

THE STANDARDISATION AND
ESTIMATION OF VITAMIN A.

F271.3
G5
044781

Crown Copyright Reserved

Privy Coun

MEDICAL RESEARCH COUNCIL

Reports on Biological Standards

IV.—THE STANDARDISATION AND ESTIMATION OF VITAMIN A

Edited by

E. MARGARET HUME & HARRIETTE CHICK



LONDON
HIS MAJESTY'S STATIONERY OFFICE

1935

Stationery Office Code Number
45-8-2

Universal Decimal Classification
577.16a

MEDICAL RESEARCH COUNCIL

The Most Hon. the MARQUESS OF LINLITHGOW, P.C., K.T., G
(*Chairman*).

The Rt. Hon. LORD MILDMAY OF FLETE, P.C. (*Treasurer*).

W. S. MORRISON, M.C., K.C., M.P.

Professor E. D. ADRIAN, M.D., F.R.C.P., F.R.S.

SIR THOMAS LEWIS, C.B.E., M.D., D.Sc., F.R.C.P., F.R.S.

Professor D. P. D. WILKIE, O.B.E., M.D., M.Ch., F.R.C.S.

Professor H. S. RAPER, C.B.E., D.Sc., M.B., F.R.S.

Professor A. J. CLARK, M.C., M.D., F.R.C.P., F.R.S.

Professor J. C. G. LEDINGHAM, C.M.G., D.Sc., F.R.C.P., F.R.S.

Professor J. A. RYLE, M.D., F.R.C.P.

Professor MATTHEW J. STEWART, M.B., F.R.C.P.

EDWARD MELLANBY, M.D., F.R.C.P., F.R.S. (*Secretary*).

COMMITTEE ON ACCESSORY FOOD FACTORS (VITAMINS)

(*Jointly with the Lister Institute of Preventive Medicine*)

EDWARD MELLANBY, M.D., D.Sc., F.R.C.P., F.R.S. (*Chairman*).

Miss HARRIETTE CHICK, C.B.E., D.Sc. (*Secretary*).

Miss K. H. COWARD, D.Sc.

J. M. HAMILL, O.B.E., M.D., D.Sc.

Professor ARTHUR HARDEN, D.Sc., F.R.S.

SIR FREDERICK G. HOPKINS, O.M., D.Sc., F.R.C.P., P.R.S.

Miss E. M. HUME.

Professor R. A. PETERS, M.C., M.D., F.R.S.

O. ROSENHEIM, D.Sc., F.R.S.

S. S. ZILVA, D.Sc.

PREFACE

During the last few years much has been done to make it possible to express vitamin potency in exact quantitative terms, by the improvement of methods of biological measurement and by the adoption of agreed standards. This meets the practical need for means of expressing the strength of the highly concentrated preparations of the various vitamins which are now available for therapeutic use, and the similar need in dietetics for means of measuring the vitamin content both of ordinary foods and of preparations in respect of which special claims are made by their manufacturers. The methods have also immediate value in introducing greater accuracy into fundamental research work in the field of nutrition.

The present international agreement on the subject of vitamin standards is the result of two conferences held in London, in 1931 and 1934, under the auspices of the Permanent Standards Commission of the Health Organisation of the League of Nations. The decisions then taken had been made possible only by a great deal of preparatory research work, very much of which was done in this country. It is of a section of that work, concerned in particular with vitamin A, that the following report gives an account.

At the first International Conference for the Standardisation of Vitamins, in 1931, a provisional unit of vitamin A was established in terms of the biological activity of a sample of carotene. It was necessary to make this standard provisional, in the then imperfect state of knowledge, as it seemed clear that experience would reveal possibilities of improvement. This was soon shewn to be the case, for immediately after the conference several isomeric forms of carotene, including β -carotene, were isolated by various investigators; and, since it was evident that the standard carotene was a mixture, it at once seemed probable that a sample of a uniform substance could be substituted with advantage at some future date. A further point was that the crystalline form of the provisional standard had been found by some workers to be difficult to manipulate, making it desirable that any future standard should be issued in solution. The second International Conference, in 1934, accordingly adopted a solution of pure β -carotene as the permanent standard in terms of which units of vitamin A were in future to be expressed.

Much of the preparatory research work on the standardisation of vitamin A, for both the International Conferences, was done by the members of a special sub-committee of the Accessory Food Factors Committee, appointed jointly by the Medical Research Council and the Lister Institute. In the course of this excellent team work all kinds of problems presented themselves, such as finding the best

MEDICAL RESEARCH COUNCIL

The Most Hon. the MARQUESS OF LINLITHGOW, P.C., K.T., G.C.I.E.
(*Chairman*).

The Rt. Hon. LORD MILDMAY OF FLETE, P.C. (*Treasurer*).

W. S. MORRISON, M.C., K.C., M.P.

Professor E. D. ADRIAN, M.D., F.R.C.P., F.R.S.

SIR THOMAS LEWIS, C.B.E., M.D., D.Sc., F.R.C.P., F.R.S.

Professor D. P. D. WILKIE, O.B.E., M.D., M.Ch., F.R.C.S.

Professor H. S. RAPER, C.B.E., D.Sc., M.B., F.R.S.

Professor A. J. CLARK, M.C., M.D., F.R.C.P., F.R.S.

Professor J. C. G. LEDINGHAM, C.M.G., D.Sc., F.R.C.P., F.R.S.

Professor J. A. RYLE, M.D., F.R.C.P.

Professor MATTHEW J. STEWART, M.B., F.R.C.P.

EDWARD MELLANBY, M.D., F.R.C.P., F.R.S. (*Secretary*).

COMMITTEE ON ACCESSORY FOOD FACTORS (VITAMINS)

(*Jointly with the Lister Institute of Preventive Medicine*)

EDWARD MELLANBY, M.D., D.Sc., F.R.C.P., F.R.S. (*Chairman*).

Miss HARRIETTE CHICK, C.B.E., D.Sc. (*Secretary*).

Miss K. H. COWARD, D.Sc.

J. M. HAMILL, O.B.E., M.D., D.Sc.

Professor ARTHUR HARDEN, D.Sc., F.R.S.

SIR FREDERICK G. HOPKINS, O.M., D.Sc., F.R.C.P., P.R.S.

Miss E. M. HUME.

Professor R. A. PETERS, M.C., M.D., F.R.S.

O. ROSENHEIM, D.Sc., F.R.S.

S. S. ZILVA, D.Sc.

PREFACE

During the last few years much has been done to make it possible to express vitamin potency in exact quantitative terms, by the improvement of methods of biological measurement and by the adoption of agreed standards. This meets the practical need for means of expressing the strength of the highly concentrated preparations of the various vitamins which are now available for therapeutic use, and the similar need in dietetics for means of measuring the vitamin content both of ordinary foods and of preparations in respect of which special claims are made by their manufacturers. The methods have also immediate value in introducing greater accuracy into fundamental research work in the field of nutrition.

The present international agreement on the subject of vitamin standards is the result of two conferences held in London, in 1931 and 1934, under the auspices of the Permanent Standards Commission of the Health Organisation of the League of Nations. The decisions then taken had been made possible only by a great deal of preparatory research work, very much of which was done in this country. It is of a section of that work, concerned in particular with vitamin A, that the following report gives an account.

At the first International Conference for the Standardisation of Vitamins, in 1931, a provisional unit of vitamin A was established in terms of the biological activity of a sample of carotene. It was necessary to make this standard provisional, in the then imperfect state of knowledge, as it seemed clear that experience would reveal possibilities of improvement. This was soon shewn to be the case, for immediately after the conference several isomeric forms of carotene, including β -carotene, were isolated by various investigators; and, since it was evident that the standard carotene was a mixture, it at once seemed probable that a sample of a uniform substance could be substituted with advantage at some future date. A further point was that the crystalline form of the provisional standard had been found by some workers to be difficult to manipulate, making it desirable that any future standard should be issued in solution. The second International Conference, in 1934, accordingly adopted a solution of pure β -carotene as the permanent standard in terms of which units of vitamin A were in future to be expressed.

Much of the preparatory research work on the standardisation of vitamin A, for both the International Conferences, was done by the members of a special sub-committee of the Accessory Food Factors Committee, appointed jointly by the Medical Research Council and the Lister Institute. In the course of this excellent team work all kinds of problems presented themselves, such as finding the best

solvent for carotene and determining the conditions under which this substance remained stable in solution. In addition, completely new aspects of the subject arose, including the possibility of measuring vitamin A by a physical method, based on spectrophotometry, which necessitated a comparison of the spectrophotometric results with those obtained by biological methods. Thus researches were made which, although each separate item was possibly of minor importance, became in the mass of great interest to workers in the field. Some of the work had been published by the individuals concerned, but much remained unpublished: it was thought, therefore, that the collection and publication of all the investigations together would constitute an important record, very useful to other experts.

It should be clearly understood, however, that this publication is not to be regarded either as a comprehensive account of the quantitative estimation of vitamin A, or as a detailed guide to the methods of making the tests. It is actually a collection of technical information, not available elsewhere, concerning the properties and use of the international vitamin A standard, and is intended for the information of competent workers already practised in the usual methods for the quantitative estimation of the vitamin.

As in all problems of active and growing importance, it will be seen that in some respects the present account is incomplete and indeed raises fresh problems of great interest. Further work, for instance, is needed to show whether the spectroscopic method of estimating vitamin A is trustworthy, and to establish more precisely the conversion factor relating this method to the results of the biological tests. It is hoped that in this and other ways the present report will stimulate further investigation.

In issuing this report, an instructive example of associated researches directed to a common object, the Medical Research Council would thank all those who have combined in its production, namely, Dr. Katharine Coward, Mr. M. Evers, Miss E. M. Hume, Mrs. K. Lathbury, Dr. I. Smedley MacLean, Dr. R. J. Macwalter, Dr. T. Moore, Dr. R. A. Morton, Dr. O. Rosenheim, and the late Dr. R. S. Morgan. To Miss Hume and Dr. Harriette Chick has fallen the task of co-ordinating and editing all the results of the investigations, and to them also the Council would wish to express their appreciation.

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

1st November, 1935.

REPORT ON THE STANDARDISATION AND ESTIMATION OF VITAMIN A

Edited by

E. MARGARET HUME AND HARRIETTE CHICK

CONTENTS

	PAGE
INTRODUCTION	6
I.—CAROTENE AS INTERNATIONAL STANDARD FOR VITAMIN A	
(A) SOLVENTS FOR CAROTENE: THEIR EFFECT ON ITS STABILITY AND EASE OF ABSORPTION.	
1. Introductory	9
2. Experimental	10
(a) Technique of Colorimetric and Biological Tests	10
(b) Maintenance of Colour as an Indication of the Preservation of Biological Activity	11
(c) Selection of a Suitable Oil as Solvent for Carotene. Colorimetric and Biological Tests on Carotene Dissolved in Various Solvents	12
3. Summary and Conclusions	23
(B) RELATION BETWEEN THE VITAMIN A ACTIVITY OF THE 1931 INTERNATIONAL STANDARD CAROTENE AND PURE β -CAROTENE.	
1. Introductory	24
2. Experimental	25
(a) Biological Tests	25
(b) Colorimetric and Spectroscopic Tests	26
3. Summary	27
II.—SPECTROPHOTOMETRIC METHOD OF ESTIMATING VITAMIN A BY MEANS OF THE EXTINCTION COEFFICIENT AT $328m\mu$	
1. Introductory	28
2. Experimental	29
(a) Results Obtained by Different Workers in Determining $E_{1\%}^{1\text{cm}}$ $328m\mu$ for Six Specimens of Cod-Liver Oil and One Vitamin A Concentrate, with a Discussion of the Different Methods Used	29
(b) Ratio between the Values obtained for Vitamin A Potency, by the Spectrophotometric and Biological Methods	33
3. Discussion	35
4. Summary	36

6 STANDARDISATION OF VITAMIN A

III.—THE USE OF COD-LIVER OIL AS A SUBSIDIARY INTERNATIONAL STANDARD FOR VITAMIN A	PAGE
1. Biological Tests on Cod-Liver Oil X	38
2. Biological Tests on Cod-Liver Oil Z	38
3. Solvents for Cod-Liver Oil in Biological Tests	40
4. Summary	40
IV.—GENERAL SUMMARY AND CONCLUSIONS	41
V.—REFERENCES	43
VI.—APPENDICES	
1. Protocols of the Biological Tests, Nos. (1) to (19)	44
2. Approved Methods of Saponification	51
3. Report of the Second International Conference on Vitamin Standardisation (London, 1934, League of Nations Health Organisation)	52
SUBJECT INDEX	60

INTRODUCTION

At the International Conference on Vitamin Standardisation, which was held in London in 1931 at the invitation of the Permanent Commission on Biological Standardisation of the League of Nations (Health Section), a certain sample of carotene was accepted provisionally for two years as the biological standard for vitamin A activity. This arrangement recognised that difficulties might arise in its use, since both the purity and stability of the sample were doubtful, and certain specific problems for further investigation were suggested by the Conference. Such investigations were undertaken by a number of workers, in some cases independently, in others at the invitation of a Vitamin A Sub-committee, specially set up by the Accessory Food Factors Committee (Medical Research Council and Lister Institute of Preventive Medicine). The body of work thus produced, when put together, forms a substantial mass of evidence in which the results obtained in different laboratories corroborate each other. Published separately, however, the contributions of single laboratories on many points would be evidentially inadequate and would tend to encumber the literature with a mass of small communications of varying individual value. It has, therefore, been thought desirable to collect these observations in

the present publication and it is hoped that their mutual support will make them a valuable contribution to the study of vitamin A standardisation. In those cases in which work by individual workers has already been published separately, the published material has been drawn on less fully, but is included, partly to make the account more complete and partly to strengthen the other investigations described for the first time in this memoir.

Choice of a Solvent for the Standard Carotene.—The greatest difficulty encountered in the use of the sample of carotene adopted as provisional standard was connected with the choice of the solvent to be used for its administration in biological tests. The results obtained in different laboratories, where the standard was dissolved in different solvents, were profoundly discrepant and a considerable body of investigation was evoked in response to the demand for guidance on this point (Section I(A)). This particular technical aspect of the problem became even more pressing, for, as the date of the second meeting of the Vitamin Standardisation Conference approached, evidence accumulated that great difficulty had been experienced in handling carotene in the crystalline state, in which it had been issued for use as standard. There was a widely voiced request that the standard carotene might, in future, be issued as a solution in some suitable oil. Those properties in a solvent for carotene, which would render the solution suitable for world distribution as well as for laboratory use, had, therefore, also to be considered.

β -Carotene as Vitamin A Standard.—In the three years following the 1931 Vitamin Conference, the researches of Karrer and of Kuhn and of their co-workers separated several different, biologically active constituents in carotene, α - β - and γ -carotene, and also identified several other substances, β -oxycarotene (Kuhn and Brockmann, 1932, 1 and 2), semi- β -carotenone (Kuhn and Brockmann, 1933), cryptoxanthine (Kuhn and Grundmann, 1933), α -dihydrocarotene (Kuhn and Brockmann, 1932, 2), which can also serve as provitamin A. Moreover, Kuhn and his co-workers have reported that the various isomers of carotene differ in their biological value; α -carotene, for instance, has only half the biological value of β -carotene (Kuhn, Brockmann, Scheunert and Schieblich, 1933), while some of the other substances mentioned appear to have even less. It was clear, therefore, to those who were concerned in collecting material for the guidance of the 1934 Conference that it would be far better to select, as standard, one of these pure substances than to continue the use of a sample known to be a mixture.

β -Carotene is the form of carotene which is of commonest occurrence and most easily prepared in large quantities. It was therefore selected for investigation with a view to its possible adoption as International Standard. To ensure continuity, it was necessary to know its biological value in terms of the existing standard and this relationship was determined independently in several laboratories

(Section I (B)), thereby supplying a more reliable value for this very important relationship, than one derived from one laboratory only.

Spectrophotometric and Colorimetric Estimation of Vitamin A.—A biological test, involving the use of a biological standard, is the last resort for testing substances which are chemically and physically too ill-defined and too difficult of isolation to permit of a quantitative chemical or physical test. As knowledge of vitamin A has progressed to a sharper definition in this respect, the possibility has increased of replacing the biological test, at least as far as vitamin A in liver oils and concentrates is concerned, by the measurement of the extinction coefficient of the characteristic $328m\mu$ band. In Section II, evidence is collected concerning the reliability and scope of the spectroscopic test as applied to vitamin A, and the relationship which the values for the extinction coefficient bear to the biological values, expressed in international units.

The Antimony Trichloride test ("blue value"), though of undoubted value for quantitative estimation of vitamin A in strictly comparable materials, was not accepted by the Vitamin A Sub-committee as a method of Standardisation and is, therefore, not discussed as such in this Report (*see* p. 40).

Cod-Liver Oil as a Subsidiary Vitamin A Standard.—Besides establishing a sample of carotene as a provisional International Standard for Vitamin A, the 1931 Conference recommended the use of a selected sample of cod-liver oil as a subsidiary standard.

The sample of cod-liver oil referred to in this report as oil X, is now available for international use. It was originally issued for trial as a standard by the Committee of Revision of the United States Pharmacopoeia, and has been examined extensively in biological tests in comparison with the International Standard carotene. When the difficulties with regard to the appropriate solvent for the carotene standard had been overcome, a harmonious series of values was obtained by workers in a large number of different laboratories in the United States and in England. Another sample of cod-liver oil (Z) had been in use for a similar purpose by the Pharmaceutical Society in England and when tested against the Standard in various laboratories in England and on the Continent has also yielded a similar series of harmonious results. Brief tables showing the results obtained in each of these series of tests are included in Section III. They constitute a strong case for the publication of these researches, which, though of doubtful significance individually, are collectively convincing; moreover, they afford the proof that the international standard when handled with proper precautions can yield results which are concordant although emanating from different laboratories.

The results of the experiments on all the above points, summarised in the succeeding pages, were submitted for the consideration of the Second International Conference on Vitamin Standardisation, held in London in June, 1934. The information therein contained was largely instrumental in guiding this Conference to the decisions embodied in its Report, which is reprinted in Appendix 3.

I. CAROTENE AS INTERNATIONAL STANDARD FOR VITAMIN A

(A) SOLVENTS FOR CAROTENE: THEIR EFFECT ON ITS STABILITY AND EASE OF ABSORPTION.

1. Introductory

Ever since it was shown that failure of carotene to show biological activity as a source of vitamin A, could be due to its instability in the solvent used (e.g., ethyl oleate), it has been clear that the right choice of a solvent for use in biological tests is of great importance (Hume and Smedley-MacLean, 1930). Such a solvent must satisfy two conditions; the carotene standard must be stable in it for the period and in the conditions of the biological tests and must be satisfactorily absorbed from it by the test animal. Unless these two conditions are fulfilled, it is obvious that the standard will be worthless for purposes of reference. Of these two conditions, the first may fortunately be controlled by colorimetric methods. The second condition, that of securing satisfactory absorption, should be studied biologically after colorimetric tests have shown that the carotene is stable in the solvent used. Biological tests, without the additional evidence of colorimetric tests, may show the same dose of carotene to be more potent in one solvent than in another, but, unless precautions have been taken to obviate differences in stability, it is impossible to say whether the difference is due to differing utilisation or differing stability of the carotene.

The literature up to 1933 is reviewed by Baumann and Steenbock (1933), who also record a number of observations on the stability of carotene in various solvents, measured spectrophotometrically. Up to the date of this investigation there was no agreement as to which solvent was to be preferred for practical purposes. Cotton seed, arachis, corn or olive oil was frequently used and the varying results recorded in the literature suggested that even the use of different samples of the same vegetable oil might cause variations in the biological activity of the dissolved carotene.

Baumann and Steenbock examined the effect of light, temperature and chemical stabilisers on the stability of carotene in a number of solvents. A refined cotton seed oil (Wesson oil brand) gave the best results, followed by sesame, coconut, olive, corn and crude wheat germ oil in the order given. Addition of hydroquinone in a concentration one-tenth of that of the carotene, did not appear to increase its stability. Ethyl laurate and ethyl sebacate gave very poor results but the stability of the dissolved carotene was greatly enhanced by addition of the hydroquinone. A more detailed study was made of the Wesson cotton seed oil and it was concluded that at room temperature there might be a loss of 26 per cent. of the dissolved carotene within a month and that storage should be at 0° C. and in the dark. Rancidity in the solvent did not appear to be detrimental to the stability of the carotene. From the point of view of stability, Wesson cotton seed oil is the solvent selected by these workers; their study did not, however, include any biological tests.

From a similar spectroscopic study, F. G. McDonald (1933) reached the same conclusion; carotene was very unstable in ethyl butyrate, laurate and palmitate and Wesson cotton seed oil was the most satisfactory solvent tested.

R. G. Turner (1934) studied the stability of carotene in ethyl laurate and olive oil, measuring the rate of fading of the yellow colour with the Lovibond tintometer. The colour faded faster in ethyl laurate than in olive oil, and the presence of stabilisers (0.1 per cent. hydroquinone to 0.2 per cent. carotene) delayed the fading and seemed to arrest it altogether after a certain point. The conclusions to be drawn are a little doubtful. Rough biological tests were carried out, one with a completely faded solution, in which no activity as provitamin A could be detected, and two with half-faded solutions, which gave values approximately half and much less than half, of the original. These biological tests show that there is correlation between fading of carotene solutions and loss of activity as provitamin A.

Mineral oil was examined as a solvent for carotene by Dutcher *et al.* (1934), who found that it definitely hindered the absorption of carotene, as shown by recovery of the carotene from the faeces.

Culhane Lathbury and Greenwood (1934), using arachis and coconut oils, obtained very different biological values for carotene, when dissolved in these solvents, and also when dissolved in different samples of the same solvent. Their experiments were not all controlled with colour tests for stability, but the solutions were kept at a low temperature in full bottles, of which a fresh bottle was opened at least once a week, during the period of the tests. Since the authors had found solutions of carotene in arachis and coconut oil stable when kept under these conditions, they drew the conclusion that different samples of the same oil may vary in the degree to which the dissolved vitamin may be utilised by the animal.

2. Experimental

This section deals with the physical and biological tests made on the specimen of carotene used as international standard from 1931 till 1935 (referred to throughout this report as 1931 standard carotene (*see p. 27*)), and on the β -carotene prepared by Karrer (*see p. 24*), which the International Conference of 1934 decided should supersede it.

(a) *Technique of Colorimetric and Biological Tests.*

Colorimetric Tests.—The physical tests made to detect deterioration, collected in Table I, Tests 1-54, consist of colorimetric measurements made by matching a solution of carotene of known strength, either against the standard glasses of the Lovibond tintometer, or against a solution of potassium bichromate, either directly or in the Duboscq colorimeter. In the latter test, the length of the column of bichromate, which, viewed from above, matched a column of the carotene solution, was measured and the factor given in the Table is the length of the former divided by the length of the latter. None of these means of measuring the fading of the carotene solution is highly accurate. Results indicating a deterioration of less than 25 per cent. are therefore disregarded in the Table.

