic representation based on photometric indications. With so little time before us, we could not do more than begin a comparative study of this nature: but, now that we are provided with the necessary organisation, we propose to continue it during the coming months.

We can only submit the beginnings of charts, chiefly of syphilitic cases put under treatment immediately on arrival, with the descending curve following treatment.

Another part of our documentation concerns cases which, judging by our experience — our observations extend over an uninterrupted period of seventeen years — can be regarded as cured.

The following points must be borne in mind: the great difficulty has been to establish a definite line of demarcation between syphilitic and normal sera, since syphilitic flocculability is simply an exaggeration of normal flocculability, just as fever is merely an exaggeration of the normal temperature in any individual. Further, normal subjacent flocculability remains invariable in any given person (producing a horizontal line in the chart), whereas the distinctive feature of superadded syphilitic flocculability is that it may be characterised by very marked fluctuations. Again, and this is most important, in a person in whom the cerebro-spinal fluid is normal, but in whom syphilis has not been completely eradicated, an upward curve invariably occurs within eight months of the last injection of arseno-benzene, whereas, once the chart line has remained low for eight consecutive months after such injection (arsenical signpost — *jalon arsenical*), with normal cerebro-spinal fluid on the expiry of that period, no subsequent rise in the curve has ever been observed during our seventeen years' experience.

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The ideal test would be in the nature of an alarm signal recording the entry of the first microbe and ceasing to register on the death of the last survivor.

As this is not feasible, we have to consider what is the best test for practical purposes. This test, in our view, should show a reaction directly after the introduction of the treponema, should be absolutely specific for syphilis, and should give the same objective result to any operator at any time.

We now come to the famous question of sensitivity.

The mere fact of a reaction showing an upward tendency more promptly or to a greater degree in a given subject is insufficient to prove that it is more sensitive; it must also drop, under the influence of treatment, below its starting-point (otherwise the starting-point does not represent an increase in flocculability). In view of what we already know concerning the variability of syphilitic flocculability as compared with the invariability of any normal case, the position of zero, as determined by nature, is placed at the lowest point to which the curve can be made to drop as the result of treatment, and only a series of observations can determine this basic line of invariability.



As regards our own experience, we have at our disposal the patient and his humours and we never take any step in serology that is not supported by clinical observation or any step in medical practice that is not borne out by the results of serological research. After many years of experiment, we are now able to lay down the conditions necessary for a proper control of the effects of various drugs. In virtue of this method, we now propound the principle that true prophylaxis of syphilis consists in rendering the patient innocuous, not for a certain period, but for life — in his own personal interest and as the only means of successfully combating the terrible scourge of hereditary syphilis. In this way we have brought about the technical organisation which we are still endeavouring to perfect for our country with the aid of Parliament, the State, the Paris Hotel de Ville and numerous organisations.

Prolonged observation appears to us essential if we are to make a really useful contribution towards a comparative study, as we have been requested to do. We ask nothing better than to preserve this study as long as may be necessary along the lines which we have organised in Paris.

All who have devoted themselves during the last twenty years to exhaustive researches on the serology of syphilis have come to the same conclusion, namely, the importance of a minute analysis of the phenomena of flocculation. The sanction of this Conference will thus mark an immense progress.

If the Health Organisation of the League of Nations would do all in its power to promote the practical application of this principle in the supervision of syphilis treatment, it will have done much to assist the methodical organisation of the various means designed to eradicate this disease all over the world.

I may add, since we propose to pursue our comparative studies during the coming months in a spirit of perfect objectivity and impartiality, that we shall be only too glad to receive suggestions and information from the different authors in regard to their technique.

Comparative studies were carried out at our Institute between January 12th and May 26th on upwards of eight hundred patients. We have only been able to submit a limited number of charts (two hundred) owing to the clerical work involved. Should you be willing for the next Conference to be held at the Paris Prophylactic Institute, I can assure you, on behalf of the Committee which I have the honour to represent here, that its laboratories and services would be placed entirely at your disposal and that everything possible would be done to facilitate your work. You will find all the sera and specimens of cerebro-spinal fluid you can possibly need, taken on the actual day, from patients under observation or undergoing treatment whose clinical history is recorded in our archives.

You may rest assured, gentlemen, that your visit would meet with a most sympathetic welcome from the French authorities, and, in order to convey to you the warm welcome which would be extended to you, I am authorised to inform you that the President of the Republic will be happy to receive you and to express in person his interest in your activities.

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## Annex 2.

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### DESCRIPTION OF THE VARIOUS METHODS USED<sup>1</sup>

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#### METHOD USED BY PROFESSOR D. DE BLASI.

##### 1. *Method:*

The classic Bordet-Wassermann method, except that on each occasion the hæmolytic system is first adjusted according to the method described below. A suspension of red blood corpuscles is used, the concentration of which is variable, but which always contains the same "hæmolysable mass" determined by reference to a colorimetric standard. The "*vis complementaris*" of the guinea-pig serum is then titrated.

##### 2. *Principles:*

If the discrepancies between the results of the serodiagnostic methods based on complement fixation are to be reduced, results must above all be capable of comparison with each other. The comparability of the results of the B.-W. reaction obtained with the same sera by several operators is none other than the comparability of the antigens used. In order that a comparison between several antigens may show which of them is the most sensitive and at the same time the most specific, *the antigens must be titrated by all the operators with the same doses of complement, of amboceptor and of "hæmolysable mass"*. But the complement must be titrated before the antigen and the two other elements must therefore be constant. One amboceptor, once it has been titrated, is used throughout in a constant dose until the supply is exhausted. But the quantity of corpuscles, or, more precisely, of the "hæmolysable mass" is not always equally constant. In fact, 5% suspensions prepared in different laboratories (sometimes even in the same laboratory, but at different times) do not always contain the same "hæmolysable mass". This explains why the results of the titration of the "*vis complementaris*" of the same guinea-pig serum may work out differently for different operators, which necessarily involves a difference between the results of the titration of the same antigen. In view of these considerations, the method was tried, in 1918, of rendering

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<sup>1</sup> The following descriptions have been supplied by the participants themselves, any appreciation of the single method must therefore be regarded as the personal opinion of the author concerned.

the "hæmolysable mass" constant with the help of a constant colorimetric standard of reference. (See the Memorandum "Sulla determinazione del sistema emolitico nella reazione di Wassermann", DE BLASI, *Bollettino della R. Accademia di Medicina di Roma*, A. XLV, 1918-1919.)

3. *The advantages of the preliminary determination of the hæmolytic system are as follows:*

(a) By consistently using the same quantity of amboceptor and the same "hæmolysable mass", the "*vis complementaris*" of guinea-pig serum is made really constant in all experiments carried out at any time in the same laboratory; (b) further, an almost perfect comparability between the hæmolytic systems used in laboratories situated at a great distance from one another could be assured if the same colorimetric standard of reference were adopted by agreement everywhere; (c) finally, once the composition of the hæmolytic system was made constant *always and everywhere*, and once the antigen was dosed with such a system, *a more satisfactory comparability of the B.-W. results would be obtained, i.e., a more reliable comparative estimate of the quality of the various antigens*, independently of the time and place where the reactions are carried out.

4. *Temperature:*

The sera are inactivated at 53° C. for half an hour in a water-bath. The mixtures "serum + antigen + complement" are kept for an hour at 37° C. in a water-bath. After adding the mixture "amboceptor + corpuscles", the tubes are replaced in the water-bath, from which they are removed as soon as the controls permit.

5. *Reading results:*

Two readings are taken: the first after the removal of the tubes from the thermostat; the second half an hour later. The results according to the second reading are taken as final. If the results of the first reading are ++ or + and those of the second ± or —, the reaction is always repeated with a fresh blood sample.

6. *Number of tubes required for the control of one dose of serum:*

One tube containing the same dose of serum as the reaction tubes.

7. *Pipettes required for each test:*

- (a) For each serum, a 1 or 0.5 c.c. pipette, graduated either in 100ths or 200ths;
- (b) Three pipettes for the saline: one 2 c.c. in 100ths, one 5 c.c. in 10ths, one 10 c.c. in 10ths;
- (c) Two pipettes for the complement: one 2 c.c. in 100ths (for the titration), one 5 c.c. in 10ths (for the distribution);
- (d) Two pipettes for the antigen: one 2 c.c. in 100ths, one 5 c.c. in 10ths;
- (e) One 1 c.c. pipette in 200ths for the amboceptor;
- (f) Two pipettes for the corpuscles: one 2 c.c. in 100ths (for the determination of the "hæmolysable mass"), one 5 c.c. in 10ths (for the distribution of the mixture "amboceptor + corpuscles").

8. *Other apparatus required:*

Small graduated glass tubes, one of which contains a formol-acetic solution of acid fuchsin, the colour of which matches that of the lackage of a dense mass of washed corpuscles, diluted in the proportion of 1:200.

*Adjustment of the Hæmolytic System.*

1. *Determination of the quantity of blood-corpuscle suspension.* — The first stage is a colorimetric process which consists in laking one volume of corpuscle suspension in 99 volumes of distilled water (1:100) and in comparing the colour of the solution with that of a standard colour solution. At first, Professor de Blasi used Gower's hæmoglobinometer, but subsequently came to prefer a formol-acetic solution of acid fuchsin and picric acid, the chromatic intensity of which corresponds to a thick red-cell suspension laked 1:200. The composition of the liquid used as a standard of reference is that given by Vernes in the *Comptes rendus de l'Académie des Sciences*, 1918, No. 14. The colorimetric procedure is described in the paper on "The Adjustment of the Hæmolytic System in the Wassermann Reaction" (*Bollettino della Reale Accademia di Medicina di Roma*, A. XLV, 1918-19).

2. *Dosing of complement.* — The second preliminary stage consists in the determination of the minimum hæmolytic doses of guinea-pig serum, using twelve different concentrations, ranging by regular degrees from 2:1000 to 13:1000. By multiplying the minimum concentration which produces hæmolysis by the empirical constant of 2.5, the concentration of complementary serum is obtained which is to be used both for the dosing of antigens and for the B.-W. test. This second stage is described in the paper on the "Method of ensuring Constancy of Effect with the Hæmolytic System" (*Archivio di scienze biologiche*, Vol. V., Nos. 1-2, 1923).

The first of the two preliminary stages mentioned ensures the use of a constant "hæmolysable mass" at all times by one and the same worker; and could do so in the case of several workers if they came to an agreement. The second stage makes it possible always to use the same quantity of complement which may be present in varying amount in the sera of different guinea-pigs. Taken together, the two processes ensure comparability of results with the hæmolytic systems used on various days in one and the same laboratory; and they could do this with the hæmolytic systems used in various laboratories, provided the colorimetric liquid which serves as a standard of reference and the titration of the complement were the same.

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METHOD EMPLOYED BY DR. E. DEBAINS.

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1. *Method employed:*

The Calmette and Massol method applied to *unheated sera* by super-activation procedure.

2. *Principles:*

The original method of Calmette and Massol (heated sera). Use of a fixed dose of antigen; increasing doses of complement — E. Debain's modification; use of human complement (unheated sera); superactivation by increasing doses of guinea-pig complement.

3. *Advantages:*

(a) A greater sensitiveness:

Alteration of the sera by heating at 55° C. is avoided;

The component parts of the hæmolytic system can be measured accurately; for each tube containing antigen there is a control tube which is strictly comparable.

(b) The strength of the reaction can be measured by this method.

This sensitiveness is particularly valuable in the detection of unsuspected latent syphilis—pseudo-syphilis.

The method, however, requires the use of fresh sera; the samples must be taken twenty-four or forty-eight hours before the test.

4. *Temperature:*

37° C. (1½ hours).

5. *Reading of results:*

After half-an-hour. (The racks are inspected; if the hæmolytic activity of a serum is low, the reading must be postponed till hæmolysis is complete in the control tubes.)

6. *Tubes necessary for each serum:*

Nine tubes are necessary for each serum: four control tubes and five containing antigen.

7. *Pipettes required:*

One pipette per serum.

8. No other apparatus is necessary.

9. *Other details concerning the Calmette and Massol method applied to unheated sera; superactivation procedure:*

*Preparation of the Antigen* (horse-heart extract). — The heart, having first been pounded, is dried in a vacuum at 30° to 35° C. in an ordinary desiccator which can work large quantities; the operation only requires a few hours. The dry matter is pulverised and sifted by machinery. The powder thus obtained is homogeneous and very fine. The fatty matter is removed by successive extractions with

acetone at room temperature. To make sure that all the fatty matter has been removed, excess of water is added to the decanted acetone ; the mixture should remain clear. The powder, freed from fat, is dried in the incubator at 37° and placed for 15 days in absolute alcohol, in the proportion of 20 gr. of powder to 100 gr. of alcohol. The liquid is decanted and filtered. For the test the alcoholic extract is measured with a pipette into a flat-bottomed cylindrical vessel, and physiological saline is added in the proportion of 25 c.c. to 1 c.c. of extract. The saline is added in two stages: first 5 c.c. of saline for each c.c. of extract are added drop by drop, imparting at the same time to the cylindrical vessel which is held in the left hand a circular motion in order that the saline may mix thoroughly with the extract. The remainder of the saline is then poured down the side of the vessel and the whole gently mixed.

*Reaction.* — Nine tubes are used for each serum: four control tubes containing 0.8 c.c. of saline, five reaction tubes containing 0.8 c.c. of colloidal suspension (antigen). Doses of complement diluted 1:25 are then delivered into the tubes according to the table given below:

	I	II	III	IV	V
Control tubes	0	0.1	0.2	0.3	—
Reaction tubes	0.1	0.2	0.3	0.4	0.5

Although the antigen has no anti-complementary action, each reaction tube is given one more dose of complement than the corresponding control tube.

Increasing doses of complement are added to all the tubes in fixed quantities of 0.8 c.c. For this purpose, five dilutions of complement in an increasing ratio of concentration are prepared, *e.g.*, for 80 sera the following solutions must be prepared:

	Saline	Guinea-pig serum
1.	160 c.c.	1 c.c.
2.	160 c.c.	2 c.c.
3.	160 c.c.	3 c.c.
4.	80 c.c.	2 c.c.
5.	80 c.c.	2.5 c.c.

After these have been in the incubator for one and a-half hours, 0.8 c.c. of a suspension of sensitised red sheep corpuscles are added.

The total volume in each tube is 2.5 c.c. (0.8 c.c. of saline or antigen plus 0.8 c.c. of complement, plus 0.1 c.c. serum, plus 0.8 c.c. sensitised red corpuscles.)

The quantity of corpuscles contained in 0.8 c.c. should be such that hæmolysis is not complete in the first control tube. This quantity has been calculated by taking into account the average hæmolytic power of human sera.

The corpuscles, well washed in saline, are centrifuged for ten minutes. At the end of that time, the volume of the deposit has

reached its maximum. The volume of this deposit multiplied by 2.8 indicates the quantity of saline to be added.

*Example:* Volume of the deposit = 50 c.c.

Total volume of the emulsion =  $50 \times 2.8 = 140$  c.c.

Quantity of saline to be added = 90 c.c.

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METHOD USED BY DR. L. W. HARRISON  
AND DR. E. J. WYLER.

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1. *Method used:*

No. 1 method, Medical Research Council, Special Report Series No. 14, as modified by Dr. E. J. Wyler.

2. *Principle:*

Complement fixation: the essentials of the technique are set out below.

3. *Advantages:*

It would appear that the principal advantage of this method consists in a high degree of specificity without undue loss of sensitiveness.

4 and 5. *Temperature and reading of results.*

Fixation takes place for 30 minutes at room temperature followed by 30 minutes in the water-bath at 37° C. The sensitised sheep cells are then added and the racks replaced in the water-bath at 37° C. The results are read as soon as the negative control serum shows complete lysis.

6. *Tubes used for each serum:*

Four.

7. *Number of pipettes used:*

In Donald's "dropping" technique which was employed at the Copenhagen Conference, graduated pipettes are not needed in the test proper. One dropping pipette is used for the sera and is washed out with saline between each specimen; one dropper is used for saline, complement dilutions, and sensitised cells; and one other dropper is used for antigen suspension.

8. *Apparatus used:*

A burette to which Donald's droppers are attached as described in Medical Research Council, Special Report Series No. 14, pages 22-27.

9. *The following are the essentials of the method:*

*Inactivation of patient's serum.* — Thirty minutes at 55° C.

*Complement.* — Guinea-pig's serum titrated as follows:

This is done on every test-day, the volume of fluid in each tube being the same as in the test proper. Two rows of tubes containing graded strengths of complement dilution are set up as follows:

- Row 1 contains 2 volumes saline;  
1 volume complement dilution;  
1 volume sensitised cells.
- Row 2 contains 1 volume saline;  
1 volume complement dilution;  
1 volume antigen suspension.

Row 1 is incubated for thirty minutes at 37° C. and the titre of the complement is then read.

Row 2 is incubated for thirty minutes at room temperature and then for thirty minutes in the water-bath at 37° C. At the end of this period, a volume of sensitised cells is added to each tube and the rack replaced in the water-bath at 37° C. for thirty minutes. Provided that the result of the final incubation of row 2 shows that the titre as ascertained in row 1 has not been reduced through the presence of antigen by more than one dose the reading obtained in row 1 is accepted.

*Antigen.* — Alcoholic human heart extract (three parts); 1% alcoholic solution cholesterol (two parts). These are mixed on the day of the test and the mixture diluted 1 in 15 with normal saline by pouring the saline quickly upon it.

The heart extract is prepared as follows: the fat having been cut away, the muscular portion of the left ventricle is minced and ground for one minute with absolute alcohol (1 grm. of heart muscle to 9 c.c. absolute alcohol) in a mortar with powdered glass. The mixture is placed in the incubator at 37° C. for twenty-four hours, being shaken at intervals. At the end of twenty-four hours it is filtered, placed in the ice-chest overnight and again filtered. The extract remains unchanged at room temperature for many months.

*Hæmolytic system.* — A standardised suspension of sheep's red blood cells sensitised with six doses of hæmolytic immune body.

*Test proper.* — Four tubes are required for each serum.

Each tube contains the same quantity of fluid as follows:

- Tube 1 (serum control) { 1 volume saline;  
1 volume patient's serum diluted 1 in 5 with saline;  
1 volume complement dilution in saline, containing three minimal hæmolytic doses.
- Tube 2 (serum control) { Same as tube 1, but the volume of complement dilution in saline contains two minimal hæmolytic doses.



Tube 3	}	1 volume patient's serum diluted 1 in 5 with saline;
		1 volume complement dilution in saline containing five minimal hæmolytic doses;
		1 volume antigen suspension.
Tube 4	}	Same as tube three, but the volume of complement dilution in saline contains three minimal hæmolytic doses.

The tubes are incubated first at room temperature for thirty minutes and then in the water-bath at 37° C. for thirty minutes, after which one volume of sensitised cells is added to each and the racks replaced in the water-bath at 37° C. The reading is taken in thirty minutes or earlier if the negative control serum shows complete lysis.

*Cerebro-spinal fluid.* — This is tested in the same way as serum (one volume of C.S.F. diluted 1 in 5), except that a further three rows of tubes are put up containing two, five and ten times this amount.

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#### METHOD EMPLOYED BY DR. E. JACOBSTHAL.

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*Method used :*

Synoptic cold method (Jacobsthal).

*Principle :*

In order to gauge the strength of the reaction, simultaneous tests of varying sensitiveness are made with the same serum, namely:

- (1) "The Jacobsthal cold method" with cholesterinised alcoholic heart extract (human, or perhaps ox, heart extract);
- (2) The "warm method" with the same extract;
- (3) The "cold method" with non-cholesterinised alcoholic heart extract;
- (4) The "warm method" with the same extract.

As a rule, one dose of each extract is employed and not graduated doses. The tests are made with "quarter doses" (total volume 1.25 c.c.). Complement diluted 1 in 10; amboceptor titrated on every test-day: 2 ½ doses. Patient's serum inactivated and diluted 1 in 10.

In the *cold method*, the serum and antigen mixture combined with complement is shaken, immersed in a salt and ice-water mixture and placed in the ice-box for one hour. The hæmolytic system having been meanwhile prepared, the sensitised cells are added at the expiration of the hour and the tubes put in the incubator at 37° C. for a further hour. In the warm method, the mixture of antigen and serum is incubated in the usual manner at 37° C. for an hour, during which the hæmolytic system is prepared. Incubation as above.

With very rare exceptions, the cold reactions are far more sensitive than the corresponding warm reactions; again, the reaction is more sensitive with cholesterinised than with non-cholesterinised extract. For this reason, No. 2 of the above-mentioned reactions is sometimes stronger and sometimes weaker than No. 3.

*The cerebro-spinal fluid* is never tested by Jacobsthal's cold method and never with cholesterinised extracts, because unspecific results may be obtained, especially in isolated cases of tuberculosis.

But in every instance a graded test is made according to Hauptmann's method in the following doses: 0.05, 0.1, 0.5 and 1.0.

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METHOD EMPLOYED BY PROFESSOR R. OTTO  
AND DR. BLUMENTHAL.

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1. *Method used:*

The original Wassermann Reaction.

2. *Principles:*

Complement fixation. The results obtained with syphilitic liver extract adjusted to give sensitive reactions are primarily taken into account.

3. *Advantages of the method:*

Detection of a wide range of cases, especially *Lues latens* and *Lues congenita*.<sup>1</sup>

4. *Temperature:*

In the incubator at 37° C.

5. *Reading results:*

After fixation for one hour in the incubator, the hæmolytic system is added. Further incubation until the controls are lysed. This requires on an average another half-hour.

6. *Number of tubes required for a test:*

Four.

7. *Number of pipettes required:*

In addition to 1 pipette each for saline, blood, complement, amboceptor, and extract, one pipette is required for each serum.

8. *Other apparatus required:*

A water-bath at 56° C. and an incubator at 37° C.

9. *Other details of the technique:*

Government-tested extracts are used as antigens, *i.e.*, the syphilitic liver extract adjusted to give sensitive reactions with an

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<sup>1</sup> In the Serological Department of the Robert Koch Institute, a parallel flocculation reaction is carried out (*cf.* R. OTTO, *Klin. Woch.* 1925, page 1312).

admixture of guinea-pig's heart extract, employed with  $\frac{1}{10}$  and  $\frac{1}{15}$  complement (cf. G. BLUMENTHAL, *Medizinische Klinik*, 1926, No. 51), and a less strongly cholesterinised extract of ox-heart.

The dose of amboceptor to be used is determined in the preliminary test by two readings (in twenty minutes and again in an hour), and comparison of the values thus obtained. (Cf. G. BLUMENTHAL, *Berliner Klinische Wochenschrift*, 1921, page 1067.)

METHOD EMPLOYED BY DR. D. PAVLOVITCH.

1. The method of serum diagnosis of syphilis practised at Copenhagen is based on the complement fixation principle of *Bordet-Wassermann*.

2. Heated sera are used, with three different antigens and a fixed dose of complement. The antigens are as follows: (1) Bordet's antigen, (2) two alcoholic extracts of ox-heart *without cholesterin*. The hæmolysin is titrated daily with guinea-pig complement taken the same day. The lay-out of the test may be seen from the following table:

Tubes	I	II	III	IV
Heated serum	0.10	0.10	0.10	0.20
Antigen I	0.25	—	—	—
Antigen II	—	0.25	—	—
Antigen III	—	—	0.25	—
Complement 1/10	0.25	0.25	0.25	0.25
NaCl Solution	0.20	0.20	0.20	0.40

One and a-half hours in the water-bath at 37° C.

Hæmolytic system. 0.5 c.c. of a 5% suspension of sheep red corpuscles is added to each tube.

Half-an-hour in the water-bath at 37° C.

The antigens are diluted by means of the *Bigger* apparatus used for the Sigma reaction.

The amount of hæmolysin necessary for the reaction is determined in two stages: first, the minimum hæmolytic dose with the fixed quantity of complement is ascertained and then the complement is titrated with two, three (and four) doses *in the presence of antigen*, according to the following arrangement:

Tubes	1	2	3	4	5
2 to 4 Minimal Hæmo-lytic Doses	0.25	0.25	0.25	0.25	0.25
Complement 1/10	0.10	0.15	0.20	0.25	0.30
Antigen	0.25	0.25	0.25	0.25	0.25

Half-an-hour at 37° C. Then 0.25 c.c. of 5% suspension of red corpuscles to each tube.

For the test, the dose is taken which, with 0.20 c.c. of the complement, produces complete hæmolysis:

3. *The advantages of this method are:*

- (a) Our non-cholesterinised antigens have very rarely given non-specific results. The reaction is perhaps rather less sensitive than the others, but we prefer to maintain specificity even at the expense of sensitiveness. The antigens are always diluted in the same manner.
  - (b) The method saves time, a great advantage in laboratories in which tests are made on a large scale, for all the titrations are carried on after the incubation of test proper at 37° C. has commenced.
4. The reaction is carried out in the water-bath at 37° C.
5. The reading is taken immediately on removal from the water-bath, *i.e.*, two hours are required to obtain the result.
6. Four tubes are used for each serum.
7. One worker uses about five pipettes of 1 c.c. divided into  $\frac{1}{100}$ ths for the distribution of the sera. After use, a pipette is washed with saline solution, and is left to drain on filter paper while the others are in use. In addition, one 5 c.c. pipette is required for the complement, one for the antigen, and one 10 c.c. pipette for the hæmolytic system.
8. The other apparatus required consists of a 37° C. and a 56° C. water-bath and a dropper.

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METHOD EMPLOYED BY DR. S. SIERAKOWSKI.

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1. *Method used:*

McIntosh and Fildes' method for the Bordet-Wassermann reaction.

2. *Principles:*

- (a) Preliminary titration of the complement on each test-day;
- (b) Dose of hæmolytic serum fixed in advance;
- (c) Fixed dose of antigen.

3. *Advantages of the method:*

- (a) Titration of the complement as a preliminary;
- (b) Titration of the complement in the presence of antigen.

4. *Temperature:*

37° C.

5. *Reading result:*

The titration of the complement takes 45 minutes; the reaction itself 45 minutes.

6. *Number of test-tubes used for each serum:*  
Using a single antigen: two tubes — one for the serum plus antigen and one for the control.
7. *Number of pipettes used for each experiment:*  
One pipette for each serum, and a few extra for the reagents.
8. *Other apparatus required:*  
Water-bath at 56° C.; water-bath at 37° C.
9. *Other details of the technique employed by Dr. Sierakowski:*  
Use of two antigens:
  - (1) Alcoholic extract of ox-heart + cholesterin 1 per cent.  
Antigen diluted 1 in 15 (0.6 heart extract + 0.4 cholesterin + 14 saline).
  - (2) Bordet-Ruelens antigen (evaporated). Diluted 1 in 40.  
Fixation in the water-bath at 37° C. for 30 minutes.  
Dose of serum: 0.1.

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#### TECHNIQUE EMPLOYED BY DR. H. BOAS.

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The method employed by Dr. Boas is the new *Kahn* method (the rapid method) exactly as described by Kahn in his book "Serum Diagnosis by Precipitation".

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#### OUTLINE OF TECHNIQUE OF KAHN TEST.

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1. *Method used:*  
The Kahn test.
2. *Principles of this method:*  
The Kahn test is a precipitation method for differentiating syphilitic from non-syphilitic serum with the aid of a specially prepared cholesterinised alcoholic extract. The test has been developed in conformity with the following requirements for optimum precipitation:
  - (a) Optimum concentration of antigenic lipoids in the antigen:  
Excessive or deficient concentration preventing precipitation. This quantitative relation between the concentration of an antigen and its sensitiveness permits the preparation of an antigen having a standard degree of sensitiveness by the variation of the lipid concentration.
  - (b) Proper physical state of the antigen suspension: The suspension must contain a minimum amount of physiological salt solution and the lipid aggregates must be dispersible.
  - (c) Correct quantitative relation between serum and antigen suspension, an excessive amount of suspension being inhibitory to precipitation.

- (d) Shaking — as a probable aid in hastening collision between the serum-antigen interacting particles (optimum speed = 275 to 285 oscillations per minute).
- (e) Total dilution of the suspension — serum mixture should be a minimum for a given amount of mixture. Increasing the volume of the mixture with physiological salt solution (or with water) tends to reduce precipitation.

### 3. *Advantages of this method:*

#### Technical.

- (a) Practical steps evolved for bringing antigens prepared from different heart muscles to a standard degree of sensitiveness.
- (b) Stability of antigen.
- (c) The use of but three reagents — antigen, salt solution and serum.
- (d) Relative simplicity of technique, minimising thereby the sources of error.
- (e) Rapidity in obtaining results.
- (f) Availability throughout the world — in the tropics, field laboratories, etc.

#### Clinical.

- (a) High degree of specificity combined with sensitiveness.
- (b) Clear-cut reactions, minimum number of border-line reactions.
- (c) Several processes aside from the regular Kahn test as an additional aid to clinicians in their diagnosis and treatment of syphilis:
  - (i) Quantitative process with serum as an aid in studying the serological effect of therapy.
  - (ii) Qualitative process with spinal fluid corresponding to regular Kahn test with serum.
  - (iii) Quantitative process with spinal fluid as an aid in studying the serological effect of neuro-syphilitic therapy.
  - (iv) Presumptive processes with serum and spinal fluid, more sensitive than the regular processes owing to the employment of an antigen (sensitised) which is more potent than standard antigen.
  - (v) Special processes, including microprocesses with serum and spinal fluid, and process with chancre fluid.

#### Research.

The Kahn test especially lends itself to the application of research by physical and biological chemists as well as by serologists.

### 4. *Temperature at which the heating is carried out:*

- (a) The serum is heated for thirty minutes at 56° C.
- (b) The test is performed at room temperature.

5. *Reading of results:*

After three minutes' shaking.

6. *Test-tubes used for one serum:*

Three tubes.

7. *Pipettes used for one test:*

Three pipettes, two of which may be used for a series of tests.

8. *Other apparatus required:*

Centrifuge for separating serum from blood-clots.

Water-bath for heating serum at 56° C. for thirty minutes.

Test-tube rack.

In laboratories where large numbers of tests (thirty or more) are made daily, a shaking apparatus is of considerable help.

9. *Other details of the method:*

I. *Apparatus.*

1. Test-tubes for performing test are about 7.5 cm. in length and 1 cm. in diameter.

2. Vials (with straight wall and flat bottom) for preparing antigen suspension are about 5.5 cm. in length and 1.5 cm. in diameter.

3. Pipettes: 10 c.c. graduated to 0.1 c.c.; 1 c.c. graduated to 0.01 c.c. and 0.2 c.c. graduated to 0.001 c.c.

4. Test-tube rack: Made of sheet copper, 3 in. wide, 11½ in. long, 2¾ in. high. Consists of three shelves, upper and middle ones containing three rows of ten holes, each of approximately ½-in. diameter. The centre-row holes are offset half an inch.

5. Shaking apparatus may be of any construction that will hold the test-tube racks employed. The required speed is 275 oscillations per minute, with a stroke of 1½ inches.

6. Water-bath (56° C.); centrifuge and centrifuge tubes may be of any make that will be found convenient in the particular laboratory.

II. *Reagents.*

1. *Antigen.* For preparation and standardisation of antigen for Kahn test, see "Serum Diagnosis" and *Jour. Inf. Diseases*, Vol. 41, page 111, 1927. An antigen once standardised maintains its titre indefinitely. Antigen should be kept in the dark at room temperature. When subjected to cold, a precipitate may be thrown down, which can be re-dissolved upon warming in a water-bath. Only chemically clean and dry glass vessels should be used for storing antigen, and the cork stoppers should be covered with thin high-grade tinfoil.

2. *Serum.* The blood specimen should be centrifuged to remove clot and cells. The serum must be entirely free from cells or other particles. Previous to its use in the test, the serum is heated in a water-bath at 56° C. for thirty minutes. When serum that has been heated is kept overnight in the icebox, re-heating for ten minutes at 56° C. is necessary before using in the test.

3. *Physiologic salt solution.* A solution is prepared of 0.9% sodium chloride (chemically pure) in distilled water.

### III. Routine (Diagnostic) Test with Serum.

This is a three-tube test. Each tube contains a different proportion of serum and antigen suspension according to the following outline:

Tube	1	2	3
Antigen suspension, c.c.	0.05	0.025	0.0125
Serum, c.c.	0.15	0.15	0.15

It is well to have everything arranged before mixing the antigen with salt solution for the test. Have racks set up, tubes numbered, sera heated and pipettes ready for reasuring antigen suspension and serum. For measuring the 0.05 c.c. quantities of antigen suspension, mark off these amounts on a 1 c.c. (graduated to 0.01 c.c.) pipette with a wax pencil. For measuring the 0.025 and 0.0125 c.c. quantities, use 0.2 c.c. (graduated to 0.001 c.c.) pipettes, on which these amounts are also indicated with a wax pencil.