The results were contributed by the following workers:—

K. Culhane Lathbury and G. N. Greenwood, Res. Labs., The British Drug Houses, Ltd., London.

I. Smedley-MacLean and E. M. Hume, Lister Institute, London.

T. Moore, Dunn Nutritional Lab., Cambridge.

Biological Tests.—The biological tests described in this section were made by the following workers:—

F. J. Dyer, K. M. Key and K. H. Coward, Pharmaceutical Society, London.

K. Culhane Lathbury and G. N. Greenwood, Res. Labs., The British Drug Houses, Ltd., London.

E. M. Hume and H. Henderson Smith, Lister Institute, London.

Some of the results are already published (Dyer, Key and Coward, 1934; Culhane Lathbury and Greenwood, 1934) and the original papers may be referred to for full details as to the technique used.

The biological technique used by Hume and Smith (Collison, Hume, Smedley-MacLean and Henderson Smith, 1929) was their usual curative one with depleted animals, the results being based partly on survival. The number of animals used was usually too small to permit of more than an approximate value for the results obtained.

The individual biological tests are numbered serially in the text and are described in correspondingly numbered protocols in Appendix 1. Where work has already been published, the reader is referred to the original paper for the protocols.

(b) Maintenance of Colour as an Indication of the Preservation of Biological Activity.

Most workers have taken it for granted that loss of colour in a carotene solution is an index of loss of biological potency, and all the evidence in the literature supports this view. The criticism has, however, been advanced that maintenance of colour is not necessarily an indication that the biological value is equally maintained. As the present Report records a number of colorimetric tests, on which considerable reliance was laid in selecting the solvent for the standard carotene, it is deemed useful, before giving these results, to describe a biological test which was specially carried out to test this criticism. This test is therefore inserted first, where it seems to have greatest logical significance.

Biological Test on a Solution of β -Carotene in Coconut Oil, Sealed in Nitrogen and Stored at 37° C.—In Table I, Test 54, a colorimetric test by Smedley-MacLean is recorded in which a 0.0067 per cent. solution of β -carotene, in coconut oil 162398, was sealed in a white glass tube in nitrogen and stored for 90 days at 37° C., without access of daylight and chiefly in the dark. This test was made without opening the sealed tube, and as far as could be judged by this rather rough method, the solution exactly matched, both before and after the 90 days' storage, a 1.1 per cent. solution of potassium bichromate.

A biological test on this material was made by Hume and Smith (1)* who compared the potency of this solution of β -carotene kept at 37° C. (hot-room solution) with that of another ampoule of the same solution, sealed in the same manner but stored at 0° C. (cold-room solution). The latter solution was just detectably darker than the former. The ampoules were finally opened after 120 days' storage and both solutions were diluted with their own volume of the same coconut oil. Colorimetric measurements of the height of the columns of carotene solution and of potassium bichromate, which exactly matched each other, were made on five separate occasions. Neither of the carotene solutions deteriorated at all in colour during the biological experiment, but every one of the five readings ascribed a slightly higher value to the cold-room solution, the average of all the readings being 0.87 for the cold-room solution and 0.81 for the

* The figures in brackets refer to the serial numbers of the protocols printed in Appendix 1, p. 44.

hot-room solution. The difference is very small, but there is little doubt that, in this case, it is significant and indicates a slight deterioration of the carotene kept at 37° C.

The result of the biological test (1) showed a very slight superiority of the cold-room solution, which, coupled with the observation of the slight fading of the hot-room solution makes it clear that the solution of β -carotene in coconut oil, kept at 37° C. for 4 months, did not suffer any greater deterioration in biological value than in colour value.

(c) *Selection of a Suitable Oil for Use as Solvent for Carotene. Colorimetric and Biological Tests on Carotene Dissolved in Various Solvents.*

In seeking a solvent in which carotene should be stable and from which it should be well absorbed, the workers had little experience to guide them. Apart from the fact that the presence of oxygen caused instability, there was little precise knowledge of what properties in a solvent tended to promote or hinder oxidation. Different samples of the same oil might differ in this respect, but the cause of their differing was, and is, uncertain. The workers had therefore to proceed in an empirical manner, testing various solvents in turn, either colorimetrically, biologically, or both, rejecting those which for various reasons proved unsuitable (arachis, olive and hardened cotton seed oils, ethyl laurate and liquid paraffin) and finally adopting coconut oil and subjecting it to a more intensive examination.

Arachis Oil.—The colorimetric results are set out in Table I, Tests 1-3.

Coward made two biological tests (Dyer *et al.*, 1934); in the first, the 1931 standard carotene dissolved in arachis oil was five times as potent as when dissolved in hardened cotton seed oil or ethyl laurate. In the second, the same 1931 standard carotene dissolved in coconut oil (161441) and in arachis oil had the same biological potency. There is no very definite evidence as to stability of the carotene in these tests, but, as in the latter it was potent in daily doses of 1 γ or 2 γ , it is obvious that there cannot have been any serious deterioration in that experiment.

Both the colorimetric and biological tests indicate that some samples of arachis oil are satisfactory, but the results of Culhane Lathbury and Greenwood (1934), already cited, show that this solvent varies greatly in individual samples, and the result of Moore (Table I, Test 3) indicates that in some cases the dissolved carotene is very unstable.

Olive Oil.—A colorimetric result is included in Table I, Test 4. Olive oil is itself yellow in colour, so that colorimetric checks on the stability of the dissolved standard carotene are not easy to make. The oil is therefore unsuitable as a diluent and only one colorimetric estimation was made with it.

Hardened Cotton Seed Oil.—Colorimetric tests are given in Table I, Tests 5 and 6. Hardened cotton seed oil may, on keeping, develop properties which render carotene dissolved in it highly unstable. In one specimen, kept in the laboratory for several years, carotene was completely decolorised after seven days at 0° C. (Table I, Test 6). In other samples it was comparatively stable.

Coward, as mentioned above under arachis oil, found that carotene dissolved in hardened cotton seed oil, had only one-fifth of the biological value of carotene in arachis oil. It is uncertain whether, in this case, the inferiority was due to instability or imperfect utilisation of the carotene, but in either case, hardened cotton seed oil is not a satisfactory solvent for carotene.

CAROTENE AS INTERNATIONAL STANDARD 13

Ethyl Laurate.—Besides the colorimetric tests by Smedley-MacLean (Table I, Tests 7-10), Moore has also reported rapid deterioration of carotene in ethyl laurate and ethyl stearate.

In a biological test, mentioned above under arachis oil, Coward found that 1931 standard carotene dissolved in ethyl laurate had one-fifth of the potency possessed in arachis oil. The stability of the carotene in the ethyl laurate is uncertain, but the inferiority of this solvent is clear. In a biological test Hume and Smith (2) also found 1931 standard carotene dissolved in ethyl laurate distinctly inferior to the same carotene in coconut oil. In spite of the usual instability of carotene in ethyl laurate, it was found possible to maintain the stability of this solution for the period of the biological test, so that, in this case, the inferiority of the carotene in this solvent must be attributed to inferior utilisation by the test animals.

Ethyl laurate thus fulfils neither of the conditions to be exacted from a solvent for carotene.

Liquid Paraffin.—In Table I, Test 11, a colorimetric test is given.

A biological test (3) by Hume and Smith, showed the potency of 1931 standard carotene in liquid paraffin to be so low that its use as a solvent for the standard could not be contemplated. The stability of the carotene in the oil was good so that the inferiority was due to failure in utilisation by the test animals.

It is of interest in this connection to note the result obtained when the cod-liver oil (Z), which was tested biologically by a number of workers (see p. 38, Section III), was tested against the international 1931 standard carotene dissolved in paraffin oil (see Table IX, Test by Trevan). The value obtained for the cod-liver oil in this test is much higher than that obtained by any other worker. The use of paraffin oil for the standard would tend to lower its apparent biological value and therefore exalt that of the cod-liver oil tested against it.

Cotton Seed Oil (Wesson Brand).—Colorimetric tests are set out in Table I, Tests 12-14. A sample of Wesson brand cotton seed oil, similar to that which had been found so satisfactory by Baumann and Steenbock (1933), was kindly furnished by Professor Steenbock. Colorimetric tests were made, but there was, unfortunately, no opportunity to carry out biological tests. These tests, Nos. 12-14, are directly comparable with those made on coconut oil 162398 (Tests 52-54), which proved to be the best sample of coconut oil examined. The performances of the two oils were practically identical and the result is in accordance with that of Baumann and Steenbock.

Special Investigation of Coconut Oil (Oleum Cocos nuciferae). Behaviour of Carotene in Solution in Three Different Specimens of Coconut Oil. Stabilising Action of Hydroquinone on Carotene Solutions.

Except Wesson brand cotton seed oil, none of the solvents thus far dealt with could be regarded as satisfactory. The results of 13 colorimetric tests on carotene, in solution in various commercial samples of coconut oil obtained from the British Drug Houses, however, appeared much more promising (see Table I, Tests 15-27). Culhane Lathbury and Greenwood (1934), Coward (see above under Arachis Oil) and Hume (see above under Ethyl Laurate) obtained satisfactory results with coconut oil in biological tests also. It was therefore decided to pursue the study of this oil further, with both kinds of tests, and three special samples of coconut oil of different origin were specially obtained for the purpose, through the kindness of Dr. Underhill. These consisted of two different Cochin oils (161441 and 162398) and one Ceylon oil (162750), referred to as Cochin I, Cochin II, and Ceylon, respectively, in Culhane Lathbury and Greenwood's paper (1934).

TABLE I.—*Colorimetric tests for the stability of carotene in various solvents. All the solutions were kept in closed vessels with some air space, unless stated to the contrary. (Hq = hydroquinone.)*

No. of Test.	Solvent.	Observer.	Kind of carotene : strength of solution %.	Nature of test for stability.	Temperature °C.	Duration, days.	Colour estimation.	Percentage deterioration.*
1	Arachis oil . .	MacLean (See note 1, p. 20.)	1931 Standard, .0067	Matching 1·1% bichromate in Dubosc colorimeter.	0	0	1·07	—
						40	0·97	0
						93	1·05	0
						211	1·00	0
2	" " . .	MacLean .	1931 Standard, .0067	" " "	0	0	1·13	—
						30	0·95	0
						209	0·78	30
3	" " . .	Moore . (See note 2, p. 20.)	1931 Standard, .005	Lovibond tintometer .	0	0	1800	—
						6	1200	33
						12	710	60
						180	280	84
4	Olive oil . . .	MacLean .	1931 Standard, .0067	Matching 1% bichromate in Dubosc colorimeter.	0	0	0·95	—
						40	0·95	0
						93	1·19	0
						211	0·92	0
5	Hardened cottonseed oil.	MacLean .	1931 Standard, .0067	Matching 1·1% bichromate in Dubosc colorimeter.	0	0	1·60	—
						40	1·20	25
						78	1·20	25
						211	0·90	40
6	" " " " (several years old).	MacLean .	1931 Standard, .0067	" " "	0	0	0·78	—
						7	< 0·05	100
7	Ethyl laurate .	MacLean .	1931 Standard, .0067	" " "	0	0	0·46	—
						20	0·50	0
						34	0·49	0
						78	0·43	0
						211	0·40	0

8	" " "	MacLean	1931 Standard, ·0067	" " "	0	0 7 30 89 209	0.70 0.53 0.46 0.37 0.11	Reading doubtful. — 0 30 80
9	" " "	MacLean	1931 Standard, ·0067	" " "	Lab. (15-20)	0 28 43 160	0.53 0.22 0.12 0.00	— 60 80 100
10	" " "	MacLean	1931 Standard, ·0067	" " "	37	0 28	0.53 0.00	— 100
11	Liquid paraffin	MacLean	1931 Standard, ·0067	" " "	0	0 34 78 211	0.60 0.56 0.57 0.55	— 0 0 0
12	Cotton seed oil, Wesson brand.	MacLean	β -carotene (Karrer), ·0067	Direct comparison with bichromate 1·1%.	Lab. (15-20)	0 77	1·09 1·09	— 0
13	" " "	MacLean	β -carotene (Karrer), ·0067	" " "	37	0 21 77	1·09 1·09 0·55	— 0 50
14	" (sealed in nitro- gen).	MacLean	β -carotene (Karrer), ·0067	" " "	37	0 90	1·00 1·00	— 0
15	Coconut oil	MacLean	1931 Standard, ·0067	Matching 1·1% bichro- mate in Dubosc colori- meter.	0	0 236	0·75 0·85	— 0
16	" " "	MacLean	1931 Standard, ·0067	" " "	Lab. (15-20)	0 30 0 236	0·75 0·58 0·58 0·55	— 25 — 0

TABLE I.—Colorimetric tests for the stability of carotene in various solvents. All the solutions were kept in closed vessels with some air space, unless stated to the contrary. (Hq = hydroquinone)—continued.

No. of Test.	Solvent.	Observer.	Kind of carotene : strength of solution %.	Nature of test for stability.	Temperature °C.	Duration, days.	Colour estimation.	Percentage deterioration.*
17	Coconut oil	MacLean	1931 Standard, .02	Matching 1.1% bichromate in Dubosc colorimeter.	37	0 28 162	3.10 2.79 0.00	— 0 100
18	" "	Moore	1931 Standard, .005	Lovibond tintometer	0	0 409	2000 1800	— 0
19	" "	Moore	1931 Standard, .005	" "	0	0 550	1600 1200	— 25
20	" "	Moore	1931 Standard, .005	" "	0	0 274 409	1900 2000 1800	— 0 0
21	" "	Moore	1931 Standard, .005	" "	0	0 375	1800 1700	— 0
22	" "	Moore	β -carotene (Kuhn), .005	" "	0	0 158 292	2400 2200 1800	— 0 25
23	" "	Moore	β -carotene (Kuhn), .005	" "	0	0 158 292	2200 2000 1800	— 0 25
24	" "	Moore	Carotene recryst. \times 10 (Moore), .0025	" "	0	0 688	2000 2000	— 0
25	" "	Lathbury (See note 3, p. 20.)	1931 Standard, .01, diluted to .0005 for tintometer with CHCl_3	" "	5	0 49	4.1 3.6	— 0

26	" "	Lathbury	β -carotene (Karrer), ·005, diluted with CHCl_3 to ·00025	" "	5	0 49	3·7 1·6	— 50
27	" "	Lathbury	β -carotene (Karrer), ·01, diluted with CHCl_3 to ·0005	" "	5	0 49	6·9 3·6	— 50
28	Coconut oil 162750	Lathbury	1931 Standard, ·01, diluted with CHCl_3 to ·0005	" "	5	0 42	4·9 5·1	— 0
29	" "	Lathbury	1931 Standard, ·01 + ·01% Hq., di- luted with CHCl_3 to ·0005	" "	5	0 42	4·4 4·8	— 0
30	" "	Hume . (See note 1, p. 20.)	1931 Standard, ·0033 + ·01% Hq.	Direct comparison with bichromate 0·55%.	0	0 57 398	0·71 0·71 0·74	— 0 0
31	" "	Hume .	1931 Standard, ·0033 + ·01% Hq.	" "	37	0 8 13 49 81 185	0·84 0·75 0·76 0·79 0·69 0·77	— 0 0 0 0 0
32	Coconut oil 161441	Lathbury	1931 Standard, ·01 diluted with CHCl_3 to ·0005	Lovibond tintometer	5	0 42	4·6 4·8	— 0
33	" "	Lathbury	1931 Standard, ·01 + ·01% Hq. di- luted with CHCl_3 to ·0005	" "	5	0 42	4·8 5·3	— 0

TABLE I.—Colorimetric tests for the stability of carotene in various solvents. All the solutions were kept in closed vessels with some air space, unless stated to the contrary. (Hq = hydroquinone)—continued.

No. of Test.	Solvent.	Observer.	Kind of carotene : strength of solution %.	Nature of test for stability.	Temperature °C.	Duration, days.	Colour estimation.	Percentage deterioration.*
34	Coconut oil 161441	Hume . . .	1931 Standard, .0033	Direct comparison with bichromate 0.55%.	0	0 234	0.74 0.65	— 0
35	" "	Hume . . .	1931 Standard, .0033	" " "	37	0 4 7 12 90	0.76 0.57 0.38 0.29 0.00	— 25 50 60 100
36	" "	Hume . . .	1931 Standard, .0033 + .01% Hq.	" " "	0	0 57 156 398	0.79 0.72 0.78 0.82	— 0 0 0
37	" "	Hume . . .	1931 Standard, .0033 + .01% Hq.	" " "	37	0 6 12 185	0.80 0.76 0.71 0.68	— 0 0 0
38	" "	Hume . . .	β -carotene (Karrer), .0033	" " "	0	0 51 240	0.76 0.63 0.23	— 0 70
39	" "	Hume . . .	β -carotene (Karrer), .0033	" " "	37	0 3 7 12	0.63 0.45 0.29 0.00	— 30 50 100
40	" "	Hume . . .	β -carotene (Karrer), .0033 + .01% Hq.	" " "	0	0 161 398	1.00 1.00 0.95	— 0 0

41	" "	Hume . .	β -carotene (Karrer), ·0033 + ·01% Hq.	" " "	37	0 6 13 59 185	0·95 1·05 0·86 0·76 0·86	— 0 0 0 0
42	Coconut oil 162398	Lathbury .	1931 Standard, ·01 diluted with CHCl ₃ to ·0005	Lovibond tintometer	5	0 42	5·2 5·8	— 0
43	" "	Lathbury .	1931 Standard ·01 + ·01% Hq. diluted with CHCl ₃ to ·0005	" " "	5	0 42	5·6 5·9	— 0
44	" "	Hume . .	1931 Standard, ·0033	Direct comparison with bichromate 0·55%.	0	0 234	0·81 0·75	— 0
45	" "	Hume . .	1931 Standard, ·0033	" " "	Lab. (15-20)	0 17 94 189	0·83 0·76 0·66 0·64	— — 25 25
46	" "	Hume . .	1931 Standard, ·0033	" " "	37	0 3 17 63 94	0·81 0·76 0·64 0·40 0·10	— 0 0 50 90
47	" "	Hume . .	1931 Standard, ·0033 + ·01% Hq.	" " "	0	0 398	0·82 0·84	— 0
48	" "	Hume . .	1931 Standard, ·0033 + ·01% Hq.	" " "	37	0 49 90 185	0·84 0·76 0·66 0·77	— 0 0 0

TABLE I.—Colorimetric tests for the stability of carotene in various solvents. All the solutions were kept in closed vessels with some air space, unless stated to the contrary. (Hq = hydroquinone)—continued.

No. of Test.	Solvent.	Observer.	Kind of carotene : strength of solution %.	Nature of test for stability.	Temperature °C.	Duration, days.	Colour estimation.	Percentage deterioration.*
49	Coconut oil 162398	Hume . .	β -carotene (Karrer), ·0033	Direct comparison with bichromate 0.55%.	0	0 224	0.84 0.84	— 0
50	" "	Hume . .	β -carotene (Karrer), ·0033	" " "	Lab. (15-20)	0 17 94 189	0.90 0.81 0.76 0.79	— 0 0 0
51	" "	Hume . .	β -carotene (Karrer), ·0033	" " "	37	0 3 17 63 94 189	0.90 0.86 0.71 0.52 0.32 0.00	— 0 0 50 60 100
52	" "	MacLean .	β -carotene (Karrer), ·0067	Direct comparison with bichromate 1.1%.	Lab. (15-20)	0 77	1.04 1.04	— 0
53	" "	MacLean .	β -carotene (Karrer), ·0067	" " "	37	0 21 77	1.04 1.00 0.71	— 0 30
54	" " (sealed in nitro- gen).	MacLean .	β -carotene (Karrer), ·0067	" " "	37	0 90	1.00 1.00	— 0

1. The results of MacLean and of Hume are expressed as the relation between the height of a column of carotene solution and the height of the column of bichromate solution of fixed strength, which it closely matches. The ratio to one another of the results obtained in a series may be taken as linear and the amount of deterioration legitimately calculated as percentages.

2. The results of Moore are expressed as Lovibond yellow units per mg. of carotene. The observations were made on dilute solutions. The ratio of the results to one another is not strictly linear and the deterioration can only roughly be reckoned as percentage.

3. The results of Lathbury are direct tintometer readings, made on suitable dilutions of the original solutions.

*Owing to the general roughness of all the methods, results showing a difference of less than 25 per cent. are not calculated, since they cannot be regarded as significant.

CAROTENE AS INTERNATIONAL STANDARD 21

Colorimetric Tests without Addition of Hydroquinone.—Tests 28, 32 and 42, by Culhane Lathbury, show that when the solutions used for her biological tests were kept for 6 weeks at 5° C., there was no deterioration of a 0·01 per cent. solution of 1931 standard carotene, in any of the three oils. A test in these conditions therefore detected no difference in the behaviour of the three oils.

In the colorimetric tests made by Hume, the carotene solutions (0·0033 per cent.) were kept in corked bottles, with considerable air space and chiefly in the dark. At 0° C. there was no appreciable loss in the colour of 1931 standard carotene in oils 161441 and 162398 after 234 days (Tests 34 and 44). In the same conditions β -carotene lost 70 per cent. of colour in oil 161441 after 240 days (Test 38), but showed no loss in oil 162398 after 224 days (Test 49), so that, even at 0° C., the lower stability of one sample of carotene in the oil 161441 became apparent when the test period was sufficiently long. At 37° C., 1931 standard carotene in oil 161441 lost 60 per cent. in 12 days and was completely decolorised after 90 days (Test 35); β -carotene was completely decolorised after 12 days (Test 39). In similar conditions 1931 standard carotene in oil 162398 had suffered no loss recognised as significant in 17 days, but had lost 50 per cent. in 63 days and 90 per cent. after 94 days (Test 46); β -carotene behaved similarly, losing 60 per cent. in 94 days (Test 51). At 37° C., therefore, the decolorisation in oil 161441 was at least five times as rapid as in oil 162398.

No colorimetric tests were made on the oil 162750, without addition of hydroquinone to the solution of carotene.

As the results with the oil 162398 were so promising, a test was made by Smedley-MacLean (Table I, Test 54), in which the most drastic conditions to which solutions of the carotene standard might be exposed during international transport were reproduced and exaggerated. A 0·0067 per cent. solution of β -carotene in oil 162398, was sealed in nitrogen and kept at 37° C. for 90 days. At the beginning and end of the test the solution matched a 1·1 per cent. solution of potassium bichromate, so that with anaerobic conditions, deterioration after 90 days at 37° was imperceptible. A biological test with this material has been described above.

Colorimetric Tests with Addition of Hydroquinone.—Hume made colorimetric tests, parallel with those described above, on solutions of carotene in all the three oils, with an addition of hydroquinone (0·01 per cent.). When this addition was made, no significant fading was detected at 0° or 37° C. after periods of 185–398 days (Tests 30, 31, 36, 37, 40, 41, 47 and 48). The protective power of a 0·01 per cent. concentration of hydroquinone upon a 0·0033 per cent. solution of carotene was thus remarkable. It completely obliterated the difference already demonstrated between the oils 161441 and 162398, a loss of 60 per cent. in colour in 12 days at 37° C. for oil 161441 being changed into no significant loss after 185 days at that temperature. It would appear, therefore, that even the less satisfactory samples of coconut oil can be rendered reliable by hydroquinone.

The effectiveness of the hydroquinone may be related to its concentration, either absolutely, or relatively, to the concentration of the carotene. Baumann and Steenbock (1933), who found it ineffective with natural oils, used a concentration of only one tenth of the concentration of the carotene; the absolute concentration of the hydroquinone was about 0·002 per cent. in most of their experiments. Turner (1934), who found it partly effective, used hydroquinone in 0·1 per cent. concentration, equal to half the concentration of the carotene. In the present instance the hydroquinone was in 0·01 per cent. concentration and had three times the concentration of the carotene.

Biological Tests on 1931 Standard Carotene dissolved in Coconut Oil, with and without Addition of Hydroquinone.—Dyer, Key and Coward (1934) and Culhane Lathbury and Greenwood (1934) compared the biological effect of 1931 standard carotene, dissolved in all the three oils with and without addition of hydroquinone. Hume and Smith (4) examined only the oil 161441 in the same way.

In these biological experiments the carotene solutions seem to have been kept in favourable conditions of temperature, in which there was no liability to deterioration, so that there was no opportunity for any protective action by the hydroquinone to be demonstrated. As might be expected, therefore, the result of the series of tests with hydroquinone simply duplicates that of the series without.

The experimental rats did not seem to suffer any inconvenience from the consumption of as much as 6γ daily of hydroquinone.

Biological Tests comparing the Value of 1931 Standard Carotene dissolved in the Three Special Samples of Coconut Oil.—Biological tests to compare the potency of 1931 standard carotene, dissolved in the three samples of coconut oil were made by Culhane Lathbury and Greenwood (1934) and by Coward (Dyer *et al.*, 1934) and by Hume and Smith.

Culhane Lathbury and Greenwood found carotene dissolved in oil 162398 the best, and in oil 162750 nearly the same, while in oil 161441, it was markedly inferior (*see* Table II, Test 1).

Dyer, Key and Coward found carotene in the oil 162398 the best and in 162750 the worst, with 161441 intermediate (*see* Table II, Test 2) but doubt is expressed whether the difference is really significant.

Hume and Smith (5) found little difference in the potency of carotene in the three oils; if there was any difference, oil 162398 was the best, oil 161441 second and oil 162750 third (Table II, Test 3).

All the three sets of workers thus found the oil 162398 slightly the best and Coward and Hume agreed as to the order in which they placed the other two oils. The only marked discrepancy was Culhane Lathbury's finding that oil 161441 was markedly inferior, which she confirmed in other experiments (7).

TABLE II.—*Comparison of the biological value of 1931 standard carotene dissolved in three different samples of coconut oil, 162398, 161441, 162750. It should be noted that only in Test 2 are the results expressed as relative potencies.*

Observer.	No. of Test.	Criterion.	Oil 162398.	Oil 161441.	Oil 162750.
Lathbury . . .	1	Percentage survival of groups of 25-30 rats. Dose, 2.0 γ carotene daily.	97%	20%	82%
Coward . . .	2	Wt. increase of groups of 10-13 rats. Results referred to formula and expressed as relative potencies. Dose, 1.0 γ carotene daily.	2.46	1.65	1.08
Hume . . .	3	Total weight increase (g.) in 42 days, of groups of 3 rats. Dose, 1.0 γ carotene daily.	130	120	115

As regards stability of the carotene during these tests, the results obtained by both Coward and Culhane Lathbury, when their solutions contained hydroquinone, exactly duplicated those obtained when they did not. Hume included hydroquinone in her carotene solutions and made colorimetric tests throughout, so that there is no doubt of the stability of the solutions in that case.

If the solutions were stable during the tests, to what cause can be attributed any difference in the potency of the dissolved carotene? No complete answer can be given to this question, but it is interesting to note that in the colorimetric tests for stability previously described, oil 162398 showed a very definite superiority over oil 161441. As no deterioration of the carotene was recorded in any of the oils in the course of the biological experiments, any greater potential instability of carotene in one oil than in another, if invoked to offer an explanation of the differing biological values, can only have become actual, either before the biological experiment began or after the animals had ingested the solutions. With regard to the latter possibility, there is no evidence; with regard to the former, there is some. Both Culhane Lathbury and Hume, before beginning their biological experiments, observed that the carotene solution in oil 162398 was the darkest in colour of the three. Here is a basis for a slight biological superiority, and it is possible that in the process of making up the solutions there might have been a greater destruction of carotene in an oil in which, in unfavourable circumstances, it was known to be less stable. Such a destruction of carotene while making up a solution may well occur, though it is doubtful whether it will account for the discrepancy.

3. Summary and Conclusions

(1) Maintenance or loss of colour by a given solution of carotene appears to be a trustworthy index of its maintenance or loss of biological value.

(2) Of the substances examined as possible solvents for the 1931 international standard carotene, coconut oil was the most favourable, in that the carotene was well utilised from it, as judged by biological tests, and comparatively stable in it, as judged by colorimetric tests.

(3) Three different samples of coconut oil, derived from different sources, were examined biologically and colorimetrically. With one exception, only small differences were detected in the biological value of the dissolved carotene, when adequate precautions were taken to preserve its stability during the biological experiment. Definite differences in the stability of carotene in the three oils, however, became apparent when the solutions were exposed to conditions likely to promote instability. There was not sufficient evidence to determine the cause of the small differences in biological value, though potential differences in stability may be suspected.

(4) Addition of hydroquinone in adequate concentration exercised a marked stabilising influence, as judged by colour, on carotene in solution in several samples of coconut oil, even in a less favourable specimen.

(5) The results summarised above were communicated to the 1934 International Conference on Vitamin Standardisation, whose decision to issue the standard, in future, as a solution of carotene in coconut oil, with addition of hydroquinone, was largely based upon them.

(B) RELATION BETWEEN VITAMIN A ACTIVITY OF THE 1931 INTERNATIONAL STANDARD CAROTENE AND OF PURE β -CAROTENE

1. *Introductory*

As soon as it became apparent that several pure chemical substances could function as provitamin A, it was clear that one of these should be selected for use as the standard, to supersede the mixture of carotenes which had been adopted by the International Vitamin Conference of 1931 and subsequently issued as the provisional International Standard for Vitamin A. This material must have consisted of β -carotene and inert material, with little or no α -carotene (*see* p. 27). When, further, Kuhn, Brockmann, Scheunert and Schieblich (1933) demonstrated that the biological value of the different pro-vitamins might be different, according to their possession of one or two β -ionone rings, it was also apparent that it would be necessary to establish as accurately as the method permits, the relative biological potency of the 1931 standard carotene and whatever pure carotenoid was selected as the future standard.

β -Carotene is the form most widely distributed in nature and most easily prepared. According to Kuhn, its biological value is double that of α - or γ -carotene, a result confirmed by von Euler (1934), to the extent of finding β -carotene active in a definitely smaller dose than α -carotene. β -Carotene seemed, therefore, the most suitable isomer to select. Professor P. Karrer very kindly prepared about a gram of pure crystalline β -carotene for the National Institute for Medical Research, and this specimen formed the basis for the

necessary investigations. It was separated from α - and γ -carotene by differential adsorption and had m.p. 182.5–183° C. (uncorr.).

2. Experimental

(a) Biological Tests

Biological tests with the techniques already referred to (see p. 10) were made by Coward, by Culhane Lathbury, and by Hume and Smith. Coward's result has already been published (Dyer *et al.*, 1934) and is summarised in Table III, Test No. 1; she found the potency of international 1931 standard carotene to that of β -carotene to be in the ratio of 1.40 to 2.44, i.e. 1 to 1.75.

Hume and Smith (6) found that 1931 standard carotene had about two-thirds of the biological value of β -carotene (see Table III, Test 2); Culhane Lathbury (7) placed this ratio at about one-half. The rats in her experiment all lost weight, and the criterion adopted was degree of loss of weight.

TABLE III.—Comparative biological value of international 1931 standard carotene and β -carotene (Karrer) dissolved in coconut oil.

Observer.	No. of Test.	Criterion.	International 1931 standard carotene. Daily dose, γ			β -Carotene. Daily dose, γ		
			0.5	0.75	1.0	0.5	0.75	1.0
Coward .	1	Mean increase in weight over 5 weeks, of groups of 10 rats. Results referred to formula and expressed as relative potencies. Carotene dissolved in coconut oil 161441.	—	—	1.40	—	—	2.44
Hume .	2	Total increase in weight (g.) of groups of 4 comparable rats in 49 days. Carotene dissolved in coconut oil 162398 Group of 2 ♂ and 2 ♀ rats Group of 1 ♂ and 3 ♀ rats	146 —	182 154	198 —	172 —	— 172	246 —

The dose of β -carotene equivalent to 1 γ of the international standard carotene, on the basis of these estimations, is therefore:—

Coward	0.57 γ
Hume	0.66 γ
Lathbury	0.50 γ
Average	0.58 γ

For biological results these represent a good measure of agreement. The average of these values, taken as 0.6 γ , was accepted by the International Conference (1934) as the figure which should be adopted. Dr. E. M. Nelson (U.S.A.), one of the participants in the International Conference (1934), reported to the Conference a figure in agreement with the above. He found that 0.66 γ of β -carotene (a specimen other than that prepared by Karrer) was biologically equivalent to 1.0 γ of 1931 standard carotene.

The amount 0.6 γ of β -carotene is thus held to have the same biological value as 1.0 γ of the 1931 standard carotene and its potency therefore represents the value of one international unit. This relation between the biological values of these two specimens of carotene is naturally the one to which crucial importance belongs. Confirmation was, however, also sought from physical tests.

(b) *Colorimetric and Spectroscopic Tests*

(i) T. Moore compared the intensity of yellow colour of 1931 standard carotene and of pure β -carotene (a specimen prepared by Kuhn) in dilute chloroform solution, in the Lovibond tintometer. He obtained a reading of 1,300 yellow units per mg. for the standard and 2,000 for the β -carotene, from which he concluded that the standard contained about 65 per cent. carotene.

(ii) W. J. Dann made a spectroscopic estimation, comparing the extinction coefficient of the peak of the main absorption band at 450 $m\mu$, in the spectrum of the 1931 standard and of β -carotene (Karrer). For the wave-length 450 $m\mu$, $E \frac{1\%}{1 \text{ cm.}}$ was 1,520 for the international standard and 2,495 for β -carotene. On the assumption that the extinction coefficients of α - and β -carotene are the same (Smith and Milner, 1934), these figures indicate that the 1931 standard carotene contained $\frac{1,520}{2,495} \times 100 = 61$ per cent. carotene.

(iii) Early in 1935 R. A. Morton and A. E. Gillam tested the 1931 standard carotene for direct absorption and observed $E \frac{1\%}{1 \text{ cm.}}$ 463 $m\mu$ to be 1,500 in chloroform, as against 2,200 for pure β -carotene. The antimony trichloride colour test showed $E \frac{1\%}{1 \text{ cm.}}$ 590 $m\mu$ to be 331 and 420 for the 1931 standard carotene and β -carotene, respectively. Chromatographic analysis showed that about 15 per cent. of the total pigment present in the 1931 standard carotene was different from β -carotene. These results indicate that the β -carotene content of the 1931 standard carotene cannot differ much from 65 per cent.

Taken together, the results of the biological and physical tests show a satisfactory degree of harmony; in the biological test the 1931 standard had 58 per cent. of the value of pure β -carotene. In the

CAROTENE AS INTERNATIONAL STANDARD ' 27

colorimetric test it contained 65 per cent. of carotene and in the spectroscopic test 61 per cent., as compared with 100 per cent. in pure β -carotene.

The 1931 standard carotene was composed of a pooled collection of samples of varying degrees of purity, contributed by eight different laboratories in seven different countries. After pooling, the material was recrystallised and the final product must have consisted of β -carotene, with no significant admixture of α -carotene since there was no dextro-rotation, but containing a certain amount of inactive material. The standard crystalline material, sealed in nitrogen-filled ampoules, was stored for two years before the tests described in this section were made and might have deteriorated to some extent in that time. Moore examined one ampoule of the standard material on 12th October, 1932, and found a value of 1,900 yellow units per mg., while another ampoule examined on 13th July, 1933, had a value of only 1,300 yellow units. R. S. Morgan, on the other hand, opened 4 different ampoules between July 1932 and September 1934 and found a deterioration of not more than 10 per cent. in any instance in colorimetric tests and no significant deterioration in biological tests.

The 1931 standard carotene used in these tests must thus have contained more than a little inactive material, and in addition, perhaps, some small admixture of a biologically less active carotene. The value found for the standard, about 60 per cent. of that of pure β -carotene, although unexpectedly low, has therefore no inherent improbability and 0.6 γ of β -carotene may reasonably be regarded as a reliable equivalent for 1.0 γ of 1931 international standard carotene, both of these values representing the value of one international unit of vitamin A activity.

3. Summary

From the results of biological, colorimetric and spectroscopic tests, 0.6 γ of pure β -carotene was accepted as being the equivalent of 1.0 γ (one international unit) of the international (1931) standard carotene, at the date (1933-1934) when the tests were made. This figure was accepted by the 1934 International Conference, and the international unit for vitamin A was defined as the biological activity of 0.6 γ of pure β -carotene.

II. SPECTROPHOTOMETRIC METHOD OF ESTIMATING VITAMIN A BY MEANS OF THE EXTINCTION COEFFICIENT AT $328m\mu$

1. *Introductory*

The application of spectrophotometry to the quantitative estimation of minute amounts of organic substances has made rapid strides in recent years and the solution of many problems in connection with the vitamins has been greatly furthered by the use of spectroscopy.

If a trustworthy and practical spectrophotometric method could be elaborated for the quantitative measurement of any vitamin it would, where applicable, eliminate the need for a biological test and, therefore, for an international standard preparation for comparison in such tests. Some time before the 1934 meeting of the International Conference on Vitamin Standardisation it became apparent that the method of estimating vitamin A, at least in fish liver oils and concentrates, by measuring the intensity of absorption at $328m\mu$, was worth examination from this point of view (Morton and Heilbron, 1928, 1 and 2; Drummond and Morton, 1929; Coward, Dyer *et al.*, 1931, 1932). Where the value of a substance as a source of vitamin A was wholly or partly due to the presence of carotene, the latter clearly would not be estimated by measurement of the intensity of the $328m\mu$ band, but the method might be used to measure that portion of the activity due to the presence of vitamin A. The portion due to the presence of carotene might then be measured by separate spectroscopic tests of the absorption in the visible (Morton and Heilbron, 1930).

Many points in connection with the use of the spectroscopic method claimed the attention of the Vitamin A Sub-committee and of the associated group of workers. The ensuing section of this Report records observations made with different kinds of spectrophotometric apparatus, and deals with (i) the degree of concordance obtained by different workers, (ii) the materials to which the test could be applied, (iii) the solvent in which the material should be examined, (iv) the need or otherwise of first saponifying the material and the best method of doing so, and (v) the relation between the values for vitamin A potency obtained by spectrophotometry and by biological tests. This last point, because of its importance, is dealt with in a separate section, for, unless the results of spectrophotometric and biological tests were found to harmonise (having due regard to the large margin of error of the latter), the spectrophotometric method could not be adopted officially.

The different workers were asked to determine $E_{1\text{ cm.}}^{1\%}$ $328m\mu$ for three different samples, X, Y and Z, of cod-liver oil and for one sample, C, of a concentrate of vitamin A, using their own methods and instruments. When the reports were received and compared, it was possible to arrive at certain preliminary conclusions. Three other samples, U, V and W, of cod-liver oil were then circulated, the workers being asked to unify their technique with regard to certain

points. From the results of collective investigations of this type, it is not possible completely to disentangle the various points which needed to be settled or to tabulate separately the data with regard to each point. The result of the preliminary investigation on the first three oils and the concentrate C are, therefore, set out as one whole in Table IV and those of the subsequent examination of the remaining oils in Table VI.

In compiling this section, the editors have had the constant assistance of Dr. R. A. Morton, who has placed the results of his work, both published and unpublished, at their disposal. Without his help they could scarcely have undertaken the task. They have also drawn largely on the summary of the collective work, prepared by Dr. K. H. Coward, at the request of the Vitamin A Sub-committee.

2. Experimental

(a) *Comparison of the Results obtained by Different Workers in Determining $E_{1\text{ cm.}}^{1\%}$ at $328\text{ m}\mu$ for Six Specimens of Cod-Liver Oil and One Vitamin A Concentrate, with a Discussion of the Different Methods used.*

The tests were undertaken by workers in seven different laboratories:—

R. K. Callow, Nat. Inst. Med. Res., London.

S. K. Crews, Res. Labs., The British Drug Houses, Ltd., London.

F. J. Dyer, Pharmaceutical Soc., London.

N. Evers and W. Smith, Res. Labs., Messrs. Allen and Hanburys, Ltd., London.

R. J. Macwalter, University College, London.

T. Moore and W. J. Dann, Dunn Nutritional Lab., Cambridge.

R. A. Morton and J. R. Edisbury, The University, Liverpool.

The materials circulated for examination were the following:—

Cod-liver oil X, a medicinal oil (Reference Oil of the United States Pharmacopoeia).

Cod-liver oil Y, a medicinal oil.

Cod-liver oil Z, a veterinary oil.

Vitamin A concentrate C, kindly supplied by the British Drug Houses, Ltd.

Cod-liver oil U, a veterinary oil,

Cod-liver oil V, a crude coast cod oil,

Cod-liver oil W, a medicinal oil,

} kindly supplied by
Messrs. Allen and
Hanburys, Ltd.

The tests were made both on the oils and on their unsaponifiable fractions.

The results are expressed as $E_{1\text{ cm.}}^{1\%}$, which refers to $\log I_0/I$ at the wave length maximum, with a solution containing 1 per cent. of the vitamin-containing substance in a 1 cm. cell, I_0 being the intensity of the incident light and I that of the emergent light.

The results obtained with the oils X, Y and Z and the concentrate C are set out in Table IV. The workers responsible for these results, after a joint discussion, drew up the following four conclusions, which are printed below in italics.

TABLE IV.—*Estimation of vitamin A, by the spectrophotometric method, in the cod-liver oils X, Y and Z before and after saponification, and in a concentrate C, by different workers each using his own method. A value furnished for oil Z by Dr. A. Jung of Basle is also included for comparison. (S.P.A. = Society of Public Analysts. The small figures in brackets indicate the number of estimations from which the average is derived.)*

Observer.	Instrument.	Solvent.	Method of saponification of oil.	Oil X, E _{1 cm.} ^{1%}		Oil Y, E _{1 cm.} ^{1%}		Oil Z, E _{1 cm.} ^{1%}		Concentrate C, E _{1 cm.} ^{1%}
				Oil.	Unsap. fraction.	Oil.	Unsap. fraction.	Oil.	Unsap. fraction.	
Dann & Moore	Hilger F ₃ Quartz Spectrograph and Echelon Cell.	Ethyl alcohol Cyclohexane	Moore's "	— 1·64	1·39 1·33	— 0·51	0·42 0·42	— 0·82	0·57 0·59	1010 ⁽²⁾
Macwalter	Hilger Spectrograph and Echelon Cell.	Ethyl alcohol	S.P.A.	—	1·44 ⁽²⁾	—	0·39 ⁽⁴⁾	—	0·59 ⁽²⁾	—
Morton & Edisbury	Hilger Quartz Spectrograph.	Ethyl alcohol Cyclohexane Chloroform	S.P.A. " "	1·74 ⁽²⁾ 1·73	1·58 ⁽²⁾ 1·49 1·32	0·54 0·52	0·50 ⁽²⁾ 0·47 ⁽²⁾	0·79 0·77	0·70 ⁽⁴⁾ —	1100 1085 <1000
Evers & Smith	Bellingham & Stanley Sector Photometer.	Ethyl alcohol Cyclohexane Chloroform	S.P.A. " "	— — —	— — —	— 0·54 0·53 ⁽²⁾	0·44 ⁽²⁾ 0·44 ⁽²⁾	— 0·83 0·80 ⁽²⁾	0·73 0·67 0·61	1030 —
Callow	Philpot Schuster Spectrometer.	Ethyl alcohol Cyclohexane Chloroform	S.P.A. "	— — —	— — —	— — —	— — —	0·74 — 0·72	0·62 ⁽²⁾ — 0·59 ⁽²⁾	1013 1025 —
Crews	Bellingham and Stanley Sector Photometer.	Cyclohexane " "	Evers' Smith Hazley	1·70 —	1·43 1·70 ⁽²⁾	0·55 —	0·40 0·55	0·80 —	0·60 0·87 ⁽²⁾	— —
Dyer	Vitameter A " new model.	Ethyl alcohol " "	Smith Hazley	2·00 ⁽²⁾ —	1·83 ⁽²⁾ —	0·61 ⁽²⁾ —	0·41 ⁽²⁾ —	0·72 ⁽²⁾ —	0·52 ⁽²⁾ —	926 ⁽²⁾ 1060
	Average 1 " 2 " 3	Ethyl alcohol Cyclohexane Chloroform	Moore's, Evers' or S.P.A.	1·74 1·69 —	1·47 1·42 1·32	0·54 0·53 0·53	0·44 0·43 —	0·76 0·81 0·76	0·63 0·62 0·60	1058 1038 1000
	Average of 1 and 2 combined			1·70	1·44	0·53	0·44	0·79	0·63	1046
A. Jung (Basle)	Zeiss Quartz Spectrograph.	Ethyl alcohol	S.P.A.	—	—	—	—	0·83	0·70	—

(i) *The values obtained when the oils were examined were always higher than those obtained when the unsaponifiable fraction from the same weight of oil was used.*—The need for correction of the gross absorption, on account of the contribution made by substances other than vitamin A was well known (Drummond and Morton, 1929; Coward, Dyer *et al.*, 1931), but there was always the possibility that some loss of vitamin might occur during saponification and thus make the apparent difference wider. Re-saponification does not cause a loss of vitamin A, and an experiment by N. Evers, under conditions suitable for analytical tests, showed that no further decrease in absorption followed upon a second saponification treatment. This made it probable that no loss occurs in the initial saponification.

The decrease in observed absorption at $328m\mu$, after elimination of glycerides etc. by saponification, is due to the removal of substances exhibiting absorption, not necessarily selective, in the same region as vitamin A. The amount of the substances giving rise to this irrelevant absorption bears no constant relationship to the amount of vitamin A present but does not increase beyond a certain amount. Reference to Table V, where the values for the absorption at $328m\mu$, for all the six oils, before and after saponification are compared, shows that the irrelevant absorption, though not varying very greatly in total amount, may be widely different in its percentage of the whole. In the case of the oils V and X, for instance, the amount of irrelevant absorption is almost the same ($E_{1\text{ cm.}}^{1\%} = 0.22$ and 0.26 respectively), but in the case of V, it is 42 per cent. of the total and in the case of X only 15 per cent. It is therefore not

TABLE V.—*Comparison of values obtained by the spectrophotometric method for the six oils U, V, W, X, Y and Z before and after saponification, showing the variation in the absolute and proportional amounts of irrelevant absorption.*

The values given in the first two columns are the average values taken from Tables IV and VI.

Oil.	$E_{1\text{ cm.}}^{1\%}$ determined on oil.	$E_{1\text{ cm.}}^{1\%}$ determined on unsap. fraction.	Difference : irrelevant absorption.	Percentage of total absorption represented by irrelevant absorption.
U	1.20	1.01	0.19	16
V	0.52	0.30	0.22	42
W	0.54	0.45	0.09	17
X	1.70	1.44	0.26	15
Y	0.53	0.44	0.09	17
Z	0.79	0.63	0.16	20

possible to make a constant allowance for the irrelevant absorption, and this source of error should only be disregarded in oils which are so rich in vitamin A that it is proportionately insignificant in amount, i.e., in oils containing more than 10,000 international units of vitamin A per gram (Morgan, Edisbury and Morton, 1935). In oils of this potency, it is therefore permissible to make the spectroscopic estimation directly on the oil; with oils less rich, the estimation should always be made on the unsaponifiable fraction.