#### *Performance of Test.*

1. *Preparation of Standard Antigen Suspension.* Mix antigen with salt solution according to required titre. Thus, if the titre is 1 c.c. antigen plus 1.1 c.c. normal saline, mix the antigen as follows: (a) Measure 1.1 c.c. saline into a standard antigen suspension vial; (b) measure 1 c.c. antigen into a similar vial; (c) pour the salt solution into the antigen, and as rapidly as possible (without waiting to drain the vial) pour the mixture back and forth six times to ensure thorough mixing; (d) allow the antigen suspension to stand for ten minutes before using. The suspension should not be used after thirty minutes' standing.

One may mix more than 1 c.c. of antigen with a proportionally larger amount of salt solution, but not less than 1 c.c. This amount when mixed with saline will be sufficient for about fifteen tests. Two c.c. of antigen mixed with saline will be sufficient for about thirty-five tests.

2. *Measuring antigen suspension.* After the antigen suspension has stood for ten minutes, shake it well and measure 0.05, 0.025, and 0.0125 c.c. amounts for each serum, delivering the suspension to the bottom of the tubes. When employing the standard rack which contains thirty tubes, measure 0.05 c.c. amounts in the tubes of the first row; 0.025 c.c. amounts in the tubes of the second row and 0.0125 c.c. amounts in the tubes of the third row.

3. *Measuring serum.* The serum should be added as soon as possible after the antigen suspension has been pipetted, to avoid undue evaporation from the suspension. When examining large numbers of sera, it is well for one worker to measure the antigen suspension and for another to follow with the sera. Add 0.15 c.c. serum to the 0.05, 0.025 and 0.0125 c.c. amounts of antigen suspension, and shake the rack of tubes vigorously for a few seconds to ensure thorough mixing of the ingredients. The rack can now be set aside



until the remaining tests are ready for the regular three-minute shaking period.

4. *Controls.* At least one positive and one negative serum control should be included with each series of tests. Every serum giving a positive reaction should be examined to establish that it is free from red cells or foreign particles which might be confused with a specific precipitate. Dilute 0.1 c.c. serum with 0.3 c.c. saline. Shake well and examine for particles. If particles are present, the serum should be recentrifuged and re-tested.

5. *Shaking.* During the three-minute shaking period, it is important not merely to agitate the rack of tubes, but to see to it that the fluids within the tubes are vigorously agitated. When shaken by hand, one may shake three one-minute periods with short rest periods. When a shaking machine is employed, its speed should be 275 oscillations per minute, with a stroke of 1.5 inches. Hand shaking should approximate this speed.

6. *Addition of saline.* After the tests have been shaken, add 1 c.c. saline to each tube of the first row of the rack (containing the 0.05 c.c. amounts of antigen suspension) and 0.5 c.c. saline to the remaining tubes. Shake sufficiently to mix ingredients

7. *Reading results.* The results are read after the addition of the salt solution. Optimum reading conditions in each laboratory should be determined by trial. The following points will be found helpful: (a) When utilising daylight for reading the tests, it is well to have but one source of light coming from a single window immediately in front of the reader. It will be found satisfactory to shade the upper and lower portions of the window, narrowing the source of light to a section several feet in height. Light from any other windows near the reader should be dimmed by lowering the window shades. (b) When holding the rack in front of the exposed section of the window, the definitely positive and the negative reactions are readily differentiated without lifting the tubes from the rack. (c) In case of weak reactions, examine each tube individually, lifting it several inches above the eye level and slanting it until the fluid is spread into a thin layer. The precipitate will then become readily visible.

Those preferring magnification will find the microscopic mirror helpful. Place mirror on reading table with concave surface upward. Hold the tube in slanted position two to three inches above the mirror and examine the image in the mirror. Both daylight and artificial light may be employed. One may also utilise an ordinary hand lens for reading the tests. A two- or three-fold magnification will be found satisfactory. Some workers prefer the use of a slit-light arrangement, the source of light being an electric bulb enclosed in a box which is provided with a narrow slit.

As far as possible, workers should limit themselves to one method of reading. The occasional use of magnification by readers who usually do not resort to it will be likely to affect the uniformity of

their reading scale. It should be emphasised that certain highly magnifying agglutinoscopes show particles in serum alone, and are thus unfit for use in this test. The magnification must be sufficiently low as to assure opalescent and clear-cut negative reactions, with entire freedom from visible particles.

8. *Interpretation of results.* A definite precipitate suspended in a clear medium is read ++++. Proportionally weaker reactions are read ++++, ++, + and ± respectively. The *final results* is the average of the readings of the three tubes, as indicated in Table I.

*Table I.* OUTLINE OF KAHN TEST AND INTERPRETATION OF RESULTS.

	Tube No.	1	2	3	Completion of test.
Serum :	Antigen suspension	3 : 1	6 : 1	12 : 1	Tests are shaken three minutes, 1 c.c. salt solution is added to first tube and 0.5 c.c. to other two tubes and results are read.
Antigen suspension	c.c.	0.05	0.025	0.0125	
Serum (heated at 56° C. for 30 minutes) c.c.		0.15	0.15	0.15	

*Interpretation of Results.*

Reaction No.				Final result (average of reactions of three tubes)
1.	++++	++++	++++ <sup>1</sup>	++++
2.	+++	++++	++++	+++
.....				
3.	++	++++	++++	+++
4.	+	+++	++++	+++
.....				
5.	—	+++	++++	+++
6.	—	++	++++	++
.....				
7.	—	±	++++	+++
8.	—	—	+++	+
.....				
9.	—	±	++	±
.....				
10.	—	±	+	—
11.	—	—	+	—
12.	—	—	—	—

<sup>1</sup> Weakly potent sera show most marked precipitation in the third tube because a small amount of *reagin* reacts best with a small amount of antigen suspension, the relatively larger amounts of suspension in the first two tubes being inhibitory to precipitation.

Strongly potent sera show ++++ precipitation in each tube, but, owing to the different amounts of antigen suspension employed, the precipitates are of unequal bulk, being greatest in the first tube and least in the last tube.

In rare instances, an atypical reaction is obtained in which precipitation is marked in the first tube and weak or negative in the second and third tubes. In such a case, a quantitative test should be made and, if the result is twenty units or more, the qualitative reaction may be considered ++++; if less than twenty units, the results of the qualitative reaction should be averaged.

9. *Recording results.* Make a permanent record of findings in all tubes of each test at time of reading. Preferably, the tests should be read independently by two separate workers. When two workers are not available, the original reading should be checked by the same worker after a short interval.

10. *Procedure with less than three tubes.* If there is insufficient serum for the three-tube test, examine and report as follows: (a) If enough serum for two tubes, employ the lesser amounts of antigen suspension; report as a two-tube test. (b) If enough for one tube, employ the least amount of antigen suspension; report as a one-tube test. (c) If less than 0.15 c.c. serum is available, a one-tube test (micro test) may be made by employing 10 parts of serum to one part of antigen suspension: thus, if 0.05 c.c. of serum is available, it is employed with 0.005 c.c. of antigen suspension; report these as micro tests.

#### IV. *Qualitative Spinal Fluid Process.*

In this process, the greater part of the spinal fluid globulins is precipitated by means of ammonium sulphate and re-dissolved in an amount of normal saline equivalent to a tenth of the original spinal fluid volume. The concentrated globulin solution thus obtained is then tested with antigen suspension.

1. *Preparation of Globulin Solution.* (a) Centrifuge spinal fluid to render it free from cells and foreign particles. (b) Add 3 c.c. of the clear fluid to a conical-shaped 10 or 15 c.c. centrifuge tube. (c) To the same tube add 2 c.c. of a saturated solution of ammonium sulphate. (The ammonium sulphate must be of highest purity, such as Merk's Reagent or Baker's Analysed.) (d) Mix vigorously and place in 56° C. water-bath for fifteen minutes. (e) Centrifuge at high speed for fifteen minutes to throw down the precipitated globulins. (f) Remove with capillary pipette the supernatant fluid *as completely as possible*. (g) Add 0.3 c.c. normal saline to the precipitate and re-dissolve it by gentle shaking. This saline should be added by lowering the pipette close to the bottom of the tube to avoid washing down ammonium sulphate. The use of a 0.2 c.c. pipette is preferred for adding saline. With this pipette, the fluid is gently drawn up and expelled to assure the complete solution of the precipitate. This re-dissolved globulin solution is now ready to be tested with antigen suspension.

2. *Preparation of Antigen Suspension.* Mix salt solution with antigen in the same manner as for the test with serum, according to the required antigen titre for spinal fluid. The antigen suspension should stand ten minutes before its use in the test and should be used within thirty minutes.

3. *Measuring of Antigen Suspension.* With a 0.2 c.c. pipette graduated to 0.001 c.c., measure 0.01 c.c. of antigen suspension to the bottom of a test-tube.

4. *Measuring of Concentrated Globulin Solution.* Measure 0.15 c.c. of concentrated solution into the antigen suspension tube, using a 0.2 c.c. pipette. Shake tests vigorously for a few seconds to mix ingredients.

5. *Controls.* Include positive and negative spinal fluid controls; also observe each concentrated globulin solution to establish that it is free from foreign particles.

6. *Shaking.* After mixing the concentrated fluid with antigen suspension, shake tube vigorously for three minutes.

7. *Addition of Salt Solution.* Add 0.5 c.c. normal saline to tube.

8. *Reading of Results.* A definite precipitate suspended in a clear medium is read ++++. Proportionally weaker precipitates are read +++, ++ and + respectively.

(The spinal fluid procedure may be carried out with 1.5 c.c. spinal fluid plus 1 c.c. saturated ammonium sulphate solution. Dissolve globulin precipitate in 0.15 c.c. normal saline (instead of 0.3 c.c.) and perform test with 0.01 c.c. antigen suspension.)

*Highly Potent Spinal Fluid.* It should be emphasised that highly potent spinal fluids give positive reactions directly without concentrating the globulins by means of ammonium sulphate. It is recommended, therefore, that spinal fluids from clinically diagnosed cases of neuro-syphilis be examined by mixing 0.15 c.c. fluid plus 0.01 c.c. antigen suspension, using the same antigen titre as for tests with serum. If the spinal fluid is definitely positive (++++, +++ or ++) in the unconcentrated state, the reaction may be interpreted as ++++ in the qualitative procedure. If the reaction is very weak or negative (+, ± or -), the spinal fluid is examined in the usual manner, after concentrating the globulins.

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#### METHOD USED BY PROFESSOR E. MEINICKE.

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1. *Method:*

The M.T.R. (Meinicke Turbidity Reaction.)

2. *Principles:*

The organ extract employed is an alcoholic horse-heart extract highly diluted with alcohol, and to which balsam of tolu and benzoic acid are added. A dilution of this extract is prepared with saline. If this saline dilution is mixed with active sera, the liquid becomes turbid in the case of syphilitic sera, whereas with negative sera the liquid remains clear. Thus turbidity is the indicator in the method. In contradistinction to other flocculation reactions, active sera are used.

3. *Advantages:*

Great simplicity and speed. The sera are not inactivated. No incubator is required, as the reaction is carried out at room

temperature. No special optical instruments are needed for reading. This is done with the naked eye. The M.T.R. can easily be carried out in any small laboratory provided with a centrifuge.

4. The test is carried out at *room temperature*.

5. The results are read in *one hour* and again after a further hour. Thus the reading is completed in two hours.

6. Three test-tubes are required for testing one serum, *i.e.*, one for the control, one for the dilution of the strong extract, and one for the dilution of the weak extract.

7. In addition to the *pipettes* used for distributing the sera, the following are required: (1) one pipette for the original extract; (2) one graduated cylinder for the saline solution; (3) one pipette for distributing the prepared extract dilution, *i.e.*, three pipettes in all.

8. No *special apparatus* is needed for the test. Only a centrifuge is required for spinning the sera as in all serum reactions.

9. *Details of the technique:*

The technique for the Meinicke Turbidity Reaction (M.T.R.) is at present as follows:

*Preparation of the extract.* — The extracts, prepared as for the D.M. reaction, are diluted with 96% alcohol in the proportion of 1:14 approximately, and balsam of tolu and benzoic acid are added. Extracts must be protected from light and kept at room temperature. In the test proper, one strong and one weak extract are used simultaneously.

*Dilution of the extract.* — The necessary quantity of extract (1 volume) is poured into a test-tube. Ten times this volume of 3% saline containing crystallised soda 0.01 per cent is poured into a second tube. The two tubes are heated at 45° C. for five to ten minutes and their contents are then very rapidly mixed by first pouring the saline into the extract and then pouring the whole from one tube into the other, back and forth two or three times.

*Sera to be tested.* — The sera should be freed from all cellular elements, preferably by centrifuging. They are used in their *active* state and therefore must not be heated.

*Test.* — Of the freshly prepared extract which has been allowed to mature for five minutes, 1 c.c. is added to 0.2 c.c. of the serum to be tested. One drop of commercial formalin is added to the *control* tube. The tubes are left for an hour at the temperature of the well-warmed laboratory (about 20° C.).

*Reading results.* — Standing at a distance of about two or three metres from a well-lighted window, the worker looks at the window frame through the fluid. If the daylight is very poor, the reading is done by artificial light (19 candle-power). A suitable apparatus can be extemporised by means of a small box fitted with two electric bulbs. At the front end of the box an opening, 15 cm. high and 20 cm.

wide, is made and covered with a plate of ground glass, in the middle of which a large black cross is drawn. To read the results, the tubes are held at a distance of about 60 cm. from the box and the operator looks through them at the cross, which is seen more or less distinctly.

*Interpretation of results.* — When the serum is *negative*, the liquid is as transparent in the tube of the test proper as in the control tube. The cross is clearly outlined and black.

When the serum is *strongly positive*, the liquid in the tube of the test proper is *very turbid* and the cross is no longer visible.

A *weakly positive* reaction is recognised by a *slight turbidity* in comparison with the control tube: the cross, seen through the tube of the test proper, looks *indistinct with blurred outlines*. The difference between the aspect of the fluid in the tube of the test proper and in the control tube is always sufficient indication of the nature of the reaction.

In doubtful cases it is advisable, in addition, to carry out a Meinicke Micro-Reaction (M.M.R.). With this method the course of the reaction itself may be followed under the microscope, and impurities and infections of the sera, which may occasionally lead to false results in all syphilis reactions, are detected.

Untersteiner has published a useful method of applying the M.T.R. in cerebro-spinal fluid examinations.

Untersteiner mixes one part of M.T.R. extract with five parts of 1 % saline, after heating them to 45°; 0.5 c.c. of this mixture are added to 0.5 c.c. of active fluid and shaken. The test tubes are left at room temperature. The results are read in one hour by the hanging drop method as described for the Meinicke Micro-Reaction.

THE TECHNIQUE OF THE MEINICKE MICRO-REACTION (M.M.R.) is as follows:

*Extracts and dilution of extracts.* — The extracts are those used for the macro-reaction (M.T.R.), but containing rather less balsam. The dilutions are similarly prepared.

Active sera are used, very small quantities are required, and the blood can therefore be procured from the finger or the ear.

*Test.* — The extracts and sera are mixed by means of a *platinum loop* and not with pipettes as in the macro-reaction. A dilution of freshly prepared extract, matured for two minutes, is poured into a porcelain capsule. One drop is taken with a platinum loop 5.5 mm. in diameter. A drop of serum is then taken with another platinum loop, smaller than the preceding, *i.e.*, 2.5 mm. in diameter. The contents of the second platinum loop are then introduced into the lumen of the first loop, thus mixing the two liquids. A drop of the mixture is then taken with the smaller loop and deposited on a cover-slip, which is then covered with a concave slide. The preparation is left for 45 to 60 minutes at laboratory temperature (20° to 22° C.).

*Reading results.* — The preparations are read with a microscope fitted with a weak eye-piece and a strong dry objective — *e.g.*, LEITZ, eye-piece 1 or 2, objective 6 or 7. The various layers of the preparation must be carefully examined.

When the reaction is *negative*, *nothing* can be seen, or only *a number of moving specks* at the limit of visibility. *Strongly positive* reactions are characterised by the presence of *large clumps* which by their weight *sink to the bottom* and must be sought in the lower strata of the fluid.

When the reactions are *weakly positive*, larger or smaller clumps can be seen, of which the larger sink to the bottom of the preparation.

These *two reactions*, the M.T.R. and the M.M.R., are *extremely simple and very easy to read*. Any worker, however little practised in handling pipettes, can conduct these tests. He requires no special instruction for the purpose, but needs only a capacity of appreciating degrees of turbidity such as is possessed by every doctor familiar with testing the urine for albumin. For the micro-reaction, an ordinary knowledge of microscopic technique is required.

*Neither method requires the use of an incubator or a regulated water-bath for serum inactivation.*

The micro-reaction can even be conducted without a centrifuge: the blood corpuscles and fibrin are merely allowed to deposit, when the clear, supernatant serum can be used. Only a few glass vessels, the platinum loops, and a microscope are essential.

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## METHOD USED BY PROFESSOR R. MÜLLER.

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### 1. *Method:*

Müller Conglomeration Test (Müller Ballungs-Reaktion).

### 2. *Principles:*

Alcoholic ox-heart extract, to which a fairly high proportion of cholesterin has been added, is concentrated in the water-bath and then stored in this concentrated form (conglomeration reagent). For use it is warmed and diluted in two stages and in fixed proportions with 0.9% saline and then placed to mature for several hours in a thermostat at 56°C. With positive inactivated syphilis sera, this antigen causes the formation of a single freely suspended globular complex of white or yellowish-white gelatinous appearance, generally with a denser nucleus which is rather dark in colour and has a delicate nebular outer zone, whereas in negative cases the whole suspension has a perfectly clear colloidal appearance. In very rare cases of grave non-syphilitic disease of various kinds, crumb-like precipitates are formed which can readily be distinguished from conglomeration.

3. *Advantages:*

The method is sensitive to a very wide range of cases of syphilis, with marked specificity. Positive reactions are quite clearly defined. Lends itself to accurate quantitative work and can be used for testing the cerebro-spinal fluid.

4. *Temperature:*

37° C.

5. *Reading results:*

The first reading is taken after nine hours in the incubator. A second reading after a further fifteen hours at room temperature.

6. *Number of tubes required for one serum test:*

Three.

7. *Number of pipettes required:*

One dropper for delivering the various sera; one pipette for saline; one pipette for preparing the antigen to be used; and one for delivering the prepared antigen into the individual test-tubes.

8. *Other apparatus required:*

Test-tubes for the test proper, a small number of test-tubes for heating the concentrated reagent and maturing the diluted antigen; two beakers for diluting the antigen. An inactivating water-bath (56° C.); an incubator (37° C.); a thermostat (56° C.) for maturing the antigen, or, failing the thermostat, a metal box with closely fitting lid which can be immersed in the 56° C. water-bath and inside which the physical conditions of a dry thermostat obtain.

9. *Details of the technique:*

The principle recommended for the preparation of the Wassermann antigen (namely, concentrating cholesterinised ox-heart extract and maturing for several hours the colloidal antigen obtained after dilution with saline), has been adhered to in the preparation of antigen for the conglomeration test. As ox-heart muscle varies considerably in suitability, and as a great deal depends on very accurate adjustment of the extract with cholesterin, and as, moreover, it is difficult to obtain the exact degree of concentration in the laboratory, it is necessary to obtain selected and correctly cholesterinised, ready-concentrated antigens from a distributing centre ("Ballungsreagens" Schering-Kahlbaum, Berlin).

The preparation of colloidal antigen from the conglomeration reagent is described below, in detail.

*Directions for the preparation of the colloidal conglomeration antigen.*—

The bottle is vigorously shaken and 8 c.c. (or, if smaller quantities are required, 4 or 2 c.c.) of the reagent are poured into a moderately long test-tube, which is then well corked and heated for half-an-hour at 56° (water-bath). The heating causes any flocculated lipid particles to re-dissolve. The reagent is now diluted with an accurate



0.9% solution of sodium chloride (the figures given here apply to Kahlbaum's NaCl crystals). 5 c.c. of saline are poured into a small beaker (I) about 45 mm. in diameter, and 50 c.c. into a second beaker (II). If, in the case of certain antigens, a small modification in the degree of dilution should prove desirable, a slip bearing a notice to that effect would be enclosed in the package. The saline in both beakers must be raised to a temperature of 17° C. (accurately to within about half a degree). The mixing must be carried out as follows: the reagent, taken from the water-bath, is poured quickly and suddenly into beaker I, the test-tube is laid aside and beaker II seized with the same hand and its contents added equally quickly and suddenly to the first mixture. This method provides the simplest means of restricting the maturing period to about one and a-half seconds, which is the optimum time for the dispersion of the colloidal antigen. The colloidal solution obtained in this manner is poured into test-tubes 18-20 mm. in diameter, with walls  $\frac{3}{4}$  mm. thick, which, when well sealed with a rubber stopper, are placed, to further mature the solution, for twenty-four hours in a 56° C. incubator (*not* water-bath). The matured colloidal antigen, when kept for about two days at room temperature, scarcely loses any of its activity. If the mixture has been correctly prepared, the conglomeration antigen should form a uniform colloidal suspension, when matured.

8 c.c. of original extract is enough for the quantitative examination of about 40 cases. If the number of cases to be examined is smaller, the same process of dilution can be carried out with 4 or 2 c.c. of original extract. The quantities of saline are then correspondingly smaller, *i.e.*, 2.5 and 1.25 c.c. respectively in beaker I and 25 and 12.5 c.c. respectively in beaker II.

In order to obtain good conglomeration antigen, the instructions given here must be strictly adhered to, more especially as regards the use of absolutely pure distilled water, concentration and temperature of the saline, interval between the two stages of dilution, period of maturing, size and sealing of the test-tubes, temperature of the incubator, etc. All glass vessels must be scrupulously cleaned. (the final rinsing should be made in distilled water).

*Directions for carrying out the conglomeration test.* — The sera are inactivated for half-an-hour in a 56° C. water-bath. For the examination of one specimen, three carefully cleaned small test-tubes (Vidal tubes) of about 8 mm. internal diameter are required. Into these are delivered respectively 0.15 c.c. (3 drops), 0.2 c.c. (4 drops) and 0.25 c.c. (5 drops) of the inactivated serum (the volume of each drop must be exactly 0.05 c.c.<sup>1</sup>); 0.05 c.c. of the ripened antigen is then added to each of these tubes from the antigen tube, which must be shaken twice before opening, so that the contents may be uniform. The contents of the Vidal tubes are well mixed by shaking for a short

<sup>1</sup> If in an exceptional case owing to lack of serum, one dose has to be omitted, it is best to leave out the middle tube (4 drops). A fairly accurate quantitative reading can then still be obtained.

time. The tubes are then placed, uncorked, in the 37° C. incubator for from seven to nine hours, after which the first reading is taken. The reaction is continued for a further nine to fifteen hours at room temperature, after which the final reading is taken.

Cerebro-spinal fluid can be tested in the same manner, but with different doses. The fluid should also be inactivated for half an hour. The doses are as follows:

Fluid:	0.05 (1 drop)	0.1 (2 drops)	0.15 (3 drops)
Antigen :	0.3	0.3	0.3
Fluid:	0.3 (6 drops)	0.45 (9 drops)	0.9 (18 drops) <sup>1</sup>
Antigen:	0.3	0.3	0.3

*N.B.* — In the examination of spinal fluid the antigen must not be dropped, but measured from a pipette.

Period of incubation in thermostat and at room temperature, as for serum examination.

*Reading results.* — With correctly prepared antigen, a positive reaction produces a globular mass of white or yellowish-white gelatinous appearance, generally with a denser nucleus, which is rather dark in colour and has a delicate nebular outer zone. This mass is freely suspended in the centre of the column of liquid. If the preparation of the antigen has not been perfect the conglomeration will often appear opaque and crumbling. The results are best expressed in terms of intensity of reaction.

The strongest reaction (IV) is that in which conglomeration already occurs at incubator temperature with the smallest dose of serum; the weakest positive reaction (I) is that in which conglomeration does not set in until the mixture has stood at room temperature, and then only with the largest dose of serum.

Grade of Reaction	Results at :					
	7-9 hours at 37° C.			9-15 hours at room temperature.		
	0.15	0.20	0.25	0.15	0.20	0.25
I	—	—	—	—	—	+
I-II	—	—	—	—	+	+
II	—	—	—	+	+	+
II-III	—	—	C <sup>2</sup>	+	+	+
III	—	—	+	+	+	+
III-IV	—	+	+	+	+	+
IV	+	+	+	+	+	+

*Interpretation of Results.* — The M.B.R., like the B.-W. and all flocculation tests, may also, in exceptional cases of *Tbc. florida*, acute suppuration, etc., give "unspecific" positive results where no syphilis is present. Leaving out of account the rarity of such unspecific results (3 to 4 per cent in *Tbc. florida*), the quantitative method of the M.B.R. admits of differentiation, in so far as reactions which

<sup>1</sup> Instead of using 0.9 of fluid, the quantity of fluid and extract can, for the sake of economy, be proportionately reduced (0.6 : 0.2 or 0.3 : 0.1).

<sup>2</sup> Clouding.

show a positive result at the *early reading* (degrees of intensity IV, III and II-III) unquestionably indicate syphilis, whether the patient is also suffering from one of the above-mentioned diseases or not (except malaria and leprosy). On the other hand, the results of the *late reading*, especially degrees of intensity I and I-II — like a weakly positive B.-W. — must only be considered as indicative of the presence of syphilis if the patient is not simultaneously suffering from some *grave non-syphilitic disease*.

At all events, it would be advisable, before evaluating such a result, to repeat the test after a short lapse of time. The highest degree of intensity at the late reading (degree of intensity II) is not so *absolutely* certain an indication of syphilis as the results of the early reading, but a reaction as strong as this is so rare in the case of even the most serious non-syphilitic diseases that, in establishing a diagnosis, result II would, under any circumstances, have to be given due consideration. Therefore, in the absence of any grave non-syphilitic disease, the presence of syphilis is indicated with complete certainty by result II and with the *highest degree of probability* by the weakest result (I). The latter result is frequently obtained in cases of *syphilis latens*, and also tabes and sclerosis, when all other reactions are negative.

*Unspecific Reactions.* — In addition to the very rare unspecific conglomeration of the lowest degree of intensity (I) already mentioned, cases of disease in which the serum is said to be in a labile condition, may quite exceptionally show precipitates or larger clumps; these appearances, however, are so distinctive that they cannot be mistaken for positive clotting reactions.

It is characteristic of unspecific reactions of this kind that the small dose of serum (especially 3 drops) often reacts more strongly than the larger doses.

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#### METHOD USED BY DR. M. NAGAYO AND DR. NOBECHI.

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##### THE MURATA METHOD, A MODIFICATION OF THE SACHS-GEORGI REACTION.

1. *Method used:*  
Murata method.
2. *Principles:*  
Formation of a precipitation layer, when the antigen solution is superposed upon the patient serum.
3. *Advantages:*
  - (a) The simplest method of all.
  - (b) Consumption of least time for test.
  - (c) Smallest quantity of serum required for the test.
  - (d) Specificity of this test is demonstrated by the curves observed with sera of leprosy or cancer patients.

4. *Temperature :*

37° C.

5. *Reading results :*

After forty-five minutes with clear sera, and after three to four hours with chylous sera (turbid).

6. *Number of tubes required :*

One tube per serum.

7. *Number of pipettes required :*

(a) One pipette of 1 c.c. for antigen.

(b) One capillary pipette for the delivery of sera, and another for the distribution of the antigen solution.

8. *Other apparatus required :*

(a) Two tubes of about 2.5 cm. diameter for the dilution of antigen.

(b) One graduated cylinder for the measurement of 10 c.c. saline solution to make the antigen dilution.

(c) Test-tube racks.

(d) A blackboard to facilitate reading of test.

9. *Other details of the method :*

Murata's method is a modification of the Sachs-Georgi reaction. It consists in a ring test with the cholesterinised crude ox-heart extract. The specific features of the test are as follows:

As well known and especially well elucidated by Neukirsch's thorough studies, the cholesterinised extract suffers from the effect of reaction temperature. If, however, the cholesterin content is reduced to a certain degree in proportion to the amount of extract, a phase is reached when the temperature effect is insignificant and yet the reaction is sufficiently intensified by the cholesterin fraction. Using this phase, Murata made the antigen sufficient for the reading of the reaction after forty-five minutes at room temperature. Murata's test is thus done without the use of an incubator.

The antigen dilution to be used as the reagent in the Murata test appears water-clear in the slender reaction test-tubes, whilst it facilitates the recognition of the ring formation in positive cases. Murata's test owes this advantageous feature chiefly to the low content of cholesterin in the antigen in proportion to the amount of extract.

The most important feature of the Murata test is its specificity to syphilis, which is based upon experimental evidence. After five years' studies on the serodiagnosis of syphilis, especially on the positive reactions with non-syphilitic cases, Murata and Tamiya, in our institute, came to the conclusion that the variation in the proportion of cholesterin to extract not only changes the sensitiveness of the antigen, but also alters its specificity.

The ring tests are based upon the result of the whole range of the gradually varying phases of mixtures of the serum with antigen.

Therefore, the contact precipitation ring formation cannot be missed in the case of a positive serum, no matter what is the optimal phase of mixture. The ring test overcomes the difficulty of zone phenomena.

The interpretation of the results of the ring test is usually much easier than that of flocculation tests. Furthermore, the performance of the test is the simplest and the most economical of time, as arbitrary amounts of serum and antigen are applied for individual tests, and such steps as those of the measurement of the reagents for individual tests or the dilution of each serum are saved. As it is a highly sensitive reaction, the results can be read within a short duration of time with the thin dilution of antigens. In the case of the flocculation tests, one has to use a thick antigen dilution such as Kahn's if the reaction time has to be shortened. Nobechi found that Kahn's antigen diluted 100 times is just sufficient for the Murata test, as far as sensitiveness is concerned.

The disadvantage of the ring test is the occurrence of the false ring, which sometimes makes the reading of the reaction difficult. The false ring, however, is mostly formed in cases with hæmolysed or chylous sera; and, if the precautions, described later in the technique concerning the sera, are observed, it will be easily avoided.

The established technique of the Murata method contains special features in the technique of the precipitation test. The detailed method is given below.

#### TECHNIQUE.

##### I. Apparatus required.

1. Pipette of 1 c.c. capacity . . . to measure the antigen.
2. Capillary pipette. . . . . to distribute the antigen dilution and the sera.
3. Mouthpiece with rubber tubing or rubber cap . . . to operate the pipettes.
4. Two test-tubes of *circa* 2.5 cm. diameter . . . . . one to contain the antigen and the other the saline solution, for the preparation of the antigen dilution.
5. Test-tubes of *circa* 0.5 cm. diameter . . . . . to carry out the tests therein.
6. Test-tube rack.
7. Small blackboard . . . . . to facilitate the reading of the result, it is held behind the test-tube rack against the window.

##### II. The Sera.

The sera are to be inactivated by placing them for thirty minutes in a water-bath at 55° C. Before the application of the test, they must be kept standing at room temperature at least thirty minutes after the completion of inactivation. The same precaution must be observed when the tubes have been kept in the ice-chest after inactivation.

Chylous and hæmolysera sometimes make the reading of the results difficult. It is therefore advocated that the blood be taken before a meal, preferably before breakfast. Sera in which spontaneous clotting occurs are to be centrifuged or kept standing before inactivation, in order to remove blood corpuscles.

### III. *The Antigen.*

(1) *Preparation of the antigen.* — The fat-free part of ox-heart muscle is stored in the ice-room overnight, and is then finely mashed by passing it through a mincing machine several times, the exuding fluid being discarded. 500 c.c. alcohol (*circa* 96 per cent) is added to 100 gr. of mashed muscle. The resulting suspension of mashed muscle is allowed to stand at room temperature for a week, and is occasionally shaken. It is afterwards put in the ice-chamber and, after being stored there for two days, it is filtered cold. To the filtrate, two parts of alcohol are added. The diluted extract is filtered, and stored at room temperature for a few months and filtered again. Thus the final extract is made up, ready for cholesterinisation.

The 1% alcoholic solution of cholesterin is made from the preparation of Merck, Darmstadt. This is added to the extract in the proportion indicated by the result of titration for sensitiveness.

(2) *Standardisation of the antigen.* — The cholesterin will precipitate if it be diluted with the saline solution alone, whereas the addition of a certain amount of extract does not cause the precipitation of the cholesterin in the dilution with the saline solution. The more extract is added the greater stability of the cholesterin solution results. The extract therefore acts as a protective colloid upon the cholesterin.

A definite amount of the cholesterin solution is put in several test-tubes, and to these varying amounts of the extracts are added; 1 c.c. of each mixture is placed in a test-tube of *circa* 2.5 cm. in diameter and is diluted with 10 c.c. of the saline solution from a test-tube of the same diameter. The dilution is carried out with as rapid a movement as possible and in a definite way. After ten minutes' interval, the test is performed with each of the dilutions, using both normal and syphilitic sera. After observation of the results at the end of forty minutes at room temperature, the mixture with the requisite sensitiveness is fixed upon as the standard antigen. In the case of Murata's own standard antigen, the border-line between normal and syphilitic is fixed to coincide with that of the Taniguchi's Wassermann test, *i.e.*, the Wa — sera with the named test will not cause precipitation rings but the  $\pm$  sera will form faint rings.