It should not be forgotten that, up to a point, the intensity of absorption at $328m\mu$ can be artificially increased by bubbling air through an oil. This source of irrelevant absorption is eliminated by saponification (Macwalter, 1934).

(ii) *Chloroform and ether are unsuitable solvents with which to dilute oils or in which to dissolve concentrates, while making the spectroscopic examination. Ethyl alcohol or cyclohexane is recommended.*

(iii) *The method of saponification used is of importance as influencing the accuracy of the results.*—The method of Smith and Hazley should not be used, since it necessitates the use of chloroform. The method of the Society of Public Analysts was found satisfactory but unnecessarily long. It was modified and abbreviated and adopted by the International Conference (1934) in the form reprinted in Appendix 3, p. 56. The methods used by Moore and Evers were also regarded as satisfactory (see Appendix 2, p. 51), though only one method was actually recommended by the Conference.

(iv) *All the instruments used should be calibrated by means of a solution of known absorption spectrum, such as potassium chromate in KOH solution (see Rössler, 1926; Baly, Morton and Riding, 1927).*

The four conclusions above mentioned, based on the preliminary examination of the oils, X, Y and Z and the concentrate C, indicated several factors tending to produce a want of concordance between the results of different workers using the spectrophotometric method to evaluate the same material. The same workers agreed to examine three more oils, U, V and W, before and after saponification. The method of saponification used was to be either that of the Society of Public Analysts, or the agreed modification, or Moore's or Evers' method. The solvent was to be ethyl alcohol or cyclohexane.

When the conditions of experiment were thus made more uniform, it is seen by reference to Table VI that, within certain limits, a good measure of agreement was obtained among the various workers. The values found directly on the three oils vary by about 4-14 per cent. above and below the average value, but the values obtained with the unsaponifiable fractions vary considerably more, by about 17-33 per cent. above and below the average. The process of saponification, though essential for obtaining a reliable result, may lead to loss of vitamin A, which is negligible in the hands of an operator with skill and experience. It is, however, claimed that the degree of accuracy obtainable in

SPECTROPHOTOMETRIC ESTIMATION

33

TABLE VI.—*Estimation of vitamin A by the spectrophotometric method, in cod-liver oils U, V and W before and after saponification. Three methods only of saponification were used and the solvents were cyclohexane or ethyl alcohol. (S.P.A.=Society of Public Analysts.)*

Observer.	Method of saponification.	Oil U, E 1% 1 cm.		Oil V, E 1% 1 cm.		Oil W, E 1% 1 cm.	
		Oil.	Unsap. fraction.	Oil.	Unsap. fraction.	Oil.	Unsap. fraction.
Dann & Moore	Moore's . . .	1.16	1.10*	0.44	0.30	0.52	0.51
	S.P.A. . . .	—	1.13	—	—	—	—
Macwalter . .	Moore's . . .	1.25	1.00	0.53	0.28	0.56	0.44
	Evers' . . .	—	0.76	—	0.24	—	0.41
Morton and Edisbury.	S.P.A. . . .	1.19	1.10	0.50	0.32	0.55	0.51
Evers . . .	Evers' . . .	1.18	1.00	0.53	0.40	0.53	0.45
	S.P.A. . . .	—	1.27	—	0.38	—	0.53
Callow . . .	Moore's . . .	1.25	0.86	0.56	0.29	0.49	0.43
	Evers' . . .	—	0.86	—	0.24	—	0.40
	S.P.A. . . .	—	0.98	—	0.24	—	0.41
Crews . . .	Moore's . . .	—	1.11	—	0.30	0.55	0.43
	Evers' . . .	—	1.10	—	0.27	—	0.40
	S.P.A. . . .	—	—	—	—	—	0.45
Dyer . . .	Moore's . . .	1.20	0.85	0.54	0.33	0.58	0.49
	Evers' . . .	—	1.07	—	0.29	—	0.51
Average	1.20	1.01	0.52	0.30	0.54	0.45

* Average of 5 estimations.

spectroscopic determinations of the absorption at $328m\mu$, with the precautions here enumerated and with sufficient practice, is as great as that obtainable in similar estimations of other materials, i.e. ± 2.5 per cent.

(b) *Ratio between the Values obtained in Estimating Vitamin A by the Spectrophotometric and Biological Methods.*

From the preceding paragraphs it is clear that different observers can obtain concordant results when measuring vitamin A by the spectrophotometric method. It remains to be considered whether the results thus obtained are also in harmony with those obtained by the biological method. If so, it should be possible to designate a constant factor by means of which results obtained by the spectrophotometric method can be converted to express potency in terms of international units of vitamin A per gram. It should, however, be recollected that, even when 10 pairs of animals are used for a comparison, the error in the biological method may be as much as ± 30 per cent., and that the large variation in the ratio between

the values obtained by the spectrophotometric and biological methods may be due rather to this inherent weakness of the biological method than to any real variation in the ratio.

Numerous estimations on the same material by the two methods are needed before the figure for this ratio can confidently be stated. Unfortunately, biological estimations are slow and arduous, and it was not possible to place at the disposal of the Vitamin A Subcommittee, biological values for all the six oils and the concentrate, which were examined spectrophotometrically. Biological results were, however, obtained by more than one observer for the oils X, Y and Z and for the concentrate C.

The following took part in the tests:—

K. H. Coward, Pharmaceutical Society, London.

E. M. Hume and H. Henderson Smith, Lister Institute, London.

K. Culhane Lathbury, Res. Labs., The British Drug Houses, Ltd., London.

T. Moore, Dunn Nutritional Lab., Cambridge.

R. S. Morgan, Res. Dept., Messrs. Lever Bros., Ltd. Port Sunlight.

The biological methods used in the first three laboratories have already been mentioned (*see* p. 10). The methods used by Moore and Morgan were also comparisons of the growth rate of depleted rats (Moore, 1933; Morgan, 1934).

Cod-liver oil X.—This oil, being the Reference Oil of the United States Pharmacopoeia, was subjected to an exhaustive investigation. The potency assigned to it by Dr. E. M. Nelson (U.S.A.), as the result of examinations in nine different laboratories, was 3,000 international units per gram. The individual values thus obtained are considered in a later section (*see* Table VIII, p. 38). Oil X was also examined by Coward (8), by Culhane Lathbury (9) and by Morgan (10); the values ascribed to it were 3,000, 2,700 and 2,840 international units per gram, respectively.

Cod-liver oil Y was examined biologically by Coward (11) and by Culhane Lathbury (12), who obtained values of 600 and 675 international units per gram, respectively.

Cod-liver oil Z was examined by Coward (13), by Culhane Lathbury (14) and by Morgan (15), who ascribed to it the values 1,600, 1,000 and 980 international units per gram, respectively. This oil had been used for about two years as a reference cod-liver oil by the Pharmaceutical Society of Great Britain, and reports on its biological activity were received from three other laboratories in England and from four on the continent (*see* Table IX, p. 39, for the individual values). The average value, based on results from all these sources, is taken as 1,500 international units of vitamin A per gram.

The vitamin A concentrate C, supplied by the British Drug Houses, was examined in four laboratories; the value found by Culhane Lathbury (16) was 1,500,000 international units per gram, by Hume and Smith (17) 900,000, by Moore (18) 1,750,000 and by Morgan (19) 1,290,000.

3. Discussion

In Table VII the values thus obtained by the biological method are set out against the average values obtained for the same materials by the spectrophotometric method; the latter figures are derived from Tables IV and VI. The results show that the ratio I.U./E between the two sets of values ranged from 1,300 to 2,380, for the four materials studied.

TABLE VII.—Comparison of results obtained in estimating vitamin A by the biological method (I.U.) and by the spectrophotometric method (E), for three cod-liver oils and a vitamin A concentrate, and a calculation of the ratio I.U./E. The values for E are the average values for the unsaponifiable fraction, taken from Table IV. (*See Table VIII.)

Observer.	Material.	Biological value international units per gram. (I.U.)	E 1% 1 cm. estimated on the unsap. fraction. (E)	Ratio I.U./E.
Coward	Oil X	3,000		
U.S.P.(Av.from 9 labs.)*		3,000		
Lathbury		2,700		
Morgan		2,840		
Average (taken as)		3,000	1.44	2080
Coward	Oil Y	600		
Lathbury		675		
Average		638	0.44	1430
Average from 10 labs.	Oil Z	1,500	0.63	2380
Lathbury	Concentrate C	1,500,000		
Hume		900,000		
Moore		1,750,000		
Morgan		1,290,000		
Average		1,360,000	1046	1300

Carr and Jewell (1933), in an investigation of the purest specimen of vitamin A which they could prepare by vacuum distillation, found $E \frac{1\%}{1 \text{ cm.}} 328m\mu = 1,600$, and a biological value of about 2 million international units of vitamin A per gram; in this case the ratio I.U./E is about 1,250.

Culhane Lathbury (1934), from data submitted to the Vitamin A Sub-committee and since published, concluded that the value for I.U./E is about 1,400.

Chevallier and Chabre (1934), after examining several cod-liver oils and concentrates by both methods, concluded that the value for I.U./E should be 750, but their biological results are expressed

in Sherman "rat units", not in international units. A Sherman rat unit, being a "biological" unit, can have no fixed factor by which it can be converted into international units, but is regarded as roughly equal to 1.4 international units. On this basis the value found by Chevallier and Chabre for I.U./E would be 1,050.

Edisbury, Morgan and Morton (1934; Morgan *et. al.*, 1935) have indicated the possibility that the value of I.U./E may not necessarily be the same for cod-liver oils and some other sources of vitamin A; a value of the order of 1,600 is suggested for the former and of 1,000 for the latter.

The figure for I.U./E recommended by the Vitamin A Sub-committee and adopted by the International (1934) Conference was 1,600, but this figure was regarded as provisional and subject to revision with the accumulation of further data.

When more data of biological tests are available it should be possible to fix the value for I.U./E with considerably more confidence. At present, for practical purposes, the figure 1,600 may be accepted provisionally.

4. Summary

(1) The results obtained in seven different laboratories in estimating $E \frac{1\%}{1 \text{ cm.}} 328 m\mu$ for six cod-liver oils and one concentrate showed that :—

(a) Estimations on cod-liver oils should be made on the unsaponifiable fraction of the oil.

(b) Cyclohexane or ethyl alcohol and not chloroform should be used as solvent.

(c) Only a method of saponification approved for the purpose should be used; three such methods are described.

(2) When these precautions were observed the results of different laboratories were reasonably concordant.

(3) The results obtained by different laboratories, when estimating biologically the vitamin A in some of the above materials, are reported, and the value for the factor to be used in converting $E \frac{1\%}{1 \text{ cm.}} 328 m\mu = 1$, into international units of vitamin A per gram, is discussed. The figure for this factor recommended by the Vitamin A Sub-committee and accepted by the International Conference (1934) was 1,600.

(4) Reasons are given why this factor is to be regarded as provisional, though it may be retained at present for practical purposes.

III. THE USE OF COD-LIVER OIL AS A SUBSIDIARY INTERNATIONAL STANDARD FOR VITAMIN A

For several years before the meeting of the 1931 International Conference on Vitamin Standardisation, the need for standard preparations had been felt, and, in default of any official standards, a few laboratories had themselves selected such preparations for regular use in all biological tests. In Great Britain, Dr. Coward, at the Pharmaceutical Society, was using as vitamin A standard a selected specimen of cod-liver oil, the oil Z referred to in the previous section, while in 1932, in the United States, the oil X of the previous section was selected by the United States Pharmacopoeia Commission as a "reference" cod-liver oil for both vitamins A and D.

The first (1931) International Conference recommended in its Report (League of Nations, 1931) "that a supply of an approved sample of cod-liver oil be obtained with the object of making a series of comparative tests to determine its suitability as an alternative standard". And further, the Conference "decided to ask the United States Department of Agriculture to obtain sufficient samples of the oil . . . to enable investigators of other countries to assay this oil in terms of the international unit of standard carotene". The oil thus obtained by the United States Department of Agriculture was the cod-liver oil X. In the years which elapsed between the Conferences of 1931 and 1934, oil X was subjected in a number of laboratories to biological tests against the international 1931 standard carotene. During this intermediate period oil X was also adopted officially by the U.S. Pharmacopoeia Commission, and issued as a subsidiary standard of reference for vitamins A and D.

The International Conference of 1934, after consideration of the arguments in favour of a subsidiary cod-liver oil standard for vitamin A, recommended "that a sample of cod-liver oil, the potency of which has been accurately determined in terms of the International Standard Preparation of β -carotene, shall be provided as a Subsidiary Standard of Reference. In view of the fact that the Reference Cod-liver Oil of the United States Pharmacopoeia, which has been accurately assayed in terms of the provisional International Standard adopted in 1931, has been in effective use in the United States of America for some time, the Conference recommends that the Board of Trustees of the United States Pharmacopoeia be approached and invited to place a quantity of their Reference Cod-liver Oil at the disposal of the Health Organisation of the League of Nations, with a view to its adoption for international use as a Subsidiary Standard for Vitamin A." (See Appendix 3, p. 55.)

In consequence of this recommendation and with the kind co-operation of the Trustees of the U.S. Pharmacopoeia, cod-liver oil X has now been made generally available as subsidiary standard, and it is, therefore, clear that accurate information as to its biological value

is a matter of importance. Further, there are very few, if any, other materials extant, with the exception of oil Z, on which as many as nine independent biological tests for vitamin A potency have been made, showing direct comparison with the international 1931 carotene standard. The actual value of oil X and the measure of agreement obtained in the results of the various workers are, therefore, of great interest.

1. *Biological Tests on Cod-Liver Oil X*

The values obtained in nine different laboratories for oil X were furnished to the 1934 Conference by Dr. E. M. Nelson, one of the participants from the United States. They are set out in Table VIII. They range from 2,350 to 3,725 international units per gram, the average value being 2,998; the value accepted is 3,000 international units per gram. The three biological tests made on oil X in English laboratories, which have already been referred to in more detail (*see* p. 34), gave values of 3,000, 2,840 and 2,700 international units per gram, which fall satisfactorily within this series.

TABLE VIII.—*Values obtained, in nine different laboratories, in biological tests of the United States Pharmacopoeia Reference Cod-Liver Oil (Oil X) for vitamin A potency.*

		<i>International Units per Gram.</i>
		3,725
		2,830
		2,350
		3,030
		3,375
		2,475
		3,450
		2,400
		3,350
		<hr/>
Average	2,998
Potency assigned	3,000

In view of the diversity of technique employed by the different workers and the uncertainty then prevailing as to suitable solvents for dissolving the standard and for diluting the cod-liver oil, it is surprising that the divergence of the results was not wider.

2. *Biological Tests on Cod-Liver Oil Z*

The cod-liver oil Z, used as a standard for vitamin A by Dr. Coward at the Pharmaceutical Society, shares with cod-liver oil X the interest of being the subject of numerous independent biological tests. These tests, in which comparison was also made with the international 1931 standard carotene, were carried out in laboratories in England and on the Continent. The range of values obtained was somewhat wider than in the case of oil X, the lowest being 980 and

COD-LIVER OIL AS SUBSIDIARY STANDARD 39

the highest, with one exception to be specially mentioned later, 2,000, international units per gram. The results, set out in Table IX, include data as to the solvent used for dissolving the standard carotene and for diluting oil Z, where such a dilution was made. The oils used as solvents included arachis, sesame, coconut, olive and maize oils; in one case liquid paraffin was used as solvent for the standard carotene. There can be little doubt that this diversity in the solvents used, contributed to the diversity in the results obtained. Particularly is this the case with the very high result (3,000-4,000 international units per gram) obtained when liquid paraffin was the solvent for the standard carotene. This result has been mentioned above in the discussion of suitable solvents for carotene (*see* p. 13), and the high result obtained is attributed to bad utilisation by the test rats of carotene from a paraffin solution. This would have the effect of lowering the apparent value of the standard and, therefore, of raising the apparent value of the oil tested. Apart from this one high result, the explanation of which seems clear, the divergence between the various results is not excessive, when the normal degree of error in biological estimations is taken into consideration.

TABLE IX.—*Vitamin A potency of Cod-Liver Oil Z, showing the variation in results obtained by different workers in estimating biologically its vitamin A potency in terms of the International Standard.*

<i>Observer.</i>	<i>Solvent used for 1931 standard carotene (and for oil Z when needed).</i>	<i>Vitamin A value of oil Z, International Units per g.</i>
Prof. L. S. Fridericia (Copenhagen).	Arachis oil	2,000
Prof. A. Scheunert (Leipzig) .	Sesame oil	1,500
R. S. Morgan (Messrs. Lever Bros., Ltd., Port Sunlight).	Arachis oil + 0.05 per cent. hydroquinone.	980
K. Culhane Lathbury (The Brit. Drug Houses, Ltd., London).	Coconut oil	1,000
A. L. Bacharach (Glaxo Laboratories, London).	High grade, partly neutralised, arachis oil + 0.1 per cent. hydroquinone.	1,200
W. A. Broom (Messrs. Boots, Ltd., Nottingham).	Maize oil	2,000
K. H. Coward (Pharmaceutical Society, London).	Arachis oil	1,400
K. H. Coward (Pharmaceutical Society, London).	Coconut oil	1,750
A. Jung (Basle)	Olive oil + 0.01 per cent. hydroquinone.	1,500 to 2,000
Mme. L. Randoin (Paris) .	Arachis oil	1,960
J. W. Trevan (Wellcome Res. Lab., Beckenham).	Liquid paraffin (for carotene standard).	3,000 to 4,000

3. Solvents for Cod-Liver Oil in Biological Tests

It is an important principle that the same oil should be used for diluting the oil to be tested and for dissolving the carotene standard, and, further, that all the test rats in any one comparison should receive the same amount of the solvent selected. Obviously then the choice of the diluent for the test material must be governed by the choice of the solvent for the standard, although it may be uncertain whether the solvent best adapted for the one is always best adapted for the other. Whatever oil be chosen as solvent, it is essential that the investigator should have some means of assuring himself that deterioration of the vitamin A has not taken place during the period of the biological test. As a rough guide to stability the use of the antimony trichloride colour reaction may be recommended. Under optimal conditions, i.e., when using an oil of high colour value dissolved in a solvent such as coconut oil which does not interfere with the test, the results so obtained should provide a reliable indication as to the stability of the vitamin. When oils of low colour value have to be tested, or when the chosen solvent itself reacts with the antimony trichloride reagent, the results obtained will naturally be more difficult to interpret. A comparison of the colour reaction given by the freshly diluted oil, and by the same solution tested at regular intervals, should however be sufficient to indicate whether gross deterioration is taking place. It may be emphasised that the antimony trichloride reaction only gives results of absolute value when applied to concentrates or other very rich sources. Thus the vitamin A of cod-liver oil may show only some 50 per cent. of its true colour value, as determined after saponification. Stability tests by the antimony trichloride method have, therefore, only relative value unless applied to the unsaponifiable fraction.

4. Summary

(1) As the result of numerous biological tests, the potency ascribed to oil X, the Reference Cod-Liver Oil of the United States Pharmacopoeia, now adopted as the Subsidiary International Standard for Vitamin A, is 3,000 international units per gram.

(2) The range of values obtained in the above series of tests and in a similar series on cod-liver oil Z, is indicated, and the degree of harmony obtained is regarded as satisfactory.

(3) The importance of using the same oil as diluent for the standard, whether carotene or cod-liver oil, and for the test material is stressed.

(4) The necessity for checking the stability of the standard, whether carotene or cod-liver oil, and of the material tested, throughout the biological test is emphasised.

IV. GENERAL SUMMARY AND CONCLUSIONS

Selection of coconut oil as solvent for standard β -carotene.—The acceptance by the 1934 International Conference (League of Nations) on Vitamin Standards and Units, of pure β -carotene as International Standard for Vitamin A, together with the widely expressed desire that, for purposes of convenience, the Standard should be distributed in the dissolved form, has made the question of an appropriate solvent for β -carotene a matter of great importance. Section I(A) of this Report contains the results of investigations concerning the suitability of a large series of oils in which carotene is readily soluble. Of these, coconut oil was selected as being the most suitable, both for maintaining the stability of the dissolved carotene and for permitting unhindered utilisation of the carotene by the experimental animals. In future, therefore, the International Vitamin A Standard will be distributed dissolved in a selected specimen of coconut oil. The Report of the 1934 International Conference is reproduced in Appendix 3.

Relative potency of 1931 standard carotene and pure β -carotene.—The adoption of pure β -carotene as vitamin A standard in place of the impure specimen of mixed carotenes previously issued, necessitated an accurate estimate of the potency of the former in terms of the latter. The results of colorimetric, spectroscopic and biological tests carried out in several laboratories showed a reasonable degree of concordance and the potency of 0.6 γ pure β -carotene was accepted as being equal to that of 1.0 γ of the previous standard and also as representing the unit of vitamin A activity. The value of the vitamin A unit was therefore maintained unchanged.

Spectroscopic method for estimating vitamin A: comparison with biological method.—An exhaustive examination of the spectroscopic method for estimation of vitamin A in cod-liver oils and concentrates by measurement of the extinction coefficient of the 328 $m\mu$ absorption band, showed that concordant results could be obtained by workers investigating the same materials in different laboratories, (see Tables IV and VI), if certain precautions and specified methods were adopted. The materials used in these collective investigations included six different cod-liver oils and one vitamin A concentrate.

A degree of concordance which was considered fairly satisfactory was also obtained when the results of the spectroscopic examination were compared with those of biological tests (Table VII). The average value for the ratio I.U. : E (ratio of international units per gram obtained from the biological test, to $E_{1\text{ cm}}^{1\%}$, the extinction coefficient for the 328 $m\mu$ band, derived from the spectroscopic test) was taken as 1600. This figure was derived from comparative tests on three cod-liver oils and one vitamin A concentrate, and reasons are given (in Section II) why it must be considered provisional at the present time, pending the results of further investigation with a variety of different test materials.

Cod-liver oil as subsidiary standard.—The use of a selected specimen of cod-liver oil as Subsidiary Vitamin A Standard was recommended by the International Conference and is a necessary and convenient means of economising the limited amount of the β -carotene standard available. One such oil (cod-liver oil Z) has previously been in unofficial use in England and another (cod-liver oil X) is officially distributed for use as a subsidiary standard in the United States by the U.S. Pharmacopoeia Commission. The vitamin A potency of both these oils has been determined by biological tests in 9 or 10 different laboratories and the good degree of concordance in the values obtained is shown in Tables VIII and IX. Arrangements have been made to permit international issue of the U.S. standard oil.

The International Unit: importance of its universal adoption in place of all other "units".—The data contained in the different sections of this Report show that standardisation of vitamin A may now be regarded as on a satisfactory, if in some respects still provisional, basis. It is to be hoped, in the interests of clarity and uniformity, that the international unit adopted officially by the League of Nations, following the Conference of the representatives of nine different countries, will now be universally adopted and will entirely supersede the many different "biological units" which at present complicate the literature and the practice of vitamin A standardisation.

There is however a distinct risk that, even where the international unit is adopted, its adoption may be nullified by the continuation of a certain practice, which perpetuates the fundamental error of the biological unit. Satisfactory adoption of the international unit entails simultaneous comparison with the international standard in every vitamin A test. It is not sufficient for each laboratory to make *once* an estimation of the degree of response of its stock animals to unit dose of the standard. The inherent difficulty lies in the variation in the growth response of the stock animals of a given laboratory even when the diet and management are controlled and remain uniform. Such animals in a test at one time, may give an average growth response of x g. weekly to a daily dose of one international unit of the vitamin A standard while, at another time, the response to the same dose may be $x+y$ or $x-y$ g. The only way in which this fundamental source of error can be avoided is by making every test a simultaneous comparison with the standard.

It has been suggested that it would be useful to include in this Report a table, giving factors relating the various units which different workers have used, to the international unit. It has however been decided not to do this. The majority of such units were "biological" units defined in terms of animal response and consequently, as explained above, of fluctuating value, while the international unit, defined as the vitamin A potency of a given weight of the standard material, has a fixed value. The publication of a series of conversion factors would suggest a fixed relationship between these units, which does not in fact exist, and would give a false impression of accuracy to a value, expressed in international units and derived from such a conversion.

V. REFERENCES

- BALY, F. C. C., MORTON, R. A., and RIDING, R. W. (1927) *Proc. Roy. Soc. A*, **113**, 709.
- BAUMANN, C. A., and STEENBOCK, H. (1933) *J. biol. Chem.*, **101**, 561.
- CARR, F. H., and JEWELL, W. (1933) *Nature*, **131**, 92.
- CHEVALLIER, A., and CHABRE, P. (1934) *Bull. Soc. Chim. biol.*, Paris, **16**, 1451.
- COLLISON, D. L., HUME, E. M., SMEDLEY-MACLEAN, I., and SMITH, H. H. (1929) *Biochem. J.*, **23**, 634.
- COWARD, K. H. (1933) *Biochem. J.*, **27**, 445.
- COWARD, K. H., KEY, K. M., DYER, F. J., and MORGAN, B. G. E. (1930) *Biochem. J.*, **24**, 1952. (1931) *Biochem. J.*, **25**, 551.
- COWARD, K. H., DYER, F. J., MORTON, R. A., and GADDUM, J. H. (1931) *Biochem. J.*, **25**, 1102.
- COWARD, K. H., DYER, F. J., and MORTON, R. A. (1932) *Biochem. J.*, **26**, 1593.
- CULHANE LATHBURY, K. (1934) *Biochem. J.*, **28**, 2254.
- CULHANE LATHBURY, K., and GREENWOOD, G. N. (1934) *Biochem. J.*, **28**, 1665.
- DRUMMOND, J. C., and MORTON, R. A. (1929) *Biochem. J.*, **23**, 785.
- DUTCHER, R. A., HARRIS, P. L., HARTZLER, E. R., and GUERRANT, N. B. (1934) *J. Nutrit.*, **8**, 269.
- DYER, F. J., KEY, K. M., and COWARD, K. H. (1934) *Biochem. J.*, **28**, 875.
- EDISBURY, J. R., MORGAN, R. S., and MORTON, R. A. (1934) *J. Soc. chem. Ind.*, Lond., **53**, 1083.
- EULER, H. v., KARRER, P., and ZUBRYS, A. (1934) *Helv. chim. Acta*, **17**, 24.
- HUME, E. M., and SMEDLEY-MACLEAN, I. (1930) *Lancet*, i, 290.
- KUHN, R., and BROCKMANN, H. (1932, 1) *Ber. dtsh. chem. Ges.*, **65**, 894. (1932, 2) *Hoppe-Seyl. Z.*, **213**, 1. (1933) *Ber. dtsh. chem. Ges.*, **66**, 1319.
- KUHN, R., BROCKMANN, H., SCHEUNERT, A., and SCHIEBLICH, M. (1933) *Hoppe-Seyl. Z.*, **221**, 129.
- KUHN, R., and GRUNDMANN, C. (1933) *Ber. dtsh. chem. Ges.*, **66**, 1746.
- LEAGUE OF NATIONS HEALTH ORGANISATION (1931). Report of the Permanent Commission on Biological Standardisation, p. 68, Annex iv, Report of the Conference on Vitamin Standardisation, held at London from June 17 to 20th, 1931. (*Series of League of Nations Publications*, 1931, iii, 10.)
- MCDONALD, F. G. (1933) *J. biol. Chem.*, **103**, 455.
- MACWALTER, R. J. (1934) *Biochem. J.*, **28**, 472.
- MOORE, T. (1933) *Biochem. J.*, **27**, 898.
- MORGAN, R. S. (1934) *Biochem. J.*, **28**, 1178.
- MORGAN, R. S., EDISBURY, J. R., and MORTON, R. A. (1935) *Biochem. J.*, **29**, 1645.
- MORTON, R. A., and HEILBRON, I. M. (1928, 1) *Nature*, **122**, 10. (1928, 2) *Biochem. J.*, **22**, 987. (1930) *Biochem. J.*, **24**, 870.
- ROSSLER, G. (1926) *Ber. dtsh. chem. Ges.*, **59**, 2606.
- SMITH, E. L., and HAZLEY, V. (1930) *Biochem. J.*, **24**, 1942.
- SMITH, J. H. C., and MILNER, H. W. (1934) *J. biol. Chem.*, **104**, 437.
- TURNER, R. G. (1934) *J. biol. Chem.*, **105**, 443.

VI. APPENDICES

APPENDIX—1

PROTOCOLS OF THE BIOLOGICAL EXPERIMENTS

(1) *E. M. Hume and H. Henderson Smith.*—The hot-room (120 days at 37° C.) and the cold-room (120 days at 0° C.) solutions of β -carotene dissolved in coconut oil 162398, without addition of hydroquinone, were tested simultaneously on depleted rats for a period of 35 days. The colour of the solutions was maintained throughout the period of the biological tests. Four litter-mate pairs of rats, comparable as regards sex, received 1.0 γ daily of the two solutions, and four similar pairs received 0.5 γ . The results were as follows :—

Daily Dose.				Total Weight Increase. g.
β -Carotene at 0° C., 1.0 γ	208
β -Carotene at 0° C., 0.5 γ	170
β -Carotene at 37° C., 1.0 γ	204
β -Carotene at 37° C., 0.5 γ	157

All rats grew or maintained weight throughout the experiment, except one receiving the 0.5 γ dose of cold-room solution, which began to decline sharply in weight two days before the end of the experiment.

A slight superiority of the solution stored at 0° C. is indicated, which is most apparent with the 0.5 γ dose.

(2) *E. M. Hume and H. Henderson Smith.*—A simultaneous comparison was made between 1931 standard carotene, dissolved in coconut oil (commercial, British Drug Houses) and in ethyl laurate, without addition of hydroquinone. The colour of the solutions was maintained throughout the biological test. The carotene was administered simultaneously to depleted rats in daily doses of 1.0, 2.0 and 3.0 γ for a period of 42 days. Each group contained three or four rats and the six groups were balanced as far as possible in litter and sex. The result was as follows :—

Daily Dose.				No. of Rats Surviving 42 Days.	
Carotene in ethyl laurate, 1.0 γ	+0	-3
2.0 γ	+2	-1
3.0 γ	+2	-2
Carotene in coconut oil, 1.0 γ	+2	-1
2.0 γ	+3	-0
3.0 γ	+3	-0

Thus, four out of ten rats survived on carotene in solution in ethyl laurate and seven out of eight on the same doses of carotene in coconut oil; the superiority of the latter is thus apparent.

(3) *E. M. Hume and H. Henderson Smith.*—A simultaneous comparison was made between 1931 standard carotene dissolved in liquid paraffin and in ethyl laurate, without addition of hydroquinone. The colour of the solutions was maintained throughout the biological test. The carotene was administered to depleted rats for a period of 42 days; three rats received a daily dose of 3.0 γ of carotene dissolved in liquid paraffin, while two received 3.0 γ , and two others 4.0 γ , in ethyl laurate. The results were as follows :—

Daily Dose.				No. of Rats Surviving 42 Days.	
Carotene in liquid paraffin, 3.0 γ	+1	-2
Carotene in ethyl laurate, 3.0 γ	+2	-0
4.0 γ	+2	-0

The one survivor on carotene dissolved in liquid paraffin showed poor growth; all rats on carotene in ethyl laurate survived and grew. Carotene in liquid paraffin was thus inferior even to carotene in ethyl laurate.

(4) *E. M. Hume and H. Henderson Smith.*—A simultaneous comparison was made between 1931 standard carotene dissolved in coconut oil 161441, with and without addition of hydroquinone to a concentration of 0.01 per cent. The colours of the solutions were maintained throughout. The carotene was administered to depleted rats for a period of 42 days; groups of three rats received daily doses of 0.5 γ and 1.0 γ of carotene in each of the two solutions. The results were as follows:—

<i>Daily Dose.</i>	<i>No. of Rats Surviving 42 Days.</i>	
Carotene in coconut oil 161441 (+0.01 per cent. hydroquinone),		
0.5 γ	+1	-2
1.0 γ	+2	-1
Carotene in coconut oil 161441 (no hydroquinone),		
0.5 γ	+1	-2
1.0 γ	+2	-1

The number of rats surviving on each dose was thus the same for each dose of the two solutions. The growth performance of the survivors was also approximately the same. The potency of carotene in oil 161441 was thus the same whether hydroquinone was added or not.

(5) *E. M. Hume and H. Henderson Smith.*—A simultaneous comparison was made of 1931 standard carotene dissolved in each of the coconut oils 162398, 161441 and 162750. Hydroquinone to the concentration of 0.01 per cent. was added to all the solutions. The colour of the solutions was maintained throughout the biological tests. The carotene was administered to groups of three depleted rats for a period of 42 days in daily doses of 1.0 γ and 0.5 γ . The results were as follows:—

<i>Daily Dose.</i>	<i>Total Gain in 42 Days of 1♂ and 2♀ Rats. g.</i>	<i>No. of Rats Surviving 42 Days.</i>		<i>Total Gain in 42 Days of Survivor, or Average of Survivors (♂).</i>
Carotene in coconut oil 162398,				
1.0 γ	130	—	—	—
0.5 γ	—	+2	-1	46
Carotene in coconut oil 161441,				
1.0 γ	120	—	—	—
0.5 γ	—	+1	-2	40
Carotene in coconut oil 162750,				
1.0 γ	115	—	—	—
0.5 γ	—	+1	-2	28

The results given by carotene in the three oils are of the same order, but every test suggests a slight superiority of oil 162398, which may not, however, be significant.

(6) *E. M. Hume and H. Henderson Smith.*—A simultaneous comparison was made between 1931 standard carotene and β -carotene. Both carotenes were dissolved in coconut oil 162398. No hydroquinone was added. The colours of the solutions were maintained throughout. The carotenes were

administered to groups of four depleted rats for 49 days, in daily doses of 0.5, 0.75 and 1.0 γ . All the rats survived the period of the experiment. The results were as follows:—

Daily Dose.	Total Gain (g.) in 49 Days of Groups of 4 Rats.	
	2 ♂ and 2 ♀	1 ♂ and 3 ♀
1931 Standard carotene,		
0.5 γ	146	—
0.75 γ	182	154
1.0 γ	198	—
β -Carotene,		
0.5 γ	172	—
0.75 γ	—	172
1.0 γ	246	—

The performance of the groups receiving 0.5 γ of β -carotene and 0.75 γ of 1931 standard carotene, are the nearest to one another. It was, therefore, reckoned that 1931 standard carotene had two-thirds the value of β -carotene.

(7) *K. Culhane Lathbury*.—A comparison was made between 1931 standard carotene and β -carotene, both being dissolved in coconut oil 161441. The carotenes were fed to depleted rats for a test period of 70 days in daily doses of 2.0 γ of the 1931 standard and 2.0 and 1.0 γ of the β -carotene. The results of seven different comparisons are shown in the table.

Daily Dose. Average Weekly Loss of Weight (g.) of Litter-Mate Groups of Rats.

	6♂	4♂	5♂	8♀	6♀	8♀	10♀
1931 Standard carotene,							
2.0 γ	3.0	2.3	3.0	2.0	2.4	2.9	2.4
β -Carotene,							
1.0 γ	—	0.8	2.6	—	2.2	3.1	—
2.0 γ	1.3	0.7	0.8	0.7	1.3	1.1	0.4

In three out of four comparisons, the loss in weight of the rats receiving 1.0 γ of β -carotene was almost the same as that of the rats receiving 2.0 γ of 1931 standard carotene. β -Carotene was, therefore, regarded as having twice the value of the 1931 standard carotene.

Note.—The loss in weight of the rats in this experiment is attributed to failure of absorption of carotene from coconut oil 161441.

(8) *K. H. Coward*.—Cod-liver oil X was compared simultaneously with the International Standard 1931 for vitamin A. Groups of 7-8 rats, which had become steady in weight on a diet deficient in vitamin A, received daily doses of 2.0 and 0.5 mg. of oil X, and of 2 γ of International Standard, for four weeks. Each dose was dissolved in 20 mg. of arachis oil. Comparison was made of the average increase in weight of the three groups of rats.

The results were as follows:—

Daily Dose.	Mean Increase in Weight in 4 Weeks.
International Standard 1931, 2.0 γ ..	23.6
Cod-liver oil X, 2.0 mg. ..	43.0
0.5 mg. ..	12.7

From her curve of response (Coward, Key, Dyer and Morgan, 1930, 1931; Coward, 1933), relating mean increase in weight of a group of rats in four weeks to dose of vitamin A administered, Coward estimated that 1.0 mg. of oil X was equivalent to 3.0 γ of International Standard. The oil X therefore contained 3,000 international units of vitamin A per gram.

(9) *K. Culhane Lathbury*.—Cod-liver oil X was compared simultaneously with the International Standard 1931 for vitamin A. Coconut-oil 162398 was used to dissolve the Standard and to dilute cod-liver oil X. A series of five comparisons was made by feeding the material to 5 pairs of litter-mate groups of depleted rats for a test period of 70 days, the 1931 Standard being administered in daily doses of 1.0 and 2.0 γ (1 and 2 international units). A suitable dosage of cod-liver oil was based on the blue value, as ascertained by the method of Smith and Hazley (1930). The oil was diluted from a blue value of 54 to blue values of 2 and 1 and all rats received a volume of 0.02 cc. The doses of cod-liver oil were thus 0.33 and 0.67 mg. Five separate results were obtained from the five comparisons and the most probable value deduced from them. The results are shown below:—

Daily Dose. Average Weekly Weight Increase (g.) of Litter-Mate Groups of Rats.

	7♂	6♂	6♂	4♀	4♀
International Standard 1931,					
1.0 γ	—	—	2.6	—	1.8
2.0 γ	4.6	5.0	—	3.1	—
Cod-liver oil X,					
0.33 mg.	3.8	—	4.2	—	1.8
0.67 mg.	—	5.0	—	3.0	—
Results calculated as inter- national units per gram. . .	<5,400	2,700	>2,700	2,700	2,700

The value ascribed to cod-liver oil X is therefore 2,700 international units per gram.

(10) *R. S. Morgan*.—Cod-liver oil X was compared simultaneously with the International Standard 1931 for vitamin A. Both were administered in solution in fresh arachis oil containing hydroquinone. The weight increase of the rats, averaged at 5 weeks after the beginning of the experimental period, was as follows:—

Daily Dose. Weight Increase (g.) Averaged at 5 Weeks of Litter-Mate Groups of Rats.

	3♂	2♂	1♀	3♀
International Standard 1931,				
0.5 γ	—	—	19.0	—
0.75 γ	—	—	—	44.3
1.0 γ	55.0	—	—	—
2.0 γ	—	77.6	—	—
Cod-liver oil X,				
0.167 mg.	—	—	26.7	—
0.250 mg.	—	—	—	35.0
0.333 mg.	57.9	—	—	—
0.667 mg.	—	77.2	—	—

The result calculated from the individual pair differences of the squares of the weight increase was 2,840 units of vitamin A per gram (*see Morgan 1934, pp. 1184-1185*).

(11) *K. H. Coward*.—Cod-liver oil Y was examined simultaneously with cod-liver oil Z, of vitamin A potency 1,500 international units per gram (*see p. 34*). Two groups of 10 rats each, which had ceased to grow on a diet

deficient in vitamin A, received daily doses of 1.0 mg. of oil Y and of 1.0 mg. of oil Z, respectively, in 20 mg. of olive oil, for three weeks. The results were as follows:—

Daily Dose.	Mean Increase in Weight in 3 Weeks.
Cod-liver oil Y, 1.0 mg.	g. 20.2
Cod-liver oil Z, 1.0 mg.	28.9

From her curve of response (Coward, Key, Dyer and Morgan, 1930, 1931; Coward, 1933), relating mean increase in weight of a group of rats in three weeks to dose of vitamin A administered, Coward estimated that 1.0 mg. of oil Y was equivalent to 0.4 mg. of oil Z. Cod-liver oil Y therefore contained 600 international units of vitamin A per gram.

(12) *K. Culhane Lathbury*.—Cod-liver oil Y was compared simultaneously with the International Standard 1931 by the same method as that described in Protocol (9). The doses of the standard were the same, but in this case the suitable daily doses of the oil, calculated from the blue value (13.5) were 1.33 and 2.67 mg. The results are shown in the table, as follows:—

Daily Dose. Average Weekly Weight Increase (g.) of Litter-Mate Groups of Rats.

	Test No.	8♂	5♂
International Standard 1931,			
1.0γ	(1)	3.1	2.7
2.0γ	(2)	4.3	—
Cod-liver oil Y,			
1.33 mg.	(3)	3.6	2.8
2.67 mg.	(4)	4.3	4.0
Results, calculated as international units per gram from comparison of:	(1) and (3) (1), (2) and (4)	>675 675	675 Definitely <1350

The value ascribed to cod-liver oil Y was therefore 675 international units per gram.

(13) *K. H. Coward*.—Cod-liver oil Z was compared simultaneously with the International Standard 1931 for vitamin A. Two groups of 12 rats each, which had become steady in weight on a diet deficient in vitamin A, received daily doses of 1.0 mg. of oil Z and of 2γ of the International Standard respectively, dissolved in 20 mg. coconut oil (161441 of the British Drug Houses), for five weeks. The results were as follows:—

Daily Dose.	Mean Increase in Weight in 5 Weeks.
International Standard 1931,	g.
2.0γ	35.3
Cod-liver oil Z,	
1.0 mg.	25.4

From her curve of response (*see* Protocol (11)) relating mean increase in weight of a group of rats in five weeks to dose of vitamin A administered, Coward estimated that 1.0 mg. of oil Z was equivalent to 1.4 mg. of International Standard 1931. Oil Z, therefore, contained 1,400 international units of vitamin A per gram.

A second test, with doses of 1.0 mg. of oil Z and 2.0γ of International Standard 1931, in 20 mg. of arachis oil and with 9 and 10 rats respectively, gave the potency of cod-liver oil Z as 1,750 international units of vitamin A per gram. The average from these two tests was taken as 1,600.

(14) *K. Culhane Lathbury*.—Cod-liver oil Z was compared simultaneously with the International Standard 1931 and with another cod-liver oil by the same method as that described in Protocol (9). The doses of the Standard were the same, 1.0 and 2.0 γ . This test being the first of the series and the doses not being appropriately based on the blue value (24.0) of the oil, the daily doses adopted (2.5 and 5.0 mg.), proved to be too large to compare with those of the Standard used. The results from the 71 rats used in the test showed, however, that 2.5 mg. contained more than 2 units and, therefore, that oil Z contained more than 800 international units per gram. Comparison with the other cod-liver oil, tested at the same time, also showed that oil Z contained more than 800 units per gram and, from experience with other cod-liver oils, a value of 1,000 units per gram was estimated to be near to the true figure.

(15) *R. S. Morgan*.—Cod-liver oil Z and the International Standard 1931 were administered simultaneously in solution in fresh arachis oil containing hydroquinone (about 0.05%).

The weight increase of the rats, averaged at 5 weeks after the beginning of the experimental period was as follows:—

Daily Dose. Weight Increase (g.) Averaged at 5 Weeks of Litter-Mate Groups of Rats.

	3♂	4♂	4♂	3♀	3♀
International Standard 1931,					
0.5 γ	24.9	—	—	11.0	—
1.0 γ	—	78.4	—	—	42.9
2.0 γ	—	—	86.9	—	—
Cod-liver oil Z,					
1.0 mg.	46.7	—	—	53.1	—
2.0 mg.	—	84.1	—	—	66.1
4.0 mg.	—	—	107.1	—	—

The value of cod-liver oil Z was calculated (*see* Protocol (10)) as 980 international units per gram.

(16) *K. Culhane Lathbury*.—The vitamin A concentrate C was compared simultaneously with the International Standard 1931 for vitamin A by the same method as that described under Protocol (9). The daily doses of the Standard were the same, but, because this sample was a concentrate, relatively larger doses, 0.6 and 1.2 γ , as judged from the blue value (60,000), were given. Five separate comparisons of litter-mate rats were made. The results are shown in the table as follows:—

Daily Dose. Average Weekly Weight Increase (g.) of Litter-Mate Groups of Rats.

	7♂	9♂	9♂	10♀	10♀
International Standard 1931,					
1.0 γ	—	4.2	—	2.9	—
2.0 γ	6.4	—	5.4	—	2.0
Concentrate C,					
0.6 γ	—	4.6	—	3.2	—
1.2 γ	6.6	—	5.8	—	2.1
Results, calculated as inter- national units per gram ..	1,500,500	Just> 1,500,000	Just> 1,500,000	Just> 1,500,000	1,500,000

It was concluded that the concentrate contained slightly more than 1,500,000 international units per gram.

(17) *E. M. Hume and H. Henderson Smith.*—The vitamin A concentrate C was compared simultaneously with the International Standard 1931 and with Karrer's sample of β -carotene. All three were dissolved in coconut oil 162398, without addition of hydroquinone, and the stability of the carotene solutions was checked by colorimetric tests. The stability of the concentrate was not checked. The total increase in seven weeks of groups of 4 rats (2 ♂ and 2 ♀), in which the litters were distributed as evenly as possible, was as follows :—

<i>Daily Dose.</i>	<i>Total Weight Increase in 7 Weeks. g.</i>
International Standard 1931, (1 int. unit) 1.0 γ	198
0.75 γ	182
0.5 γ	146
Concentrate C, 2.0 γ	250
1.0 γ	187
β -Carotene, (1.7 int. unit) 1.0 γ	246
0.5 γ	172

Thus 1.0 γ of the concentrate C is regarded as approximately equal to 0.75 γ of International Standard, and 2.0 γ of the concentrate to 1.0 γ of β -carotene; 1.0 γ of the concentrate therefore has a potency of 0.75 international unit by the first comparison, and of 0.83 international unit by the second comparison. The general condition of the rats favoured the concentrate rather more than is suggested by the weight increase and a value of 900,000 international units per gram was therefore ascribed to the concentrate.

(18) *T. Moore.*—The concentrate C was compared with Karrer's sample of β -carotene. Both were dissolved in coconut oil without addition of hydroquinone. The stability of both solutions was checked colorimetrically, the carotene directly with the Lovibond tintometer and the concentrate by means of the $SbCl_3$ test. Groups of 3 or 4 rats received the test doses for three weeks and their weight increase was as follows :—

<i>Daily Dose.</i>	<i>Average Daily Gain. g.</i>
β -Carotene, 1.25 γ	1.43
Concentrate C, 1.82 γ	1.76
1.21 γ	1.38
0.61 γ	0.86

Thus 1.25 γ of β -carotene gave about the same response as 1.21 γ of concentrate C; the concentrate therefore had about the same biological activity as β -carotene, which has been found to contain 1,750,000 international units per gram.