To titrate the new antigens, the mixtures of the cholesterin solution with varying amounts of new extract are prepared and diluted with saline solution as described above. A positive serum, preferably with a clinical diagnosis of syphilis, is taken and diluted with saline solution five times, ten times, fifteen times, etc. Applying these dilutions of the serum, the sensitiveness of the mixtures of the cholesterin solution with the new extract as antigen is compared

with that of the standard antigen. The mixture showing exactly the same sensitiveness is taken as suitable for the antigen. One part of cholesterol solution to 17.5, 18, 18.5, or 19 parts of extract is usually found to be the correct proportion of the mixture in the preparation of the standardised antigen. If two mixtures with different proportions are equally sensitive, the one which contains the greater proportion of extract is selected in order to be as much farther away as possible from the phase to cause the non-specific reactions. If a certain mixture is found a little too sensitive, slight reduction of the extract (the protective colloid) in this method will be sufficient to adjust its sensitiveness, and *vice versa*. By this means the delicate adjustment of the sensitiveness of the antigen is done with ease.

1 c.c. of the antigen is measured into a test-tube of *circa* 2.5 cm. diameter, and 10 c.c. of the physiological saline into a second tube of the same diameter. The latter is added to the former with as rapid a movement as possible and the mixture is returned to the second tube. The antigen dilution thus prepared is ready for use in the test after standing ten minutes, and may be used within an hour after preparation. The "made-up" antigen dilution has a faint opalescence, but in the slender test-tubes it appears quite clear. Should it appear opaque, it is not suitable for use. Unclean glass-ware is in most instances responsible for abnormal cloudiness.

#### IV. *The Test.*

Into a test-tube of *circa* 0.5 cm. diameter, a quantity of inactivated serum is introduced, so as to reach the height of *circa* 1.5 cm. The antigen dilution is then superposed upon the serum layer. For the purpose of obtaining the clear-cut contact surface between the two media, the following precautions are taken. *At the end of distribution of serum*, the inner wall of the test-tube over the serum layer must be moistened by a circular motion of the tip of the capillary pipette, and then the latter is withdrawn along one side of the test-tube so as to leave a wet line. The last drop of the serum is let run down along the wet line to open the path for the antigen. The delivery of the antigen is begun with a drop of antigen slowly running down along the wet line described above. After ascertaining that the first drop of antigen has reached the upper surface of the serum layer, a few more drops are added with the same precaution, and then five to ten more drops in succession. After distributing the antigen, it should be noted that the contact surface in each tube is sharp. As far as our experience goes, this drop method is the best and easiest one for successful ring tests and the mode in which the first drop of the antigen is introduced is the key to success.

It is not necessary to change the capillary pipette for each serum; but, after one serum is distributed, the tip of the pipette is wiped with a piece of gauze, the inside of it is rinsed out with saline solution a few times and the adhering trace of saline solution wiped off with another piece of gauze, and the pipette is ready for use for the second serum.

### V. *Reading the Result.*

The results are read at the end of forty-five minutes' standing at room temperature. If a white ring appears at the contact surface the reaction is positive, and if this is not the case the reaction is regarded as negative. According to the intensities of the white ring formation, the reactions are recorded as +++, ++, + and ±. With some sera the false ring is formed, which frequently interferes with the reading. The true ring as well as the false one appears on the contact surface; but, whilst the former is caused in the antigen layer and its lower-limit line is sharp, the latter is formed in the serum layer and its upper-limit line is clear-cut. In doubtful cases, at the second reading after two to four hours, the true ring will increase its density and width, whereas the false will remain as it was or even become less marked.

Reading must be done sitting at a light window without shade, with the blackboard held behind the test-tube rack. Controlling the light by lifting or lowering the blackboard helps sometimes in the recognition of a weak reaction. The observer's eyes must be on the same level as the contact surface.

No control system is required, as the antigen is very accurately standardised.

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## METHODS OF PROFESSOR H. SACHS AND DR. E. WITEBSKY.

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### A. SACHS-GEORGI REACTION (LENTOCHOL).

1. *Method employed:*  
Lentochol reaction.
2. *Principle:*  
Direct flocculation of cholesterinised extract.
3. *Advantages:*  
Simple flocculation and a high degree of specificity for syphilis.
4. *Temperature:*  
37° C.
5. *Reading results:*  
Eighteen to twenty-four hours.
6. *Number of test-tubes required for one serum test:*  
Two to three test-tubes (naturally, for quantitative purposes more are required).
7. *Number of pipettes required:*  
One pipette for each serum, one for saline, and one or two pipettes for extract dilutions.
8. *Apparatus required:*  
An agglutinoscope for reading results is useful though not absolutely necessary.



9. *Other details of technique:*

I. *Extracts.* — Cholesterinised organ extracts are used for the flocculation reaction. The choice of the organ and of the method of extraction are of secondary importance, but the extracts must be sufficiently sensitive and, as far as possible, specific for syphilis. These requirements can only be fulfilled after extensive empirical tests have been carried out with each of the extracts.

Professor Sachs supplies suitable cholesterinised extracts (Institute for the Experimental Study of Cancer, Heidelberg). These extracts are made from ox-heart to which cholesterin has been added.

They are diluted immediately before use with five times their volume of physiological saline. This dilution is made in two stages. One part of cholesterinised ox-heart extract is quickly mixed with one part of saline. The mixture is shaken for an interval which varies from ten to twenty seconds, after which the four further parts of saline are quickly added. It is extremely important that the instructions with regard to the interval between the first and second additions of the saline should be strictly followed. These instructions are specially given for each individual extract.

II. *Patient's serum.* — Patient's serum is heated, immediately after the blood has been taken, for half-an-hour in the water-bath (inactivation at 55° C.). As with all methods of serological diagnosis of syphilis, the serum should, as far as possible, be free from pigment and should be tested as fresh as possible.

III. *Saline.* — A solution is prepared of chemically pure 0.85 % sodium-chloride in *distilled* water. The solution must be clear. If it contains floating particles of cotton wool or other substances, it must be filtered through ordinary filter paper.

IV. *Technique.* — The flocculation reaction is carried out in tubes 100 mm. in length and 15-16 mm. internal diameter. The walls of the tube must not be thick, for thickness would render the reading of the results difficult or even impossible.

Each serum is tested with two extracts. As a control, each serum is mixed with a corresponding quantity of alcohol at 96 per cent. The serum controls are actually put up as follows: 0.5 c.c. of a 1-in-5 dilution of serum mixed with 0.25 of a 1-in-6 dilution of 96 per cent alcohol in saline. Three tubes are therefore required for each serum, preferably placed one behind the other in the rack. The various patients' sera are placed one beside the other. In this way three parallel rows are obtained:

- A. The first principal test with Extract A.
- B. The second principal test with Extract B.
- C. The control with alcohol.

Inactivated patient's serum is diluted 1 in 5. The figures given below refer to tests made with 0.5 c.c. of a dilution of serum 1 in 5 and with 0.25 of extract diluted 1 in 6. These quantities may be doubled.

When working with 0.5 c.c. of a dilution of serum 1 in 5 there are two alternatives:

- (a) Either 1.2 c.c. of physiological saline is placed in the tubes in row C, and a 1-in-5 dilution is obtained by adding 0.3 c.c. of inactivated patient's serum, 0.5 c.c. of the solution thus obtained being then delivered to each tube in rows A and B;
- (b) Or 0.1 c.c. of inactivated patient's serum is placed in each tube in rows A, B and C, and a 1-in-5 dilution is obtained by adding 0.4 c.c. of saline.

In either case, at the ends of rows A and B there must be one tube containing only 0.5 c.c. of saline (extract control).

After setting out the sera in this way and entering the particulars in the records, the extracts are added. For this purpose 0.25 c.c. of extract dilution prepared in the manner indicated above is added to each tube in rows A and B immediately after preparation.

0.25 c.c. of a 1-in-6 dilution of alcohol (96 per cent) with saline is added to each tube in row C (serum control).

The tubes are now shaken one by one, and then all together in the rack, which is placed in the incubator. This must be kept at a constant temperature of 37° to 37.5° C.

V. *Reading results.* — Results are read:

- (a) After four to five hours in the incubator;
- (b) And after eighteen to twenty-four hours in the incubator

The second reading is decisive irrespective of the earlier reading. In the majority of cases the result can be gauged at the earlier reading. Nevertheless, the second reading cannot be dispensed with on account of the rare cases in which the reaction is delayed or in which labile sera may produce a reversible flocculation which will have disappeared by the time the second reading is taken.

After shaking the tubes for a few seconds, the reading is taken with a Kuhn and Woithe agglutinoscope. These agglutinoscopes can be obtained from Paul Altmann, Luisenstr., Berlin, N.W., and from F. & M. Lautenschläger, Luisenstr., Berlin, N.W. The agglutinoscope must be adjustable to suit the observer, *i.e.*, it must be possible to alter the distance between the lens and the tube so that the flocculi are perfectly visible. It is easy to see whether the apparatus is serviceable by taking the reading with the naked eye in doubtful cases. Where there is even a slight reaction it is possible to see the flocculi with the naked eye; the observation can then be made much more accurately with the help of the agglutinoscope.

Specific flocculi can be distinguished by the fact that they are regularly distributed over the whole field of vision. They may range in size from very small granules to large masses. Irregular, doubtful-looking granules should not be interpreted as a positive flocculation. Obviously, a result must not be considered positive if flocculation is present in row C (serum control) and in the end tubes in rows A or B (extract control). If flocculation is present in the tubes at the

end of rows A and B, the inference is that the extract or the extract dilution is unsuitable for use in the test. If flocculation is present in a serum control tube (row C), the result must be rejected on account of auto-flocculation. In such a case, an attempt must be made to clarify the serum by means of centrifuging or filtration, or a fresh blood specimen must be taken from the patient.

The result is recorded in the usual approximate manner: a positive reaction being indicated by a larger or smaller number of “+” signs according to the degree of flocculation, a doubtful reaction being indicated by “±” and a negative reaction by “—”.

VI. *Advantages of the flocculation reaction.* — The advantages of the flocculation reaction when correctly carried out are, according to personal experience, the following: The reading of the results is easy, sensitiveness is sufficient, and the danger of a non-specific lability reaction is reduced to a minimum as compared with other serological tests for syphilis.

VII. *Sources of errors.* — To avoid errors it is necessary:

- (a) To use cholesterinised extracts of irreproachable quality and to adhere to the prescribed interval between the two dilutions;
- (b) To use an absolutely pure saline solution of 0.85% (purest sodium chloride, distilled water);
- (c) To keep the incubator temperature constant;
- (d) To make sure that the agglutinoscope is serviceable (suitable distance between the lens and the tube).

VIII. *The quantitative flocculation test.* — The flocculation test may equally well be carried out quantitatively with decreasing amounts of serum on the same lines as those set out above. The total volume must, of course, be the same in all the tubes. By a quantitative test on these lines, it is undoubtedly possible to determine up to a certain degree the reacting power of a serum. But it is doubtful whether a quantitative test — always somewhat complicated — can enhance the practical value of serological tests. It is true that in rare cases a positive reaction may be adduced which has been masked by inhibition through excess (where the quantity of serum generally used was too large). The quantitative method entails no modification of the technique; it merely takes advantage of possibilities inherent in the process.

## B. CITOCHOL REACTION.

1. *Method:*  
Citochol Reaction.
2. *Principles:*  
Rapid flocculation of concentrated cholesterinised extracts.
3. *Advantages:*  
Simple technique, rapid results (without special apparatus), comparatively high specificity, greater sensitiveness than with the Lentochole Reaction.

4. *Temperature:*  
Room temperature.
5. *Reading results:*  
After half an hour.
6. *Number of test-tubes required:*  
Two to three (according as one or two extracts are used).
7. *Number of pipettes required:*  
One pipette for every serum. Also one pipette for saline, and one or two for extract dilutions.
8. *Other apparatus:*  
None (possibly an agglutinoscope).
9. *Further particulars:*

Alcoholic benzoin solutions have proved comparatively unstable, and consequently must be prepared at frequent intervals; therefore, the principle of plain cholesterinised ox-heart extract first laid down by Sachs and Georgi has been reverted to in more recent experiments, in which some of the measures adopted in Kahn's carefully modified method have been taken into consideration. This method secures rapid flocculation. The interaction of a low dilution of concentrated cholesterinised extract with serum in strong concentration plays an important part in the production of a quick result. As in Bruck's method, a suspension of precipitated particles of extract is used instead of extract emulsion.

We tried concentrating our cholesterinised extracts for the Sachs-Georgi Reactions by steaming and using them in this highly concentrated form, with the result that *rapid flocculation could actually be obtained.*

The next step was to evaporate alcoholic ox-heart extract prepared according to the old method (*i.e.*, by the extraction of one part by weight of the moist substance with five parts by volume of alcohol), and to re-dissolve the triple concentration in alcohol. To these concentrated extracts cholesterin is added so as to obtain a cholesterin content of 0.3 to 0.6 per cent according to the nature of each individual extract. These cholesterinised ox-heart extracts are called *Citochol* extracts (citocholesterin), because they are used in the rapid reaction which will be described below, and we call the resulting method of flocculation the "*Citochol Reaction*" in contradistinction to the Sachs-Georgi Reaction, which, because of its slow reaction time, we call the "*Lentochol Reaction*".

The Citochol Reaction is carried out as follows:

1. The Citochol extracts are quickly diluted by mixing one part of extract with two parts of 0.85 % saline. The 1-in-3 dilution prepared in this way is left standing at room temperature for ten minutes before use.

2. For carrying out the Citochol Reaction, 0.1 c.c. of serum inactivated for thirty minutes at 55° C. are mixed with 0.05 c.c. of the

diluted extract. The mixture is then left standing at room temperature for half an hour, after which 0.5 c.c. of 0.85% saline are added.<sup>1</sup>

Thirty seconds' shaking may be substituted for the half-hour standing at room temperature.

3. The reading follows immediately, either macroscopically or in an agglutinoscope (possibly also as a turbidity reaction).

4. The Citochol Reaction can be carried out simply as a micro-reaction. For this purpose one to two parts of inactivated sera are mixed with one part of extract dilution on a slide or in a watch-glass or capsule and rapidly and evenly mixed. The result can be read on the slide after the lapse of a few minutes like a bacterial agglutination or a blood-group determination.

So far, results obtained by this procedure have been favourable, but there is the same drawback as in all rapid reactions, viz., under given circumstances unspecific results are obtained more frequently, though there may be increased sensitiveness.

This differential factor ought to be taken into account in the sero-diagnosis of syphilis by the flocculation of cholesterinised extracts; the Lentocholesterol Reaction possesses a very high degree of specificity, but a lesser degree of sensitiveness. The Citochol Reaction is more sensitive, but does not perhaps possess an equally high degree of specificity.

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#### METHOD USED BY DR. K. NOREL.

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##### THE SIGMA ( $\Sigma$ ) REACTION.

The Sigma Reaction has the following advantages:

- (1) Simple technique;
- (2) The results are expressed in Sigma units so that different tests are comparable;
- (3) When using standardised antigen and a standard flocculation test the reaction can be reproduced by different workers at different times in different places.

The original technique of Professor Dreyer and Dr. Ward at Oxford (1921) has been modified and simplified in the Danish State Serum Institute in Copenhagen; it is given in detail below.

With this modification, over 40,000 sera have been tested in Copenhagen, the Wassermann Reaction being performed simultaneously. It was found that the Sigma Reaction was equal to the Wassermann test in sensitiveness and the decrease in strength of the Sigma Reaction followed in a satisfactory manner the clinical picture during treatment.

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<sup>1</sup> It is advisable to set up as controls parallel rows in which the extract dilution is replaced by a corresponding dilution of alcohol. Formalin controls are also useful.

A. *Reagents and Apparatus required.*

1. Antigen (alcoholic extract of calf's heart).
2. Alcoholic solution of cholesterin, 1 per cent.
3. Normal saline solution.
4. Stands with one row of holes.
5. Agglutination dwarf test-tubes, internal diameter 5.5 to 6.2 mm., external diameter 6.6 to 7.2 mm., length 58 mm., conical point, broad collar.
6. Graduated measuring cylinders: (a) to contain 25 c.c., height 11 cm., inside diameter 2.2 cm.; (b) to contain 100 c.c., height 16 cm., inside diameter 3.5 cm.
7. Bigger's dropping apparatus.
8. 0.5 and 1 c.c. pipettes, graduated in hundredths of 1 c.c.
9. A hand-lens magnifying six times.
10. A special artificial light apparatus (Sigma agglutinoscope).
11. A water-bath (54° C.) for inactivation of the sera.
12. A dry-air incubator (38.5° C.) for the incubation.
13. Rubber-stoppered tubes for collecting blood samples.

B. *Technique (Elaborated by Norel, The State Serum Institute, Copenhagen).*

The blood samples must be drawn aseptically; 2 to 3 c.c. blood (= 1 c.c. serum) is required. The serum is pipetted into sterile dwarf test-tubes plugged with cotton-wool (not with a rubber or cork stopper). The serum is heated for 90 minutes in the water-bath (54° C.) The inactivated serum is pipetted into seven to ten of the conical test-tubes according to the following table:

Tube No	1	2	3	4	5	6	7	8	9	10
Pure serum.....	0.4	0.2	0.1	0.05						
Serum dilution 1:10.....					0.2	0.1	0.05			
Serum dilution 1:100.....								0.2	0.1	0.05
Normal saline....			0.1	0.15		0.1	0.15		0.1	0.15
α-Suspension ....	0.1									
β-Suspension . . .		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
(Degree of dilution)	1:1 <sup>1</sup> / <sub>4</sub>	1:2 <sup>1</sup> / <sub>2</sub>	1:5	1:10	1:25	1:50	1:100	1:250	1:500	1:1000

The two serum dilutions are prepared in two dwarf test-tubes, containing 0.9 c.c. saline; into the first tube is pipetted 0.1 c.c. serum; and into the second 0.1 c.c. of this 1-in-10 dilution.

The *antigen* is employed in two different dilutions:  $\alpha$ -suspension and  $\beta$ -suspension. The suspensions must be prepared immediately or only about half an hour before the tests.

2 c.c. antigen is mixed with 0.2 c.c. cholesterin solution; this cholesterinised antigen is emulsified with saline by the help of Bigger's dropping apparatus in the following way: 1 c.c. cholesterinised antigen is poured into a measuring cylinder placed under the dropping tube (the tube is made of a piece of capillary tubing, 6 mm. external diameter, 0.5 mm. internal diameter); the height from the bottom of the cylinder to the point of the dropping tube is 36 cm.; the drops have a size of about 0.1 c.c. each; 34 c.c. must drop off in the course of nine minutes. The two antigen dilutions are:

$\alpha$ -suspension: Into 1 c.c. cholesterinised antigen is dropped  $10^{2/3}$  c.c. saline;

$\beta$ -suspension: Into 1 c.c. cholesterinised antigen is dropped 34 c.c. saline.

The antigens are prepared only in the quantities mentioned. If, for instance, 70 c.c.  $\beta$ -antigen is required, two lots, each of 35 c.c., are prepared.

The resulting suspensions should not be shaken, but are mixed by gently inverting the measuring cylinders.

The  $\alpha$ - and  $\beta$ -suspensions are added to the serum dilutions according to the table, the  $\alpha$ -suspension by a pipette, the  $\beta$ -suspension preferably by a Dreyer syringe.

Each sample must now be shaken thoroughly, covering the tube with the thumb, in order from right to left—*i.e.*, beginning with the tube containing the *highest* serum dilution. Dry the thumb on a cloth before passing from one tube to the next.

Place the stand in the dry-air incubator (38.5° C.) for twenty to twenty-two hours.

### C. Reading of Results.

Place the stand on the agglutinoscope and read by help of the hand-lens. Compare the flocculation in the tubes with the Standard Flocculation Tube. Take the tube showing this degree of flocculation and find the corresponding strength in Sigma units, indicated on the label of the antigen bottle.

The figures given there have been checked with Professor Dreyer's Standard. If none of the tubes are showing exactly the standard flocculation, the value must be interpolated. In view of the possible presence of an inhibition zone, it is essential to examine at least the first five tubes in the series before pronouncing a serum negative.

Regarding the details in relation to standardisation, see Medical Research Council Report, No. 78.

### Literature.

Dreyer and Ward: *The Lancet*, Vol. I, 1921, p. 956.

„ „ *Medical Research Council Report*, No. 78.

League of Nations: Health Organisation's Investigations of Syphilis,  
1924, document C.5.M.5.

Mörch: *The Lancet*, Vol. II, 1924, p. 58.

Bigger: *The Lancet*, Vol. II, 1924, p. 743.

Norel: *Zeitschr. f. Imm. & Exp. Therapie*, 1926, p. 475.

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### VERNES' REACTION.

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1. *Method:*

The syphilimetric method.

2. *Principles:*

To produce flocculation in the blood serum and cerebro-spinal fluid by means of a chosen reagent and to carry out the investigations under such close co-operation between clinic and laboratory (instanced, up to the present, by more than 100,000 records at the Institut Prophylactique) that the presence or absence of flocculation indicates, not only the presence or absence of syphilis, but also, in the former case, the rises and falls occurring in the course of the infection.

3. *Advantages:*

(a) The elimination of the personal element, so that different workers testing the same sample in separate laboratories necessarily obtain the same results.

(b) *The measurement* — by means of an optical apparatus, the V.B.Y. photometer — of the variations produced by the fluctuations of the infection; the registration of these variations on a chart according to definite rules, and the use of the graphs in the conduct of treatment as a means of ensuring the successful prevention of syphilis, which consists in rendering the patient non-infectious not only for a time but for the remainder of his life. This is to his personal interest and is the only way of preventing hereditary syphilis.

4. *Temperature:*

Preliminary heating of the sera at 55° C.; performance of the test at room temperature of 19° to 21° C.; flocculation in the water-bath at 25° C.

5. *Reading of results:*

After four hours.

6. *Test-tubes used for each serum:*

Four.

7. *Number of pipettes per test:*

Nil.

8. *Other apparatus required:*

A rheometer; a mixing apparatus with propeller to regulate the size of the particles in the suspension, and a V.B.Y. photometer.



9. The technique employed at the Danish State Serum Institute for the examination of sera and cerebro-spinal fluids is that described in Part II of the Publications of the Paris Prophylactic Institute, pages 59 to 64 and 67 and 68.

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Appendix.

METHODS DESCRIBED AT THE CONFERENCE BY  
DR. A. SCALTRITTI

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FLOCCULATION WITH THE USE OF OX OR PIG HEART LIPOIDS  
PRECIPITATED BY CADMIUM CHLORIDE.

*Procedure for obtaining the Lipoid.*

The cardiac muscle of fresh ox or pig heart, freed from fat, is finely minced, and spread in thin layers on glass plates, which are placed in front of a revolving fan and dried as quickly as possible. When drying is complete, the muscle is pounded in a mortar and passed through a very fine sieve in order to obtain an impalpable powder.

The powder is treated with sulphuric ether for about three days, the ether being changed repeatedly until it remains colourless. This operation is carried out at room temperature.

The ether is now decanted and the powdered heart placed in the incubator at 37° C. for two hours, *i.e.*, long enough to dispel any remaining ether.

The dry powder, free of sulphuric ether, is then extracted in absolute alcohol in the proportion of one part of powder to six of alcohol for three days at room temperature, the mixture being shaken several times a day. At the end of that time, it is filtered through a double filter-paper. A transparent or very faintly yellowish liquid is thus obtained. It is from this solution that we precipitate the lipoids which, we believe, are required for the flocculation of the syphilitic sera. It is prepared once a week.

*Method of Preparation.*

As much of a super-saturated solution of cadmium chloride in absolute alcohol is prepared beforehand as will be needed for precipitation. As a rule, the proportion is 0.1 grammes of  $\text{Cl}_2\text{Cd}$  to every c.c. of absolute alcohol. The cadmium chloride is finely powdered in a glass mortar. It is then mixed with the alcohol and dissolved as completely as possible. The solution is placed in a small tube and spun for two to three minutes, until the alcohol becomes clear. It is then ready for use. 0.25 c.c. of this solution is placed in a small tube of the kind commonly used for Wassermann reactions. 2 c.c. of the alcoholic solution mentioned above is measured into another

tube. This solution is quickly poured on the first solution of  $\text{Cl}_2\text{Cd}$  and *vice versa*; the operation is repeated twice in order to mix the cadmium chloride as completely as possible with the lipid solution. In this way a caseous precipitate is obtained. It is allowed to settle for half-an-hour and is then gently "centrifuged", allowing the centrifuge to make only a few revolutions.

The supernatant liquid is now carefully decanted and the precipitate thoroughly mixed with the small quantity of alcohol remaining at the bottom of the tube. The tube is then filled with 80% alcohol, the precipitate becoming altered in appearance, and more nearly resembling snow. The tube is gently shaken to break up any lumps and is then set aside to allow the lipid to settle. The alcohol is decanted and replaced by fresh alcohol, which is again decanted after being left to stand for a few moments. The tube is then half-filled with 80% alcohol, and filled up with  $8\frac{1}{2}/1000$  to  $9/1000$  saline, care being taken not to shake the tube. If the lipid should rise from the bottom of the tube, a slight centrifugal movement will make it settle again to the bottom.

Finally, the saline solution is decanted, leaving the lipid almost free from cadmium chloride.

1 c.c. of physiological saline  $8\frac{1}{2}/1000$  or  $9/1000$  is then added and the mixture is emulsified by briskly shaking the tube, which must be well stoppered. To obtain a good emulsion ten minutes' shaking is needed. The emulsion is now ready. As many tubes as are required are prepared, remembering that for each reaction tube 0.05 c.c. is needed.

#### *Technique of the Reaction.*

Patient's serum inactivated at  $56^\circ\text{C}$ . for twenty to thirty minutes: 0.1 c.c.

Lipoid emulsion: 0.05 c.c.

The tubes are shaken briskly, preferably in a mechanical shaker, for a quarter of an hour.

By that time flocculi begin to appear in the case of strongly *positive* sera. In any case the tubes must be left at room temperature for eighteen to twenty hours, to allow time for the reaction to take place with weaker positive sera. If the test tubes are then examined with a lens in a suitable light, one can distinguish the tubes in which there is flocculation from those in which it is absent. This inspection should occupy but little time.

1 c.c. of  $8\frac{1}{2}-9/1000$  saline is then added to each tube, which is gently shaken to obtain a good mixture.

Flocculation should now be more evident than at the first examination. The tubes are then briskly "centrifuged" for one or two minutes. The supernatant fluid is decanted, care being taken to prevent any lipid passing over with it. Another c.c. of saline is added and the same operation is repeated.

Finally, 0.5 c.c. of saline is added, and the tube is gently rocked to and fro. It will then be seen that the flocculated lipid no longer

forms an emulsion with the saline. There are degrees in this reaction from a thick, compact floccular mass to small loosened flakes floating in a transparent medium.

The non-flocculated lipoids readily form emulsions and become homogeneous. Reactions are classified as strongly, medium, or weakly positive, according to the intensity of flocculation.

In every case, a control tube containing 0.1 c.c. of saline and 0.05 c.c. of lipoid emulsion is put up and subjected to the same treatment as the tubes of the test proper.

#### PROCEDURE TO OBTAIN THE LIPOID USED IN THE BORDET-WASSERMANN REACTION.

Ox or pig heart (preferably the latter) is finely minced and dried with a revolving fan. It is then pounded and passed through a sieve. The powder is then treated with acetone and dried in an incubator at 37° C. for three or four hours until the acetone has evaporated.

The powder is soaked in absolute alcohol for three days at room temperature in the proportion of one part of powder to ten of alcohol. It is filtered through a double filter-paper. The result is an amber-coloured liquid from which the lipoids are precipitated by an alcoholic solution of  $\text{Cl}_2\text{Cd}$ .

(The lipoid solution is prepared every four days.)

(For the preparation of the supersaturated alcoholic solution of  $\text{Cl}_2\text{Cd}$ , see the flocculation technique described above.)

1 ½ c.c. of lipoid solution is poured quickly upon 0.25 c.c. of cadmium chloride solution and *vice versa*. The precipitate is allowed to settle. This is then washed in the same manner as the lipoid in the flocculation reaction previously described.

The lipoid having been obtained practically free from cadmium chloride, it is emulsified with 2 c.c. of saline by brisk shaking in a well-stoppered tube.

The bulk of fluid is brought up to 20 c.c. with saline at 8 ½/1000 and shaken for a further five minutes until a perfectly homogeneous emulsion is obtained. 0.2 c.c. of the emulsion is used in each reaction tube. This is much less than the anticomplementary dose.

As to the technique of the Bordet-Wassermann Reaction, we always employ that of Bordet-Ruelens.

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## Annex 3.

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### LIST OF THE INSTITUTIONS WHICH SENT BLOOD AND CEREBRO-SPINAL FLUID SPECIMENS FOR THE CONFERENCE.

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#### **Austria**

- Professor Kerl, Vorstand der Klinik für Geschlechts- und Hautkrankheiten, Allgemeines Krankenhaus, Wien IX.
- Professor Wagner-Jauregg, Vorstand der Psychiatrischen Klinik, Allgemeines Krankenhaus, Wien IX.
- Professor Arzl, Vorstand der Klinik für Haut und Geschlechtskrankheiten, Allgemeines Krankenhaus, Wien IX.
- Professor Pappenheim, Vorstand der Nervenabteilung des Versorgungshauses, Wien XIII. Lainz.
- Herrn Docenten Brandweiner, Vorstand der dermatologischen Abteilung der Poliklinik, Wien IX. Mariannengasse.
- Herrn Professor Redlich, Primarius im Maria Theresienschlüssel, Wien XIX, Hofzeile.
- Professor Oppenheim, Vorstand der dermatologischen Abteilung des Wilhelminenspitales, Wien XVI. Montleardgasse.
- Professor Porias, Vorstand der dermatologischen Abteilung des Rainerspitales, Wien XIII.
- Professor Mucha, Vorstand der Heilanstalt in Klosterneuburg bei Wien.
- Professor Kren, Vorstand der dermatologischen Abteilung des Krankenhauses der Stadt Wien, Wien XIII. Lainz.

#### **Denmark (Copenhagen)**

- Professor Bie, Blegdamshospitalet.
- Overlæge Bing, Kommunehospitalets II. Afd.
- Overlæge Bisgaard, St. Hans Hospital.
- Dr. H. Boas, Kommunens vederlagsfri Consultation.
- Overlæge Brun-Pedersen, Marinehospitalets Hudafd.
- Professor Ehlers, Kommunehospitalets IV. Afd.
- Professor Faber, Rigshospitalets Afd. B.
- Professor Gammeloft, Rigshospitalets Fødeafd..
- Professor Hauch, Rigshospitalets Fødeafd.

Dr. Henningsen, Kommunens vederlagsfri Consultation.  
Overlæge Jacobæus, Balders Hospital.  
Overlæge Jersild, Rudolph Berghs Hospital.  
Dr. A. Kissmeyer, Kommunens vederlagsfri Consultation.  
Dr. H. Levy, Kommunens vederlagsfri Consultation.  
Dr. Lomholt, Kommunens vederlagsfri Consultation.  
Overlæge Meulengracht, Bispebjerg Hospital.  
Overlæge L. Nielsen, Frederiksberg Hospitals Afd. C.  
Dr. Pontoppidan, Kommunens vederlagsfri Consultation.  
Professor Rasch, Rigshospitalet.  
Overlæge Reyn, Finsens medicinske Lysinstitut.  
Overlæge Thalbitzer, St. Hans Hospital.  
Overlæge Tobiesen, Øresundshospitalet.  
Overlæge Vogelius, St. Johannes Stiftelse.  
Professor Wimmer, Kommunehospitalets VI. Afd.  
Overlæge Würtzen, Øresundshospitalet.

#### **France**

Dr. Vernes, Directeur de l'Institut Prophylactique, Paris.  
Professor Desbains, Hôpital de Versailles.

#### **Germany**

Professor Otto (Berlin), and his colleagues from:

Rudolf Virchow-Krankenhaus (Hautklinik und Infektions-  
krankenabteilung), Berlin.

Charité-Krankenhaus (Hautklinik und Poliklinik für Krebskranke),  
Berlin.

Staat-Krankenhaus Moabit (Innere Abteilung), Berlin.

Heilstätten Wittenau in Berlin-Wittenau; und

Viktoria-Luise-Kinderheilstätte, Hohenlychen Kreis Templin,  
Berlin.

Dr. Jacobsthal, Allgemeines Krankenhaus, Hamburg.

Professor Nocht, Institut für Schiffs- und Tropenkrankheiten,  
Hamburg.

#### **Great Britain**

Colonel L. W. Harrison and colleagues of the Venereal Diseases  
Treatment Centre, St. Thomas's Hospital, London.