(19) *R. S. Morgan.*—The concentrate C was compared simultaneously with the International Standard 1931; both were administered in solution in fresh arachis oil containing hydroquinone. The weight increase of the rats, averaged at 3 weeks after the beginning of the experimental period, was as follows :—

Daily Dose. Weight Increase (g.) Averaged at 3 Weeks of Litter-Mate Groups of Rats.

	2♂	3♂	9♂	3♂
International Standard 1931,				
0.5γ	26.5	—	—	—
0.75γ	—	20.3	—	—
1.0γ	—	—	44.0	—
2.0γ	—	—	—	67.8
Concentrate C,				
0.417γ	19.7	—	—	—
0.625γ	—	35.9	—	—
0.835γ	—	—	49.9	—
1.670γ	—	—	—	60.5

The value of concentrate C was calculated (*see* Protocol (10)) as 1,290,000 international units per gram.

APPENDIX—2

APPROVED METHOD OF SAPONIFICATION

Besides the method of saponification for liver oils adopted by the International Conference on Vitamin Standardisation 1934 (*see* p. 56), two other methods were regarded by the Vitamin A Sub-committee as satisfactory and are described below. That of Dann and Moore is specially modified to meet the specific need for which it is intended, being planned for the saponification of very small quantities of oil such as are needed for the spectroscopic examination.

It is pointed out by J. R. Edisbury and R. A. Morton that the method accepted by the Conference is capable of further improvement, and their suggestions are also included below under (c).

(a) Method of W. J. Dann and T. Moore (Cambridge)

About 500 mg. of oil are weighed into a small (5 c.c.) flask; 0.22 c.c. of KOH solution (made by dissolving 80 g. KOH in 54 c.c. water) is added and 1 c.c. of alcohol. The mixture is heated on a water bath, with shaking, for about 1 minute (by which time saponification should be complete) and is then poured into a graduated separating funnel containing 50 c.c. of ether. The flask is washed 4 times with 2 c.c. of distilled water and the funnel shaken vigorously. The aqueous layer is discarded and the ethereal layer washed with 25 c.c. of water by very gentle rotation; the water is again discarded. The ethereal layer is filtered through a 1 inch layer of anhydrous sodium sulphate, contained in a sintered glass funnel, into a flask of rather more than 100 c.c. capacity, suitable for the evaporation. The separating funnel and the sulphate are washed with 20 c.c. ether. The ether is evaporated carefully on a water bath under a filter pump and the residue dissolved in 10 c.c. of alcohol or cyclohexane, delivered from a pipette. This volume gives a solution suitable for spectroscopic observation with most cod-liver oils. For halibut-liver oil and for others of high potency a further dilution is necessary.

(b) Method of N. Evers (Res. Lab. Messrs. Allen and Hanburys, Bethnal Green, London)

A quantity of oil is weighed, such that when the unsaponifiable matter is dissolved in 50 c.c. of cyclohexane, a concentration suitable for examination in the spectrophotometer is obtained. A quantity of 0.5 c.c. of approximately 10 N aqueous KOH is added, together with 5 c.c. of 95 per cent. alcohol. The whole is heated under a reflux condenser for 15 minutes and washed

into a small separating funnel with 5 c.c. of water; 20 c.c. of spectrographically pure cyclohexane are added and the mixture is shaken thoroughly. The soap solution is further extracted with 10, 10 and 5 c.c. of cyclohexane successively. To the mixed cyclohexane solutions, 10 c.c. of water are added and the separator is rotated without shaking and allowed to stand for a few minutes, when the water is run off. The cyclohexane solution is washed by shaking it twice with 10 c.c. of water; it is then filtered into a 50 c.c. flask and made up to 50 c.c. with cyclohexane.

(c) **Improvements to the Method accepted by the International Conference, 1934, suggested by J. R. Edisbury and R. A. Morton (Liverpool)**

The amount of oil taken for saponification should be 0.2 to 1.0 g., according to potency. It is saponified with 10 c.c. N/2 alcoholic potash for 5 to 10 minutes, then diluted with 20 c.c. water and extracted twice with 25 c.c. *freshly distilled* ether. The combined ethereal extracts are washed once with water (swirling), once with dilute alkali or carbonate and then twice more with water (gently shaking), 10 to 20 c.c. being used for each wash. For the colour test an aliquot portion of the ethereal solution is evaporated and blown dry twice with nitrogen or carbon dioxide at 70–80° C., after the addition of a few drops of pure ethyl alcohol. The residue is made up to suitable volume with pure dry chloroform and examined without delay.

For the examination of the ultraviolet absorption, a second aliquot portion of the ethereal extract is evaporated in a stream of nitrogen or carbon dioxide and the wet residue* is dissolved in alcohol, which has been tested spectrographically for transparency. The use of porous pot to prevent "bumping" should be avoided, since adsorption of appreciable quantities of vitamin A on the pot may occur.

APPENDIX—3

REPORT OF THE SECOND INTERNATIONAL CONFERENCE, 1934, ON VITAMIN STANDARDISATION OF THE LEAGUE OF NATIONS (HEALTH ORGANISATION)

PRELIMINARY NOTE

An International Vitamin Conference, attended by most of the experts responsible for the present report, was held in London in June, 1931, under the auspices of the Permanent Commission on Biological Standardisation of the League of Nations Health Organisation. The report of the earlier Conference,† published in 1931, recommended for international adoption standards and units for vitamins A, B₁, C and D, which were to be "provisional for two years". Since certain of the standard preparations recommended for adoption were not available for general use until 1932, the second Conference was postponed until 1934, at which date two years' experience of the practical application of the provisional standards was available.

The following report should therefore be regarded as a revision of the report of the 1931 Conference. In making its decisions, the present Conference had also at its disposal the results of many important new investigations connected with vitamin standardisation. It is to be noted that, in this report, the standards and units recommended are no longer termed "provisional". While the advance of scientific knowledge may at some future date make changes in the adopted standards and units desirable, it was not felt necessary, as before, to assign provisional periods for their use.

* If cyclohexane is to be used, the residue must be dried or the solution in cyclohexane filtered through a paper wetted first with cyclohexane.

† "Report of the Conference on Vitamin Standards." Publications Department of the League of Nations No. C.H.1055(1), Geneva, 1931.

The time may shortly arrive when the cumbersome method of comparing the vitamin activity of test materials with the activity of a chosen standard will be obsolete, and vitamin potency will be expressed in terms of the exact amount of pure active vitamin present. The Conference, however, considered it desirable that, for the present, vitamin potency should, in the case of all the vitamins dealt with in its report, continue to be estimated by means of biological tests in comparison with a chosen standard, even in those cases in which pure substances possessing high vitamin potency have been isolated, thus making it possible to estimate with considerable accuracy, by chemical and physical methods, the amount of these substances present in materials under test.

As in the 1931 Report, standards and units are recommended for only four of the known vitamins—namely, vitamins A, B₁, C and D. Consideration was given to the possibility of adopting standards for vitamins B₂ and E, but it was felt that our knowledge of the nature and possible complexity of these vitamins, and of the pathological results to which their absence gives rise, is still insufficient to justify the adoption of standards and units for them.

The standards for vitamins A and C have been altered. Those chosen for these vitamins in 1931 were found to have certain defects which impaired their usefulness; the substitution of more clearly defined and more easily reproducible chemical substances is a definite step in advance. No change has been recommended in the standards for the vitamins B₁ and D. The former has proved highly convenient in practice—of all the standards chosen by the 1931 Conference the vitamin B₁ standard has perhaps proved most satisfactory—and a large stock, sufficient to last for many years, is available at the central distributing institution, the National Institute for Medical Research, London. In view of all the circumstances its replacement by a purer vitamin B₁ preparation does not seem at present desirable. The vitamin D standard also remains unaltered, with the proviso that it may be replaced when exhausted (or if it should become for any reason unsatisfactory) by crystalline vitamin D in suitable solution. Large quantities of the original standard solution of irradiated ergosterol are still available. Certain recently observed anomalies, in the antirachitic action on certain species, of different substances containing equal numbers of international vitamin D units, to which brief reference is made in the report, suggest that a re-examination of the suitability of the present vitamin D standard may become necessary at some future date.

The units of vitamin activity remain the same in all cases. Where a change has been made in the standard material, the old units have been re-stated in terms of the newly chosen substance. The desirability of leaving the original units unaltered is emphasised by the fact that certain of the units recommended by the 1931 Conference have been adopted in the pharmacopoeias of certain countries.

The majority of the members of the Conference have in recent years carried out experiments bearing directly on problems of vitamin standardisation, the results of which proved to be of the greatest value to the Conference. In addition to the members of the Conference, many workers in different countries, whose names do not appear in the Report, helped to carry out the recommendations for research made by the 1931 Conference, and placed valuable information at the disposal of the present meeting. Thanks are due to such workers, without whose active and untiring co-operation the Conference could not have undertaken the task set before it.

REPORT OF THE CONFERENCE

List of Participants

Chairman: Dr. E. MELLANBY, Secretary, Medical Research Council of Great Britain, London.

Professor J. C. DRUMMOND, Professor of Biochemistry, University College, London.

Professor H. v. EULER, Professor of Biochemistry, University of Stockholm.

- Professor L. S. FRIDERICIA, Director, Institute of Hygiene, University of Copenhagen.
- Professor B. C. P. JANSEN, Professor of Physiological Chemistry, University of Amsterdam.
- Professor P. DE MATTEI, Director, Institute of Pharmacology, University of Pavia.
- Dr. E. M. NELSON, Senior Chemist, Bureau of Chemistry and Soils, U.S. Department of Agriculture, Washington, D.C.
- Professor E. POULSSON, Director, State Vitamin Institute, Oslo.
- Mme. RANDOIN, Directeur du laboratoire de physiologie de la nutrition à l'École des Hautes-Études, et Ministère de l'Agriculture, Paris.
- Professor H. STEENBOCK, Professor of Agricultural Chemistry, University of Wisconsin, Madison, U.S.A.
- Professor A. SZENT-GYÖRGYI, Institute of Medical Chemistry, University of Szeged, Hungary.
- Dr. H. CHICK, Lister Institute of Preventive Medicine, London. }
 Dr. W. R. AYKROYD, Health Section, League of Nations } Technical
 Secretariat, Geneva. } Secretaries.

The following also were present :—

- Dr. M. H. BROWN, Connaught Laboratories, Toronto, Canada.
- Dr. K. H. COWARD, Laboratory of the Pharmaceutical Society of Great Britain, London.
- Sir Henry H. DALE, Director, National Institute for Medical Research, London.
- Dr. P. HARTLEY, National Institute for Medical Research, London.
- Miss E. M. HUME, Lister Institute of Preventive Medicine, London.
- Dr. A. JUNG, Institute of Physiological Chemistry, University of Basle.
- Dr. Ch. LORMAND, Directeur du Laboratoire national de contrôle des médicaments, Paris.
- Dr. R. MENDEZ, Technical Institute of Pharmacy and Biology, Madrid.
- Dr. R. A. MORTON, Department of Chemistry, University of Liverpool.
- Dr. R. A. PETERS, Professor of Biochemistry, University of Oxford.
- Dr. O. ROSENHEIM, National Institute for Medical Research, London.
- Dr. M. TSURUMI, Member of the League of Nations Health Committee (Japan).
- Mr. T. A. WEBSTER, National Institute for Medical Research, London.
- Dr. S. S. ZILVA, Lister Institute of Preventive Medicine, London.

The meeting was opened by Sir George BUCHANAN who welcomed the members on behalf of the Health Committee of the League of Nations, after which Sir Henry H. Dale gave a brief review of the present position with regard to international vitamin standardisation.

I.—VITAMIN A

(a) *International Standard*

The Conference recommends that pure β -carotene be adopted as the International Standard for Vitamin A. The Standard Preparation shall conform to the requirements stated in Note 1 in regard to its chemical and physical constants.

(b) *Definition of Unit*

The Conference recommends that the unit for Vitamin A provisionally adopted at the 1931 Conference shall be maintained. It has been established that one such unit is contained in 0.6 microgram (0.6 γ) of pure β -carotene.

The International Unit for Vitamin A recommended for adoption shall be defined as the Vitamin A activity of 0.6 microgram (0.6 γ) of the International Standard Preparation.

Daily doses of 2 to 4 International Units of Vitamin A, when administered to young rats suitably prepared on a Vitamin A-deficient diet, have been found adequate to restore growth; somewhat larger doses are required for the cure of xerophthalmia.

(c) *Mode of Preparation*

It is recommended that the Health Organisation of the League of Nations shall be requested to obtain a sample of β -carotene as defined by the Conference (see Note 1), and that the National Institute for Medical Research, London, acting for this purpose as central laboratory on behalf of the Health Organisation of the League of Nations, shall undertake the care, storage and distribution of the International Vitamin A Standard so obtained.

(d) *Mode of Distribution*

The Conference recommends that the International Standard Preparation shall be issued in the form of a standard solution in oil, the strength of the solution being such that 1 gramme contains 500 International Units, or 300 micrograms (300 γ) of β -carotene. (See Note 2.)

(e) *Adoption of Subsidiary Standard of Reference*

The Conference recommends that a sample of cod-liver oil, the potency of which has been accurately determined in terms of the International Standard Preparation of β -carotene, shall be provided as a Subsidiary Standard of Reference.

In view of the fact that the Reference Cod-liver Oil of the United States Pharmacopoeia, which has been accurately assayed in terms of the provisional International Standard adopted in 1931, has been in effective use in the United States of America for some time, the Conference recommends that the Board of Trustees of the United States Pharmacopoeia be approached and invited to place a quantity of their Reference Cod-liver Oil at the disposal of the Health Organisation of the League of Nations, with a view to its adoption for international use as a Subsidiary Standard for Vitamin A.

In the event of the Reference Cod-liver Oil of the United States Pharmacopoeia not being available for international adoption, the Conference recommends that another sample of cod-liver oil be selected, its potency in terms of the International Standard Preparation of β -carotene accurately determined by biological comparison and independently by spectrophotometric measurements (see (f) below), and that this selected sample be then adopted as a Subsidiary International Standard for Vitamin A.

(f) *Spectrophotometric Test for the Assay of Vitamin A in Liver Oils and Concentrates of High Potency*

It has been found that, within certain defined conditions, measurement of the coefficient of absorption (E) at 328 $m\mu$ affords a reliable method for measuring the Vitamin A content of liver oils and concentrates. As a means of converting values obtained for $E_{1\text{cm.}}^{1\%}$ 328 $m\mu$ into a figure representing the International Units of Vitamin A per gram of the material examined, the factor 1600 is recommended for adoption. (See Note 3.)

Note 1

Properties of Pure β -Carotene (C₄₀H₅₆).

- (a) Melting-point, 184–184.5°C., corrected.
- (b) Optically inactive.
- (c) Absorption spectrum showing bands :—
 - in carbon disulphide at 519, 485.5 $m\mu$
 - in chloroform at 495, 465 $m\mu$
 - in cyclohexane at 486, 456 $m\mu$

Note 2

Nature of the Oil to be used as Diluent.

The oil used for the preparation of the standard solution of β -carotene, and for the further dilution of the standard and for use in the biological assay of Vitamin A, shall be a colourless, Vitamin A-free, vegetable oil, from which the

dissolved carotene can be properly absorbed from the alimentary canal of the test animals. 0.01 per cent. of hydroquinone shall be added to the oil to prevent oxidation and ensure stability.

In order to establish its suitability, the oil to be used as diluent for the above-named purposes must be submitted to the following test. A 0.003% solution of β -carotene in the oil is prepared, and the colour of the solution compared with that of a 0.5% solution of potassium bichromate; about 6 c.c. of the carotene solution in oil are placed in a flat-bottomed tube (5 cm. \times 2 cm.) which is closed with a hard cork so as to leave an air space of about 6 c.c. capacity. The tube is then maintained at a temperature of 37° C. in the dark for seven days, and the colour of the solution again compared with that of the standard solution of potassium bichromate. An oil may be considered satisfactory for the purposes indicated if the loss in colour does not exceed 10 per cent. Some samples of coco-nut oil have been found to fulfil the requirements of the above test.

Note 3

The Spectrophotometric Test.

(a) The measurement must be made on the unsaponifiable fraction, unless the material is of potency greater than 10,000 International Units per gram.

Method of saponification. Since the error associated with the saponification may be considerable, the following method is recommended, as yielding trustworthy results.

One gram of oil is saponified with 10 c.c. N/2 freshly prepared alcoholic KOH, by boiling until clear (time needed about 5 minutes). 20 c.c. water are added, the whole transferred to a small separator and extracted with two quantities of 25 c.c. ether (peroxide-free). The ethereal extracts are washed first with water (10–20 c.c.), then with 10–20 c.c. N/2 KOH, and again with water, while rotating gently without shaking. The ethereal solution is then shaken thoroughly with two quantities of 10 c.c. water, after which it is filtered into a flask, the ether evaporated almost to dryness and the residue dissolved in ethyl alcohol or cyclohexane and made up to the concentration required for the particular instrument in use. A preliminary test on the original oil will indicate the amount both of oil and of solvent which will be necessary.

(b) Pure ethyl alcohol or cyclohexane must be the solvent used. Pure cyclohexane, suitable for spectrographic examination, should have the following properties: $d_{40}^{20} = 0.7784$; B.P. 81.4° C.; F.P. 6.5° C.; it should be almost completely transparent in the region of 328 $m\mu$ and exhibit no trace of discontinuous absorption.

(c) The intensity of absorption at 328 $m\mu$ may be determined to within ± 2.5 per cent. by any of the recognised methods of spectrophotometry.

(d) The factor, 1600, is the average figure derived from a series of comparative and independent tests on the unsaponifiable fractions of liver oils and on concentrates of high potency. It is desirable that, when a figure expressing the biological potency of a preparation has been derived by the use of this calculation, the fact should be stated.

II.—VITAMIN B₁

(a) *International Standard*

The Conference confirms the adoption, as International Standard, of the adsorption product of Vitamin B₁ prepared in the Medical Laboratory, Batavia (Java), by the method of Seidell, as described by Jansen and Donath.

(b) *Terminology*

The International Standard Preparation shall be known as the "standard adsorption product of Vitamin B₁".

(c) *Method of Preparation*

The International Standard was prepared by extracting rice polishings with water, sufficient sulphuric acid being added to make the pH 4.5. Salicylic

APPENDICES

57

acid to a concentration of 0.2 per cent. and toluene were then added to prevent bacterial decomposition. The process of extraction was continued for two days, after which the solution was filtered. For each 100 kg. of the original rice polishings, 3 kg. of fuller's earth (specially selected for its adsorptive powers) were added to the solution, which was then stirred for twenty-four hours. Subsequently, the solution was filtered off and the fuller's earth, after being washed with water and alcohol, was dried; 3 kg. of the fuller's earth adsorbate represents the Vitamin B₁ from 100 kg. of rice polishings.

(d) Definition of Unit

The International Unit recommended for adoption is the Vitamin B₁ activity of 10 milligrams of the International Standard adsorption product.

A daily dose of 10 to 20 milligrams of this preparation is required to maintain normal growth in a young rat on a diet deficient in Vitamin B₁ but complete in all other respects. The curative "day dose" for a pigeon (300 grams weight) exhibiting head retraction on a diet of polished rice is about 10 to 30 milligrams (method of Kinnersley and Peters). The Standard contains Vitamin B₄, but relatively small amounts of Vitamin B₂.

(e) Mode of Distribution

The International Standard adsorption product of Vitamin B₁ is kept at the National Institute for Medical Research, London, *acting for this purpose as central laboratory on behalf of the Health Organisation of the League of Nations.*

No special precautions are necessary in keeping this preparation, except that it should be stored in a dry place. In the presence of moisture, bacterial decomposition readily takes place.

(f) Recommendations for Future Research

The potency of the standard adsorption product should be tested relatively to that of the crystalline Vitamin B₁ preparations at present available, with the aim of ultimately adopting pure crystalline Vitamin B₁ as International Standard. The following, who have isolated such crystalline preparations, should be asked to make this comparison, employing the biological methods usual in their own laboratories:—

Professor B. C. P. JANSEN, Amsterdam.

Professor R. A. PETERS, Oxford.

Dr. A. SEIDELL, Washington.

Professor U. SUSUKI, Tokio.

Dr. R. R. WILLIAMS, New York.

Professor A. WINDAUS, Göttingen.

In further research, the comparative effect of parenteral and oral administration should be examined. In the case of the standard adsorption product, the preparation of suitable extracts should be investigated.

III.—VITAMIN C

(a) International Standard

The Conference recommends the adoption of l-ascorbic acid as International Standard. The Standard Preparation shall conform to the requirements stated in Note 4, below, in regard to its chemical and physical constants.

(b) Definition of Unit

The unit recommended for adoption is the Vitamin C activity of 0.05 milligram l-ascorbic acid.

This is about 1/10th of the daily dose necessary to prevent development of gross macroscopic scorbutic lesions in a young guinea-pig maintained on a scurvy-producing diet.

(c) *Mode of Preparation*

It is recommended that the Institute of Medical Chemistry, Szeged, should be asked, through Professor SZENT-GYÖRGYI, to prepare a batch of 500 grams of the Standard Preparation, and that Professor W. W. HAWORTH, University of Birmingham, be invited to co-operate in controlling the purity of the standard material.

(d) *Place of Distribution*

It is recommended that the National Institute of Medical Research, London, acting for this purpose as central laboratory on behalf of the Health Organisation of the League of Nations, shall be requested to undertake the storage and distribution of the Vitamin C standard.

(e) *Method of Use*

The standard may be kept at room temperature in a sealed tube or in an evacuated desiccator, and the required amount must be dissolved in freshly boiled and cooled glass-distilled, water, immediately before daily administration to the test animals.

Note 4*Properties of the Vitamin C Standard.*

The Standard Preparation of *l*-ascorbic acid should have the following characteristics:

(a) Melting-point $192^{\circ}\text{C}.$, uncorrected, taken in an open capillary, without appreciable darkening before melting.

(b) Specific rotation $[\alpha]_{\text{D}}^{20} = +20.0^{\circ}$ in water (conc. 14.0 g. per 100 c.c. solution).

$[\alpha]_{\text{D}}^{20} = +22.4^{\circ}$ in water (conc. 2.2 g. per 100 c.c. solution).

(c) The absorption spectrum in slightly acid aqueous solution is characterised by a single intense band with head at $245\text{ m}\mu$. The molecular extinction coefficient for a solution containing 20 milligrams per litre is 10,000. (The ascorbic acid is to be dissolved in freshly distilled water to which 0.1 c.c. N/1 sulphuric acid has been added for every 100 c.c. water).

(d) 10 milligrams *l*-ascorbic acid should require 11.4 c.c. N/100 aqueous iodine, the titration being carried out with starch as indicator.

(e) The substance must be ash-free and its elementary analysis must agree with the figures for $\text{C}_6\text{H}_8\text{O}_6$. It normally crystallises in rectangular plates which show straight extinction with high birefringence. [n_{α} (D line) 1.47 ± 0.005 ; n_{β} (D line) 1.68 ± 0.005].

IV.—VITAMIN D

(a) *International Standard*

The Conference recommends that the standard solution of irradiated ergosterol, prepared at the National Institute for Medical Research, London, and at present issued as International Vitamin D Standard, shall be retained.

When the present International Standard Solution is exhausted, or if it should become unsatisfactory for any reason, the Conference recommends that it should be replaced by an equivalent solution of pure crystalline Vitamin D in olive oil, of such strength that 1 milligram contains 0.025 micrograms (0.025 γ) of crystalline Vitamin D. (For a definition of crystalline Vitamin D, see Note 5 below.)

(b) *Definition of Unit*

The Unit of Vitamin D recommended for adoption by the 1931 Conference shall remain unaltered: namely, the Vitamin D activity of 1 milligram of the international standard solution of irradiated ergosterol, which has been found equal to that of 0.025 micrograms (0.025 γ) of crystalline Vitamin D.

The assay of materials for Vitamin D content should be carried out by comparative tests on rats, and their value in international units should be derived from the results of such tests. If other species are employed for these tests, the values cannot be expressed in international units (see (d) below).

(c) *Mode of Distribution*

The National Institute for Medical Research, acting for this purpose as central laboratory on behalf of the Health Organisation of the League of Nations, shall be asked to continue the storage and distribution of the International Vitamin D Standard. The mode of distribution recommended by the 1931 Conference shall remain unaltered either in the case of the existing standard solution, or the solution of crystalline Vitamin D by which it may be replaced in future.

(d) *Recommendations for Further Research—Cod-liver Oil as Subsidiary Vitamin D Standard*

The Conference recognises that amounts of cod-liver oil and irradiated ergosterol which contain an equal number of Vitamin D units, as determined by tests with rats, may not possess equal anti-rachitic activity when tested on certain other species—*e.g.*, poultry. The utility of cod-liver oil as a secondary standard of reference is obvious, and such standard cod-liver oils are at present distributed in several countries. In view of the fact that inconveniently large quantities of cod-liver oil would have to be handled for international purposes as Vitamin D Standard, thereby giving rise to storage problems, the Conference does not recommend the adoption of a sample of cod-liver oil as a subsidiary International Standard for Vitamin D.

The Conference recommends, however, that national centres of distribution should prepare subsidiary reference samples of cod-liver oil, of known Vitamin D activity, for distribution to workers in the countries concerned. In order to preserve a uniform value for the International Unit, the Vitamin D activity of such oils should be compared with that of the International Standard by means of tests on rats and their potency should be stated in International Units. It is recommended that the experience gained with such subsidiary cod-liver oil standards should be devoted to an attempt to elucidate the questions involved in the anomalous action on certain species of different sources of Vitamin D, and that members of the Conference be asked to co-operate in these investigations in their respective countries.

(e) *Biological Methods for Estimation of Vitamin D*

In using the International Standard Solution for the determination of the anti-rachitic potency of unknown preparations, it is recommended that not fewer than twenty similar rats (preferably more) be used for a determination, half of these to receive the standard and the remaining litter-mates the unknown substance. Provided this precaution is observed, it is considered permissible to use either prophylactic or therapeutic methods of investigation,

Note 5

Properties of Crystalline Vitamin D (Calciferol or Vitamin D₂), C₂₈H₄₄O.

(a) Colourless acicular crystals, odourless; melting-point, 114.5–117°C. (open capillary).

(b) Specific rotation:

in alcohol $[\alpha]_D^{20} = +101^\circ$ to $+102.5^\circ$; $[\alpha]_{5461}^{20} = +119^\circ$ to $+122^\circ$

in chloroform $[\alpha]_D^{20} = +52^\circ$; $[\alpha]_{5461}^{20} = +62^\circ$

(c) Absorption spectrum: in alcohol or other suitable non-absorbing solvent, a smooth curve with a maximum at 265 m μ ($E_{1\text{ cm.}}^{1\%} = 470$ to 485).

SUBJECT INDEX

- Antimony trichloride test as guide in dosage of cod-liver oils, 47
 — — — — — to stability of solutions of vitamin A, 40
 — — — — — method of vitamin A estimation (blue value), 8
- Arachis oil, tests of suitability of, as solvent for standard carotene, 12, 14, 47-49
- Biological units for vitamin A in relation to international unit, 42
 Blue value : *see* Antimony trichloride test
- Carotene, behaviour of, in 3 different specimens of coconut oil, 13, 15-20
 — — — — — , colorimetric tests of stability of, in various solvents (tabulated results), 14-20
 — — — — — dissolved in various solvents, colorimetric and biological tests on, 12
 — — — — — , maintenance of colour as indication of preservation of biological activity of, 11
 — — — — — , selection of suitable oil as solvent for, 12
 — — — — — , 1931 standard, dissolved in coconut oil with and without hydroquinone, biological tests on, 22, 45
 — — — — — , — — — — — 3 special samples of coconut oil, biological tests on, 22, 45
 — — — — — , — — — — — , preparation of, 27
 — — — — — , — — — — — , and pure β -carotene, relation between vitamin A activity of, 24-27, 41, 46, 47
- β -Carotene standard, preparation of, 24
 — as vitamin A standard, 7
- Coconut oil, behaviour of carotene in 3 different specimens of, 13, 15-20, 44, 45
 — — — — — , biological tests on 1931 standard carotene dissolved in 3 special samples of, 22, 45
 — — — — — , selection of, as solvent for β -carotene, 41
- Cod-liver oil to be used in biological tests, solvents for, 40
 — — — — — as subsidiary vitamin A standard, 8
 — — — — — , vitamin A activity of 6 specimens of, determined by different workers spectrophotometrically, 29, 30
 — — — — — X, biological tests on, 38, 46, 47
 — — — — — , spectrophotometric tests on, 29-35
 — — — — — , use of, as subsidiary international standard for vitamin A, 8, 37, 42
 — — — — — Z, biological tests on, by different workers, 38, 39, 48-49
 — — — — — , spectrophotometric tests on, 29-35
 — — — — — , use of, by Pharmaceutical Society as subsidiary standard for vitamin A, 8
 — oils U, V, W, and Y, spectrophotometric tests on, 29-35
- Colorimetric tests of stability of carotene in various solvents (tabulated results), 14-20
- Concentrate C (vitamin A), tests on, 34, 50
- Cotton seed oil, hardened, tests of suitability of, as solvent for standard carotene, 12, 14
 — — — — — (Wesson brand), tests of suitability of, as solvent for standard carotene, 13, 15
- Cyclohexane as solvent for oils and concentrates to be examined spectrophotometrically, 32
- Ethyl alcohol as solvent for oils or concentrates to be examined spectrophotometrically, 32
 — — — — — laurate, tests of suitability of, as solvent for standard carotene, 13, 14, 15, 44

- Hydroquinone, stabilising action of, on carotene solutions, 13, 21, 44, 45
- International unit for vitamin A : importance of its universal adoption, 42
 — — — — —, in relation to biological units, 42
 — Conference on Vitamin Standardisation, 1934, report of, 52
- Olive oil, tests of suitability of, as solvent for standard carotene, 12, 14
- Paraffin, liquid, tests of suitability of, as solvent for standard carotene, 13, 15, 44
- Protocols of biological experiments, 44-50
- Saponification of cod-liver oils as preliminary to spectrophotometric vitamin A estimation, 31
 —, methods of, 51, 56
- Sherman rat unit, relation of, to International Unit for vitamin A, 36
- Solvent for standard carotene, choice of, 7, 12
- Solvents for dilution of oils or concentrates to be examined spectrophotometrically, 32
 — — carotene, effects of, on its stability and ease of absorption, 9, 10, 11
 — — cod-liver oil in biological tests, 40
 —, various, colorimetric tests of stability of carotene in (tabulated results), 14-20
- Spectrophotometric method of estimating vitamin A, 8, 28-36, 41
- United States Pharmacopoeia, reference cod-liver oil of: *see* Cod-liver oil X
- Vitamin A activity of 1931 standard carotene and of pure β -carotene, 24-27, 41, 46
 — — estimation, spectrophotometric, 28-36, 41
 — — —, spectrophotometric and biological, ratio between values obtained by, 33-36
 — — concentrate C, tests on, 34, 50
 — —, international unit for: importance of its universal adoption, 42
 — —, — — —, in relation to biological units, 42
 — — standardisation, report of International Conference (1934) on, 52

Privy Council

MEDICAL RESEARCH COUNCIL

(Formerly Medical Research Committee, National Health Insurance.)

LIST OF PUBLICATIONS

(The prices given are net : those in brackets include postage.)

November, 1935.

The following publications relating to the work of the Medical Research Council can be purchased directly from **H.M. Stationery Office**, at the following addresses : Adastral House, Kingsway, London, W.C.2 ; 120 George Street, Edinburgh 2 ; York Street, Manchester 1 ; 1 St. Andrew's Crescent, Cardiff ; 80 Chichester Street, Belfast ; or through any bookseller.*

In addition, numerous memoirs upon work aided by the Medical Research Council have appeared in Scientific Journals : particulars of these may be seen in the Annual Reports.

ANNUAL REPORTS

Medical Research Committee, Nos. 1-5, 1914-15 to 1918-19.

Medical Research Council, 1919-20 and subsequently.

(Price of each report from 1920-1 to 1925-6, † 3s. 6d. (3s. 8d.)) ; from 1926-7 to 1928-9, 3s. (3s. 2d.) ; 1929-30 and 1930-31, 2s. 6d. (2s. 8d.) ; 1931-32, 2s. (2s. 2d.) ; 1932-33, 2s. 6d. (2s. 9d.) ; 1933-34, 3s. (3s. 2½d.)

SPECIAL REPORTS, &c.

Alcohol :

No. 31. Alcohol : its Absorption into and Disappearance from the Blood under different conditions. By E. Mellanby. [1919.] *Out of print.*

No. 34. The Influence of Alcohol on Manual Work and Neuro-muscular Co-ordination. By H. M. Vernon. [1919.] *Out of print.*

No. 56. The effects of Alcohol and some other Drugs during Normal and Fatigued Conditions. By W. McDougall and May Smith. [1920.] 1s. (1s. 1d.)

No. 168. Alcohol and Inheritance : An Experimental Study. By F. M. Durham and H. M. Woods. [1932.] 1s. 3d. (1s. 4½d.)

(Book). Alcohol : its Action on the Human Organism. Second Edition. [1924.] 1s. paper covers, 1s. 6d. cloth bound.

Anaerobic Bacteria : see WOUND INFECTIONS.

Animals, Diseases of :

No. 121. Borna Disease and Enzootic Encephalo-Myelitis of Sheep and Cattle. By S. Nicolau and I. A. Galloway. [1928.] 5s. (5s. 2½d.)

See also TUBERCULOSIS (Nos. 94, 122, 149, 184, and 189).

Bacteriology (MISCELLANEOUS) :

No. 35. The Reaction of Culture Media, by S. R. Douglas, J. W. H. Eyre, P. P. Laidlaw, and C. G. L. Wolf. Second Edition, revised by P. P. Laidlaw. [1927.] 6d. (7d.)

No. 49. On the Destruction of Bacteria in Milk by Electricity. By J. M. Beattie and F. C. Lewis. [1920.] *Out of print.*

No. 51. The Laboratory Diagnosis of Acute Intestinal Infections, including the Principles and Practice of the Agglutination Tests. By the Committee upon Pathological Methods. [1920.] 4s. 6d. (4s. 8d.)

* For overseas agencies see p. xii. † 1923-4 out of print.

- No. 64. Catalogue of the National Collection of Type Cultures. Third Edition. [1931.] 2s. (2s. 1d.).
- No. 169. The Haemolytic Streptococci : Their Grouping by Agglutination. By F. W. Andrewes and Ethel M. Christie. [1932.] 1s. 3d. (1s. 4½d.).
- No. 203. The Pathogenic Aerobic Organisms of the Actinomyces Group. By Dagny Erikson. [1935.] 1s. (1s. 1½d.)
See also MILK (No. 206), SURGERY (No. 138) and IMMUNITY.
- A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE. (*See last page.*)
- Blood Physiology :**
- No. 72. The Acid-base Equilibrium of the Blood. By the Haemoglobin Committee. [1923.] 2s. (2s. 1d.).
- Blood Vessels, Diseases of :**
- No. 193. Dissecting Aneurysms. By T. Shennan. [1934.] 2s. 6d. (2s. 8½d.).
See also SHOCK, SURGICAL.
- Borna Disease :** *see ANIMALS, DISEASES OF.*
- Brain Surgery :** *see SURGERY.*
- Bright's Disease :** *see NEPHRITIS.*
- Burns :**
- No. 141. The Tannic Acid Treatment of Burns. By W. C. Wilson. [1929.] 1s. (1s. 1d.).
- Cancer :**
- No. 99. An Investigation into the Statistics of Cancer in Different Trades and Professions. By Matthew Young and W. T. Russell. [1926.] 1s. 6d. (1s. 7d.).
See also RADIUM.
- Catgut :** *see SURGERY (No. 138).*
- Cerebro-spinal Fever :**
- No. 2. Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-spinal Fever during the Epidemic of 1915. [1916.]
Out of print.
- No. 3. Bacteriological Studies in the Pathology and Preventive Control of Cerebro-spinal Fever among the Forces during 1915 and 1916. By M. H. Gordon, Martin Flack, P. W. Bassett-Smith, T. G. M. Hine, and W. J. Tulloch. [1917.]
Out of print.
- No. 17. (I.) A Report upon the Seasonal Outbreak of Cerebro-spinal Fever in the Navy at Portsmouth, 1916-17. By Paul Fildes and S. L. Baker. (II.) The Treatment of Cerebro-spinal Meningitis by Antimeningococcus Serum at the Royal Naval Hospital, Haslar, 1915-16-17. By G. P. Adshead. [1918.] 2s. 6d. (2s. 8½d.).
- No. 50. Cerebro-spinal Fever. Studies in the Bacteriology, Preventive Control, and Specific Treatment of Cerebro-spinal Fever among the Military Forces, 1915-19. By M. H. Gordon and others. [1920.] 4s. (4s. 3d.).
- No. 124. The Meningococcus. By E. G. D. Murray. [1929.] 3s. 6d. (3s. 8½d.).
- Chemotherapy :** *see STREPTOCOCCAL INFECTIONS.*
- Child Life (ANTENATAL AND POSTNATAL INVESTIGATIONS) :**
- No. 10. The Mortalities of Birth, Infancy, and Childhood. By A. K. Chalmers, W. A. Brend, L. Findlay, and J. Brownlee. [1917.] *Out of print.*
- No. 74. The Relation between Home Conditions and the Intelligence of School Children. By L. Isserlis. [1923.] 1s. (1s. 1d.).
- No. 81. The Effect of Maternal Social Conditions and Nutrition upon Birth-weight and Birth-length. By M. Bruce Murray. [1924.] 1s. (1s. 1d.).
- No. 82. Maternal Syphilis as a cause of Death of the Foetus and of the New-born Child. By J. N. Cruickshank. [1924.] 1s. 6d. (1s. 7½d.).

- No. 86. The Estimation of Foetal Age, the Weight and Length of Normal Foetuses, and the Weights of Foetal Organs. By J. N. Cruickshank, M. J. Miller, and F. J. Browne. [1924.] 2s. 6d. (2s. 7½d.).
- No. 101. Poverty, Nutrition, and Growth: Studies of Child Life in Cities and Rural Districts of Scotland. By D. Noël Paton, Leonard Findlay, and others. [1926.] 10s. (10s. 4½d.).
- No. 109. A Clinical and Pathological Study of 1,673 Cases of Dead-Births and Neo-natal Deaths. Compiled by E. L. Holland and J. E. Lane-Clayton. [1926.] 3s. 6d. (3s. 7½d.).
- No. 114. Social Conditions and Acute Rheumatism. [1927.] 2s. 6d. (2s. 8d.).
- No. 117. The Toxaemias of Pregnancy: A Clinical and Biochemical Study. By J. N. Cruickshank, J. Hewitt, and K. L. Couper. [1927.] 4s. (4s. 2d.).
- No. 118. The Cause of Foetal Death in 144 Cases. By A. C. Palmer. [1928.] 3s. (3s. 2d.).
- No. 145. The Causes of Neo-natal Death. By J. N. Cruickshank. [1930.] 1s. 6d. (1s. 7½d.).
- No. 157. Nutritional Anaemia in Infancy: The Influence of Iron Deficiency on Infant Health. By H. M. M. Mackay, L. Goodfellow, and A. Bradford Hill. [1931.] 2s. (2s. 2d.).
- No. 162. Intelligence and Disease. By Shepherd Dawson assisted by J. C. M. Conn. [1931.] 1s. (1s. 1½d.).
- No. 190. A Study of Growth and Development: Observations in Successive Years on the Same Children. By R. M. Fleming. With a Statistical Analysis by W. J. Martin. [1933.] 1s. 6d.

See also NUTRITION; RICKETS.

Dental Disease:

- No. 70. The Structure of Teeth in relation to Dental Disease. By J. Howard Mummery. [1922.] 2s. (2s. 1d.).
- No. 97. The Incidence of Dental Disease in Children. By the Committee for the Investigation of Dental Disease. [1925.] 1s. 6d. (1s. 7½d.).
- No. 140. Diet and the Teeth: An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. [1929.] 17s. 6d. (18s.).
- No. 153. Diet and the Teeth: An Experimental Study. Part II. A.—Diet and Dental Disease. B.—Diet and Dental Structure in Mammals other than the Dog. By May Mellanby. [1930.] 2s. 6d. (2s. 8½d.).
- No. 159. The Influence of Diet on Caries in Children's Teeth (Interim Report). By the Dental Committee. [1931.] 6d. (7d.).
- No. 171. Facial Growth in Children, with Special Reference to Dentition. Part I, by Corisande Smyth. Part II, by Matthew Young. [1932.] 1s. 6d. (1s. 8d.).
- No. 191. Diet and the Teeth: an Experimental Study. Part III. The Effect of Diet on Dental Structure and Disease in Man. By May Mellanby. [1934.] 5s. (5s. 4d.).

Diphtheria:

- No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] 2s. (2s. 1½d.).
- (Book). Diphtheria: its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1923.] 12s. 6d. (13s. 3d.).
- See also* EPIDEMIOLOGY (Nos. 75, 137 and 195).

Dust: *see* VENTILATION, ETC.

Dysentery:

- Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean:—
- No. 4. I.—Amoebic Dysentery and the Protozoological Investigation of Cases and Carriers. By Clifford Dobell. [1917.] *Out of print*.

- No. 5. II.—Report upon 878 Cases of Bacillary Enteritis. By L. Rajchman and G. T. Western. [1917.] *Out of print.*
- No. 6. III.—Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16. By Paul Fildes and others.
- IV.—Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] 4s. 6d. (4s. 7½d.).
- No. 7. V.—Report upon 2,360 Enteritis “Convalescents” received at Liverpool from various Expeditionary Forces. By E. Glynn and others. [1918.] 6s. (6s. 2d.).

- No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals: with special reference to the Incidence and Treatment of Amœbic Dysentery Carriers. By Clifford Dobell, H. S. Gettings, Margaret W. Jepps, and J. B. Stephens. [1918.] 1s. 3d. (1s. 4d.).
- No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. By W. Fletcher and Doris L. Mackinnon. [1919.] 9d. (10d.).
- No. 30. An Investigation of the Flexner-Y Group of Dysentery Bacilli. By H. S. Gettings. [1919.] 1s. (1s. 1d.).
- No. 40. Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. By L. S. Dudgeon and others. [1919.] 3s. (3s. 1½d.).
- No. 42. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli. By F. W. Andrewes and A. C. Inman. [1919.] 2s. (2s. 1½d.).

See also FOOD POISONING.

Encephalitis :

- No. 108. The Sheffield Outbreak of Epidemic Encephalitis in 1924. [1926.] 1s. 9d. (1s. 10½d.).

Enteric Infections :

- No. 9. A Report upon the Use of Atropine as a Diagnostic Agent in Typhoid Infections. By H. F. Marris. [1917.] 1s. (1s. 1d.).
- No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October, 1916. By W. W. C. Topley, S. G. Platts, and C. C. Imrie. [1920.] 3s. (3s. 1½d.).
- No. 179. Chronic Enteric Carriers and their Treatment. By C. H. Browning with others. [1933.] 1s. 6d. (1s. 7½d.).

See also BACTERIOLOGY ; FOOD POISONING.

Epidemiology :

- No. 75. The Schick Test, Diphtheria and Scarlet Fever. By S. F. Dudley. [1923.] 1s. (1s. 1½d.).
- No. 111. The Spread of Droplet Infection in Semi-isolated Communities. By S. F. Dudley. [1926.] 1s. 6d. (1s. 7½d.).
- No. 120. An Inquiry into the Relationship between Housing Conditions and the Incidence and Fatality of Measles. By J. L. Halliday. [1928.] 1s. (1s. 1d.).
- No. 137. Scarlet Fever, Diphtheria, and Enteric Fever, 1895–1914 : A Clinical-Statistical Study. By E. W. Goodall, M. Greenwood, and W. T. Russell. [1929.] 2s. (2s. 1½d.).
- No. 180. Epidemiological Study of Scarlet Fever in England and Wales since 1900. By Hilda M. Woods. [1933.] 1s. 3d. (1s. 4½d.).
- No. 192. Housing Conditions and Respiratory Disease. Morbidity in a Poor-class Quarter and in a Rehousing Area in Glasgow. By C. M. Smith. [1934.] 9d. (10d.).
- No. 195. Active Immunization against Diphtheria : its effect on the Distribution of Antitoxic Immunity and Case and Carrier Infection. By S. F. Dudley, P. M. May, and J. A. O'Flynn. [1934.] 3s. (3s. 2½d.).

See also SMALL-POX ; TUBERCULOSIS ; ETC.

Flying, Medical Problems of:

Reports of the Air Medical Investigation Committee:—

No. 28. The Sense of Balance and Stability in the Air. By Henry Head. [1919.] 9d. (10d.). (Included in No. 53.)

No. 37. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands. By C. H. Kellaway. The Ear in relation to certain Disabilities in Flying. By S. Scott. [1919.] 1s. (1s. 1d.).

No. 53. The Medical Problems of Flying (including reports on oxygen want, selection of candidates for flying, sense of balance, and flying strain). [1920.] 6s. (6s. 4d.).

No. 84. The Application of the Air Force Physical Efficiency Tests to Men and Women. By L. D. Cripps. [1924.] 1s. 6d. (1s. 7½d.).

Food Poisoning:

No. 24. A Report on the Investigation of an Epidemic caused by *Bacillus aertrycke*. By H. Marrian Perry and H. L. Tidy. [1919.] 9d. (10d.).

No. 91. An Investigation of the Salmonella Group, with Special Reference to Food Poisoning. By W. G. Savage and P. Bruce White. [1925.] 3s. 6d. (3s. 8d.).

No. 92. Food Poisoning: a Study of 100 Recent Outbreaks. By W. G. Savage and P. Bruce White. [1925.] 2s. 6d. (2s. 8d.).

No. 103. Further Studies of the Salmonella Group. By P. Bruce White. [1926.] 5s. (5s. 2½d.).

Goitre:

No. 154. Iodine Supply and the Incidence of Endemic Goitre. By J. B. Orr. [1931.] 4d. (5d.). See also NUTRITION (Nos. 123, 201).

Haemoglobin: see BLOOD.

Hearing:

No. 166. Reports of the Committee upon the Physiology of Hearing. I.—The Localization of Sounds in the Median Plane. By J. H. Shaxby and F. H. Gage. II.—Some Factors in Auditory Localization. By H. E. O. James and Marion E. Massey. [1932.] 1s. (1s. 1½d.).

Heart:

No. 8. Report upon Soldiers returned as Cases of "Disordered Action of the Heart" (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] 1s. (1s. 1d.).