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## A P P E N D I X

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Comparison of the Kahn with the Bordet-Wassermann Method (No. 1 Medical Research Council Report Series No. 14) in Tests of Cerebro-spinal Fluids, carried out by Dr. Kahn and Dr. Wyler in London after the Conference, July 10th to 25th, 1928.

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(The Kahn Test was carried out by Professor Kahn in the Pathological Laboratory of the Venereal Diseases Treatment Centre, St. Thomas's Hospital, and the B.-W. Test was carried out by Dr. Wyler in the Ministry of Health's Special Laboratory, Medical School, St. Thomas's Hospital. Five hundred and fifty-five cerebro-spinal fluids were tested.)

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**Table A.**

SUMMARY OF 317 TESTS IN WHICH BOTH WORKERS REPORTED THE SPECIMENS AS "CLEAR".

Diagnosis	Totals	Kahn					Wyler (B.-W.)					
		Positive			Doubt-ful.	Neg.	Positive			Doubt-ful.	Neg.	
		++	+	Total ++ & +	±	-	++	+	Total ++ & +	±	-	
<i>Syphilis :</i>												
I	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	2	—	—	—	—	2	—	—	—	—	2
II	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	3	—	—	—	1	2	—	—	—	—	3
III	U.T.	2	—	—	—	—	2	—	—	—	1	1
	T.	12	3	—	3	—	9	1	1	2	1	9
Latent	U.T.	4	1	—	1	—	3	1	—	1	—	3
	T.	12	1	—	1	1	10	1	—	1	3	8
Congenital	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	10	4	—	4	—	6	3	1	4	—	6
Tabes	U.T.	12	6	—	6	—	6	4	1	5	3	4
	T.	73	30	3	33	6	34	21	8	29	19	25
Neuro- } G.P.I.	U.T.	66	35	—	35	—	31	33	1	34	6	26
	T.	51	32	4	36	4	11	26	8	34	9	8
Tabo- paresis	U.T.	1	1	—	1	—	—	—	—	—	1	—
	T.	2	1	1	2	—	—	2	—	2	—	—
Other forms	U.T.	6	2	—	2	—	4	1	1	2	—	4
	T.	36	13	—	13	3	20	6	9	15	3	18
Totals	U.T.	91	45	—	45	—	46	39	3	42	11	38
	T.	201	84	8	92	15	94	60	27	87	35	79
Total Syphilis	(U.T. & T.)	292	129	8	137	15	140	99	30	129	46	117
Controls		25	—	—	—	2 <sup>1</sup>	24	—	—	—	3 <sup>1</sup>	21

<sup>1</sup> U.T. — untreated. T. — treated.  
<sup>2</sup> No. 377, Mental, doubtful signs of D.p.  
<sup>3</sup> No. 30, Facial Palsy, non-syphilitic; No. 591, Dementia præcox; No. 205, Mental, physical signs only suggestive of D.p., and No 277, Mental, doubtful signs of D.p.

**Table B.**

SUMMARY OF 238 TESTS IN WHICH ONE OR BOTH WORKERS REPORTED THE SPECIMENS AS "CLOUDY", AS CONTAMINATED, OR AS CONTAINING "SOME BLOOD".

Diagnosis		Totals	Kahn					Wyer (B.-W.)				
			Positive			Doubt-ful.	Neg.	Positive			Doubt-ful.	Neg.
			++	+	Total ++ & +	±	-	++	+	Total ++ & +	±	-
<i>Syphilis :</i>												
I	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	1	—	—	—	—	1	—	—	—	—	1
II	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	3	—	—	—	—	3	—	—	—	—	3
III	U.T.	2	1	—	1	—	1	1	—	1	—	1
	T.	13	2	—	2	—	11	1	1	2	—	11
Latent	U.T.	—	—	—	—	—	—	—	—	—	—	—
	T.	6	—	—	—	—	6	—	—	—	—	6
Congenital	U.T.	1	—	—	—	—	1	—	—	—	—	1
	T.	2	1	—	1	—	1	1	—	1	1	—
Tabes	U.T.	16	5	—	5	—	11	7	—	7	2	7
	T.	48	19	4	23	7	18	14	13	27	7	14
G.P.I.	U.T.	25	14	2	16	—	9	13	3	16	2	7
	T.	59	38	3	41	4	14	31	14	45	8	6
Neuro-Tabo- paresis	U.T.	2	1	1	2	—	—	2	—	2	—	—
	T.	4	3	—	3	—	1	2	—	2	1	1
Other forms	U.T.	9	4	—	4	—	5	2	1	3	1	5
	T.	25	6	—	6	1	18	4	1	5	5	15
Totals	U.T.	55	25	3	28	—	27	25	4	29	5	21
	T.	161	69	7	76	12	73	53	29	82	22	57
Total Syphilis	(U.T. & T.)	216	94	10	104	12	100	78	33	111	27	78
Controls		22 <sup>1</sup>	—	—	—	—	22 <sup>1</sup>	—	—	—	—	22 <sup>1</sup>

<sup>1</sup> Includes 2 cases "Not yet diagnosed".



**Table C.**

SUMMARY OF THE 555 TESTS SHOWN IN TABLES A AND B.

Diagnosis	Totals	Kahn					Wyler (B.-W.)						
		Positive			Doubtful.	Neg.	Positive			Doubtful.	Neg.		
		++	+	Total ++ & +	±	-	++	+	Total ++ & +	±	-		
<i>Syphilis :</i>													
I	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	3	—	—	—	—	3	—	—	—	—	3	
II	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	6	—	—	—	1	5	—	—	—	—	6	
III	U.T.	4	1	—	1	—	3	1	—	1	1	2	
	T.	25	5	—	5	—	20	2	2	4	1	20	
Latent	U.T.	4	1	—	1	—	3	1	—	1	—	3	
	T.	18	1	—	1	1	16	1	—	1	3	14	
Congenital	U.T.	1	—	—	—	—	1	—	—	—	—	1	
	T.	12	5	—	5	—	7	4	1	5	1	6	
Neuro-	Tabes	U.T.	28	11	—	11	—	17	11	1	12	5	11
		T.	121	49	7	56	13	52	35	21	56	26	39
	G.P.I.	U.T.	91	49	2	51	—	40	46	4	50	8	33
		T.	110	70	7	77	8	25	57	22	79	17	14
	Tabo- paresis	U.T.	3	2	1	3	—	—	2	—	2	1	—
		T.	6	4	1	5	—	1	4	—	4	1	1
	Other forms	U.T.	15	6	—	6	—	9	3	2	5	1	9
		T.	61	19	—	19	4	38	10	10	20	8	33
	Totals	U.T.	146	70	3	73	—	73	64	7	71	16	59
		T.	362	153	15	168	27	167	113	56	169	57	136
Total Syphilis	(U.T. & T.)	508	223	18	241	27	240	177	63	240	73	195	
Controls		47 <sup>1</sup>	—	—	—	1 <sup>2</sup>	46 <sup>1</sup>	—	—	—	4 <sup>3</sup>	43 <sup>1</sup>	

<sup>1</sup> Includes 2 cases "Not yet diagnosed".

<sup>2</sup> No. 377, Mental, doubtful signs of D. p.

<sup>3</sup> No. 30 Facial Palsy, Non-syphilitic; No. 591 Dementia præcox; No. 205, Mental, physical signs only suggestive of D. p. and No. 277, Mental, doubtful signs of D. p.

## Details of Results included in Table A in

(In the cases marked \* the diagnosis, or the classification into "treated" or "untreated" of the results to the institution)

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis
		C. S. F.	C. S. F.	
18.VII.28	251	++	+	D.p. Untreated.
20.VII.28	330	++	+	Tabes. "
13.VII.28	561	++	+	Other forms. Neuro-S. "
11.VII.28	26	++	+	D.p. Treated.
11.VII.28	48	++	+	Autres formes. Neuro-S. "
11.VII.28	61	++	+	Cong. S. "
12.VII.28	109	++	+	Tabes. "
18.VII.28	232	++	+	Other forms. Neuro-S. "
18.VII.28	254	++	+	D.p. "
19.VII.28	279	++	+	S. III. "
19.VII.28	291	++	+	Other forms. Neuro-S. "
20.VII.28	314	++	+	D.p. "
20.VII.28	337	++	+	Tabes. "
20.VII.28	345	++	+	D.p. "
25.VII.28	424	++	+	D.p. "
11.VII.28	529	++	+	Other forms. Neuro-S. "
12.VII.28	539	++	+	Tabes. "
12.VII.28	554	++	+	Other forms. Neuro-S. "
13.VII.28	571	++	+	D.p. "
24.VII.28	659	++	+	Other forms. Neuro-S. "
11.VII.28	55	+	++	Tabo-paresis. "
13.VII.28	180	+	++	Tabes. "
10.VII.28	458	+	++	Tabes. "
12.VII.28	542	+	++	D.p. "
18.VII.28	223	+	±	D.p. "
18.VII.28	231	+	±	D.p. "
20.VII.28	299	+	±	Tabes. "
13.VII.28	179	±	+	Tabes. "
18.VII.28	246	±	+	Tabes. "
19.VII.28	285	±	+	Other forms. Neuro-S. "

which the Kahn and B.-W. Results differed.

"untreated", has been changed as a result of further inquiry after submission which provided the specimens.)

Remarks	Cell Count. (Wyler)	Globulin <sup>2</sup>			B.-W. of serum taken same time as fluid <sup>2</sup>	
		Kahn	Wyler <sup>1</sup>		Date tested	Result
			N	P		
		++	+	+	11.VII.28	++
		++	-	+	7.VII.28	++
		++	+	+	13.VII.28	++
	5	+	-		18.VII.28	±
	2	++	-		18.VII.28	±
	0	±	-	-	18.VII.28	++
		++	+	+	20.VII.28	++
		±	-	+	5.VII.28	±
Malaria and Tryparsamide.		++	+	+	11.VII.28	A.C.
	1	+	D	-	7.VII.28	++
		+	-	+	7.VII.28	±
Malaria Jan. 1928.		++	+	+	7.VII.28	++
		++	-	+	7.VII.28	+±
Malaria April 1926.		++	D	+	7.VII.28	-
Malaria 1927.		++	+	+	7.VII.28	++
		++	No test		13.VII.28	-
		++	-	D	13.VII.28	+
		++	+	+	13.VII.28	±
Malaria Dec. 1927, followed by Hg., Iod., Sulfarsenol and Tryparsamide.		++	+	+	13.VII.28	++
		+	-	+	26.VII.28	++
	13	++	+	+	18.VII.28	++
		++	D	+	2.VII.28	++
		+	-	+	11.VII.28	++
Malaria Aug. 1927.		++	-	+	13.VII.28	++
Malaria Tr. (Also Tabetic)	4	++	+	+	5.VII.28	+±
	1	+	D	D	5.VII.28	+±
Optic atrophy.		+	-	D	7.VII.28	++
	3	±	-	D	2.VII.28	-
	0	±	D	D	5.VII.28	±
Paresis Ext. rectus. Agyll-Robertson pupils.		++	+	+	7.VII.28	++

<sup>1</sup> N = Nonne; P = Pandy; D = doubtful.  
<sup>2</sup> Carried out by Dr. T. E. Osmond by No. 1 Method.  
 Explication of abbreviations:  
 S.C. = slow lysis of serum control.  
 A.C. = anticomplementary.  
 - N.C. = negative not clear.

<sup>3</sup> It is to be noted that Professor Kahn graded his globulin test results: "+, +, ±, and -" whilst Dr. Wyler graded his results: "+, D, and -".

Details of Results included in Table A in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis
		C. S. F	C. S. F.	
24.VII.28	397	±	+	Tabes. Treated.
12.VII.28	543	±	+	D.p. "
19.VII.28	635	±	+	Other forms. Neuro-S. "
24.VII.28	377	±	-	*Control.
17.VII.28	194	±	-	S. II. "
11.VII.28	31	-	±	S. III. Untreated.
17.VII.28	200	-	±	D.p. "
17.VII.28	205	-	±	*Control.
19.VII.28	277	-	±	*Control.
20.VII.28	304	-	±	Tabes. "
20.VII.28	306	-	±	Tabes. "
24.VII.28	379	-	±	D.p. "
24.VII.28	386	-	±	D.p. "
24.VII.28	387	-	±	D.p. "
11.VII.28	47	-	±	Tabes. Treated.
11.VII.28	60	-	±	Tabes. "
11.VII.28	64	-	±	D.p. "
12.VII.28	93	-	±	*D.p. Untreated.
12.VII.28	115	-	±	Tabes. Treated.
13.VII.28	159	-	±	Other forms. Neuro-S. "
19.VII.28	280	-	±	Latent S. "
19.VII.28	292	-	±	S. III. "
19.VII.28	293	-	±	Tabes. "
19.VII.28	295	-	±	Tabes. "
19.VII.28	296	-	±	Tabes. "
24.VII.28	375	-	±	D.p. "
10.VII.28	469	-	±	Tabes. "
11.VII.28	522	-	±	Tabes. "
13.VII.28	565	-	±	Tabes. "

the Kahn and B.-W. Results differed (Continued).

Remarks	Cell Count. (Wylér)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wylér		Date tested	Result
			N.	P.		
Malaria Feb. 1928.		+	-	+	11.VII.28	++
Progressive muscular atrophy.		+	-	-	30.VII.28	++
Mental (doubtful signs of D.p.).		++	+	+	23.VII.28	++
		-	-	-	9.VII.28	-
	0	-	-	D	2.VII.28	-
? Gumma of Lung. Previous test of C.S.F. negative.	0	±	-		18.VII.28	-
Juvenile D.p.	0	±	-	-	2.VII.28	-
Mental; physical signs only suggestive of D.p.		±	-		2.VII.28	-
Mental; doubtful signs of D.p.	1	±	-	-	7.VII.28	-
		±	-	-	7.VII.28	++
		±	-	-	7.VII.28	++
Definite signs of D.p.		+	-	+	9.VII.28	-
Definite signs of D.p.		-	-	-	9.VII.28	-
Unequivocal signs of syphilis of central nervous system.		-	-	-	9.VII.28	-
	2	+	D	-	18.VII.28	±
	0	+	-	D	18.VII.28	++
Malaria Jan. 1927.	19	++	D	D	18.VII.28	+
Unequivocal signs of syphilis of central nervous system.		++	-	D	18.VII.28	++
	2	+	-	-	20.VII.28	A.C.
Paralysis Right external rectus.		++	No test		20.VII.28	++
	0	++	-		7.VII.28	++
? Early tabes, but previous Lange, 0000000000 and cells, 1.		-	-	-	7.VII.28	++
		+	-	D	7.VII.28	++
		+	-	-	7.VII.28	++
		-	-	-	7.VII.28	±
One course Tryparsamide.		+	D	+	9.VII.28	++
		±	-	-	11.VII.28	++
Optic atrophy.		+	+	+	13.VII.28	±±
		+	-	-	13.VII.28.	++

Details of Results included in Table A in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C S. F.		
19.VII.28	625	—	±	Latent S.	Treated.
19.VII.28	637	—	±	D.p.	”
20.VII.28	339	++	±	*Tabo-paresis.	Untreated.
11.VII.28	499	++	±	D.p.	”
13.VII.28	564	++	±	Tabes.	”
10.VII.28	8	++	±	Tabes.	Treated.
11.VII.28	56	++	±	Tabes.	”
12.VII.28	111	++	±	Tabes.	”
12.VII.28	116	++	±	Other forms. Neuro-S.	”
18.VII.28	245	++	±	Tabes.	”
25.VII.28	402	++	±	Tabes.	”
25.VII.28	429	++	±	D.p.	”
17.VII.28	587	++	±	?Tabes.	”
19.VII.28	627	++	±	Tabes.	”
20.VII.28	648	++	±	D.p.	”
25.VII.28	422	±	++	D.p.	”
19.VII.28	634	±	++	*Tabes.	”
12.VII.28	110	++	—	Tabes.	”
20.VII.28	308	++	—	Tabes.	”
24.VII.28	359	++	—	S. III.	”
13.VII.28	181	—	+	Other forms. Neuro-S.	”
13.VII.28	184	—	+	?Tabes.	”
18.VII.28	238	—	+	Tabes.	”

the Kahn and B.-W. Results differed (*Concluded*).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
Malaria. Perforation septum nasi. Giddiness, nystagmus, Knee Jerks +.	2	+	D	—	20.VII.28	++
		+	D	D	No serum.	
		++	D	+	7.VII.28	±
		+	—	+	13.VII.28	++
		+	D	D	13.VII.28	++
		±	—		No serum.	
R. Plois, L. Hemiplegia.	30	++	+	+	18.VII.28	±
		+	D	D	20.VII.28	++§C
		+	D	D	20.VII.28	++
		1	+	—	—	5.VII.28
Malaria. Optic atrophy. W.R. of blood negative prior to this. Malaria.		+	D	+	11.VII.28	±
		++	—	+	11.VII.28	++
		++	+	+	16.VII.28	++
		±	—	D	No serum.	
		++	+	+	23.VII.28	±
Paralysis r. hand 1927. Pupil light reflex nil. Knee Jerk nil.		++	+	+	11.VII.28	++
		++	+	+	11.VII.28	++
		++	—	—	20.VII.28	++
		+	—	—	20.VII.28	—
		+	No test		No serum.	
S. meningitis. Previously diagnosed D.p. Optic atrophy.	2	++	—	+	7.VII.28	++
		++	+	+	2.VII.28	++
		+	—	—	2.VII.28	++
		0	+	—	D	5.VII.28

## Details of Results included in Table B in

(In the cases marked \* the diagnosis, or the classification into "treated" or of the results to the institutions

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
18.VII.28	252	++ cloudy	+ cloudy	D.p.	Untreated.
25.VII.28	430	++ cloudy	+	D.p.	"
24.VII.28	655	++ cloudy	+ cloudy	Other forms. Neuro-S.	"
10.VII.28	14	++ cloudy	+ cloudy	D.p.	Treated.
12.VII.28	76	++ cloudy	+ cloudy	S. III.	"
13.VII.28	151	++ cloudy	+ cloudy	D.p.	"
13.VII.28	161	++	+ cloudy	Tabes.	"
13.VII.28	187	++	+ cloudy	Tabes.	"
13.VII.28	188	++	+ cloudy	Tabes.	"
18.VII.28	211	++ cloudy	+ cloudy	Tabes.	"
19.VII.28	270	++ cloudy	+ cloudy	Tabes.	"
20.VII.28	342	++ contains blood	+ some blood	D.p.	"
20.VII.28	343	++ cloudy	+	D.p.	"
20.VII.28	346	++ cloudy	+ cloudy	D.p.	"
24.VII.28	368	++ cloudy	+ cloudy	D.p.	"
25.VII.28	413	++ cloudy	+ cloudy	D.p.	"
25.VII.28	414	++ cloudy	+ cloudy	D.p.	"
25.VII.28	416	++ cloudy	+ cloudy	D.p.	"
10.VII.28	462	++ cloudy	+ cloudy	D.p.	"
11.VII.28	513	++sl. cloudy	+ cloudy	D.p.	"
11.VII.28	515	++sl. cloudy	+ cloudy	Tabes.	"
12.VII.28	555	++	+ cloudy	Other forms. Neuro-S.	"
12.VII.28	89	+ cloudy	++ cloudy	D.p.	Untreated.
11.VII.28	537	+ cloudy	++ cloudy	Tabo-paresis.	"
10.VII.28	40	+	++ cloudy	D.p.	Treated.
12.VII.28	94	+ cloudy	++ cloudy	D.p.	"
24.VII.28	372	+ cloudy	++ cloudy	D.p.	"
24.VII.28	394	+ cloudy	++ cloudy	? Tabes.	"
12.VII.28	557	+	++ cloudy	Tabes.	"
12.VII.28	559	+	++ cloudy	? Tabes.	"



which the Kahn and B.-W. Results differed.

“untreated”, has been changed as a result of further inquiry after submission which provided the specimens.)

Remarks	Cell Count. (Wylér)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wylér		Date tested	Result
			N.	P.		
Malaria April 1927.	0 2	++	+		11.VII.28	++
		++	D	+	11.VII.28	++
		+	No test		26.VII.28	++
		++	-		18.VII.28	++
		++	-		18.VII.28	++
		++	-	+	20.VII.28	±
		++	No test		20.VII.28	++
		+		„	2.VII.28	++
		++		„	2.VII.28	++
		+		„	5.VII.28	++
Malaria Sept. 1926.		++	D	+	5.VII.28	±
		++	No test		7.VII.28	++
Malaria Feb. 1928.		++		„	7.VII.28	++
Malaria June 1926.		++		„	7.VII.28	++
Malaria Oct. 1927.		++		„	7.VII.28	++
Malaria and Tryparsamide.		++		„	11.VII.28	++
Malaria and Tryparsamide		++		„	11.VII.28	++
Malaria and Tryparsamide.		++		„	11.VII.28	++
		++	+	+	11.VII.28	++
Malaria 1924.		++	No test		13.VII.28	±
Malaria 1928.		++		„	13.VII.28	++
		++		„	13.VII.28	++
		++	+		18.VII.28	++
		++	No test		13.VII.28	++
Malaria June 1927.	6	++	+		18.VII.28	++
		++	+		18.VII.28	++
		+	No test		9.VII.28	++
		+		„	9.VII.28	-
		++	+	+	No serum.	
		++	No test		13.VII.28	++

Details of Results included in Table B in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
19.VII.28	262	+cloudy, contaminated, greenish	± cloudy, some blood	D.p.	Untreated.
24.VII.28	395	+ cloudy	± cloudy	Tabes.	Treated.
12.VII.28	108	± cloudy	+ cloudy	Tabes.	"
13.VII.28	570	±	+ cloudy	D.p.	"
20.VII.28	301	± cloudy	- cloudy, infected	D.p.	"
10.VII.28	464	± cloudy, contaminated.	- some blood, inactivated.	Tabes.	"
10.VII.28	465	± cloudy, contaminated.	- cloudy	Tabes.	"
12.VII.28	106	- cloudy	± cloudy	Tabes.	Untreated.
13.VII.28	137	-	± cloudy	D.p.	"
19.VII.28	288	- cloudy	± cloudy	Tabes.	"
11.VII.28	54	- sl.cloudy	± cloudy	Tabes.	Treated.
12.VII.28	97	- cloudy	± cloudy	Congl. S.	"
13.VII.28	136	- cloudy, bloody	± hæmoglobin	Other forms. Neuro-S.	"
24.VII.28	369	- cloudy	± cloudy	D.p.	"
25.VII.28	403	- cloudy	±	D.p.	"
25.VII.28	449	- cloudy	± cloudy	D.p.	"
13.VII.28	577	- cloudy	± cloudy	D.p.	"
17.VII.28	611	-	± cloudy	Other forms. Neuro-S.	"
24.VII.28	656	- cloudy	±	Other forms. Neuro-S.	"

the Kahn and B.-W. Results differed (Continued).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
		++		„	11.VII.28	++
		±		„	9.VII.28	-
Argyll-Robertson pupils. Knee Jerks absent.		++	D	+	20.VII.28	++ S.C.
Malaria Aug. 1926. Typical. G.P.I. Dull, irritable, untidy.		+	No test		(1.VII.28 (see No. 364.)	++
		++		„	7.VII.28	-
Knee and Achilles Jerks absent.		++		„	11.VII.28	-
Also hemiparesis. K.J's and A.J's absent.		+	-	D	11.VII.28	±
Clinically typical.		++	+	+	20.VII.28	++ S.C.
Definite signs of D.p.		+	-	-	20.VII.28	-
C.S.F. ++ on 24.IV.20.		+	No test		7.VII.28	- N.C.
K.J's absent. Malarial Tr. followed by Silv.-Salv. and Sulfarsenol.	0	±	-		18.VII.28	+
Intra-cisternal injections, though no clinical cerebro-spinal symptoms.	0	±	-		18.VII.28	±
Ataxia, Romberg A.R. pupils; K.J. ++. No sensory changes.		++	No test		20.VII.28	++
Malaria May 1925. At that time C.S.F. positive W.R. Cells ++, Globulin ++.		++		„	7.VII.28	++
Malaria and Tryparsamide.		+	+	+	11.VII.28	±
Malaria April 1926.		++	No test		11.VII.28	-
Malaria and Salvarsanised serum.		++		„	16.VII.28	++
Paralysis 3rd cranial.		-		„	20.VII.28	±
A.R. pupils, K.J. +; W.R. of C.S.F. 1926, ++, 1927, ±.		+	-	D	26.VII.28	++

Details of Results included in Table B in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis
		C. S. F.	C. S. F.	
24.VII.28	654	++ bloody	± some blood	Other forms. Neuro-S. Treated.
11.VII.28	39	++ sl. cloudy	± cloudy	D.p. ”
11.VII.28	44	++ sl. cloudy	± cloudy	D.p. ”
11.VII.28	53	++ sl. cloudy	± cloudy	Tabes. ”
18.VII.28	226	++	± cloudy	Other forms. Neuro-S. ”
24.VII.28	396	++	± some blood	Tabo-paresis. ”
13.VII.28	562	++	± cloudy	Tabes. ”
13.VII.28	566	++ cloudy, bloody	± some hæmoglobin	Other forms. Neuro-S. ”
13.VII.28	569	++	± cloudy	Tabes. ”
11.VII.28	43	± sl. cloudy	++ cloudy	D.p. ”
13.VII.28	182	±	++ cloudy	Other forms. Neuro-S. ”
24.VII.28	390	±	++ cloudy	Tabes. ”
18.VII.28	243	- cloudy heavily contaminated,	++ cloudy	Tabes. Untreated.
10.VII.28	496	- cloudy, contaminated.	++ cloudy	Tabes. ”
12.VII.28	98	- cloudy	++ cloudy	D.p. Treated.
13.VII.28	144	-	++ cloudy	*Tabes. ”
18.VII.28	250	- cloudy	+ cloudy	D.p. Untreated.
12.VII.28	70	-	+ cloudy	D.p. Treated.
13.VII.28	134	- cloudy	+ cloudy	Tabes. ”
13.VII.28	183	- cloudy	+ some blood	D.p. ”
12.VII.28	541	- sl. cloudy	+ cloudy	D.p. ”

the Kahn and B.-W. Results differed (*Continued*).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
A.R. pupils, right side Hemiplegia. K.J's ++. Right Babinski.		++	No test		26.VII.28	—
Malaria Nov. 1927.	0	++	—		18.VII.28	++
Malaria June 1927.	1	++	D		18.VII.28	++
	0	++	—		18.VII.28	++
Cerebralendarteritis; Aneurism Aorta; Choroiditis.	0	+	—	D	5.VII.28	—
		++	No test		11.VII.28	++
K.J's absent. Ataxic gait.		++	„		13.VII.28	++
Cerebral.		++	„		13.VII.28	++
Charcot's disorganisation of rt. knee joint. K.J. rt. leg absent.		+	„		13.VII.28	+±
Malaria Oct. 1927.	2	++	+		18.VII.28	++
Diplopia. Headache.		+	No test		2.VII.28	++
K.J's absent. Ataxia. Rombergism.		+	„		9.VII.28	++
Optic atrophy. Ataxia. Romberg.		++	—		5.VII.28	++
A.R. pupils. Ptosis. Nystagmus.		++	No test		No serum.	
Typical.	3	++	+	+	18.VII.28	++
		++	D	+	20.VII.28	++
Stationary.		+	—		11.VII.28	++
Malaria Dec. 1925.	0	++	—		18.VII.28	—
Ataxia. K.J's absent. Incontinence urine.		++	+		20.VII.28	±
Probably tabo-paresis.		+	No test		2.VII.28	++
Malaria Aug. 1925.		++	„		13.VII.28	++

**Details of Results included in Table B in which**

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
17.VII.28	592	—	+ cloudy	Tabes.	Treated.
17.VII.28	604	—	+ cloudy	Tabes.	”
19.VII.28	629	— cloudy, contami- nated, greenish.	+ cloudy	Tabes.	”
24.VII.28	653	— cloudy	+ cloudy	Tabes.	”

the Kahn and B.-W. Results differed (*Concluded*).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
Aneurism. Lange, luetic curve 1924 and 1925 ; now almost neg. W.R. of C.S.F. pos. since 1922.		+	No test			No serum.
		±	"			No serum.
		++	-	-	20.VII.28	-
Optic atrophy.		+	No test		26.VII.28	±

**COMMENTS ON THESE, TESTS PRESENTED BY  
COLONEL L. W. HARRISON, Dr. KAHN AND  
Dr. WYLER**

REPORT BY COLONEL L. W. HARRISON.

As it had been found impracticable in the case of the cerebro-spinal fluid to obtain a sufficient number of specimens in sufficient amounts to afford a reliable comparison with all the different tests employed at Copenhagen, it was decided after the Conference to take the opportunity of Dr. Kahn's presence in London for a few weeks to carry out a comparison of the Kahn with the Bordet-Wassermann test (Harrison-Wyler method).

By the courtesy of the British Ministry of Health, the services of Dr. Wyler and the special laboratory at St. Thomas's Hospital were placed at the disposal of the League of Nations Health Organisation for the purpose; and, by that of the Treasurer and Almoner of St. Thomas's Hospital, laboratory facilities were afforded to M. Kahn in the V.D. Department of that institution.

At the request of Colonel Harrison, 555 specimens of cerebro-spinal fluid were collected by the chief physicians of the clinics indicated in the list at the end of this report, to whom the League of Nations Health Organisation would gratefully acknowledge its indebtedness for valuable assistance in this important investigation.

Most of the specimens of cerebro-spinal fluid were accompanied by specimens of blood serum from the same patients. It was found impracticable in the time available for either Dr. Kahn or Dr. Wyler to undertake the testing of these sera, and this work was carried out by Dr. T. E. Osmond, Pathologist to the V.D. Department of St. Thomas's Hospital, for whose help, given at a time of great pressure of other work, the League of Nations Health Organisation desires to express its thanks. Dr. Osmond employed No. 1 Method of the Medical Research Council Special Report Series No. 14, which is the same as that used by Dr. Wyler at the Copenhagen Conference.

It was not found practicable to carry out cell counts, as was hoped, on any but a small proportion of the specimens



of cerebro-spinal fluid, nor could any specimens be tested by a colloidal method such as the Lange, mastix or benzoin.

Owing possibly to the hot weather and the fact that most of the specimens had to travel by post, about half were found at the time of testing to be cloudy. In addition, a certain number were rejected as too heavily contaminated. It was felt that cloudiness of a specimen might affect the test or its reading, and for this reason the results have been analysed in two sections — cloudy and clear respectively — in Tables A and B. An analysis of the combined results is shown in Table C.

*Analysis of the 317 tests of clear fluid shown in Table A.*

		Agreements			
		Untreated	Treated	Total	
++	or +	42	76	118	
	±	0	6	6	
	—	38	75	113	
		80	157	237	
		Disagreements			
	Kahn	B.-W.	Untreated	Treated	Total
(a)	++ or +	±	3	13	16
(b)	±	++ or +	0	8	8
(c)	±	—	0	1	1
(d)	—	±	8	16	24
(e)	++ or +	—	0	3	3
(f)	—	++ or +	0	3	3

Thus there was substantial agreement between the two tests in approximately 75 per cent. There were no important disagreements or non-specific reactions in control cases.

The disagreements show broadly that the Kahn test gave stronger reactions with 20 specimens [lines (a), (c), (e)] and the B.-W. stronger reactions with 35 [lines (b), (d), (f)]. If the 25 disagreements between ± and — [lines (c) and (d)] are ignored, the Kahn test gave 19 stronger reactions (++ or + against ± or —) than the B.-W. [lines (a), (e)], and the B.-W. gave 11 stronger reactions than the Kahn [lines (b), (f)].

In treated cases of syphilis, however, a ± reaction is usually regarded as important. If, in such cases, it is

accepted as evidence of persistent infection, the Kahn test gave no sign of such persistence (by a ++, + or ± reaction) in 19 treated cases where the B.-W. test gave one or other of these reactions [lines (d) and (f)] and the reverse occurred in 4 [lines (c) and (e)].

*Analysis of the 238 tests of cloudy fluid shown in Table B.*

		Agreements			
		Untreated	Treated	Total	
++	or +	26	67	93	
	±	0	4	4	
	—	21	54	75	
		47	125	172	
		Disagreements			
	Kahn	B.-W.	Untreated	Treated	Total
(a)	++ or +	±	2	9	11
(b)	±	++ or +	0	5	5
(c)	±	—	0	3	3
(d)	—	±	3	9	12
(e)	++ or +	—	0	0	0
(f)	—	++ or +	3	10	13

Thus, in this series of cloudy specimens, there was substantial agreement in approximately 72 per cent.

There were no disagreements or non-specific reactions in "Controls".

The disagreements show broadly that the Kahn test gave stronger reactions than the B.-W. in 14 cases and the B.-W. stronger reactions than the Kahn in 30.

If the 15 disagreements between ± and — [lines (c) and (d)] are ignored, the Kahn test gave 11 stronger reactions [lines (a) and (e)] than the B.-W. and the B.-W. test gave 18 stronger reactions [lines (b) and (f)] than the Kahn.