No. 147. The Electrocardiogram. By W. H. Craib. [1930.] 1s. 3d. (1s. 4½d.).

Heredity: see IMMUNITY (No. 196).

Immunity:

No. 194. The Chemistry of Antigens and Antibodies. By J. R. Marrack. [1934.] 2s. 6d. (2s. 8d.).

No. 196. The Inheritance of Resistance to Infection in Animal Species. A Review of the Published Experimental Data. By A. Bradford Hill. [1934.] 1s. 3d. (1s. 4½d.).

See also EPIDEMIOLOGY.

Industrial Health:

The Annual Reports of the Industrial Health (formerly Fatigue) Research Board, and special reports on particular subjects, are published for the Council in separate series. The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 38, Old Queen Street, Westminster, S.W.1.

Influenza :

No. 36. Studies of Influenza in Hospitals of the British Armies in France, 1918. [1919.] 3s. 6d. (3s. 8d.).

No. 63. Studies in the Aetiology of Epidemic Influenza. By J. McIntosh. [1922.] 2s. 6d. (2s. 7d.).

Jaundice :

No. 113. Spirochaetal Jaundice. By G. Buchanan. [1927.] 4s. (4s. 2d.).

Light Treatment :

No. 131. Irradiation and Health. Two Experimental Studies. By Dora Colebrook. [1929.] *Out of print.*

Malaria : see QUININE.**Maternal Mortality : see CHILD LIFE and STREPTOCOCCAL INFECTIONS.****Measles : see EPIDEMIOLOGY (No. 120).****Milk :**

No. 206. The Bacteriological Grading of Milk. By G. S. Wilson. [1935.]
See also TUBERCULOSIS, No. 189.

Miners' Dietsaries : see NUTRITION.**Miners' Diseases, etc. :**

No. 89. Report on Miners' "Beat Knec," "Beat Hand," and "Beat Elbow." By E. L. Collis and T. L. Llewellyn. [1924.] 1s. 6d. (1s. 7d.).
See also JAUNDICE (No. 113).

Miners' Nystagmus : see VISION.**Nephritis :**

No. 43. Albuminuria and War Nephritis among British Troops in France. By H. MacLean. [1919.] 2s. 6d. (2s. 8d.).

No. 142. A Classification of Bright's Disease. By Dorothy S. Russell. [1929.] 8s. 6d. (8s. 10d.).

No. 178. A Study of Nephritis and Allied Lesions. By J. Gray. [1933.] 2s. 6d. (2s. 8½d.).

Nerve Injuries :

Reports of the Committee upon Injuries of the Nervous System :—

No. 54. The Diagnosis and Treatment of Peripheral Nerve Injuries. [1920.] 2s. (2s. 1½d.).

No. 88. Injuries of the Spinal Cord and Cauda Equina. [1924.] 1s. 6d. (1s. 7½d.).

Nutrition :

No. 13. An Enquiry into the Composition of Dietsaries, with special reference to the Dietsaries of Munition Workers. By Viscount Dunluce and Major Greenwood. [1918.] *Out of print.*

No. 87. Report on the Nutrition of Miners and their Families. By the Committee upon Quantitative Problems in Human Nutrition. [1924.] 1s. 3d. (1s. 4d.).

No. 105. Diets for Boys during the School Age. By H. C. Corry Mann. [1926.] 2s. 6d. (2s. 7½d.).

No. 123. Iodine in Nutrition : a Review of Existing Information. By J. B. Orr and I. Leitch. [1929.] *See also GOITRE (No. 154).*

No. 135. The Carbohydrate Content of Foods. By R. A. McCance and R. D. Lawrence. [1929.] *Out of print.*

No. 146. The Antiscorvy Vitamin in Apples. By Mary F. Bracewell, E. Hoyle, and S. S. Silva. [1930.] 9d. (10d.).

No. 151. A Study in Nutrition. An Inquiry into the Diet of 154 Families of St. Andrews. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. *Out of print.*

No. 155. Studies of Nutrition : The Physique and Health of Two African Tribes. By J. B. Orr and J. L. Gilks. [1931.] 2s. (2s. 2d.).

- No. 158. The Quantitative Estimation of Vitamin D by Radiography. By R. B. Bourdillon, H. M. Bruce, C. Fischmann, and T. A. Webster. [1931.] 1s. (1s. 1½d.).
- No. 165. Studies in Nutrition. An Inquiry into the Diet of Families in Cardiff and Reading. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. [1932.] 6d. (7d.).
- No. 167. Vitamins: A Survey of Present Knowledge. By a Committee appointed jointly by the Lister Institute and Medical Research Council [1932.] 6s. 6d. (7s. 0½d.).
- No. 175. Vitamin Content of Australian, New Zealand, and English Butters. By M. E. F. Crawford, E. O. V. Perry, and S. S. Silva. [1932.] 1s. (1s. 1d.).
- No. 187. The Chemistry of Flesh Foods and their Losses on Cooking. By R. A. McCance and H. L. Shipp. [1933.] 2s. 6d. (2s. 8½d.).
- No. 201. The Determination of Iodine in Biological Substances. By C. O. Harvey. [1935.] 1s. (1s. 1½d.).

See also CHILD LIFE; RICKETS; DENTAL DISEASE; STANDARDS (No. 202).

Pituitary Extract: see STANDARDS.

Pneumonia:

- No. 79. Bacteriological and Clinical Observations on Pneumonia and Empyemata, with special reference to the Pneumococcus and to Serum Treatment. By E. E. Glynn and Lettice Digby. [1923.] 5s. (5s. 3d.).

Pneumothorax, Artificial: see TUBERCULOSIS.

Print, Legibility of: see VISION.

Protozoan Infections:

- No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] 2s. (2s. 1½d.).

Psychology:

- No. 170. Studies in the Psychology of Delinquency. By G. W. Pailthorpe. [1932.] 2s. (2s. 2d.).

Quinine:

- No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1s. (1s. 1d.).

Radium:

- No. 62. Medical Uses of Radium: Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5s. (5s. 3d.).
- No. 90. Medical Uses of Radium: Summary of Reports from Research Centres for 1923. [1924.] 1s. (1s. 1d.).
- No. 102. Ditto for 1924. [1926.] 1s. 6d. (1s. 7d.).
- No. 112. Ditto for 1925. [1926.] 1s. 3d. (1s. 4d.).
- No. 116. Ditto for 1926. [1927.] 1s. (1s. 1½d.).
- No. 126. Ditto for 1927. [1928.] 1s. (1s. 1½d.).
- No. 144. Ditto for 1928. [1929.] 1s. (1s. 1d.).
- No. 150. Ditto for 1929. [1930.] 9d. (10d.).
- No. 160. Ditto for 1930. [1931.] 1s. (1s. 1d.).
- No. 174. Ditto for 1931. [1932.] 1s. 3d. (1s. 4½d.).
- No. 186. Ditto for 1932. [1933.] 1s. (1s. 1d.).
- No. 197. Ditto for 1933. [1934.] 9d. (10d.).
- No. 204. Ditto for 1934. [1935.] 1s. (1s. 1½d.).

Respiration:

- No. 198. Test for Respiratory Efficiency. By A. Moncrieff. [1934.] 1s. (1s. 1½d.).

Rheumatism: see CHILD LIFE (No. 114).

Rickets:

- No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*
- No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4s. (4s. 2d.).

- No. 68. Rickets : the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2s. 6d. (2s. 7½d.).
- No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4s. (4s. 3d.).
- No. 77. Studies of Rickets in Vienna, 1919-22. [1923.] 7s. 6d. (7s. 10½d.).
- No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with other factors of Diet and Environment in producing Rickets. By E. Mellanby. [1925.] 3s. 6d. (3s. 8d.).

Salvarsan : *see* VENEREAL DISEASES; STREPTOCOCCAL INFECTIONS; STANDARDS, BIOLOGICAL (No. 128).

Scarlet Fever : *see* EPIDEMIOLOGY (Nos. 137, 180).

Scurvy : *see* NUTRITION (No. 146).

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Conditions :—

- No. 25. Wound-Shock and Haemorrhage. [1919.] 4s. (4s. 5½d.).
- No. 26. Traumatic Toxaemia as a Factor in Shock. [1919.] 1s. (1s. 1d.).
- No. 27. Blood Volume Changes in Wound-Shock and Primary Haemorrhage. By N. M. Keith. [1919.] 9d. (10d.).

Small-pox :

- No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] 3s. 6d. (3s. 8½d.).
- No. 106. Small-pox and Climate in India. Forecasting of Epidemics. By Sir Leonard Rogers. [1926.] 2s. (2s. 1½d.).
- No. 143. Diagnostic Value of the "Vaccinia Variola" Flocculation Test. By W. L. Burgess, James Craigie, and W. J. Tulloch. [1929.] 1s. 3d. (1s. 4d.).
- No. 156. Further Investigations on the Variola-Vaccinia Flocculation Reaction. By James Craigie and W. J. Tulloch. [1931.] 3s. (3s. 2½d.).

Spectroscopy :

- No. 177. Apparatus for the Rapid Study of Ultra-Violet Absorption Spectra. By J. St. L. Philpot and E. H. J. Schuster. [1933.] 1s. 3d. (1s. 4½d.).

Spinal Deformities : *see* SURGERY (No. 161).

Standards, Biological :

- No. 69. I.—Pituitary Extracts. By J. H. Burn and H. H. Dale. [1922.] 1s. 6d. (1s. 7d.).
- No. 128. II.—Toxicity Tests for Novarsenobenzene (Neosalvarsan). By F. M. Durham, J. H. Gaddum, and J. E. Marchal. [1929.] 1s. 9d. (1s. 10d.).
- No. 183. III.—Methods of Biological Assay depending on a Quantal Response. By J. H. Gaddum. [1933.] 1s. (1s. 1d.).
- No. 202. IV.—The Standardisation and Estimation of Vitamin A. Edited by E. M. Hume and H. Chick. [1935.]
- See also* VENEREAL DISEASES (No. 44) and NUTRITION (No. 158).

Statistics (MISCELLANEOUS).

- No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. By Major Greenwood. [1918.] 1s. 6d. (1s. 7d.).
- No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] 3s. (3s. 1½d.).
- No. 95. Internal Migration and its Effects upon the Death-rates : with Special Reference to the County of Essex. By A. B. Hill. [1925.] 3s. 6d. (3s. 8d.).

Streptococcal Infections :

- No. 119. A Study of some Organic Arsenical Compounds with a view to their use in certain Streptococcal Infections. By L. Colebrook. [1928.] 1s. 3d. (1s. 4d.)
- No. 205. The Source of Infection in Puerperal Fever due to Haemolytic Streptococci. By Dora C. Colebrook. [1935.]

Surgery :

- No. 125. A Study of Intracranial Surgery. By H. Cairns. [1929.]
Out of print.
- No. 138. The Preparation of Catgut for Surgical Use. By W. Bulloch, L. H. Lampitt, and J. H. Bushill. [1929.] 4s. (4s. 3d.)
- No. 161. The Intervertebral Discs. Observations on their Normal and Morbid Anatomy in relation to certain Spinal Deformities. By O. A. Beadle. [1931.] 2s. (2s. 2d.)

See also BURNS ; SHOCK, SURGICAL.

T.N.T. Poisoning :

- No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. By Benjamin Moore. [1917.] 1s. (1s. 1½d.)
- No. 58. T.N.T. Poisoning and the Fate of T.N.T. in the Animal Body. By W. J. O'Donovan and others. [1921.] 3s. (3s. 1½d.)

Tuberculosis :

- No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] 3d. (3½d.)
- No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.]
Out of print.
- No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Munition Workers. By Major Greenwood and A. E. Tebb. [1919.] 1s. 6d. (1s. 7d.)
- No. 33. Pulmonary Tuberculosis : Mortality after Sanatorium Treatment. By Noel D. Bardswell and J. H. R. Thompson. [1919.] 2s. (2s. 2d.)
- No. 46. An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland : Part III. By John Brownlee. [1920.] 2s. 6d. (2s. 7½d.)
- No. 67. Report on Artificial Pneumothorax. By L. S. T. Burrell and A. S. MacNalty. [1922.] 2s. 6d. (2s. 8d.)
- No. 76. Tuberculosis in Insured Persons accepted for Treatment by the City of Bradford Health Committee. By H. Vallow. [1923.] 6d. (7d.)
- No. 83. Tuberculosis of the Larynx. By Sir St. Clair Thomson. [1924.] 2s. 6d. (2s. 8d.)
- No. 85. An Inquiry into the After-Histories of Patients treated at the Brompton Hospital Sanatorium at Frimley during the years 1905-14. By Sir P. H.-S. Hartley, R. C. Wingfield, and J. H. R. Thompson. [1924.] 1s. 6d. (1s. 7d.)
- No. 94. Tuberculin Tests in Cattle, with special reference to the Intradermal Test. By the Tuberculin Committee. [1925.] 3s. (3s. 3d.)
- No. 122. The Intradermal Tuberculin Test in Cattle : Collected Results of Experience. By J. B. Buxton and A. S. MacNalty. [1928.] 1s. 6d. (1s. 7½d.)
- No. 149. Tuberculosis in Man and Lower Animals. By H. H. Scott. [1930.]
Out of print.
- No. 152. Studies of Protection against Tuberculosis : Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. [1931.] 9d. (10½d.)
- No. 164. The Value of Tuberculin Tests in Man with special reference to the Intracutaneous Test. By P. D'Arcy Hart. [1932.] 2s. (2s. 6d.)

- No. 172. Tuberculosis Disease in Children : Its Pathology and Bacteriology. By J. W. S. Blacklock. [1932.] 3s. (3s. 6d.).
- No. 182. Tuberculous Bacillaemia, A Review. By G. S. Wilson. [1933.] 2s. 6d. (2s. 8d.).
- No. 184. The Eradication of Bovine Tuberculosis. By L. Jordan. [1933.] 2s. (2s. 2d.).
- No. 189. Tuberculous Infection in Milk. A Report by the Department of Health for Scotland. [1933.] 9d. (10d.).

Venereal Diseases :

- No. 14. The Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print* : see No. 129.
- No. 19. The Laboratory Diagnosis of Gonococcal Infections. Methods for the Detection of *Spironema pallidum*. By the Bacteriological Committee. *New Edition*. [1923.] 1s. 6d. (1s. 7½d.).
- No. 21. The Diagnostic Value of the Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print*.
- No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] 2s. (2s. 1d.).
- No. 41. (I.) An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds. By Paul Fildes and R. J. G. Parnell. (II.) A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of "914". By R. J. G. Parnell and Paul Fildes. [1919.] 2s. (2s. 1d.).
- No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. I. [1919.] 1s. (1s. 1d.).
- No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. By Paul Fildes, R. J. G. Parnell, and H. B. Maitland. [1920.] 1s. (1s. 1d.).
- No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. By H. M. Turnbull. [1920.] 2s. 6d. (2s. 7½d.).
- No. 55. (I.) Results of the Examination of Tissues from Eight Cases of Death following Injections of Salvarsan. By H. M. Turnbull. (II.) The Influence of Salvarsan Treatment on the Development and Persistence of Immunity, as indicated by Measurements of Agglutinins. By E. W. Ainley Walker. [1920.] 3s. (3s. 1½d.).
- No. 66. Toxic Effects following the Employment of Arsenobenzol Preparations. By the Salvarsan Committee. [1922.] 2s. (2s. 1½d.).
- No. 78. The Serum Diagnosis of Syphilis: The Wassermann and Sigma Reactions compared. [1923.] 5s. 6d. (5s. 9d.).
- No. 107. The Effect of Treatment on the Wassermann Reactions of Syphilitic Patients. By E. E. Glynn, R. E. Roberts, and P. M. Bigland. [1926.] 3s. 6d. (3s. 8d.).
- No. 129. The Wassermann Test. Technical Details of No. 1 Method M.R.C. (Modified). By E. J. Wyler. [1929.] 9d. (10d.).
- No. 132. The Treatment of Syphilis: A Survey of Records from St. Thomas's Hospital. By L. W. Harrison. [1929.] 2s. (2s. 1½d.).

Ventilation, etc. :

- No. 32. The Science of Ventilation and Open-air Treatment. Part I. By Leonard Hill. [1919.] 10s. (10s. 5½d.).
- No. 52. The Science of Ventilation and Open-air Treatment. Part II. By Leonard Hill. [1920.] 6s. (6s. 4½d.).
- No. 73. The Kata-thermometer in Studies of Body Heat and Efficiency. By Leonard Hill and others. [1923.] 5s. (5s. 2½d.).

No. 100. Methods of Investigating Ventilation and its Effects. By H. M. Vernon and others. [1926.] 2s. (2s. 1½d.).

No. 199. Physical Methods for the Estimation of the Dust Hazard in Industry. By H. L. Green and H. H. Watson. [1935.] 1s. (1s. 1½d.).

Vision :

No. 65. First Report of the Miners' Nystagmus Committee. [1922.]

Out of print.

No. 80. Second Report of the Miners' Nystagmus Committee. [1923.] 9d. (10d.).

No. 110. The Legibility of Print. By R. L. Pyke. [1926.] 4s. (4s. 2d.).

No. 176. Third Report of the Miners' Nystagmus Committee. [1932.] 9d. (10d.).

Reports of the Committee on the Physiology of Vision :

No. 104. I. Illumination and Visual Capacities. By R. J. Lythgoe. [1926.] 2s. 6d. (2s. 7½d.).

No. 127. II. Dark Adaptation (a Review of the Literature). By Dorothy Adams. [1929.] 5s. (5s. 2½d.).

No. 130. III. Two Studies in the Psychology of Reading. By M. D. Vernon and R. W. Pickford. [1929.] 2s. (2s. 1½d.).

No. 133. IV. Experiments on Binocular Vision. By N. M. S. Langlands. [1929.] 2s. 6d. (2s. 8d.).

No. 134. V. The Adaptation of the Eye: its Relation to the Critical Frequency of Flicker. By R. J. Lythgoe and K. Tansley. [1929.] 2s. 6d. (2s. 7½d.).

No. 136. VI. Some Experiments on Peripheral Vision. By Myer Salaman. [1929.] 2s. 6d. (2s. 7d.).

No. 139. VII. A Re-determination of the Trichromatic Mixture Data. By W. D. Wright. [1929.] 1s. 3d. (1s. 4d.).

No. 148. VIII. The Movements of the Eyes in Reading. By M. D. Vernon. [1930.] 9d. (10d.).

No. 163. IX. Psychological Factors in Peripheral Vision. By G. C. Grindley. [1931.] 1s. (1s. 1d.).

No. 173. X. The Measurement of Visual Acuity. By R. J. Lythgoe, [1932.] 1s. 6d. (1s. 7½d.).

No. 181. XI. Individual Differences in Normal Colour Vision. By W. O'D. Pierce. [1933.] 2s. (2s. 1½d.).

No. 185. XII. Colour Vision Requirements in the Royal Navy. [1933.] 1s. (1s. 1½d.).

No. 188. XIII. Determination of the Sensitiveness of the Eye to Differences in the Saturation of Colours. By L. C. Martin, F. L. Warburton, and W. J. Morgan. [1933.] 1s. (1s. 1d.).

No. 200. XIV. Characteristics of Dichromatic Vision. By F. H. G. Pitt. [1935.] 1s. 3d. (1s. 4½d.).

Vitamins : see NUTRITION.

Wassermann Test : see VENEREAL DISEASES.

Wound Infections :

No. 12. The Classification and Study of the Anaerobic Bacteria of War Wounds. By J. McIntosh. [1917.] *Out of print.*

No. 39. Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems arising therefrom. By the Committee upon Anaerobic Bacteria and Infections. [1919.] 6s. (6s. 3½d.).

No. 57. Studies in Wound Infections. By S. R. Douglas, A. Fleming, and L. Colebrook. [1920.] 4s. 6d. (4s. 8½d.).

MEDICAL RESEARCH COUNCIL

A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE

By VARIOUS AUTHORS

- Vol. I. History; Morphology; Physiology.
 Vol. II. Cocci; Haemophilic Bacteria.
 Vol. III. Economic Bacteriology; Plague; Anaerobes; Food Poisoning.
 Vol. IV. Enteric Group; Vibrios; Pasteurella.
 Vol. V. Glanders; Diphtheria; Tuberculosis; Leprosy; Brucella; Anthrax.
 Vol. VI. Immunity.
 Vol. VII. Virus Diseases; Bacteriophage.
 Vol. VIII. Fungi; Streptothricaceae; Spirochaetes; Normal Flora; Swine Erysipelas.
 Vol. IX. Bacteriological Methods; General Index.

LONDON

PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE

1929-31

Sets of nine volumes, £8 8s. per set; post free, £8 14s. 9d.

Single volumes, £1 1s. each; post free, £1 1s. 9d.

Obtainable from any of the sale offices or overseas agencies of H.M. Stationery Office or through any bookseller.)

OVERSEAS AGENCIES OF H.M. STATIONERY OFFICE

[A selection of Government publications may usually be inspected at the offices of His Majesty's Trade Commissioners.]

IRISH FREE STATE: Messrs. Eason & Son, Ltd., 40-41, Lower O'Connell Street, Dublin.

CANADA: The Imperial News Company, Ltd., 235, Fort Street, Winnipeg; 517, Burrard Street, Vancouver; Wm. Dawson Subscription Service, Ltd., 70, King Street East, Toronto; The Benjamin News Company, 975, St. Antoine Street, Montreal.

AUSTRALIA: Messrs. McGill's News Agency, 183-5, Elizabeth Street, Melbourne, Victoria; Messrs. Angus & Robertson, Ltd., 89, Castlereagh Street, Sydney, New South Wales; Messrs. Albert & Son, Ltd., 180, Murray Street, Perth, Western Australia; Messrs. Oldham Beddome & Meredith Pty., [1932] Ltd., 36, Elizabeth Street and 96, Collins Street, Hobart, Tasmania.

NEW ZEALAND: Messrs. Whitcombe & Tombs, Ltd., Auckland, Christchurch, Dunedin, and Wellington.

SOUTH AFRICA: The Central News Agency, Ltd., Cape Town, P.O. Box 9; Johannesburg, P.O. Box 1033; Durban, P.O. Box 938; Port Elizabeth, P.O. Box 356; Pretoria.

INDIA: Messrs. Thacker & Co., Ltd., Bombay; Messrs. Higginbothams, Ltd., Madras and Bangalore; The Book Company, Ltd., College Square, Calcutta.

GOLD COAST: The Methodist Book Depot, P.O. Box 100, Cape Coast. Branches at Accra, Kumasi, Sekondi, and Swedru.

U.S.A.: The British Library of Information, 270, Madison Avenue, New York.

DENMARK: Mr. A. Busck, Kjøbmagergade 49, Copenhagen.

FINLAND: Akateminen Kirjakauppa, Helsinki.

HOLLAND: N.V. Martinus Nijhoff' Boekhandel, Lange Voorhout 9, 's Gravenhage.

GERMANY: Messrs. A. Asher & Co., Behrenstrasse 17, Berlin, W.8.

NORWAY: Cammermeyers Boghandel, Karl Johans Gate 41-43, Oslo.

SWEDEN: A-B, C.E. Fritzes Kungl. Hofbokhandel, Fredsgatan 2, Stockholm.

CHINA: Messrs. Edward Evans & Sons, Ltd., P.O. Box 970, 200, Kiukiang Road, Shanghai.

JAPAN: Maruzen Co., Ltd., 6, Nihonbashi, Tori-Nichome, Tokyo.