Considering only the treated cases and accepting a ± reaction as significant of persisting infection, the Kahn test gave no sign of such persistence (by a ++, + or ± reaction) in 19 treated cases where the B.-W. test gave one of these reactions [see lines (d) and (f)] and the reverse occurred in 3 [see lines (c) and (e)].

Comparing the two series of results in clear and cloudy specimens respectively, it will be seen that there is a quite definite difference. Thus, in the specimens from syphilitic cases, the percentages of reactions were as follows:

*Cases of syphilis, treated and untreated.*

	<i>Clear specimens</i>		<i>Cloudy specimens</i>	
	Kahn	B.-W.	Kahn	B.-W.
++ or +	46.9	44.1	48.1	51.3
±	5.1	15.7	5.5	12.5
—	47.9	40.0	46.3	36.1

If the treated cases of syphilis only are considered and in these the ± reactions are added to the ± and ++, on the grounds of a ± reaction being significant in a treated case, the percentages of reactions were as follows.

*Cases of treated syphilis.*

	<i>Clear specimens</i>		<i>Cloudy specimens</i>	
	Kahn	B.-W.	Kahn	B.-W.
++, + or ±	53.2	60.6	54.6	64.5
—	46.7	39.3	45.3	35.4

In both cases, a higher percentage of positive reactions was obtained in the cloudy series, but the preponderance of such reactions was more pronounced in the case of the B.-W. test than in that of the Kahn.

A possible explanation is that the specimens in the cloudy series happened to be inherently more syphilitic, but their physical condition of cloudiness interfered with the reading of the Kahn reaction.

COMMENTS OF DR. R. L. KAHN.

(University of Michigan Hospital, Ann Arbor, Michigan, U.S.A.)

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1. I read Colonel Harrison's report of the spinal fluid comparison with great care, and I am greatly indebted to Colonel Harrison for giving a considerable amount of time and labour in making this series of spinal fluid studies possible and in preparing this very clear report.

2. There is one point about which Colonel Harrison and I disagree in principle; namely, as to the value of a plus-minus ( $\pm$ ) spinal fluid reaction in the diagnosis and treatment of neurosyphilis. In the examination of a serum for the detection of syphilis, a plus-minus ( $\pm$ ) reaction is of some value. A clinician may not suspect syphilis and a reaction of this type may give him a clue as to the possible presence of this disease. In the examination of a spinal fluid for the detection of neurosyphilis, however, the situation is different. Every case of syphilis is a possible case of neurosyphilis. Stated differently, every case of syphilis gives a clinical  $\pm$  reaction for neurosyphilis. Therefore, a serological  $\pm$  reaction in such a case adds nothing to the clinical syndrome. A serological  $\pm$  reaction means that it is neither positive nor negative and does not help the clinician in making any decision in diagnosis or in treatment of neurosyphilis.

3. This point of view led me to eliminate the  $\pm$  reaction from the spinal fluid procedure with the Kahn test. In the "Outline of Technique" of this test (see Annex 2) the  $\pm$  reaction was not listed. It is true that I reported a small number of  $\pm$  reactions with spinal fluids. The reason for this was that I was working under new conditions and, in addition, I did not know the results of my spinal fluid examinations until the end of the comparison. For these reasons I was moved to practise ultra-conservatism in my reports, and positive reactions which were in any way questionable I reported  $\pm$ .

4. In my opinion, two groups of figures taken from Colonel Harrison's tables are significant in this comparison:

*Group I. — Variations in B.-W. (Harrison) and Kahn Tests.*

In 317 clear (non-contaminated) spinal fluids:

B.-W. positive (++) or (+), Kahn negative (or doubtful)	11
Kahn positive (++) or (+), B.-W. negative (or doubtful)	19

In 238 cloudy (contaminated) spinal fluids:

B.-W. positive (++) or (+), Kahn negative (or doubtful)	18
Kahn positive (++) or (+), B.-W. negative (or doubtful)	11

*Group II. — "Totals" of ++, +, ± and — Reactions of B.-W. and Kahn Tests.*

Figures taken from Table A (clear fluids):

B.-W.				Kahn			
++	+	±	—	++	+	±	—
99	30	46	117	129	8	15	140

Figures taken from Table B (cloudy fluids):

B.-W.				Kahn			
++	+	±	—	++	+	±	—
78	33	27	78	94	10	12	100

Considering that the ± reactions are of little value and counting them with the negative reactions, the B.-W. test gave a total of 177 ++ and 63 + reactions, while the Kahn test gave a total of 223 ++ and 18 + reactions. Thus the Kahn test gave 46 ++ reactions against 45 + reactions of the B.-W. test.

It is to be noted also from Colonel Harrison's tables that not one of the Kahn ± reactions were in cases which were frankly non-syphilitic, whereas the B.-W. test gave a few ± reactions in such cases.

5. There are several technical aspects regarding the Kahn test with spinal fluid which I should like to note here. The test involves precipitating the globulins from the spinal fluid by means of saturated ammonium sulphate and mixing antigen suspension with the dissolved globulin solution. In order to minimise the influence of this salt on the final reaction between the globulin solution and antigen suspension, two steps are employed which tend to lessen the sensitiveness of the reactions: (1) The use of 40 instead of 50 per cent saturation of the spinal fluid with ammonium sulphate, thus reducing the amount of specific globulin in the test; (2) the use of an amount of physiological salt solution in the antigen suspension

greater than that indicated by the antigen titer for tests with serum, thereby reducing the sensitiveness of the antigen suspension.

It was observed that, by employing small test tubes (7.5 by 1 cm.) instead of large ones for mixing the spinal fluid with the saturated ammonium sulphate and centrifuging in the usual manner, it was possible almost completely to remove the supernatant fluid, thus reducing the amount of the salt in the dissolved globulin solution to a minimum. This made possible the use of a 50 instead of 40 per cent. saturation of the spinal fluids with ammonium sulphate. It also made possible the use of an antigen titer similar to that employed in tests with serum. By this method, reactions have been obtained of higher sensitiveness than heretofore without affecting their specificity.

It was also found that sensitised antigen can be employed in the spinal fluid procedure instead of standard antigen — using the same sensitised antigen titer as in tests with serum. The resulting reactions indicate a still higher degree of sensitiveness with freedom from false reactions. Recently, a still more sensitive spinal fluid reaction was obtained with sensitised antigen after increasing the globulin concentration of these fluids to twenty instead of ten times the original concentration of the fluid. This procedure also showed no tendency toward false reactions. It is thus evident that the Kahn reaction makes possible the use of spinal fluid procedures of marked sensitiveness and specificity in the detection of neurosyphilis.

6. I should like to take this opportunity of expressing my obligation and thanks to Colonel Harrison for the cordiality and helpfulness which he kindly extended to me during this comparison. I should also like to thank Dr. J. E. Wyler for helpful co-operation and especially Dr. T. E. Osmond for giving me the freedom of his laboratory and for making my work a most pleasant experience.

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REMARKS CONCERNING DR. KAHN'S STATEMENT  
BY DR. E. J. WYLER.

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*Dr. Kahn's 2nd paragraph.*

It is stated that, "in the examination of a serum for the detection of syphilis, a  $\pm$  reaction is of some value. A clinician may not suspect syphilis and a reaction of this type may give him a clue as to the possible presence of this disease."

Practical experience has shown that  $\pm$  reactions, whether with serum or with spinal fluid, are not merely of 'some value', and that their significance, particularly when recurring in subsequent tests, is very considerable, not only for diagnostic purposes, but also in following the effect of treatment. This has been abundantly confirmed in cases where simultaneous tests with flocculation reactions have yielded similar border-line or positive results.

It is also necessary to emphasise that a  $\pm$  result is never, in this laboratory, regarded as positive for diagnostic purposes, but as an indication for further clinical and serological investigation. It is interpreted as positive only in known cases of syphilis.

*Dr. Kahn's 3rd paragraph.*

It is stated that ". . . in addition, I did not know the results of my spinal fluid examinations until the end of the comparison. For these reasons, I was moved to practise ultra-conservatism in my reports, and positive results which were in any way questionable I reported  $\pm$ ."

I was of course also unaware of the clinical nature of the specimens until the end of the comparison, and, from the character of the investigation, had also to be conservative in my reports.

*Dr. Kahn's 4th paragraph.*

Group I appears to illustrate the fact that the Kahn test is under a disadvantage where cloudy fluids are concerned. This is of practical importance.

Under Group II, it is shown that "the Kahn test gave 46 ++ reactions against 45 + reactions of the B.-W. test". It is, however, even less possible thus strictly to compare a ++ complement fixation result with a ++ flocculation test result than to compare a ++ obtained with one B.-W. technique with that obtained by another B.-W. technique, and the statement is therefore not evidence of the greater delicacy of the Kahn test. A strictly accurate comparison of positive results can be obtained only by comparing results called *positive* with one another, irrespective of hieroglyphics. In this laboratory, a result of ++ or + indicates that the serum or spinal fluid is positive for diagnostic purposes.

When the figures of Group II are compared in this way, irrespective of the number of plus signs, it is seen that the Kahn test gave 241 *positive* results against 240 *positive* results by the B.-W. test. But when the ± results, which, in our view, are of great practical importance, are included in the positive results (and as stated above, they are so interpreted in known cases of syphilis), then it is seen that the Kahn test gave 268 *positive* results against 313 *positive* results by the B.-W. test.

With reference to the final sentence of paragraph 4 in which it is stated that the B.-W. test gave "a few" ± reactions in non-syphilitic cases, it is necessary to point out that, out of four such results obtained among the 555 tests, two only were frankly non-syphilitic (= 0.36%), whilst two were clinically doubtful as against one ± result with the Kahn test which was also clinically doubtful. I would here again emphasise that a ± result is never accepted as positive for diagnostic purposes in this laboratory.

*Dr. Kahn's 5th paragraph.*

Dr. Kahn's investigations with a view to increasing the sensitiveness of the reaction with spinal fluid are of the greatest interest and importance. It must, however, be remarked, in point of laboratory technique, that, while the procedure is far simpler and quicker than the B.-W. test in regard to sera, much of the simplicity and speed is lost where spinal fluids are concerned.

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**LIST OF THE NAMES AND ADDRESSES OF DOCTORS WHO  
SENT SPECIMENS OF C.S.F. FOR THE COMPARISON OF  
THE KAHN AND WASSERMANN TESTS**

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# LEAGUE OF NATIONS

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Health Organisation

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PERMANENT COMMISSION ON STANDARDISATION  
OF SERA, SEROLOGICAL REACTIONS  
AND BIOLOGICAL PRODUCTS

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## MEMORANDA

ON THE

International Standardisation  
of Therapeutic Sera and Bacterial Products

BY

Professor C. PRAUSNITZ  
(BRESLAU)

Series of League of Nations Publications

III. HEALTH  
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## INTRODUCTION.

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The Health Section is very much indebted to Professor C. Prausnitz for undertaking the arduous task of reviewing the Permanent Standardisation Commission's work on therapeutic sera and vaccines. The account of the Commission's own activities has been supplemented by valuable references to the literature on the subject. The object with which this survey has been prepared is to afford guidance in the use of the international standard preparations and, further, to provide all those who are interested in the introduction of such standard preparations with a conspectus of the work at the stage now reached. Those wishing to study the question in greater detail should refer to the special publications of the Permanent Standardisation Commission.

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LEAGUE OF NATIONS.

C.H. 832  
Errata.

ERRATA.

On page 34, lines 30 to 33 should read as follows:

"that these scientists in their experiments on guinea pigs, employed the method of intraperitoneal injections; therefore, in the case of these animals, which are comparatively insensitive to the toxin, the conditions were favourable for poisoning by endotoxin."

On page 50, line 9, counting from the bottom, instead of "B. Perfringens", read "vibrio septique."

end in view, the first International Conference on the Standardisation of Sera and Serological Tests was held on December 12th to 14th, 1921, meeting in London at the invitation of the British Ministry of Health. In order to deal with the questions under

# THE INTERNATIONAL STANDARDISATION OF THERAPEUTIC SERA AND BACTERIAL PRODUCTS.

*Work of the Permanent Commission on Standardisation of Sera,  
Serological Reactions and Biological Products.*

BY C. PRAUSNITZ.

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The results so far achieved in the international standardisation of medicaments, therapeutic sera and bacterial products through the agency of the League of Nations Health Organisation have already led to the adoption of uniform standards for a number of preparations having important practical uses. A comprehensive survey of the work done on pharmacological preparations has already been made by Professor KNAFFL-LENZ, and the following pages are intended to give a general account of the results obtained in the international standardisation of therapeutic sera and vaccines. In this branch, international standard preparations for

Anti-diphtheritic serum,  
Anti-tetanic serum, and  
Anti-dysenteric serum

are now available for international use. The means whereby international agreement could be secured in such matters are of interest to all authorities or other organisations contemplating the adjustment of their own national standards or of other preparations to the international standard. It is particularly with the theoretical and practical conditions governing the preparation, preservation and general use of the standard products that they are most directly concerned.

## HISTORICAL REVIEW.

It was at its second session (Geneva, October 20th to 22nd, 1921) that the Health Committee decided to investigate this group of questions. The first subjects to be taken up were the standardisation of diphtheria, tetanus, dysentery, meningococcus and pneumococcus sera, and the serodiagnosis of syphilis. With this end in view, the first International Conference on the Standardisation of Sera and Serological Tests was held on December 12th to 14th, 1921, meeting in London at the invitation of the British Ministry of Health. In order to deal with the questions under

review, four Sub-Committees were appointed to consider respectively : diphtheria and tetanus serum, dysentery serum, meningococcus and pneumococcus serum, diagnosis of syphilis. They recommended that the necessary preliminary work should be assigned to the institutes having special experience in the particular branches concerned. By comparative experimental research, these were to work out agreed uniform methods of titration and agreed specifications of international therapeutic units ; an endeavour was also to be made, on the same lines, to select and compare those methods of sero-diagnosis of syphilis which are especially suitable for practical employment. The State Serum Institute at Copenhagen was chosen as the Health Organisation's central laboratory, to which each Institute was to send the records of its experiments, as well as the cultures and reagents, for distribution to the other institutes concerned.

For the discussion of all relevant matters, the Health Committee set up a Permanent Standardisation Commission, which has since held a series of sessions. As various problems began to approach solution, the range of subjects was gradually extended. The most important sessions were :

The International Conference on the Standardisation of Sera and Serological Tests, held from December 12th to 14th, 1921, at the British Ministry of Health, London.

Meeting of the Sub-Commission on Anti-diphtheritic and Anti-tetanic Sera, held at Geneva in September 1922.

International Conference on the Standardisation of Sera and Serological Tests, held from November 20th to 26th, 1922, at the Pasteur Institute, Paris.

Technical Conference for the Study of Certain Methods of Biological Standardisation, held at Edinburgh from July 19th to 21st, 1923.

Technical Laboratory Conference, held at Copenhagen from November 19th to December 3rd, 1923.

Conference for the Standardisation of Anti-dysentery Sera, held at Geneva in September 1924.

International Conference on the Biological Standardisation of Certain Remedies, held at Geneva from August 31st to September 3rd, 1925.

Meeting of the Permanent Standardisation Commission, held at Geneva from October 11th to 13th, 1926.

Meeting of the Permanent Commission on Standardisation of Sera, Serological Reactions and Biological Products, held at Frankfurt-on-the-Main from April 25th to 28th, 1928.

Laboratory Conference on the Serodiagnosis of Syphilis, held at Copenhagen from May 21st to June 4th, 1928.

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# GENERAL PRELIMINARY REMARKS ON THE TITRATION OF THERAPEUTIC SERA.

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Since the potency of sera cannot be tested by chemical or physico-chemical means, the usual method is to observe accurately their effects. We may distinguish, in the first place, between "antitoxic" and "anti-infectious" sera.

## (a) TITRATION OF ANTITOXIC SERA.

Antitoxic sera may be suitably taken as a starting-point for discussion. Here matters are still comparatively simple, seeing that toxin and antitoxin can be accurately titrated one against the other, for, since both substances specifically neutralise each other, the principle operating here, and, so far as we are aware, here only, is the law of multiple proportions.

As an example of antitoxic sera, we will take diphtheria serum, which has been most carefully investigated. To begin with, it was titrated by determination of the quantity of serum required to protect the guinea-pig against a lethal dose of living virulent diphtheria bacillus. It was seen, however, that the difficulties caused by differences in individual resistance are particularly great precisely when living culture is used. For the results to have even some slight practical value, this technique would have required the use of large numbers of animals on each occasion, and, even then, the inevitable differences in the resistance of particular breeds and in the virulence of the cultures would have been so great that the results obtained in the various laboratories of the world would never have been comparable *inter se*. BEHRING'S innovation in carrying out serum tests with *diphtheria toxin* (broth culture filtrate) was therefore a first step in the right direction. Taking this as a starting-point, EURLICH determined the potency of his serum as the quantity which, when mixed with ten lethal doses of diphtheria toxin and subcutaneously injected into 250-gms. guinea-pigs, only caused a slight local reaction, which had disappeared by the fourth day. It soon appeared, however, that even this method did not yield equivalent potency values in different laboratories, the reason proving to be the heterogeneous and the variable composition of the diphtheria toxin. EURLICH'S inference that diphtheria toxin consists of toxic components of different avidity for antitoxin was of heuristic value, while the demonstration of the fact that toxin, though losing some of its toxicity on ageing, still requires the same quantity of antitoxin for its neutralisation, was important as a basis for subsequent work. This explains at once the observed inconstancy in the ratio of the minimum lethal dose to the neutralised dose, both in a number of different toxins and one and the same toxin at different times. The toxin-neutralising substances of antitoxic serum appear, on the contrary, to be homogeneous. If the serum is properly preserved, they remain stable, so far as we know, for a very long time. These considerations induced EURLICH to choose *the neutralising power of antitoxin as the unit* instead of the minimum lethal dose of the toxin. A serum with which he happened to be working at the time was arbitrarily chosen as the antitoxin standard. In order to test the antitoxin content of unknown

sera, the diphtheria-toxin-neutralising power of the standard serum is compared with that of the serum to be tested.

Constancy of the standard antitoxin is ensured when all factors liable to disturb the stability of organic colloids, *i.e.*, light, warmth, oxygen and water, are, so far as possible, eliminated. The serum is therefore first dried in the desiccator, then freed from the last traces of water over phosphoric anhydrid ( $P_2O_5$ ) in an air-exhausted tube, and kept in an ice-chest in darkness. It having been the experience of the Frankfort Institute that various parts of the serum acquire solubility in water in varying degrees on desiccation, the practice of that Institute is to discard the large flat flakes formed on the surface and the finest powdery substance. In order to remove them, the dry serum is roughly ground in a mortar and then passed through a double sieve, so that the large flakes are caught by the coarse upper sieve, whilst the fine powder drops through the fine lower sieve. The medium-sized flakes which lie on the lower sieve are then (*cf.* Figure 1) loaded

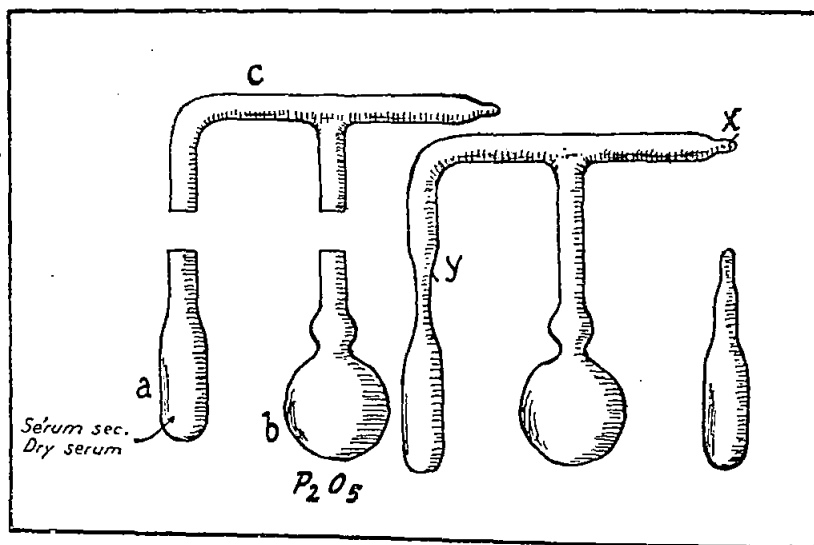


Fig. 1

into a tube (a); and the spherical glass vessel (b) is loaded with  $P_2O_5$  powder. The apparatus is then fused together, connected up with a vacuum pump and sealed by fusion at x half an hour after exhaustion. The container is left in cold storage for two or three months, being shaken up from time to time in order to bring successive dry layers of the powder to the surface. Finally, when the last traces of water can be assumed to have been removed, the serum tube is sealed off at y. For use, the contents of the tube are dissolved in a mixture of two parts of glycerine and one part of ten per cent NaCl solution. The quantity of diluent required is indicated in each case by the Institute. This serum will keep for at least two months if stored in a cool, dark place.

This standard serum solution is now used from time to time for the adjustment of the "test toxin". For this purpose, a toxin is used which has become fairly constant as a result of at least one year's storage; such a toxin preserved under toluene can normally be used for two months without deteriorating.

Decreasing quantities of the test toxin are mixed in a series of tubes containing each 1 "I.U." (immunising unit) of the standard

serum suspended in 1 c.c. of physiological NaCl solution ; the mixture in each tube is then raised to a volume of 4 c.c. by adding physiological solution. These mixtures are injected, always *subcutaneously*, into 250-gms. guinea-pigs. The toxin is thus no longer measured by its toxicity, but by its neutralising capacity in relation to 1 I.U. of the standard antitoxin. EHRLICH uses the symbol "L<sub>0</sub>" to denote the greatest quantity of toxin which, when mixed with 1 I.U., may be injected into the guinea-pig without inducing either local or general reaction due to diphtheria poisoning. He uses the symbol "L<sub>+</sub>" to denote the smallest quantity of toxin which, when mixed with 1 I.U., kills guinea-pigs on the fourth day. The determination of "L<sub>+</sub>" can be carried out with finer precision, and this method is hence always used nowadays. It enables constant results to be secured. OTTO and HERTSCH claim that, with accurate quantitative work and proper care of the animals, the margin of error can be kept below 2 per cent. *The value of the method thus depends essentially upon the use of the standard serum as a unit of measurement.*

This method has been generally acknowledged to rank first as a means of determining the antitoxin content of diphtheria serum. Certain research workers believe, however, that the effect of the diphtheria bacillus on the human organism is not due to toxin production alone, but that other biological influences—possibly of an endotoxic nature—are also at work as contributory causes.

R. KRAUS and his collaborators determine the "curative power" of diphtheria serum, believing that, apart from the I.U. content, the *speed of reaction* with the toxin ("avidity") is also a factor in the therapeutic efficacy of serum in man. As to the parallelism of these two properties, a lengthy controversy has arisen between the Vienna workers and those of the Frankfort and the Robert Koch Institutes.

The question entered on a new stage with the promising investigations of RAMON into specific *diphtheria flocculation*, which occurs in a diphtheria toxin-antitoxin mixture. If to a fixed volume of toxin decreasing quantities of serum are added, flocculation will begin only in the mixture where toxin and antitoxin neutralise each other. A very large measure of correspondence is found to exist between the results of this test and the I.U. content as determined by the EHRLICH method ; since, however, appreciable divergencies have been observed (though seldom) between the two, the flocculation method can, in the opinion of the Standardisation Commission, only be used at present in tests for general guidance.

But it derives special value also from the fact that the speed with which the initial flocculation occurs affords a *reliable measure of the speed of mutual neutralisation of antigen and antibody*. According to the experiments of MADSEN and SCHMIDT, the speed of the reaction as determined by the Ramon process and by intravenous injections in rabbits is, apart from the antitoxin content, a determining factor in curing rabbits injected with diphtheria toxin. Large doses of antitoxin of slowly reacting sera saved a smaller number of animals than smaller doses of antitoxin of quickly reacting sera. The significant fact was also established that the speed of reaction and therapeutic efficacy of the sera may be impaired if antitoxin is purified through precipitation by certain salts. The present apparent increase in the severity of human diphtheria in Europe should enable this highly important group of questions to be elucidated.

By way of conclusion, it should be said that, in the present state of our knowledge, the EHRLICH process of indirect titration by the aid of preserved standard sera is likely to afford the highest degree of accuracy in the determination of the antitoxin content of antitoxic sera. Beyond this, the idea of a standard serum as an arbitrarily fixed, but permanently stable, unit has also proved fruitful in connection with the anti-infectious sera.

(b) TITRATION OF ANTI-INFECTIOUS SERA.

This is a much more intricate question than that of testing antitoxic sera, since a larger number of immunity reactions can be brought into play. It may be justifiably assumed that practically every such reaction is due to the action of a particular antibody—agglutinins, precipitins, complement-fixing antibodies, bacteriolysins, bacteriocidins, bacteriotropins (= immune opsonins), anti-endotoxins, anti-aggressins, and other unknown antibodies. The production curve of the first three substances, which are evidently closely related, does not always run parallel with the protective and curative value of the serum. *Agglutinins* have been little used for titration; the same applies to *precipitins*, if we except RAMON'S flocculation test (p. 13). For certain purposes, on the other hand (especially the testing of meningococcus sera), BORDET'S *complement-fixation test* has been widely recommended, because the antibodies concerned increase typically in the course of immunisation and remain stable for a long time, whilst their specificity can be accurately demonstrated. As an antigen for these tests, the Frankfort Institute of Experimental Therapy uses meningococcus suspensions dissolved by treatment with antiformin, neutralised by the addition of sulphuric acid and sodium sulphite and kept under toluene until the potency becomes constant. Such extracts, which will keep for years, are tested against the standard serum, in comparative series, simultaneously with the serum to be titrated.

The oldest method of titrating anti-infectious sera is the *bacteriolysis* test by the PFEIFFER method. The technique is based upon the determination of the quantity of serum which, when intraperitoneally injected into 200-gms. guinea-pigs simultaneously with a "normal loopful" (= 2 mg.) of bacterial growth on agar, causes complete dissolution of the bacteria—usually within one hour. In trained hands, it operates with a margin of error not exceeding 5 per cent, but has the drawback that it can only be used with a small number of bacteria and that it requires, furthermore, cultures of adequate virulence. In their investigation of the so-called "anti-endotoxic serum against *B. typhosus*", PFEIFFER and BESSAU found that, in the abdominal cavity of guinea-pigs, the process of bacteriolysis was also followed by destruction of the endotoxic poisons; in contrast, however, with the toxin-antitoxin reaction, *this destruction of endotoxic poisons does not follow the law of multiple proportions*. The process is thus clearly not one of toxin neutralisation; PFEIFFER regarded this phenomenon rather as a breaking-down of the endotoxins by fermentation, brought about by the same antibodies as cause bacteriolysis.

An alternative method, described by NEISSER and WECHSBERG, is to use as an indicator, not the dissolution of bacteria in the abdominal cavity of animals, but the *bactericidal action in vitro*; but in this "bactericidal antibody" test there are a number of sources of error which can only be avoided by great care in matters of technique.

The *bacteriotropin* test has proved successful with one or two immune sera. Two techniques are now available for the purpose: (a) the WRIGHT method, which was primarily intended for clinical purposes. Serum, bacterial emulsion and leucocyte suspension are incubated for 15 minutes at 37°C.; the mean number of bacteria ingested per 100 leucocytes is then counted on smear preparations. (b) The NEUFELD method, in which the percentage of phagocytling polymorphonuclear leucocytes is ascertained. By way of example, we may take the official testing of antimeningococcus sera for bacteriotropins, which is carried out in Germany as follows (Institute of Experimental Therapy Frankfort): (1) 300 to 350-gms. guinea-pigs are intraperitoneally injected with a mixture of 10 c.c. broth and 0.5 gr. aleuronat; the next day the animals are slaughtered, and the abdominal cavity is washed out with 50 c.c.

aqueous solution of 0.1 per cent sodium citrate and 0.85 per cent NaCl at body temperature. The liquid (freed from the coarser particles) is centrifuged for three or four minutes only at a low speed of revolution in order not to damage the leucocytes, and then poured off; the sediment is again washed once with physiological NaCl solution and as gently centrifuged; it is then suspended in physiological NaCl solution at body temperature. (2) The bacterial growth must be abundant; it is ground up to a homogeneous emulsion with a mixture of equal parts of broth and physiological NaCl solution, until no visible clumps are left. The controls must show that the bacteria are not phagocyted in the absence of serum, and that they have not degenerated (do not stain badly). (3) One drop of bacterial emulsion is added in each case to 0.1 c.c. of decreasing dilutions of the inactivated serum, as well as to a control with 0.1 c.c. of physiological NaCl solution. The mixtures are left to stand at 37° C. for three-quarters of an hour, after which two drops of leucocyte suspension are added, and are then kept for three-quarters of an hour at 37° C., being repeatedly shaken by reversing the container. The liquid contents of the tubes are then poured off from the deposit of leucocytes, and the sediment is microscopically examined for the presence and degree of phagocytosis.

None of the above-mentioned methods of titrating anti-infectious sera is entirely satisfactory, since none can be used for all sera. A number of therapeutic sera still give good curative effects in animals, even after the particular antibodies concerned have been completely exhausted by digestion with the homologous bacteria and removed by centrifugation; it must therefore be assumed that, apart from the known antibodies, yet other immunising factors are brought into operation. There are, moreover, a number of immune sera, *e.g.*, anthrax-serum, which are highly effective in animal tests but do not give any one of the reactions described.

It has therefore been found a reliable plan, for a number of infectious sera, to test their *protective* and *curative* power on laboratory animals. For this purpose, it is essential to have a standard dry serum, preserved in the manner described above and a culture (or virus as the case may be) of constant virulence. Mixtures of uniform numbers of bacteria, or of uniform virus dilutions, with decreasing quantities of standard serum on the one hand, and of the serum to be tested on the other, are injected into the laboratory animals in comparative experimental series. In order to eliminate the sources of error introduced by the frequently appreciable variations in the individual resistance of the animals, large numbers of animals must be used. There is, moreover, no hope of achieving such accuracy as in the toxin-antitoxin determination. This method does, nevertheless, permit of an approximate estimation of titre and of the elimination of such animals, at any rate, as have reacted inadequately to the preliminary immunising treatment.

## I. ANTI-DIPHThERITIC SERUM.

As a result of the work of EHRlich described in the previous chapter, it became the practice of all countries to titrate their serum uniformly by this method; in most institutes it was used exclusively. Whilst, in former times, the Frankfort Institute had supplied this standard serum to most producing and testing institutes of the world, this practice had ceased since the beginning of the world war. It was only in Frankfort and Washington that the original standard was still preserved. After 1914,

some of the institutes had obtained supplies of standard serum from Washington, whilst others had reverted to the original BEHRING method, adjusting their sera to diphtheria toxins which were maintained as constant as possible. When, therefore, the Standardisation Commission began its work (London, 1921), the first thing to be ascertained was whether the two standard preparations had remained equivalent during the intervening seven years. It was decided to organise comparative tests of the two preparations at the institutes at Frankfort, Washington and elsewhere. Considerations of possible curative effects of the diphtheria serum other than antitoxic were deliberately excluded to begin with. The examination of the two, Frankfort and Washington, standards by the Frankfort, Copenhagen, Rome and Washington Institutes showed that the two samples were almost completely identical. The following abridged protocol of one of the tests performed at Copenhagen (Table I) may be reproduced as an illustration.

Table I.

COMPARISON OF THE FRANKFORT AND WASHINGTON DIPHTHERIA "STANDARD" SERA.

Date	Toxin	1 I.U. of standard antitoxin	L+
2.III.1922	Danish test toxin	Frankfort (recd. 21.III)	0.82
6.III.1922	" " "	"	0.80
22.III.1922	" " "	"	0.82
27.III.1922	" " "	"	0.82
2.III.1922	" " "	Washington (recd. 2.III)	0.82
6.III.1922	" " "	"	0.80
22.III.1922	German text toxin	Frankfort (recd. 21.III)	<0.59
27.III.1922	" " "	"	0.57
8.IV.1922	" " "	"	0.57
22.III.1922	" " "	Washington (recd. 2.III)	<0.59
27.III.1922	" " "	"	0.57

The Sub-Commission for the Investigation of Diphtheria and Tetanus Therapeutic Sera (Geneva, 1922) therefore recommended that the EHRLICH process for titrating diphtheria serum should be adopted in principle and carried out on the basis of the two, practically identical, Frankfort and Washington standards. In order to ensure the constancy, in the future, of the sera used in various countries, Professor MADSEN was requested by the Standardisation Commission (Paris, 1922) to undertake the annual comparison of the two units at his institute, which would act as the Health Organisation Central Laboratory. According to the Standardisation Commission's

reports (Geneva, 1926), the standards kept in Copenhagen, London and Washington have proved completely equivalent. The Standardisation Commission (Frankfort, 1928) had before it reports on this subject from the four institutes mentioned, and from the Paris Pasteur Institute as well, which showed the same equivalence of their standards.

As regards the RAMON method of testing sera by the flocculation reaction, the Standardisation Commission (Geneva, 1926) expressed the opinion that it yielded satisfactory results for purposes of current titration. At the session at Frankfort, 1928, it was decided to investigate it more closely. As regards the question of speed of flocculation, cf. p. 9.

## II. ANTI-TETANUS SERUM.

Theoretically, the case is similar to that of diphtheria serum ; the practical difficulties are greater, however, since three different units (Frankfort, Paris and Washington) and a variety of testing methods were in use. Matters are still further complicated by the much greater liability of tetanus toxin to deterioration.

Originally, VON BEHRING used a dried serum obtained by precipitation as unit. He mixed 1 c.c. of a 1 per cent dilution (in physiological NaCl solution) of the serum to be tested with 38 c.c. of distilled water and 1 c.c. of the toxin used for testing purposes (described by him as a "1/10th of normal" test toxin) ; after being left to stand at room temperature for 30 minutes, 1/100th part, or 0.4 c.c., of this mixture was subcutaneously injected into a mouse. If the animal remained healthy, the serum contained at least 1/10th I.U. per c.c., seeing that 1 c.c. of 1 per cent serum was equivalent to 1 c.c. of the "1/10th of normal" test toxin. It is on this foundation that the Frankfort method of testing sera has been built up.

(a) In order to obtain a toxin as free from spores and as stable as possible, the crude toxin (dextrose broth culture) is precipitated, in *Frankfort*, by the aid of ammonium sulphate, and the deposit dissolved in physiological NaCl solution and centrifuged for one hour at 4,000 r.p.m. The supernatant fluid is again precipitated, and the precipitate dissolved and centrifuged ; this operation is repeated twice. Finally, the clear fluid is dried *in vacuo* in earthenware dishes, pulverised and dissolved in distilled water (in the proportion of 1 part of powder to 2 of water). Accurately measured quantities of this solution are placed into Ehrlich vacuum tubes and dried and preserved in the same manner as the standard diphtheria serum. This product will even stand being kept at 37° C. for a month and may be regarded as constant. For use, the contents of the tube are dissolved in the requisite quantity of water,<sup>1</sup> this yielding the "normal test-toxin solution". It having been the experience of the Frankfort Institute that tetanus dry serum does not keep as well as the diphtheria standard serum after being dissolved in glycerinated saline solution, it is stored exclusively in *ad hoc* vacuum ampoules until required for use. For use, the contents of the tube are dissolved in the requisite quantity

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<sup>1</sup> The dry serum at present in use is dissolved in 33 c.c. of water.

of physiological NaCl solution,<sup>1</sup> in order that 1 c.c. of the solution may contain 1/100th I.U. For testing sera, 1 c.c. of such a dilution of the serum to be tested as should, according to the indication of the factory, contain 1/100th I.U. is mixed with eight increasing doses of the normal test-toxin solution (at present, 0.8 to 1.5 c.c.), and then filled up to a uniform volume of 4 c.c. in all the tubes with physiological saline. Simultaneously, 1/100th I.U. of the standard serum is mixed in the same proportions with the same quantity of the test toxin and physiological NaCl solution. All sixteen mixtures are protected from the light and left to stand at room temperature for half an hour. Of each mixture, 1/10th part, or 0.4 c.c. (equivalent to 1/1000th I.U., plus 0.08 to 0.15 c.c. normal test-toxin solution), is subcutaneously injected into the right hind leg of a 15-gms. mouse. If the experiments yield exactly the same results in both series, the serum to be tested has at least the requisite titre. The test thus determines both  $L_0$  and  $L_+$ . In *Germany* "fourfold" and "sixfold" sera have been supplied up till now: that is to say, sera containing 4 and 6 I.U. per c.c.

(b) The *Danish* method also involves the use of mice, but, as distinct from the German test, it determines the  $L_0$  value. The toxin used is a liquid toxin which is kept under toluene at 2°-4° C. and remains sufficiently stable under these conditions. Titration in regard to the standard serum is made every two months. The toxin is injected into 20-gms. mice at the root of the tail. The threshold value, at which no deviation of the tail from the normal occurs within five days, is taken as  $L_0$ .<sup>2</sup>

(c) A method somewhat different in principle though similar in its results is that introduced in the *United States* by ROSENAU and ANDERSON. The laboratory animals used are 350-gms. guinea-pigs (four animals for each toxin-antitoxin mixture). Four c.c. of the particular mixtures are subcutaneously injected into the abdomen on a level with the umbilicus. The "unit" is defined as ten times the smallest quantity of serum which, when used together with the official test toxin dose, after standing at room temperature for an hour, keeps the animal alive for four days. The test dose contains something under 100 minimum lethal doses, seeing that a mixture of 1/10th unit and 100 minimum lethal doses of the test toxin must kill a guinea-pig in exactly four days. It is hence not quite correct to describe the American method as a determination of  $L_+$ .

(d) In *France*, it was the practice to determine the *curative value*. The guinea-pigs were given, first, decreasing quantities of serum (1/500,000 down to 1/10,000,000 c.c.) and, from 10 to 20 hours later, a single lethal dose of tetanus toxin; the reciprocal of the serum dilution (usually 1,000,000), which enabled the animals to survive, was taken as the titre of the serum.

(e) In *Italy*, since the TIZZONI test has been abandoned, 0.1 American unit, plus 100 minimum lethal doses, are given to two guinea-pigs in each case in 3 c.c. subcutaneous injections, this being GOSRO's method.

These very considerable differences made the need for the establishment of an international tetanus serum unit particularly urgent. The Standardisation Commission (London, 1921) accordingly requested the Frankfort and Washington Institutes to distribute samples of their standard sera to all the institutes participating in the investigation. Thereupon a discussion was to take place concerning the best method of titration and

<sup>1</sup> At the present time, 27 c.c.

<sup>2</sup> It must be borne in mind that the volume to be injected should not exceed 0.5 c.c.



an attempt was to be made to establish an international unit for the serum. In particular, the most suitable method of keeping and periodically controlling this international unit was to be considered. The preliminary work was carried out by GOSIO, KOLLE, MCCOY, MADSEN and MARTIN, and the results were laid before the Sub-Commission on Diphtheria and Tetanus Serum, which met in Paris under the chairmanship of MARTIN (September 1922). An extract from the records of these investigations is given in abridged form in Table III below. Before this, however, we reproduce a detailed protocol of the Copenhagen Institute to show the accuracy with which test toxin can be adjusted against standard antitoxin by the Danish method (Table II).

Table II.

ADJUSTMENT OF A DANISH TEST TOXIN AGAINST THE AMERICAN ANTITOXIN UNIT BY THE DANISH METHOD.

Decreasing doses of Toxin No. 146 with 0.1 American unit.

Quantity of toxin	Condition of animals after <sup>1</sup> :				
	1 day	2 days	3 days	4 days	5 days
0.135	0	+	++	dead	
0.130	0	?	++	+++	dead
0.125	0	0	++	+++	dead
0.120	0	0	+	+++	dead
0.115	0	0	?	++	++
0.110	0	0	0	0	0
0.105	0	0	0	0	0
0.100	0	0	0	0	0
0.095	0	0	0	0	0
0.090	0	0	0	0	0

By this method, the three standard sera of Copenhagen, Paris and Washington were compared with each other (Table III).

<sup>1</sup> 0 = no tetanus; ? = doubtful tetanus; + = slight tetanus; ++ = moderate tetanus; +++ = severe tetanus.

Table III.

COMPARISON OF THE FRANKFORT, PARIS AND WASHINGTON STANDARD ANTITOXINS BY THE DANISH METHOD.

Danish test toxin.

Date of test	Source of antitoxin	Toxin (c.c.)	Neutralised by + antitoxin
6.v. 1922	German (3.iv.17) <sup>1</sup>	0.034	0.001 German I.U.
3.v. 1922	" (21.iii.22)	0.034	" " "
6.v. 1922	" "	0.034	" " "
10.v. 1922	" "	0.034	" " "
15.viii.1922	" (1.viii.22)	0.039	" " "
19.viii.1922	" "	0.04	" " "
31.viii.1922	" (19.viii.22)	0.04	" " "
5.ix. 1922	" "	0.04	" " "
9.ix. 1922	" "	0.04	" " "
13.ix. 1922	" "	0.04	" " "
10.x. 1922	" "	0.04	" " "
28.x. 1922	" "	0.04	" " "
27.v. 1922	French (19.v.22)	0.034	3.75 French Units
3.vi. 1922	" "	0.034	3.75 " "
10.v. 1922	American (26.iv.22)	0.034	0.06-0.065 Amer. Units
16.v. 1922	" "	0.034	0.06-0.065 " "
31.viii.1922	" (30.viii.22)	0.04	0.068 " "
5.ix. 1922	" "	0.04	0.068 " "
9.ix. 1922	" "	0.04	0.07 " "
18 ix. 1922	" "	0.04	0.07 " "
17.x. 1922	" "	0.04	0.07 " "
28.x. 1922	" "	0.04	0.07 " "

According to this method of test, therefore, 1 German unit = approximately 66 American = 3,750 French units. Moreover, the ratio of 1 : 66 has since remained practically constant, as will be seen from the reports submitted to the Standardisation Commission (Geneva 1926) by the Frankfort, Copenhagen and Washington Institutes.

Whilst these experiments, performed on mice and with the  $L_0$  value as a basis, agree well one with the other, the comparison of  $L_0$  and  $L_+$  values on mice and guinea-pigs revealed notable differences. To begin with, the  $L_0$  and  $L_+$  of the Danish test toxin was determined on mice and guinea-pigs against the German standard serum. (The  $L_0$  value is the dose producing no tetanus by the fourth day ;  $L_+$  the dose which kills the animal on the fourth day.)

<sup>1</sup> The figures in parentheses give the date on which the particular samples of serum reached Copenhagen.

Table IV.

GERMAN STANDARD SERUM AGAINST DANISH TEST TOXIN.

Date of test	L <sub>0</sub>		L <sub>+</sub>	
	Mice	Guinea-pigs	Mice	Guinea-pigs
9.ix.1922	0.04	—	—	—
13.ix.1922	0.04	—	—	0.048
10.x. 1922	0.04	0.04	0.048	0.048
28.x. 1922	0.04	0.04	0.048	0.048

The American standard serum was then tested against the L<sub>0</sub> and L<sub>+</sub> values of the Danish toxin as determined, on mice and guinea-pigs, in relation to the German standard serum.

Table V.

Date of test	Test for L <sub>0</sub> with 0.04 c.c. toxin		Test for L <sub>+</sub> with 0.048 c.c. toxin	
	Mice	Guinea-pigs	Mice	Guinea-pigs
9.ix.1922	70	—	—	—
18.ix.1922	70	—	—	80
17.x. 1922	70	<74	>76	76-80
28.x. 1922	70	72	80	76-80

This series shows the extraordinary difficulty of securing accurate results with the L<sub>+</sub> process, as the point on the scale at which the animal dies exactly on the fourth day is frequently indeterminate. In this, differences in the animals themselves, as well as in the conditions under which they happen to be kept, etc., play an important part. MADSEN found the determination of the L<sub>0</sub> value on mice both simple and reliable; even the slightest trace of tetanus can be detected by deviation of the tail from the axis of symmetry.

Reports on further comparative tests of the German and American standard serum were sent in by GOSIO, KOLLE and McCoy as follows :

GOSIO : 1 German unit = 65-70 American units.

KOLLE : 1 German unit = about 66 American units.

McCoy : 1 German unit = somewhat less than 66.6 American units.

It was plain that there was urgent need of eliminating these disturbing differences between the various units and of setting up a new international unit. But the enormous disparity of the various units was in itself a difficulty in the way of such agreement, since the medical profession in each country was accustomed to its old range of doses. KOLLE expressed his readiness to propose that the German unit should be changed in such a manner that the new unit would be one-half of the American ; this would have the further advantage that the number of serum units to be administered to patients would then be of the same order of magnitude in the case of diphtheria and tetanus serum. MARTIN proposed that this new German unit should be made the international unit. This rule has now been accepted by the European members of the Permanent Standardisation Commission. On the packets intended for export, the American producers indicate the content in terms of the "new" units as well as in American units.

The Copenhagen Institute was requested to keep a permanent check on the equivalence of the various standard sera by annual comparative examinations. A review of the investigations carried out in this direction during recent years and of the comparative tests performed by the American method (p. 14) at the National Institute for Medical Research in London and at the Hygienic Laboratory at Washington shows that the results are entirely coincident (cf. Table VI). This is all the more noteworthy since the testing methods adopted in the three countries differ appreciably, as already mentioned. The solution thus reached, therefore, constitutes a very notable advance in the direction contemplated.

In view of the experiments here described, and of the complete identity of results obtained in further comparative experiments in Frankfort, London and Paris, the Standardisation Commission submitted the following proposal to the Health Organisation in 1928 :

" The unit fixed at the Geneva Conference (1926) is suitable for international use and can now be finally adopted. "

Table VI.

COMPARATIVE TITRATION OF VARIOUS TOXINS AND ANTITOXINS BY THE DANISH AND THE AMERICAN METHOD IN COPENHAGEN, LONDON AND WASHINGTON.

Date of test	Place of test	Method	Source of :		Neutralisation with :		Remarks
			Toxin	Antitoxin	Toxin	+ Antitoxin	
1927 12.III	Copenhagen	Danish	Dan. 146	Amer. Stand. Serum	0.110	0.1 Amer. Unit	L <sub>o</sub> = 0.110 L <sub>o</sub> = 0.110
18.III.	"	"	"	"	0.110	0.1 "	
18.III.	"	"	"	Dan. Stand. Serum A	0.110	0.2 Int. Unit	0.2 Int. Unit = 0.1 Amer. Unit "
26.III.	"	"	"	"	0.110	0.2 "	
1.X.	"	"	"	"	0.125	0.2 "	L <sub>o</sub> = 0.125 L <sub>o</sub> = 0.125 L <sub>o</sub> = 0.125
6.X.	"	"	"	Engl. Stand. Serum	0.125	0.2 "	
"	"	"	"	"	0.125	0.2 "	
1928 11.II.	"	"	"	Dan. Stand. Serum A	0.135	0.2 "	L <sub>o</sub> = 0.135 L <sub>o</sub> = 0.135
17.II.	"	"	"	Eng. Stand. Serum (Jan. 1928)	0.135	0.2 "	
"	"	"	"	German Stand. Serum (Dec. 1927)	0.135	0.2 "	L <sub>o</sub> = 0.135

Table VI (continued).

Date of test	Place of test	Method	Source of :		Neutralisation with :		Remarks
			Toxin	Antitoxin	Toxin	+ Antitoxin	
23.II.	Copenhagen	Danish	Dan. 146	Dan. Stand. Serum A	0.135	0.2 Int. Unit	Lo = 0.135
"	"	"	"	Engl. Stand. Serum	0.135	0.2 "	Lo = 0.135
"	"	"	"	German Stand. Serum	0.135	0.2 "	Lo = 0.135
1927 26.IV	Washington	American	Amer. Toxin G.	Amer. Stand. Serum	0.00078	0.1 Amer. Unit	0.1 Amer. Unit = 0.2 Int. Unit
"	"	"	"	Dan. Stand. Serum	0.00078	0.2 Int. Unit	
1928 Feb.	London	American	Eng. Toxin E.	Dan. Stand. Serum	0.095	0.24 Int. Unit <sup>1</sup>	
"	"	"	"	Amer. Stand. Serum	0.095	0.24 "	
"	"	"	"	Engl. Stand. Serum	0.095	0.24 "	
"	"	"	Eng. Toxin L.C.	Dan. Stand. Serum	0.0475	0.22 "	
"	"	"	"	Amer. Stand. Serum	0.0475	0.22 "	
"	"	"	"	Engl. Stand. Serum	0.0475	0.24 "	

<sup>1</sup> The value which the American Laboratory had given in terms of American Units was converted into International Units (Ratio 1 : 2).

### III. ANTI-DYSENTERY (SHIGA-KRUSE) SERUM.

#### 1. PRELIMINARY REMARKS.

For a variety of reasons, the problem is essentially more intricate in this case than in that of diphtheria and tetanus therapeutic serum.

The multiplicity of the causative agents of dysentery, for whose designation not a few letters of the alphabet have been used up, suggests polyvalence in dysentery therapeutic sera as a first desideratum. It is difficult, however, to draw the line, and production has usually been limited to that of therapeutic sera against the type which behaves, both in culture and serological reactions, in a particularly characteristic and uniform manner, and stands in a class apart as causing in most cases a more severe form of disease, *i.e.*, the Shiga-Kruse bacillus. Since in dysentery, as in diphtheria, the agents are localised almost exclusively on and in the intestinal mucous membrane, the morbid process must be attributed to the effects of bacterial *toxins*, whilst penetration of the agents into the body itself need not, for all practical purposes, be considered. In respect of toxin production, again, the Shiga-Kruse bacillus occupies a special place among dysentery agents owing to its ability to produce exotoxins, which are neutralised by specific antitoxin in accordance with the law of multiple proportions.

Of the earlier investigations into the Shiga toxin, we may briefly mention the well-known work (1903) of VAILLARD, DOPTER, TODD, ROSENTHAL, CONRADI, NEISSER and SHIGA, which demonstrated the possibility of obtaining soluble toxins from Shiga bacilli by a number of different processes. The laboratory animals most frequently used in tests for the presence of these toxins were rabbits, guinea-pigs and mice. Whilst DOPTER described alterations in the grey matter of the central nervous system which are characteristic in animal tests, FLEXNER laid particular stress on the severe lesions of the large intestine, resembling those of human dysentery. As early as 1907, DOERR had described both the nervous and the intestinal symptoms in mice and rabbits and had found that, when heated, the toxin lost its effect on the nervous system earlier than that on the intestine. Whilst KRAUS and DOERR, especially, regarded the Shiga toxins as exotoxins, PREIFFER, together with UNGERMANN and BESSAU, contended that the endotoxins played a prominent part in the syndrome of *human* Shiga dysentery. BESSAU showed that Shiga toxin can produce two forms of poisoning in a rabbit, *i.e.*, the "paretic" and the "marantic". The paretic type is caused by an exotoxin, since this action of the toxin is specifically neutralised by the antiserum according to the law of multiple proportions, a fact which confirms KRAUS's conclusions. The marantic type, on the other hand, is brought about by endotoxins, since the action of the serum on it does not conform to the law of multiple proportions. This would also seem to apply to the intestinal lesions. In guinea-pigs, however, he could never detect the paretic type of poisoning and only found signs of acute and chronic endotoxin poisoning. The limits set to the action of the serum were hence as narrow in this case as in that of anti-infectious sera, *e.g.*, typhoid serum.

But what is wholly unprecedented and difficult to explain at present is the fact that the exotoxin of the Shiga bacillus is not a product given off primarily in liquid cultures, like the toxins of the diphtheria, tetanus, and other toxin-producing bacteria; it can,

in fact, also be obtained, by various processes of extraction, from the fresh bacillary bodies. In respect of localisation, it therefore corresponds to an endotoxin.

Among the further attempts to separate the two toxins, the work of OLITSKY and KLIGLER (1920) is of fundamental importance. These authors contend that the paralyses are produced by an exotoxin, and the intestinal symptoms by an endotoxin. They claim to have separated the two components :

1. By using cultures of different ages (young cultures are said to produce only nerve symptoms, older white-of-egg broth cultures, mainly intestinal, but also associated nerve, symptoms) ;
2. By heating (heating for one hour at 75° C. is said to destroy only the exotoxin, whilst the endotoxin is only destroyed at 90° C.) ;
3. By saturating the whole toxin with antitoxin serum, in which case it is said that the exotoxin is neutralised and not the endotoxin.

The investigations carried out by various workers at the request of the Standardisation Commission have, however, brought no confirmation whatever of these apparently highly characteristic differences between exotoxins and endotoxins.

For practical purposes, however, the important question is, of course, which of the various groups of symptoms observed in laboratory animals correspond to human dysentery. In human dysentery produced by Shiga bacilli the nervous phenomena are quite in the background ; the dominant feature of the clinical picture being the intestinal symptoms, which may be accompanied by marantic symptoms. Since, therefore, Shiga poisoning in man has a greater resemblance with the effect on the guinea-pig than with that on rabbits, PFEIFFER and BESSAU held that there was little prospect of an antitoxic Shiga serum succeeding in practice, and that any therapeutic value which such a serum might display would have to be attributed to anti-infectious action. SELTER took a similar view. It does not seem possible to settle this question at present. At the same time, the most recent work does point to the fact—as will be seen in the following pages—that the exotoxin plays the predominant part among the Shiga bacillus toxins.

It will therefore be sound policy to concentrate at first on the reliable titration of antitoxin in Shiga serum and, when this has been achieved, to ascertain by trial whether a Shiga antitoxin of high potency is suitable for administration to patients.

It is on these lines that the Standardisation Commission is proceeding in the work now to be described. At the first International Conference (1921) it was decided that :

1. The participating institutes should exchange their sera, cultures and toxins, and that further experiments should be carried out, using differing methods of titration and different species of animals.

2. The antitoxic as well as the anti-endotoxic potency of the sera should be tested, and that this could be carried out equally well with toxins and with killed Shiga cultures ; the Frankfort Institute would supply for these experiments a serum titrated in respect of antitoxin by the intravenous injection of toxin-antitoxin mixtures into mice ; tests should



be carried out on mice, rabbits and guinea-pigs ; the anti-bacterial potency of the sera was to be tested at the same time, employing living cultures for the purpose.

3. The preparation of polyvalent sera against different Shiga strains was unnecessary.

4. The preparation and titration of sera against other dysentery bacilli should not be discussed for the time being.

The following took part in the investigations connected with this scheme :

The Hygienic Institute at Basle (DOERR) ; the Robert Koch Institute, Berlin (NEUFELD) ; the Institute for Experimental Therapy, Bucharest (CANTACUZÈNE, JONESCU-MIHAESTI and COMBIESCU, CONTREA) ; the Institute for Experimental Therapy, Frankfort-on-the-Main (KOLLE, SCHLOSSBERGER, PRIGGE, HARTOCH) ; the State Serum Institute, Copenhagen (MADSEN, K. A. JENSEN) ; the Medical Research Council, London (DALE, DOUGLAS ; (with the assistance of the Lister Institute (MACCONKEY, PETRIE) and the Wellcome Physiological Laboratories (O'BRIEN, SÜDMERSEN, RUNGE, EAGLETON, TREVAN, BLAKE, OKELL) ; the Pasteur Institute, Paris (DOPTER, MARTIN, DUMAS) ; the Kitasato Institute, Tokio (SHIGA, KAWAMURA and TSUCHIYA) ; the Epidemiological Institute, Warsaw (HIRSZFELD, PRZESMYCKI, SEYDEL, SIERAKOWSKI), and its branch Institute at Cracow (GIESZCZYKIEWICZ, LIPINSKI).

The work of these institutes has enabled the most important points to be cleared up, and has now provided a sound basis for the standardisation of dysentery serum and the elucidation of a series of important questions relating to the nature of dysentery toxin. This work will now be reviewed in the light of reports received from the various research workers engaged in the investigations.

## 2. THE DYSENTERY TOXIN (SHIGA).

For the preparation of dysentery toxin, the following processes deserve special consideration :

(1) *Broth Toxin*. DOERR had already drawn attention, in the past, to the importance of the alkalinity of the broth. HIRSZFELD found that, when the Shiga bacillus grows in alkaline broth, the reaction of the latter becomes at first more acid and, later, strongly alkaline ; neutral broth becomes alkaline from the outset. Only when the pH value exceeds 8.0, or—best of all—if it reaches 8.5-8.6, are appreciable *toxin titres* obtained. He regards the reaction at the time of seeding as of minor importance, since a neutral or an even faintly acid broth will attain this degree of alkalinity with suitable strains of bacilli, even though more slowly than if more strongly alkaline to begin with. According to HIRSZFELD and his collaborators, there is no advantage to be

gained by adding white of egg to the broth, a view which conflicts with that of OLITSKY and KLIGLER. pH values over 8.6 give a poor growth. Good toxins can also be obtained by the addition of the Chapoteaut peptone recommended by the Pasteur Institute in Paris, Teruchi's preparation, Witte-peptone and other admixtures. Incubation at 32° C. leads to the same result, but more slowly than at 37° C. The toxic principle can be precipitated with ammonium sulphate. Filtration involves a considerable loss of toxin. Toxin production is stimulated by an abundant supply of air, and hence by culture in thin layers of broth (SHIGA and his collaborators). Endeavours to induce bacterial surface growth are made by a special process in DOERR'S "cultures flottantes". Formol is a suitable product for killing the bacilli in the broth culture, as it hardly damages the toxin.

(2) A very uniform and good toxin can also be obtained, however, by *autolysis* with physiological NaCl solution, or distilled water, from the bacilli washed off from quite young agar cultures.

(3) A good toxin solution can be obtained merely by 15 minutes' *washing* of agar-culture bacilli in physiological NaCl solution and subsequent removal of the bacterial bodies by centrifuging.

(4) This result is also obtained if the bacilli have been previously *killed* by heat (KOLLE, HELLER and DE MESTRAL ; BESREDKA). For this purpose toluene is better than heating to 60° C. (SHIGA and his collaborators).

(5) In the Wellcome Institute the pure *washed bacterial bodies* are used. A suspension in distilled water of 24-hour agar-culture bacilli is heated to 58° C. for 15 minutes ; the bacterial bodies are then separated out by centrifuging, dried and ground up.

(6) In the Frankfort Institute, the "*integral toxin*" is preferred. It is obtained, from a suspension of agar culture bacilli in distilled water, by drying, first in the Faust-Heim apparatus, and then *in vacuo*.

The toxins obtained by autolysis are very powerful ; the dry toxins obtained from bacilli are as strong, but less soluble ; the ammonium sulphate precipitate from broth culture is somewhat weaker ; the least toxic is the broth filtrate (SHIGA and his collaborators). The lethal dose for intravenous injection into mice of a series of toxins prepared at the Frankfort Institute by the various methods (KOLLE, SCHLOSSBERGER and PRIGGE) was as follows :

Broth toxin .. .. .	0.125 c.c.
Agar culture washings, dried .. .. .	1 mg.
Dried toxin, Wellcome method .. .. .	0.05 mg.
"Integral toxin" .. .. .	0.05 mg.

It is, of course, important to have toxins of the greatest possible constancy and strength for titration, as well as for mouse tests, in which the volume of fluid to be injected is limited owing to the smallness of the animal.

Broth toxins become attenuated. It is therefore a good plan to preserve dysentery toxins in a *dry* state, in which case they appear to remain stable almost indefinitely. (Such a toxin of MACCONKEY has not changed in ten years.) In order to obtain a good solution with dry toxins, it is frequently necessary to grind them up in an agate mortar and to add the diluent drop by drop (CANTACUZÈNE).

According to FUKUHARA, as broth toxins age their power of neutralising antitoxin decreases, *pari passu*, with the increase of the minimum lethal dose, from which it has been inferred that the structure of the toxin is comparatively homogeneous and that no toxoids are present; this is confirmed by SHIGA and his collaborators. In the case of one particular toxin, the minimum lethal dose (rabbits) had increased, after ten months' storage, to 2.2 times, and the  $L_+$  to 2.4 times, the original figure. On the other hand, SCHLOSSBERGER and HARTOCH found that the quantities of serum required for the neutralisation of the minimum lethal dose of toxins prepared by different methods may vary in the ratio of one to three (cf. Table VII).

Table VII.

NEUTRALISATION TITRES OF VARIOUS DYSENTERY TOXINS.

Toxin	Quantity of standard serum required for the neutralisation of 5 m.l.d.
Dry toxin, Wellcome method .. ..	1 : 500 c.c.
Dry toxin from culture washings .. ..	1 : 300 c.c.
"Integral toxin" .. .. .	1 : 350 c.c.
Broth toxin .. .. .	1 : 160 c.c.

They therefore considered it probable that several component toxins of different avidity for the serum antibodies are present in varying proportions in dysentery toxins.

Careful experiments were made by DOERR and the Warsaw, Cracow and Japanese workers to verify OLITSKY'S and KLIGLER'S claims, but with negative results throughout. The clear separation of a typical exotoxin with neurotropic properties from a purely enterotropic endotoxin successfully accomplished by the American scientists could in no case be confirmed. The two toxic properties were always interconnected; frequently, one and the same toxin, when heated and administered to

rabbits in equal quantities, produced only nervous symptoms in some and affected only the large intestine in others. In the experiments of the Japanese, both the filtrate and the autolysate toxin were completely inactivated by heating to 75° C., whilst the bacterial suspension was only destroyed at 80° C., and even then not always completely. Sub-neutralised toxins may produce both nervous and intestinal symptoms in rabbits. Toxins of young and old broth cultures alike affect both organic systems, the only thing being that the effect on the nerves becomes apparent at an earlier stage of the disease in rabbits. These results have an important bearing on the question of titration, for they justify the contention that the nerve symptoms must be taken into account in titrating therapeutic serum for human use (HIRSZFELD and his collaborators).

### *The Action of Dysentery Toxins on Various Animal Species.*

Guinea-pigs and rats are so little receptive as to be unsuitable for practical experiments (DOERR, DOPTER, KOLLE, SHIGA and his collaborators). In the case of rabbits and mice, on the contrary, almost complete parallelism of phenomena is found to exist (DOERR). The *dosis cerle letalis* for 1,500-gms. rabbits is about five times as great as for 15-gms. mice. Judged by dose referred to body weight, the susceptibility of mice is, according to DOERR, about 1/20th that of rabbits; according to HIRSZFELD and his collaborators, from 1/20th to 1/50th; and according to JONESCU-MIHAESTI, COMBIESCU and CONDRAIA, 1/20th in the case of fresh, and 1/50th in the case of older "stabilised" broth toxins.

Another point of fundamental importance is DOERR's demonstration that the various toxins can replace each other in tests for the determination of such values. For instance, if the  $L_0$  of a toxin prepared by the Wellcome process is 0.1 mg., and if the  $L_0$  of a toxin obtained from dried washings is 2.5 mgs., then half of the sum of the two values will also be the  $L_0$  value of a mixture of equal parts of each.

The facts which suggest that the *composition of dysentery toxin is, on the whole, homogeneous* are the following: (1) The lack of corroboration of OLITSKY's and KLIGLER's contention that the neurotropic and enterotropic action of the toxin can be separated; (2) the similarity of the toxins obtained in wholly different ways (fresh bacterial bodies, the residue thereof after washing as well as the washings themselves, agar culture autolysates, broth cultures, their filtrates and ammonium sulphate precipitates); (3) the parallelism of the action of the various toxins on rabbits and mice; (4) the cumulative action of the toxins in animal tests; (5) the similarity in the results—to be discussed hereafter—obtained in the titration of immune sera produced with the various toxins; (6) the similarity of the results

—also to be discussed hereafter—obtained, with both mice and rabbits, in the titration of immune sera against test toxins produced in different ways.

### 3. THE PREPARATION OF ANTI-DYSENTERY SERA.

Opinions differ as to the best way of obtaining high-potency immune sera from horses. DOPTER recommends the subcutaneous injection of eight-day broth filtrates ; he obtained less satisfactory results by injecting broth filtrates or living cultures, at first subcutaneously and, later, intravenously. SHIGA operates subcutaneously with virulent agar-culture suspensions killed by heating at 60° C. for half an hour, or, better, by toluene ; in his view, broth culture is less, and filtrate least, satisfactory ; the injection of the pure bacilli produces less local inflammation. The Lister Institute uses a “ pure toxin ” for preference. In Warsaw, immunisation is usually effected by subcutaneous injection of the broth culture and intravenous injection of killed or attenuated bacilli. HIRSZFELD immunised part of his horses with a six-week broth culture which had been killed with phenol and then several times filtered. He found combined immunisation of horses with mixtures of Shiga and Flexner cultures unsatisfactory. On the other hand, a polyvalent serum is prepared at Bucharest by subcutaneous treatment with Shiga broth toxin, combined with intravenous injection of living agar-culture suspensions of Shiga, Flexner, Y and Strong strains.

### 4. THE TITRATION OF ANTI-DYSENTERY SERA.

(1) The *agglutinin* test is wholly unsuitable, as there is no parallelism between the production curve of these bodies and the antitoxins (HIRSZFELD and collaborators, O'BRIEN and collaborators).

(2) Of the remaining processes, the determination of the *bactericidal*—anti-infectious serum action (SHIGA)—should be mentioned. It is carried out on 11-12gms. Japanese mice by the intraperitoneal injection of a mixture of two minimum lethal doses of living culture with decreasing quantities of serum, incubated at 37° C. for half an hour. The issue is decided one way or the other according as the animals are dead or alive after 24 hours. The process depends on the virulence of the culture (the greater the virulence, the greater the quantity of serum required). For this reason, it is regarded, even by Japanese scientists, as unsuitable for universal standardisation. In practice, it is liable to fail frequently and is not absolutely indicative of antitoxin titre. HIRSZFELD and his collaborators, as well as KOLLE and his collaborators, have reported on it in

the same terms ; but, unlike NEUFELD, the latter detected some parallelism, at least, with the antitoxin titre.

(3) *The best method is therefore that of determining the antitoxin titre.*

For the titration of the antitoxin content, two methods are available at present : the mixture test, corresponding to the process commonly employed with diphtheria and tetanus, and the curative test originally described by KRAUS and DOERR. DOERR has now abandoned the latter, for he found, when proceeding by separate intravenous injection of toxin and serum, that, even with an interval between injections of only 90 seconds, the quantity of serum required for curative purposes was 600 times as great as that needed for neutralisation when simultaneously injected in a mixture. When separately injected, the toxin is thus so rapidly bound by the organism that an accurate determination of the "curative value" is impossible.

Since, therefore, the *injection of toxin-antitoxin mixtures* is the only process which need be discussed, it must first be decided which laboratory animal is most suitable.

#### (a) *Choice of Laboratory Animals.*

As already stated (p. 26), rabbits and mice are the only laboratory animals sufficiently receptive to Shiga toxin. The choice between these two species has been the subject of lengthy discussion. So long as the, technically simpler, intraperitoneal injection was used on mice, no comparison was possible with the well-tried method of intravenous injections into rabbits. The technique of intravenous injections into mice, introduced by SACHS and GEORGI, was therefore a notable advance towards the solution of the problems under investigation. Nowadays, all institutes have adopted this method. Both species have their advantages and their drawbacks. The technique of accurate injection is naturally more difficult when mice are used. The maximum volume which can be injected into mice—0.5 c.c.—is so small that the loss of a single drop of the fluid involves a much greater relative error than it would with the quantities—20 or 30 times as great—which can be injected into rabbits. Furthermore, the symptoms of disease can be better observed with rabbits than with mice, in which paralysis usually occurs only a short time before death. But the main difficulty in the way of titration is the considerable *differences in individual sensitiveness to toxin*, which may vary in the ratio of 1 : 20 in different animals. These differences are reported both in rabbits and in mice. Age, weight and breed are decisive factors in both species. On the whole, the best criterion for uniformity of animal tests is body weight, but the "normal weight" may be reached at different ages, according to the way in which the animals are tended and fed. In rabbits, the

sensitiveness to toxin increases with age and reaches its maximum when the body weight is approximately 1,500 gms. As regards differences in breed, the small, black, short-eared rabbits are the most resistant (JONESCU-MIHAESTI and COMBIESCU). Even with mice, however, more or less comparable results can only be obtained with the adult animal, the normal weight for the European mouse being 15-20 gms. Considerable differences are, however, to be observed between various breeds. Thus, MADSEN reports that, whereas HIRSZFELD found in Warsaw one breed as sensitive, and another three times as sensitive, to the Copenhagen test toxin as the Copenhagen mice, the mice of the Pasteur Institute in Paris were meanwhile less sensitive to the same toxin than the Copenhagen animals.

There is a divergence of opinion among the various observers as to whether individual differences are greater in rabbits or in mice. Whilst DOERR regards rabbits as more uniform, KONDO (Frankfort) and O'BRIEN, SÜDMERSEN and RUNGE (London) report marked differences in these very animals. The English authors lay stress on the disturbing effects of the chronic parasitic diseases, such as coccidiosis, which are frequent in rabbits. According to O'BRIEN and RUNGE, as many rabbits are required as mice to achieve accurate results. In mice, the time of year plays an important part. Thus O'Brien obtained the following figures for equal doses of toxin :

Percentage of surviving animals							
1927							1928
March	June	July	Sept.	Oct.	Nov.	Dec.	Jan.
64	86	100	82	81	64	58	54

Similar results can, in any case, be obtained with both species of animals (DOERR, O'BRIEN and his collaborators). Whilst DOERR, HIRSZFELD and SHIGA preferred rabbits at first, the majority of research workers subsequently favoured the use of mice with the KOLLE technique. In the Standardisation Commission, it was agreed to use mice for titration, because they can always be obtained cheaply, easily and in large numbers, and because individual differences can be eliminated to some extent by injecting an appreciable number of animals with each mixture of toxin and serum. The Copenhagen Institute uses six mice of 16-18 gms. for each mixture. DOUGLAS recommends that investigators should only use mice which they have bred themselves and which are kept, so far as possible, at the same level of resistance by uniform care and attention ; after injection the animals are to be placed in a room of even temperature. O'BRIEN places an untreated mouse in each cage with four inoculated animals as a control to show whether the animals have been properly cared for.

(b) *Number of Mice to be used.*

How many mice should be inoculated with each mixture of toxin and serum has not yet been finally settled. The minimum number is unquestionably three ; for practical reasons, however, this number appears to be too small ; for if, owing to some accident, two of the three animals injected with the mixture should die instead of one, the survival rate would work out at 33 per cent instead of the correct figure of 66 per cent (JENSEN). Hence, according to English official regulations, at least six mice and, in critical tests, even more if possible, should be used for each mixture. Recently, O'BRIEN and his collaborators, having found some discrepancies in their results, have stipulated for even larger series, if not only the minimum antitoxin content of a serum but its true content is to be accurately determined. In the Wellcome Institute, TREVAN has carried out extensive statistical investigations and has constructed a curve (Figure 2) indicating the probability of death with various doses. He has also worked out similar curves for the pharmacological products dealt with by the Standardisation Commission (cf. KNAFFLLENZ).

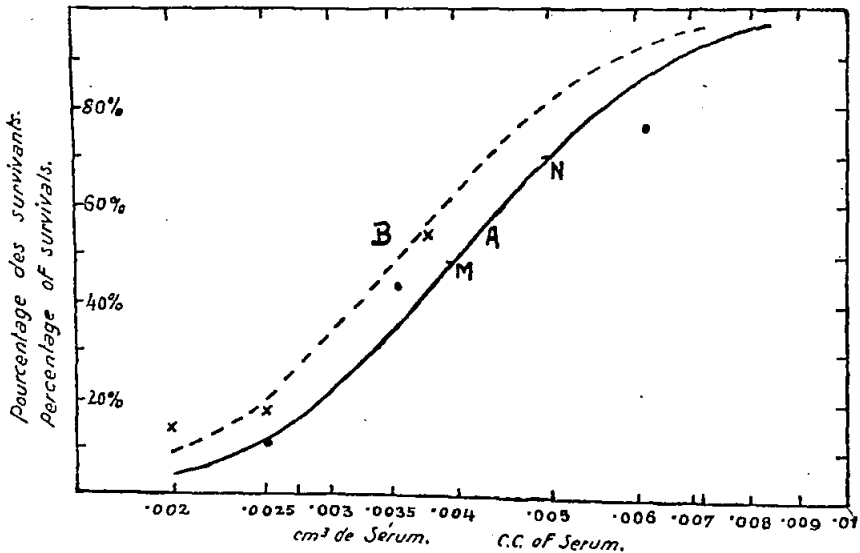


Fig. 2

The gently swinging S-curve (Figure 2) has been constructed from a series of individual points determined in the usual way, but by tests on several hundred animals. The curve has been artificially steepened by the use of the logarithmic scale for the abscissæ (quantities of serum added to a test dose of dysentery toxin), whilst the arithmetic scale was used for the ordinates



(percentage of surviving animals at each stage). The second "B" curve (320 animals) will be seen to tally well with observed results. Apart from their purely theoretical interest, such curves enable a substantially greater degree of accuracy to be attained in comparing the antitoxin content of two sera. For instance, if a given concentration of standard serum protects 70 per cent against the test dose of toxin (point N of the curve) and if the same concentration of the serum to be tested only protects 49 per cent (point M), the ordinate values of these two points on the curve correspond respectively to abscissa values of 0.005 and 0.004, and the serum to be tested would therefore be four-fifths as strong as the standard serum.

TREVAN has also made a statistical computation of the relationship between the number of animals used and the accuracy of serum titration. Where the standard serum is compared with a serum to be tested, by titration against the test dose of toxin adopted by the Standardisation Commission, the result can fluctuate :

With 10 animals, between 0.4 times and 1.8 times the true value  
 " 20 " " 0.7 " " 1.4 " " " "  
 " 60 " " 0.8 " " 1.3 " " " "

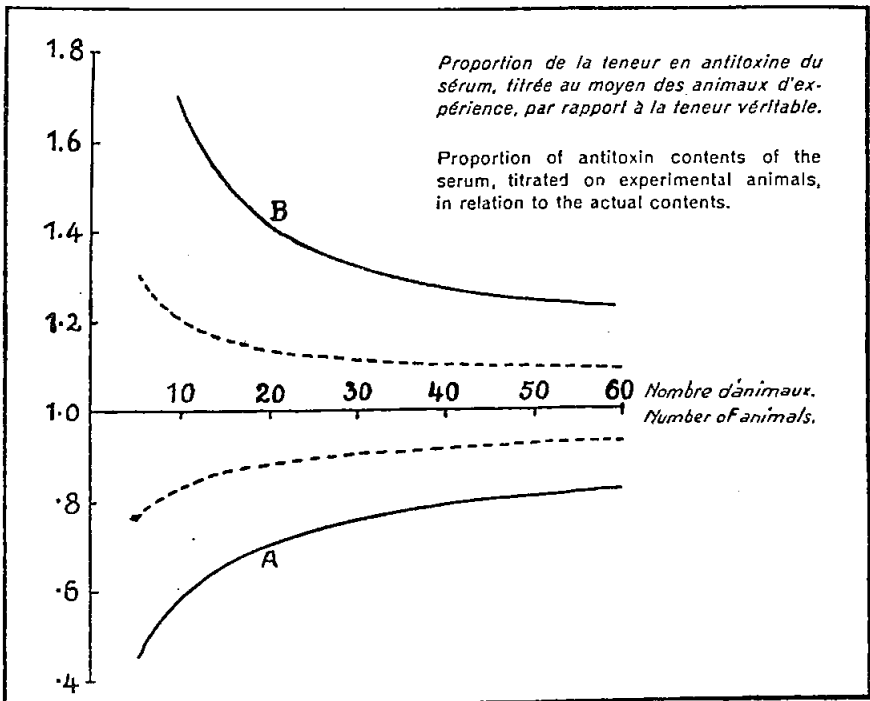


Fig. 3.

The margin of error is shown by the unbroken line in Figure 3. The dotted curves show the increased accuracy obtained by the use of five times the test dose of toxin provisionally adopted by the Standardisation Commission—the so-called “League of Nations dose”.

As O'BRIEN has said, our object must be to make the curve as steep as possible (Figure 2). He hopes to achieve this by improving the toxin. The maximum accuracy can, in any case, only be obtained if every laboratory has large supplies of uniform dry toxin available (put up in separate sealed ampoules, each containing the current dose), if the animals are so far as possible uniform, and if comparative titration of the standard serum and of the serum to be tested is carried out in each case on the same day and on the greatest possible number of mice. There are obviously limits to the number of mice which can be used if importance is attached to the careful selection of animals which are as uniform as possible. According to the experience of the Copenhagen Institute and of DOUGLAS (cf. p. 29), this is one of the very first conditions to be fulfilled.

(c) *The Dose of Toxin to be used for Titration.*

At first there were considerable differences of opinion as to the most suitable multiple of the minimum lethal dose. KOLLÉ originally used 2-4 m.l.d., but held that a larger number would be desirable. The Wellcome Institute used 10, the Lister Institute 100 and the Japanese 20. Later on, DOERR rightly pointed out that the determination of the m.l.d. was highly inaccurate owing to the large differences of individual resistance, and therefore recommended a multiple of the *dosis certe letalis*. The dose actually fixed upon is about 5 d.c.l., but this question is no longer of such great moment as was originally assumed. What the Standardisation Commission has done has been to adopt a mean value, defining it, not indeed in terms of lethal doses, but in terms of neutralising power exercised by a standard antitoxin, thus following the precedent of diphtheria and tetanus serum. As stated above, the Wellcome laboratory workers (TREVAN, and also BLAKE and OKELL) found that even greater precision could be obtained by the use of five times the “League of Nations dose”. The increase of the dose of test toxin cannot, however, be carried beyond certain limits; first, owing to the solubility of the preparations, seeing that 0.5 c.c.—which is already a comparatively large quantity—is the absolute maximum for injection into a mouse; and, secondly, because the toxin is not a pure substance but contains other components producing unspecific effects, and possibly endotoxins as well, so that, if substantially higher doses of toxin were used, these associated effects might prove very disturbing. It will therefore be

advisable to adhere, for the time being, to the "League of Nations dose" of dysentery toxin; if it should prove possible, later on, to produce test toxin in a purer form, the use of larger doses might then be contemplated.

(d) *Selection of the Toxin to be used for Titration.*

Leaving aside this question of the doses, however, it has been shown that all toxins, when titrated with sera of different potency, yield values which are strictly comparable *inter se* (MADSEN and JENSEN, KOLLE and his collaborators, NEUFELD, SHIGA and his collaborators). Thus, SCHLOSSBERGER and HARTOCH, testing four different toxins against three sera, found the following L<sub>+</sub> values (Table VIII) :

Table VIII.

ADJUSTMENT OF FOUR DIFFERENT DYSENTERY TOXINS (L<sub>+</sub>) AGAINST THREE DIFFERENT SERA.

Toxin	Standard serum dilution			
	Dose	English	Japanese	German
Dry toxin obtained by the Wellcome process .. ..	0.1 mg.	1 : 50	1 : 2000	1 : 150
Toxin from dry culture washings .. .. .	3.0 mg.	1 : 60	1 : 2000	1 : 125
"Integral toxin" .. .. .	0.35 mg.	1 : 50	1 : 3000	1 : 125
German test toxin (broth) ..	0.25 cc.	1 : 50	1 : 2000	1 : 125

The practically complete equivalence of the results of the titration of the three sera, in spite of the use of such different toxins, proves that the choice of test toxin has no fundamental importance. The Standardisation Commission therefore made no hard-and-fast proposal on this point, but merely recommended the use of a stable toxin. As such, one of the dry toxins should be given first consideration.

These interesting results lend further support to the view that the Shiga bacillus exotoxins are, for all practical purposes, the essential component of the toxin. They also justify the present policy of placing the titration of dysentery therapeutic serum exclusively on this basis.

(e) *The Relation of Shiga Toxin to Antitoxin.*

The answer to the question whether the neutralisation of a toxin by serum follows the law of multiple proportions is of fundamental importance, as already pointed out, in determining the nature of dysentery toxin. From earlier investigations, it had seemed that the answer was in the affirmative. The work undertaken at the instance of the Standardisation Commission has, however, given rise to certain doubts. HIRSZFELD and his collaborators found, when using high multiples of the m.l.d. (20-50), that the law no longer held good for rabbits. According to KOLLE and his collaborators, on the contrary, it holds good for all kinds of Shiga toxins, both in the rabbit (KONDO) and in the mouse. O'BRIEN, SÜDMERSEN and RUNGE found that it operated with quantities up to 4 m.l.d. SHIGA and his collaborators noted certain discrepancies, but offered an apparently sound explanation. They point out that, if the  $L_{+}$  value cannot be determined with perfect accuracy when a small dose of toxin is used, the error is negligible. If, however, multiple doses are used, this may result in a substantial excess of toxin. Taking matters as a whole, they, too, consider that the law applies. It is, of course, conceivable—especially so with broth toxins—that products other than the exotoxin may intervene with disturbing effect. It will therefore be well to avoid using unduly large quantities in dosing test toxins.

It is quite possible that, where higher multiples of the lethal dose are used, the disturbing component may consist of substances in the nature of a genuine endotoxin; this would be entirely in keeping with PFEIFFER'S, UNGERMANN'S and BESSAU'S results, which must in no case be disregarded. It will be remembered that these scientists used intraperitoneal injections into rabbits and guinea-pigs, so that the conditions were such as might lead to endotoxin poisoning, at all events in the last-named species with its relatively low receptivity for the exotoxin. Cases in which individual rabbits or mice of an experimental series die with "acute" symptoms a few hours after intravenous injection, and only show extensive vascular distension on post-mortem examination (HIRSZFELD and his collaborators), are, to my mind, also very probably attributable to the action of endotoxin. It is precisely these comparatively large individual differences of receptivity than tally with past experience of endotoxin poisoning. On the other hand, the relation between the exotoxin of Shiga bacilli, and its specific antitoxin, has proved so far to be the only one which lends itself to accurate titration. Without prejudice to the results to be obtained later in the clinical testing of high potency endotoxin sera on man, it will hence be proper for the time being to titrate dysentery therapeutic sera on the lines mentioned.

(f)  $L_0$  and  $L_+$  Values  
*(Dosis neutralisata and dosis non-neutralisata).*

According to what has been said above, it is clear that the titration of dysentery serum—like that of diphtheria and tetanus serum—should be based not on the minimum lethal dose of toxin, but on a standard serum as unit. For even using the *dosis certe letalis*, it is not always possible to obtain exactly comparable serum values with toxins prepared in different ways. Even though, as mentioned above, SCHLOSSBERGER's and HARTOCH's tests yielded proportionately equivalent values (Table VIII, p. 33), MADSEN showed that one and the same serum could give varying unit-values relatively to different toxins and, in some circumstances, marked divergences. He therefore used a toxin prepared by the Wellcome method and having a d.c.l. of 0.025 mg., and a broth toxin having a d.c.l. of 0.02 c.c. Operating with 10 d.c.l., he obtained the following neutralisation values ( $L_+$ ) :

	Sera				
	German	Japanese	Polish	English Standard	Lister Institute
10 d.c.l. dry toxin	150 units	250	125	60	250
10 d.c.l. broth toxin . . . .	300 units	500	250	125	550

If, therefore, different toxins are taken as a basis, the serum values may differ appreciably—indeed, on this showing, by 100 per cent. *Bul the relation of the sera inter se is the same for all the toxins used.* Consequently, the only possible basis of titration here, again, is the use of a serum preserved by the EHRLICH process, dried without antiseptics and stored *in vacuo*. The dry serum is dissolved for use in the prescribed quantity of a mixture of two parts of glycerine and one part of 0.85 per cent NaCl solution. The Frankfurt Institute uses such a serum for titration of mice in the dysentery-serum tests which have been optional in Germany since 1916. The reliability of such standard sera is demonstrated, among other things, by the fact that a standard serum prepared in Copenhagen and tested against three different toxins, together with the standard serum of the National Institute for Medical Research in London, proved to be absolutely equivalent to the latter. Similarly, three samples of standard serum sent from Copenhagen at different times to the National Institute of Medical Research were shown to be equally potent when tested at the latter establishment.

The question whether the adjustment should be made relatively to  $L_0$  or to  $L_+$  has to be considered in a somewhat different manner with dysentery toxin than with diphtheria or tetanus toxin. The great individual differences in the receptivity of mice for Shiga toxin have this result—that one or

two animals in titration series receiving the smaller dose of toxin will still be found to contract paralysis or even to die, whilst, with the larger dose, one or two will survive. If  $L_0$  and  $L_+$  were to be defined in the original EHRLICH manner, two widely diverging values would be obtained, and an accurate adjustment of toxin to serum would be impossible. The practice now generally adopted in the titration of dysentery serum is to make the term "neutral" cover such mixtures as enable two-thirds or more of the mice to survive, whilst the term "non-neutral" mixtures extends to those which kill two-thirds or more of the mice. It is on this basis that the test toxin is adjusted to the standard serum. The "*dosis neutralisata*" of a serum is thus understood to mean the quantity which, when mixed with the test toxin dose adjusted in this manner, enables two-thirds or more of the mice to survive; whilst the "*dosis non-neutralisata*" means the quantity of serum which, when mixed with the test toxin dose, kills two-thirds or more of the mice. Every titration of serum involves the determination of both values, between which the hypothetical "exact" value must be conceived to lie.

(g) *Establishment of the International Unit and of the Method of Test.*

The investigations described above having led to a better understanding of the question and to a practicable method of titration, the next step was to compare the standards in use in the various countries. SCHLOSSBERGER and HARTOCH (1924) found that the ratio between the English, German, and Japanese standard sera then in use was 1 : 3 : 45 (cf. Table VIII, p. 33). According to KOLLE, SCHLOSSBERGER and PRIGGE, the Polish serum was from 4 to 4.2 times as strong as the German. These ratios were confirmed by other research workers. In the Standardisation Commission (Geneva, 1924), it was agreed that :

1 German unit = 36 international units.

The following *directions for the performance of tests* were also finally laid down at the Geneva Conference :

Titration should be effected exclusively by determination of the antitoxin content as tested against dry toxin from Shiga bacilli. Mixtures of the same quantity of toxin with decreasing serum concentrations should be filled up to 0.5 c.c. and, after standing at 37° C. for three-quarters of an hour, intravenously injected into three (more recently six) mice in each case. In Europe, the mice are to weigh 15-18 gms.<sup>1</sup>; in Japan, 12-15 gms.

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<sup>1</sup> According to English official regulations, 15-20 gms. mice.

The animals should be kept under observation for seven days. Animals dying within eighteen hours should not be taken into account.<sup>1</sup>

The standard serum should be kept in dry form in air-exhausted ampoules. On each ampoule indications should be given as to the quantity of serum neutralising the test toxin dose (“*dosis neutralisata*”) and the quantity which leaves one lethal dose unneutralised (“*dosis non-neutralisata*”); that is to say, that with the first-named quantity of serum not less than two-thirds of the mice treated must survive, whilst with the last-named quantity at least two-thirds must die. For the standard serum supply in Denmark, the *dosis neutralisata* = 1/200 c.c., the *dosis non-neutralisata* = 1/300 c.c.

For each serum test, two rows of tubes should be prepared, one with a standard serum, the other with the serum to be tested. The row of standard-serum tubes consists of four grades of mixtures, *i.e.*, constant quantities of toxin with: the *dosis neutralisata*, the *dosis non-neutralisata*, a quantity of serum above the former, and a quantity below the latter, dose; for the unknown serum, a larger number of grades is obviously required.

The State Serum Institute at Copenhagen should prepare a sufficient quantity of dry test toxin and standard dry serum; the latter should be obtained from four horses immunised with “Behring-Werke” Shiga strain sent from the Frankfort Institute. A few days after bleeding, the serum should be dried without a disinfectant in the Krause apparatus and preserved *in vacuo* over  $P_2O_5$  at 2° C. For use, a 1% solution should be prepared in a mixture of two parts of glycerine and one part of physiological NaCl solution.

The dry test toxin should be prepared from a forty-eight-hour agar culture of the same Shiga strain; the suspension of bacilli in distilled water should be heated at 58° C. for twenty minutes, the bacilli should then be removed by centrifuging, dried in the desiccator, ground up, and stored in air-exhausted ampoules after drying over  $P_2O_5$ . In order to obtain homogeneous emulsions of the dry toxin, CANTACUZÈNE’s suggestion should be adopted of grinding up the powdered toxin in an agate mortar and adding the requisite quantity of diluent drop by drop.

Fresh solutions of dry toxin should be made every day.

A number of graded quantities of test toxin should be tested to determine the quantity which, when mixed with 1/200 c.c. standard serum, produces “neutrality”, *i.e.*, allows two-thirds or more of the animals to survive.

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<sup>1</sup> Experience shows that such deaths are not attributable solely to the action of the toxin but to complications caused by shock, trauma and other unspecific influences.

Table IXa.

ADJUSTMENT TO "NEUTRALITY".

1/200 c.c. standard serum	Percentage of mice surviving
+ test toxin 0.50 mg.	0
0.45 "	0
0.40 "	17
0.36 "	<u>67</u>
0.32 "	83
0.28 "	83

According to this, the test dose of toxin would therefore be  $\leq 0.36$  mg.

The quantity of test toxin which just gives "non-neutralisation" with the *dosis non-neutralisata* of the standard serum, i.e., causes  $\frac{2}{3}$  or more of the animals to die, is then determined.

Table IXb.

ADJUSTMENT TO "NON-NEUTRALITY".

1/300 c.c. standard serum	Percentage of mice surviving
+ test toxin 0.36 mg.	17
0.32 "	<u>67</u>
0.28 "	83

According to this, the test dose of toxin would therefore be  $\geq 0.36$  mg.

The dose of test toxin satisfying the requirements of both test series is accordingly 0.36 mg.

As regards the I.U., it was decided, on MADSEN's proposal, that 1 c.c. of the standard serum (1 per cent) solution should contain 200 international units, so that one gramme of dry standard serum would contain 20,000 I.U. Here, again, as in the case of tetanus serum, the aim in view was to fix the magnitude of the I.U. in such a manner that the therapeutic dose would contain a number of I.U. of the same order of magnitude as in diphtheria serum. A good commercial serum should contain at least 500 I.U. per c.c. The sera of 2,000 I.U. and over have been prepared in various countries.



(h) *Results of Comparative Titration in Various Countries.*

Comparative tests on these lines were made in various countries with the Danish standard serum and the Danish dry toxin (test dose 0.33 mg.) (cf. Table X).

Table X.

ADJUSTMENT OF THE SAME DRY TOXIN TO THE SAME STANDARD SERUM IN VARIOUS COUNTRIES.

Investigator	Concentration of standard serum									
	1 : 150		1 : 200		1 : 250		1 : 300		1 : 350	
	Number of animals	Died %	Number of animals	Died %	Number of animals	Died %	Number of animals	Died %	Number of animals	Died %
MADSEN .. ..	20	20	20	20	26	46	26	73	19	82
O'BRIEN .. ..	..	20	..	17.5	..	40	..	70	..	..
DOERR (Mouse)..	3	0	4	25	8	63	8	63	5	109
„ (Rabbit)..	1	0	1	0	1	0 <sup>1</sup>	1	100	1	100
HIRSZFELD ..	3	30	7	15	..	..	6	82	3	100
SHIGA.. .. .	..	..	3	0	3	66	3	66	3	100

All tests, except the one series of DOERR, were carried out on mice. The values obtained at the same time by KOLLE in Frankfort and MACCONKEY in London were similar.

In order to place the process on a sounder basis, these tests were repeated, four times in the course of one year, by a large number of institutes (cf. Table XI).

<sup>1</sup> The animal was paralysed, but recovered.



measure of agreement secured may, on the whole, be regarded as entirely satisfactory. A large number of further tests carried out in the various participating institutes produced essentially the same results.

The international unit and the method of titration described have been officially adopted in England (Therapeutic Substances Act, 1925, 15 & 16 Geo. V, cap. 60, Second Schedule, Part IV, B). In Germany and France they have also been accepted by the testing institutes (Institute for Experimental Therapy, Frankfort ; Pasteur Institute, Paris).

As a result of eight years' work in various countries, uniformity has thus been attained in the production of therapeutic serum for Shiga dysentery. This has provided the basis on which antitoxic dysentery serum can be tested for its value in human treatment. Whether it will be necessary, later on, to determine the anti-infectious value in addition to the antitoxic titre will depend upon the results of the clinical use of the serum. Opinions are still widely divergent on this point.

#### IV. MENINGOCOCCUS AND ANTI-MENINGOCOCCUS SERUM.

##### (a) THE AGENTS.

The position in regard to the three kinds of serum previously discussed was comparatively simple, to this extent, that they were connected with uniform pathogenic agents with marked toxin-producing properties. Matters are quite different with the meningococci. The first difficulty resides in the antigenic multiplicity of the types found in various countries. The First Standardisation Conference (London, 1921) therefore decided that the various laboratories should exchange and compare their strains obtained from meningitis cases and other diagnostic sera. For this purpose, it prescribed the agglutination test (24 hours at 37° C.) and recommended, in addition, an investigation of agglutinin absorption.

The results, on which MADSEN submitted a report, based on the investigations of WULFF and VOLLMOND, to the Second Standardisation Conference (Paris, 1922), show the extraordinary intricacy of the problem. In Denmark, a particular type ("A") predominated in 1919-1922. Seventy-five out of 82 strains of meningococcus obtained from the blood or cerebro-spinal fluid of meningitis cases belonged to this type, and it was frequently found in the pharyngeal secretions both of meningitis patients and of healthy persons. Antigenically divergent strains (five types) only occurred in a few isolated cases.

GORDON, in England, had previously isolated four types which he designated as I, II, III, IV. Types I and III are closely related to each other and are therefore classed by GRIFFITH and SCOTT (London) in a single group I, while Gordon's type II and IV are put into a single group II.

At the Pasteur Institute in Paris, four types were isolated and labelled A, B, C and D.

Outside of Denmark, Madsen's "A" type has only been found in patients on one occasion, and that in Germany (NEUFELD). It is very similar to Gordon's type II, but not identical with it.

The English groups I, II (GRIFFITH and SCOTT) correspond, according to DÖPFER, to the French types A and B respectively. The French type C and the very rare D are not to be identified with any of the English types.

MADSEN produced immune sera with the English groups I and II and tested other strains from Germany, Palestine, Poland, Sweden, Switzerland and Turkey. Most of these could be classified in the two groups: 11 in group I and 14 in group II.

The Second Standardisation Conference (Paris, 1922) proposed to carry on the investigation. In order, as a first step, to secure international uniformity of nomenclature, a primary division was to be made into groups designated by Roman numerals (according to the now accepted subdivision into groups I and II), and a subdivision into sub-groups designated by small Latin letters. The primary division into groups was to be based upon agglutination approaching the limiting titre, in a high dilution of monovalent serum which are relatively free from heterologous agglutination. The secondary division into types might be based upon agglutination and the absorption tests. Strains which cannot be accurately identified were to be classed under a group "X". According to recent research work by M. KRISTENSEN and MOLTKE, however, the relationship of meningococcus strains would appear to be even more complex. An appreciable number of strains of American, Danish, English and French origin, taken from the "National Collection of Type Cultures" of the Lister Institute, as well as twenty-six other Danish cultures, were tested by agglutination and agglutinin absorption with several sera prepared from a number of these cultures. Hardly in a single case was it possible to make a clear division into definite groups or types, but almost without exception one strain was found to pass over by insensible degrees into another. We may ask ourselves whether these conditions have developed in this extreme form in the last few years, during which meningitis has only been occurring sporadically. It is, indeed, quite conceivable that strains of greater antigenic uniformity might occur in larger numbers in the course of more extensive epidemics. Under present circumstances, at all events, the prospects of producing anti-meningococcus sera of *practical value* are rendered highly doubtful by the existence of this antigenic multiplicity.

#### (b) THE TITRATION OF THE THERAPEUTIC SERA.

The difficulty of titrating meningococcus sera is due, apart from the lack of uniformity of the meningococci, to the uncertainty which still subsists as to the method suitable for titration. Meningitis is neither a pure poisoning by endotoxins nor wholly and solely an infection. It is doubtful whether the morbid process is predominantly infectious. In any case, there would be justification for using both the anti-endotoxic and the bactericidal or anti-infectious action of the therapeutic serum for titration purposes. Unfortunately, however, none of the various processes of titration is entirely satisfactory.

1. There is usually no parallelism between *agglutinin* production and the protective substances of the serum.

2. *Complement-fixation* gives results of practical value, but whether these antibodies are identical with the therapeutically active principle is doubtful.

3. A modification of the *Pfeiffer test* consists in intraperitoneally injecting guinea-pigs with decreasing quantities of unheated serum and, twenty-four hours later, with one-sixth of an agar culture of meningococci. The progression of bacteriolysis is then watched in the usual way (DOPTER).

4. The *anti-endotoxins* are determined, according to GORDON, by the intraperitoneal injection into mice of mixtures of decreasing doses of dried meningococci and constant quantities of serum. DOPTER considers young guinea-pigs (80 to 100 gms.) more suitable.

5. The determination of the *curative value* is impracticable owing to the low pathogenicity of most strains of meningococcus for laboratory animals.

6. It seems that the *bacteriotropin test*, by the NEUFELD method, might be used with great advantage (cf. p. 10).

In Germany, the simultaneous determination of complement-fixation (with polyvalent antiformin extract) (p. 10) and of the tropins has been officially prescribed.

The Second Standardisation Conference (Paris, 1922) had before it reports by MADSEN, MÖRCH, DOPTER, NEUFELD and WADSWORTH. When intravenously immunising horses with large doses of meningococci, MADSEN and MÖRCH observed a characteristic increase in the agglutinins complement-fixing antibodies and tropins, when homologous strains were used, and similar, though smaller, increases with heterologous meningococcus strains as well (cf. Figure 4). The first two groups of

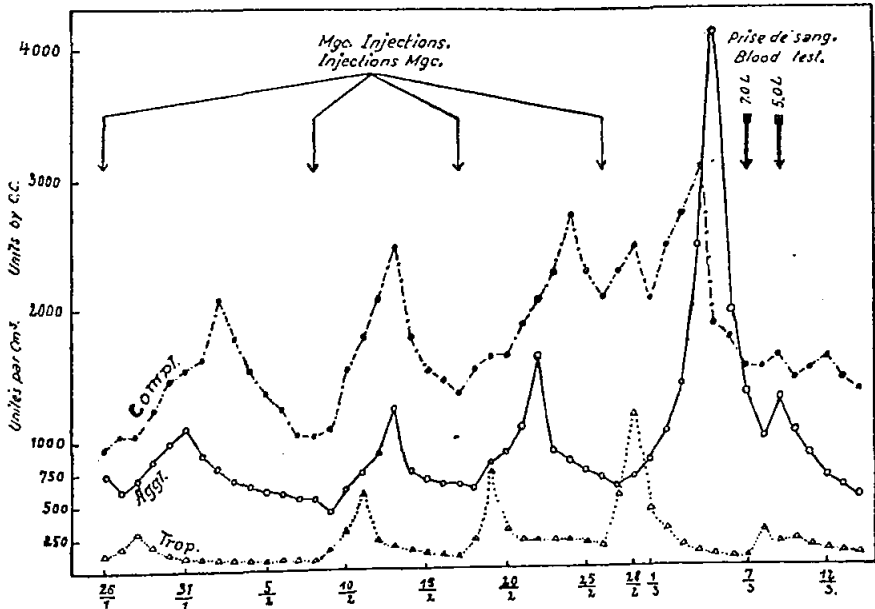


Fig. 4.

antibodies followed a more or less parallel course and reached their maximum, on each occasion, five days after the injection. The tropins, on the other hand, had already begun to decrease sharply by the third day after the injection (unspecific factors?). In fractional precipitation with ammonium sulphate, the agglutinins precipitate with the

euglobulins, the tropins with the pseudoglobulins and the complement-fixing antibodies with both fractions ! For purposes of precipitation, use is made of alcoholic extracts of meningococci suspended *ad hoc* in physiological NaCl solution. It was difficult to obtain constant values by the complement-fixation process, even using dried cocci ; neither does tropin determination always give consistent results, even when one and the same strain is used.

NEUFELD is of opinion that the agglutinin test, if carried out with a uniform technique, could give at least some indication of titre.

According to WADSWORTH, there are two systems of tests in the United States. The test at the Washington Hygienic Laboratory, as laid down by the Federal Authorities (Regulations dated April 8th, 1921), and the New York State Rules (Regulations of November 1st, 1922), which conform to the Rockefeller Institute process. The Washington test covers the agglutinins, the tropins and complement-fixation ; the New York test is limited to the agglutinins (emulsion of about 1,000 million meningococci in 1 c.c. ; the agglutination mixtures are kept at 55° C. for 16-24 hours). In addition, minimum requirements are laid down regarding the number and kind of horses to be immunised, the strains to be used and the period of immunisation.

DOTER considers the determination of the bacteriolytic capacity of the serum to be the most practical.

The Second Standardisation Conference (Paris, 1922) decided that the investigations should be continued through the agency of the Copenhagen Institute, acting as the central laboratory. The various methods of test were to be further investigated with monovalent and polyvalent sera, using both unheated (Paris Pasteur Institute process) and heated sera. Only strains obtained from the cerebro-spinal fluid of cases of meningitis were to be used for titration. Special attention was to be accorded to cultures from cases in which serological treatment had not been effective.

The Standardisation Conference in Geneva (1926) and in Frankfort (1928) made no new contribution to this question. There seems to be no immediate prospect of international agreement on the testing of anti-meningococcus serum.

## V. PNEUMOCOCCUS AND ANTI-PNEUMOCOCCUS SERA.

### (a) THE AGENTS.

Difficulties similar to those connected with anti-meningococcus serum arise here again, owing to the multiplicity of the types of agents and the uncertainty which still exists as to the best method of titration. The question of types (groups) is, indeed, simpler. Following the same policy applied in the case of meningococci, the First Standardisation Conference (1921) proposed the exchange of cultures between the institutes participating in the work (Robert Koch Institute, Berlin ; State Serum Institute, Copenhagen ; Ministry of Health Laboratory and National Institute for Medical Research, London ; Rockefeller Institute, New York ; and Pasteur Institute, Paris). From the reports submitted to the Second Standardisation Conference (Paris, 1922) by GRIFFITH and GLYNN, as well as by MADSEN, it appeared that the classification of pneumococci into four groups, I, II, III and IV (IV being a general heading for all unclassified strains) which the Rockefeller Institute workers had carried out in continuation of the work of NEUFELD, also applied to the Danish and English types of agent. As regards the agent lobar pneumonia, the following subdivision was made :

Table XII.

	United States	Denmark	England :	
			Griffith, London	Glynn, Liverpool
Total number of cultures examined ..	450	110	150	105
Percentage distribution {	I ..	33.3	36 (22%) <sup>1</sup>	44.8
	II ..	29.3	30 (7%)	20.9
	III ..	13.0	9 (22%)	1.9
	IV ..	24.5 <sup>2</sup>	35 (3%)	32.4 <sup>2</sup>

AS NEUFELD had previously found in Germany, the main types of agent are thus uniform in the United States and Europe, and similarly distributed.

At the Copenhagen Institute, S. CHRISTENSEN found agglutination and agglutinin absorption equally practicable for the identification of pneumococcus types. He could not distinguish by complement-fixation except when using washed bacteria, but the various types were frequently observed to pass over one into the other.

MADSEN and S. CHRISTENSEN, having several times repeated the examination of twenty-six patients, could only recover the type of pneumococcus first isolated; GRIFFITH, on the other hand, frequently isolated several types from one and the same person at the same or at different times. In order to detect the principal agent, he recommends that the sputum should be enriched by passages through mice.

As regards *mutation of types* among pneumococci, GRIFFITH submitted a report on the conversion by culture of the "S" type into "R"; NEUFELD had also obtained similar results. GRIFFITH's latest investigations into the conversion of one type into another could not be taken into account, at the time, by the Standardisation Commission.

In order to obtain highly potent *diagnostic sera*, GRIFFITH recommends the use of cocci removed from broth cultures by centrifuging, and intravenously injected into rabbits (whether alive or dead is immaterial). The deposit of 100 c.c. culture is given *refracta dosi* in two or three consecutive days, and, after five to seven days' rest, the injection is repeated. In a few weeks sera of a titre of 1 : 640-1 : 1,280 are obtained. Only cocci of the highest virulence produce specific sera. "R" strains are unsuitable, their antigenic properties being insufficiently differentiated. As regards test technique, he too recommends that of the Rockefeller Institute. NEUFELD found that, immediately after production, freshly prepared rabbit serum would frequently have a more or less marked agglutinating effect on heterologous pneumococci as well; after several weeks the heterologous component ceases to act (although thermostable), whilst the homologous titre remains unchanged.

The Standardisation Conference (Paris, 1922) decided to carry out similar investigations in tropical and other countries where climatic and social conditions differed; cases of lobar pneumonia were to be studied first, but observations of other pneumococcal infection were also to be recorded.

<sup>1</sup> The figures in parentheses show the fatality rate in the various groups.

<sup>2</sup> These figures include atypical group II.

With regard to *nomenclature*, it was recommended that the three chief types, I, II and III, should be retained, but that, in order to obviate the misunderstandings which might be caused by the appellation "Group IV", all strains not conforming to types I, II and III should be classified together under a group "X". The Copenhagen Institute was selected to act as distributing centre for cultures and diagnostic sera.

(b) THE TITRATION OF ANTI-PNEUMOCOCCUS SERA.

For the production of good therapeutic sera, as well as for that of the diagnostic sera, the highest possible degree of virulence is required in the pneumococci (NEUFELD).

Up to the present, sera have been titrated almost exclusively with reference to their *anti-infectious* efficacy in mice, either by prophylactic tests or by the injection of mixtures of culture and serum. The first Standardisation Conference (London, 1921) had expressed the view that tests for the agglutinating power of anti-pneumococcus serum were of no value, and that the best method was the measurement of the bactericidal power. Investigations were also to be made to determine whether this titration could best be carried out by the intraperitoneal or the subcutaneous method, and whether by preventive or simultaneous injection. The question of monovalence or polyvalence of the sera was also to be studied. As a result of these proposals, the second Standardisation Conference (Geneva, 1922) had before it reports by CORONI (Pasteur Institute, Paris), GRIFFITH, NEUFELD and WADSWORTH (Washington).

In Paris, titration was carried out subcutaneously on 15-20-gms. mice (several animals for each grade owing to the appreciable differences of susceptibility) as follows: preliminary treatment with 0.1 c.c. of serum; the next day, injections of 10, 100 and 1,000 lethal doses of culture respectively. Experiments are being carried on with a view to introducing the method of simultaneous injection. According to CORONI, the serum prepared against a strain belonging to one particular group occasionally proved highly effective against other groups as well.

In the United States, two methods of test are in use, as in the case of anti-meningococcus serum. In the test at the Washington Hygienic Laboratory prescribed by the Federal Government (Regulations of April 6th, 1921), two comparative series are laid out with the standard serum and the serum to be tested. Details are as follows: preliminary treatment with the serum of three mice in each case, twenty-four hours later, injection of culture. In New York State a standard serum is also used for comparison in all cases. 0.2 c.c. of this serum protects mice of 16-22 gms. against a subsequent injection of 0.1 c.c. of a No. 1 pneumococcus culture, 0.00001 c.c. of which kills untreated mice in fort-eight hours. The animals having received preliminary treatment must remain alive for not less than four days. Commercial sera, on the other hand, are tested by the mixed injection process. A mixture of 0.5 c.c. of the particular serum dilution with 0.5 c.c. of the culture (both dilutions being made with broth) is administered to mice of 16-22 gms. by very slow intraperitoneal injection (two minutes being taken for the injection); another series of mice are also injected with corresponding quantities of standard serum and culture.

NEUFELD and HAENDEL inject decreasing quantities of serum intraperitoneally into mice, and administer a constant "medium" dose of virulent cocci three hours later. Comparative experiments with this and the New York State method showed a satisfactory measure of correspondence. Here, the action of the serum conforms approximately to the law of multiple proportions. On the other hand, subcutaneous serum titration only gives the threshold value, but not the exact titre. At the Copenhagen Serum Institute (KRISTENSEN),



the mixed injection process is used. Decreasing dilutions of serum are left to stand at room temperature for five minutes with constant quantities of culture and are then intraperitoneally injected into mice—virulence control with 1/100th of the quantity of culture. The titre in this case is the quantity of serum which allows about half of the mice to survive; for a more accurate titration, the relation between the serum doses received by the animals that die and the time elapsing until death occurs is plotted out in the form of a curve. By this method, the Copenhagen Institute has determined, for a number of sera, a scale of values more or less corresponding to that computed for the same sera by GRIFFITH (London) and WADSWORTH (Albany, N.Y.).

So far as the *mechanism of the serum action on human patients* is concerned, NEUFELD remains convinced that the tropins probably came into play. GRIFFITH considers it possible that the serum inhibits the injurious action of the microbes on the leucocytes by destroying their capsules, whilst, at the same time, progressively attenuating the virulence of subsequent generations of pneumococci in the animal organism.

The Second Standardisation Conference came to the conclusion that the titration of the sera on mice is possible in two ways: either (1) by injecting intraperitoneally 0.2 c.c. of serum mixed with decreasing doses (0.4-0.1 c.c.) of a highly virulent broth culture, or (2) by injecting intraperitoneally decreasing quantities of serum (0.001-0.0001 c.c.), and, three hours later, a moderate dose of culture, for example 0.0001 c.c. The virulence of the culture should be such that 0.0000001 c.c. will kill control mice with certainty within twenty-four hours. In either case, a standard serum should be used for purposes of comparison. To sum up, it may be said that the effect of these investigations has been to establish the possibility of a reliable standardisation of pneumococcus serum.

The Conference also decided, on the motion of NEUFELD and GRIFFITH, to undertake further research into the mode of action of the therapeutic sera on patients. Finally, at the proposal of GRIFFITH, it was decided that the effect of anti-streptococcus serum on cases of human pneumococcus infection should be studied.

## VII. ANTI-STREPTOCOCCUS SERUM. SCARLET-FEVER SERUM.

A detailed report on the titration of anti-streptococcus sera was submitted to the Standardisation Commission by MORGENROTH (October 19th, 1923). Up till then, the testing of such sera had been carried out on mice with one and the same strain of streptococcus, regardless of the fact that many of the strains occurring in man are insensitive to these antibodies. An attempt was made to explain this on the hypothesis that the strains of streptococcus most commonly used for serum production had lost their original antigenic character by frequent passages through mice, and had become similar one to the other in respect of antigenic properties. Judging by MORGENROTH'S investigations, however, this does not seem to be correct, for two strains freshly isolated from human cases, and pathogenic for mice, kept their antigenic properties unchanged throughout in spite of seventy passages through mice. The real explanation of these observations is more likely to be that a large proportion of the streptococci used for the immunisation of the horses were antigenically similar to begin with. As regards three of these strains, MORGENROTH was, in fact, able to prove it. His technique consisted in subcutaneously injecting a series of mice with decreasing quantities of serum, and administering ten times the *dosis certe letalis* of culture intraperitoneally on the

following day ; the *dosis certa letalis* lies somewhere between 1 : 1,000,000 and 1 : 10,000,000 c.c. The widespread belief that frequent mouse passages are necessary to maintain virulence is also mistaken, according to his experience. It is precisely in passages that strains initially virulent for mice lose their virulence completely ; only strains of high original virulence retain this property when subjected to regular animal passages, and can recover it by a few passages after temporary attenuation in artificial media. Much better results are, however, obtained in this connection by UNGERMANN'S method of preserving streptococci in fluid serum under liquid paraffin.

MORGENROTH worked out a new process for testing sera against streptococci of low virulence as well. It is based on the ability of all fresh cultures of hæmolytic streptococci isolated from human cases to induce extensive phlegmons and chronic general infection when subcutaneously injected into mice. It seems possible to protect mice against the occurrence of these phlegmons by general treatment with antistreptococcus serum. This method is likely to be suitable, not only for the titration of the sera, but also for rapid identification of the type of streptococcus. It also seems possible, in cases of intravenous inoculation, to convert the otherwise acute and fatal disease into a chronic intermittent infection by general treatment with anti-streptococcus serum. On this basis plans were drawn up for subsequent work with the object of determining the various types, as in the case of the meningococci and pneumococci, and of investigating later the practical possibilities of using and titrating anti-streptococcus serum. The premature death of the great scientist arrested the progress of this undertaking, and the Standardisation Commission has proceeded no further as yet with this group of questions.

The proposal was made in Geneva, however (1926), to have the question of "the scarlet-fever streptococcus serum" studied by the American, English and Polish Institutes acting in conjunction with the Copenhagen Institute. It was contemplated that the group of questions should be jointly investigated at a later session. The Commission expressly stated that it would give no opinion as to the etiology of scarlet fever.

At the Standardisation Conference at Frankfort (1928) the dry standard serum of the United States, which the Hygienic Laboratory at Washington was prepared to supply, was taken as the initial basis with a view to securing uniformity in these investigations. The unit of the serum was fixed at the equivalent of ten times the quantity of serum neutralising the test-toxin dose in intracutaneous injections into man ; the test toxin dose was to be five times the quantity of streptococcus toxin which, when intracutaneously injected into persons sensitive to toxin (*i.e.*, Dick-positives) produces in the majority of cases a reaction of not less than 10 mm.

In this connection, a brief reference may be made to the promising research work of O'BRIEN, OKELL and PARISH at the Wellcome Laboratories. Previously, scarlet fever serum had been tested by the following methods :

- (a) On man :
- (1) The Schultz-Charlton test ;
  - (2) The above-mentioned process of neutralisation of the test toxin dose in intracutaneous injections of toxin-serum mixtures ;
  - (3) Determination of the time elapsing after an injection of serum, before a Dick-positive person becomes Dick-negative (CRUICKSHANK).

- (b) On animals : (4) Intracutaneous test on goats (WADSWORTH and KIRKBRIDE);  
 (5) OKELL'S and PARISH'S new rabbit test.  
 (c) *In vitro* : (6) The RAMON flocculation test.

In the investigations at the Wellcome Institute, the first process proved inaccurate ; the results of the sixth process did not correspond to the antitoxin content of the sera, as had already been found by the Frankfort Institute. The fourth process cannot be applied to European goats. With methods (2) and (3), on the other hand, some approach, at least, to an estimate of titre could be made, if the tests were performed on a fairly large number of human beings ; but the difficulty of securing an adequate number of scarlet fever cases or of healthy Dick-positive persons for these purposes must not be underrated. The new process, in which the rabbit is used as a test animal, seems therefore to constitute a notable advance.

By this process, the protective power of the serum is measured. The animals are intravenously injected with decreasing quantities of serum and, from four to six hours later, with 10 c.c. of a trypsin digested broth culture of scarlet-fever streptococci which kills 63-70 per cent of untreated rabbits within six days. The animals previously treated must remain alive for six days after the injection of the culture, though as a matter of fact they usually suffer from articular metastases later on. The serum would therefore seem only to afford protection against the toxin, but not to have any certain anti-infectious action. Table XIII gives an instance of such a test.

Table XIII.

TITRATION OF VARIOUS SERA FOR CONTENT OF ANTIBODIES AGAINST SCARLET-FEVER STREPTOCOCCI, ACCORDING TO OKELL AND PARISH.

Source of serum	Number of rabbits	Percentage of protected rabbits, the quantity of serum used being (in c.c.)			
		≥ 5	1	0.25	0.1
No serum, normal horse serum, diphtheria-tetanus serum .. .. .	120	6	..	..	..
Animals having recovered from scarlet fever (Dick —) .. .. .	6	50	..	..	..
Unconcentrated scarlet-fever serum P .. .. .	9	66	0	..	..
Unconcentrated scarlet-fever serum Q .. .. .	15	77	33	0	..
Concentrated scarlet-fever serum R .. .. .	15	88	66	33	..
Concentrated scarlet-fever serum S .. .. .	12	100	100	66	33

Routine tests are carried out in the Wellcome Laboratories on six rabbits in each case, with a standard serum and the serum to be tested.

The authors report that the results of tests by this method agreed well with the clinically observed curative value of the serum.

This test can also be carried out by the mixed injection method ; even in curative tests, the serum still remains active from one to two hours after injection of the culture.

### VIII. ANTI-GAS-GANGRENE SERA.

The Standardisation Conference held at Frankfort in 1928 took up the question of the titration of the Welch-Fraenkel serum, and decided to discuss the methods of test in use in the various countries at its next session. The preliminary work in that connection is now in hand.

The following are the principal processes hitherto used for the titration of anti-sera against the various types of gangrene agents :

1. In the case of toxin-producing agents, titration of the antitoxin on doves, guinea-pigs and the isolated heart of the frog (the most extensive investigations having taken place in this connection with symptomatic anthrax serum). The adjustment must always be made, in this case as well, by comparison with a standard serum.

2. Agglutinins, precipitins and complement-fixation.

3. Anti-infectious action, preferably by the mixed injection method, using standard serum as a control.

The following new processes have recently been reported from the Wellcome Laboratories :

MASON and GLENNY prepared a stable toxin from the Welch-Fraenkel culture by complete saturation of the broth culture filtrate with ammonium sulphate, and drying of the precipitate. This is tested by the mixture method—using standard serum as a control—both on mice and for hæmolysis *in vitro*. With sufficiently active toxins, the results agreed admirably with each other (see Table XIV).

Table XIV.

TESTING OF THE WELCH-FRAENKEL CULTURE ON MICE  
AND BY HÆMOLYSIS.

Mice Mortality percentage	Degree of hæmolysis
100	Complete, or practically complete
50-80	Partial
30-50	Marked traces
10	Traces
0	0

Further experiments by BUTTLE and TREVAN showed that it was possible to titrate the toxin of the Welch-Fraenkel bacillus, and still more accurately the toxin of *B. perfringens*, against the specific antitoxins on the uterus of the surviving rabbits. The results obtained by this method agreed well with the L+ value as determined by intravenous injections into mice. According to SORDELLI and FERRARI, the therapeutic serum can be satisfactorily titrated against the toxin of *B. histolyticus*, but are not in accordance with the law of multiple proportions.

## SERODIAGNOSIS.<sup>1</sup>

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### BLOOD GROUP DETERMINATION.

The confusion caused by the current nomenclatures of Moss and JANSKY (Group I Moss = Group IV Jansky) had long been regarded as a serious source of difficulties both in the investigation and routine examination of human iso-agglutinins. The last Standardisation Conference (Frankfort, 1928) took the very welcome step of ruling that the old designations of v. DUNGERN and HIRSZFELD (AB, A, B and O) should be obligatory in lieu of the two systems mentioned. It was also recommended that, not only should the diagnostic sera be designated by the letter of the group from which they were obtained, but that the designation should also indicate the blood corpuscle groups with which they reacted, thus: "Test serum A (anti-B)" and "Test serum B (anti-A)". The former test serum was to be put up in colourless glass and the latter in brown glass containers. It was decided to compare the processes currently employed in various countries in the production, preservation and titration of the test sera, with a view to the ultimate adoption of international standards. The Copenhagen Institute was selected as the central laboratory for this work.

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<sup>1</sup> With regard to Serodiagnosis of Syphilis, see documents indicated on page 59.

## C. BACTERIAL ANTIGENS, VACCINES.

### 1. TUBERCULIN.

At its session of October 1924, the Health Committee decided to take up the question of the titration of tuberculin. A Committee, under the chairmanship of TSURUMI, was instructed to study the methods of testing employed in the various laboratories, to compare their results and to decide whether any one of them could be recommended as a standard process. At the request of the Committee, CALMETTE and DE POTTER submitted a detailed report based on their own experience, the findings of which are briefly reproduced below.

#### *The Preparation of Tuberculin.*

CALMETTE and DE POTTER received reports from thirty-four institutes and sixteen different countries, showing wide divergencies in the method of preparation. The initial cultures used were: in twelve cases human; in six bovine; in eleven human and bovine; in three human, bovine and avian; in one human, bovine and equine; in one human, bovine, porcine and avian tuberculosis bacillus strains. The composition of the broth was also very different, as well as its initial reaction (pH ranging from 6 to 8 ! ) and the period of incubation at 38° C. (from 1 to 18 months ! ). Sterilisation at the end of incubation was only carried out in a few laboratories, and the concentration prescribed by Koch was not made everywhere. Filtration was effected in most cases, sometimes before and sometimes after concentration, usually through paper only, and in but three cases through a bacterial filter. One institute neither filtered nor concentrated, but merely poured off the supernatant fluid from the deposit. Where sterilisation took place, it was carried out at temperatures sometimes not exceeding 60° C, sometimes rising to 120° C. Finally, certain institutes added half per cent phenol.

#### *The Method of Test.*

The testing of tuberculin is officially prescribed in Brazil, Germany (since 1899), France, Italy, Japan and Czechoslovakia. The following processes are used :

(1) The classical KOCH process, as modified by DÖNITZ, has been prescribed in Germany and is carried out at the Frankfort Institute. Fifty guinea-pigs of 350-400 gms. are subcutaneously injected with 0.5 mg. of a 12-14 days' broth culture of *B. tuberculosis*, homogeneously suspended in 0.5 c.c. physiological NaCl solution. As soon as the animals have become tuberculous, a fact which can be ascertained from the steady loss of weight (usually towards the end of the fourth week), a preliminary test is undertaken in order to determine whether they are ripe for the main test. From two to four animals are subcutaneously injected with increasing doses of tuberculin (0.3 to 0.5 c.c.). The animals should die with characteristic symptoms, failing which it is necessary to wait until a later preliminary test yields this result. The real test experiment is then undertaken, being carried out on two parallel series of six guinea-pigs each. The first series is given increasing subcutaneous doses of standard tuberculin in order to determine the

minimum lethal dose ; the second series receives corresponding doses of the preparation to be tested, the doses used being 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 c.c. The animals must die within twenty-four hours and must display the characteristic signs on post-mortem examination. An essential condition for this test is the use of cultures of *B. tuberculosis* of constant virulence. Very considerable and disturbing differences, both in the course of the tuberculous process and in the degree of sensitiveness to tuberculin, are nevertheless frequently observed. It was formerly believed that these irregularities might be obviated by using only those guinea-pigs which steadily lost weight. It has been found much more satisfactory, however, to inject a fairly large number of animals intracutaneously with standard tuberculin about four weeks after infection, and only to use the animals giving a good and uniform allergic reaction for the subsequent main lethal shock dose test, which takes place three weeks later (HETSCH, SCHLOSSBERGER and WICHMANN).

(2) The *intracerebral* test of VON LINGELSHHEIM and BOREL represents a modification of the above method. It is certainly more sensitive but, according to NEUFELD, not absolutely specific. Furthermore, it can only be carried out with purified preparations, since even glycerine and peptone are sufficient to produce a violent cerebral shock.

(3) LONG has recently recommended the "*spermatocyte tuberculin test*". Male guinea-pigs of about 400 gms. are infected with a strain of such low virulence as to induce only caecation of the local lymph gland after the lapse of one month ; 0.1 c.c. of the tuberculin dilution to be tested is then injected into one testicle. The reading may be taken either at the end of thirty-six hours or only after one month, the testicle being excised and microscopically examined in sections. After thirty-six hours the signs to be observed are inflammatory oedema, coagulation-necrosis of the spermatocytes and spermatids, dilatation of the seminiferous tubules and unrestrained cell division of all sperm cells. In the following weeks all these cells, except the spermatogonia, die. When the testicle is examined after one month, only the basal membrane and a single cell layer of spermatogonia is hence found in the atrophied tubules. When the late symptoms are observed, the results are more easily readable. Two tuberculous animals are injected in each case with 0.01, 0.001, 0.0001 and 0.00001 c.c. of undiluted tuberculin, two healthy control animals receiving 0.01 c.c. The reaction should be sharply defined and absolutely specific.

(4) The *intradermal* injection of dilutions of the standard tuberculin and of the tuberculin to be tested into tuberculous guinea-pigs. The process has this advantage, that an appreciable number of injections can be made into one and the same animal, so that the drawback of individual differences of sensitiveness in various animals disappears. This method would seem to give more reliable results with tuberculous cattle than with guinea-pigs.

(5) *The test on tuberculous human beings.* Owing to the painfulness of the intradermal injections, only the less precise *skin test* will usually be possible.

Whilst in the first and second methods the measure is given by the lethal tuberculin shock, methods (4) and (5) rely upon the determination of allergy. To that extent they lend themselves best to the particular use of old tuberculin, which is nowadays practically the only form of its employment that need concern us. *A priori*, it would therefore be natural to prefer these latter processes ; unfortunately, however, they are much inferior to the DONITZ method in point of accuracy. In particular, they do not enable the threshold values to be determined with precision (HETSCH, SCHLOSSBERGER and WICHMANN).

Of the methods of test *in vitro*, the following have been proposed :

(6) *Complement-fixation*. The requisite anti-serum is obtained from cattle or horses by repeated intravenous injections of living tubercle bacilli.

(7) *The flocculation test*, according to DREYER and VOLLUM. It is difficult to obtain a serum which precipitates well ; this can best be achieved by the intravenous injection of horses with the " Diaplyte vaccine " ; that is to say, tubercle bacilli freed from fat by means of acetone, and killed by formalin. Decreasing dilutions of tuberculin are added, in small test-tubes, to constant quantities of the serum to be precipitated, and the mixture is then made up to the same volume with physiological NaCl solution. After being gently shaken, the tubes are then placed in a water bath adjusted to 37° C., but are only immersed to the extent of two-thirds, in order to ensure thorough mixing of the contents by continuous convection.

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#### *Critical Review of the Methods.*

It should be stated, to begin with, that every method is not suitable for use with every kind of tuberculin. Preparations containing an admixture of disinfectant are useless for flocculation tests. The complement-fixation test fails with albumose-free preparations, which otherwise react satisfactorily ; on the other hand, it gives unspecific reactions with other acid-fast bacilli, and reactions which, though weaker, are yet positive, even with concentrated unseeded glycerinated broth.

With the test-tube process it is doubtful, moreover, whether the components of the tuberculin reacting with the serum are identical with those which suscite human allergy.

It must, however, be conceded to CALMETTE and DE POTTER that, even in the case of the animal test, we cannot be certain whether the resultant toxic action on the animal is comparable to the " antigenic " (more correctly allergising) action of tuberculin on tuberculous human beings. This would be most likely to hold good for intradermal reactions in tuberculous animals, but it is precisely here that the intradermal test on tuberculous guinea-pigs is known to be capable of yielding a result differing utterly from that obtained in tuberculous cattle. It would seem to be uncertain, therefore, how far the behaviour of a tuberculin preparation in man can be inferred from the reaction in this or that animal.

CALMETTE and DE POTTER compared the intradermal method in guinea-pigs, the complement-fixation test and the DREYER and VOLLUM flocculation process, with the skin reaction in tuberculous human beings, using a considerable number of tuberculins, which they had prepared themselves with a variety of tubercle bacillus strains cultivated in different media, as well as eighteen commercial preparations purchased by the Health Committee. In their hands, the intradermal test on guinea-pigs was the most successful. Table XVII (page 55) gives a survey of the results which they obtained in testing commercial preparations comparatively with their highly active standard tuberculin.

This survey plainly shows how little the effect on tuberculous human beings tallies with the results of the flocculation and complement-fixation tests, whereas it corresponds satisfactorily to the intradermal test on tuberculous guinea-pigs. Unfortunately, CALMETTE and DE POTTER did not carry out comparative tests with the Koch-Dönitz processes.

With regard to the intradermal test, they are of opinion that this test, when carried out on tuberculous guinea-pigs or cattle, gives the



Table XVII.

TESTS OF VARIOUS COMMERCIAL TUBERCULINS BY VARIOUS PROCESSES,  
PERFORMED BY CALMETTE AND DE POTTER.

Preparation	Comple- ment- fixation (units in 1 c.c.)	Flocculation test (end-point of effective dilution)	Intradermal test on guinea-pigs (end-point of effective dilution)	Skin-test on man, effected with a 10-% dilution
I. Standard ..	130	1 : 10,000 + + +	1 : 2,500	Very good
II. Human ..	115	1 : 10,000 +	1 : 100	Very good
III. Albumen- free ..	200	1 : 10,000 + + +	1 : 10	Useless
IV. Human ..	90	1 : 1,000 + + +	1 : 2,500	Very good
V. Bovine ..	70	1 : 1,000 + +	1 : 1,500	Weak, just usable
VI. Albumen- free ..	40	1 : 5,000 +	1 : 500	Weaker than V, but still usable
VII. Precipitated	0	1 : 100 + +	1 : 2,000	Very good
VIII. Human ..	90	1 : 10,000 + + +	1 : 2,000	Very good
IX. Bovine ..	40	1 : 2,500 +	1 : 2,000	Very good
X. Bovine ..	200	1 : 1,000 +	1 : 1,000	Very good
XI. Human ..	60	1 : 100 + +	0	Useless
XII. Human ..	70	1 : 100 +	1 : 100	Inconstant re- sults even when con- centrated
XIII. Human ..	55	1 : 100 + +	1 : 100	Useless
XIV. Human ..	70	1 : 100 +	1 : 10	Useless
XV. Human ..	70	1 : 10,000 +	1 : 500	Inconstant like XII
XVI. Bovine ..	150	1 : 1,000 + + +	1 : 500	Inconstant like XII
XVII. Avian ..	80	1 : 100 + +	1 : 500	Useless
XVIII. Mixed ..	115	1 : 10,000 + + +	1 : 1,000	Very good
XIX. Avian ..	100	1 : 500 +	1 : 10	Useless

best results ; it enables the efficacy (*pouvoir toxigène et pouvoir antigène*) of several tuberculins to be determined relatively to the standard preparation on one and the same animal ; it is absolutely specific, as it gives no reaction either with concentrated culture filtrates of other acid-fast bacilli, or with mallein or concentrated unseeded glycerinated broth ; it has the further advantage that it is both swift and cheap. The authors point out, however, that the definitive test of a tuberculin preparation must necessarily be carried out in all cases on tuberculous individuals of the species for which the preparation is to be used.

In consideration of this report, the Standardisation Commission (Geneva, 1926) decided, as a first step, to compare the standard tuberculins of the Frankfort, Paris and Tokio Institutes. As was reported at the Commission's Frankfort session (1928), these investigations were carried out at the Frankfort Institute and at the National Institute for Medical Research in London. They showed the various standard

preparations to be equally active. The London Institute prepared a large supply of standard tuberculin and placed it at the disposal of the Health Organisation. Comparisons of this preparation with the standards of the various countries are being continued at the Copenhagen Institute. *The Commission regards this part of the question as settled and will endeavour to secure the acceptance of an international standard tuberculin at its next session.*

## 2. DIPHTHERIA VACCINE (DIAGNOSTIC AND PROPHYLACTIC).

At its session at Frankfort (1928), the Standardisation Commission decided to investigate the question of the titration of the various vaccines used for protective diphtheria vaccination (anatoxin and other toxin derivatives) and to lay down a uniform vaccination technique. The Pasteur Institute in Paris offered to supply anatoxin for the purpose. It is proposed to ascertain in due course whether RAMON'S flocculation method can also be used for the standardisation of diphtheria vaccine. Meanwhile, investigations have been carried out by the Copenhagen and Paris Institutes which may be expected to lead to proposals for the standardisation of these preparations.

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