Estimation of Vitamin A

A VITAMIN or accessory food factor is discovered from the effects produced in living animals by its removal from the diet. Progress in this field has been dependent upon the invention of methods of preparing many of the different constituents of a diet in a pure state, without loss of their own essential qualities, so that characteristic symptoms may be produced by the removal from the diet of hitherto unknown factors which are frequently associated with the better-known constituents. Following their discovery, interest was aroused in their distribution in Nature.

The work, at first qualitative, rapidly assumed a quantitative aspect, necessitating the devising of quantitative biological methods. At the same time, search for quantitative chemical or physical methods of assay was unremitting, since the biological method at its best is usually relatively inaccurate. Thus for vitamin A, two methods have been much used recently to supplement or even replace the biological 'growth' test, namely, the depth of the blue colour given by the antimony trichloride reagent in the 'Carr-Price' test and the intensity of absorption at 3280 A. as measured by the spectrophotometer. Before either of these methods can replace the biological, however, it must be proved that it estimates the same thing and with greater accuracy. Owing to the inherent errors of the biological test, this proof is not easy to obtain; the first need is to improve the biological method, and for this the provision of a stable standard of reference with which all samples may be compared is an absolute essential.

The International Conferences for the Standardisation of Vitamins held in London in 1931 and 1934 under the auspices of the Permanent Standards Commission of the Health Organisation of the League of Nations adopted first carotene and then β-carotene as standard for vitamin A. A considerable amount of the preparatory work for the second Conference was carried out by a special sub-committee of the Accessory Food Factors Committee, some of which has now been brought together and edited by Miss Hume and Dr. Chick*.

With the choice of carotene as standard for vitamin A, it became of the greatest importance to select a suitable solvent in which to administer it to the animals, since it had been previously shown to be inactive when given in an unsuitable solvent such as ethyloleate, in which it is unstable. Apart from instability in the solvent, there was also to be considered the question of absorption from the alimentary tract, since Lathbury and Greenwood[1] had found different biological values for carotene when dissolved in different samples of arachis and coco-nut oil, although colorimetric tests had shown that carotene was stable in certain samples of these solvents. Preliminary investigations indicated that, of a number of oils examined, coco-nut was the most satisfactory. Three samples of different origin were therefore obtained and submitted to extensive examination. All observers found that the carotene (1931 standard) had the highest potency in one of these oils; but there was less agreement about the suitability or otherwise of the other two. Colorimetric tests indicated that the carotene was most stable in the most satisfactory oil as determined biologically and least stable in the oil which one observer found to be the least suitable by the biological test. Addition of hydroquinone to 0·01 per cent obliterated the difference between these two oils in the colorimetric test. It was concluded that a selected coconut oil was the most suitable solvent for the standard, and the 1934 International Conference decided to issue the standard in solution in coconut oil with addition of hydroquinone.

The next step was to select and obtain in the pure state one of the carotene isomers to replace the 1931 standard carotene, which appears to have consisted of a mixture of β-carotene and inert material with little or no α-carotene. β-carotene is the form most widely distributed in Nature and most easily prepared, and seemed therefore the most suitable isomer to select. The results of the biological tests, carried out to determine its growth-promoting activity in terms of the 1931 standard, showed quite good agreement. The dose of β-carotene equivalent to 1γ (one unit) of the 1931 standard was found to be 0·57γ by Coward, 0·66γ by Hume and 0·50γ by Lathbury. The average of these values taken as 0·6γ was accepted by the International Conference 1934 as the figure which should be adopted as equivalent to one unit. Colorimetric and spectroscopic tests confirmed this figure.

The report then describes the spectrophotometric tests which were carried out by a number of different observers, to determine the suitability of this method as a means of estimating vitamin A. The results obtained in seven different laboratories in estimating $E\ \frac{1\ \text{per cent}}{1\ \text{cm.}}$ 3280 A. for six cod liver oils and one concentrate showed that

* Medical Research Council—Special Report Series, No. 202. Reports of Biological Standards, 4: The Standardisation and Estimation of Vitamin A. Edited by E. Margaret Hume and Harriette Chick. Pp. 61. (London: H.M. Stationery Office, 1935.) 1s. net.

estimations on cod liver oils should be made on the unsaponifiable fraction, and that cyclohexane or ethyl alcohol should be used as the solvent and not chloroform. From the results of the biological tests on a number of oils for which the extinction coefficient was also determined, it was concluded that the most likely factor for converting $E\,\frac{1\text{ per cent}}{1\text{ cm.}}$ 3280 A. into international units of vitamin A per gram is 1600. This figure is to be considered provisional and subject to revision with the accumulation of further data. Other conversion factors have in fact been published: for example, Lathbury[2] found a factor of 1400 in her examination of an extensive series of oils and concentrates; and even lower figures have been suggested.

The report does not refer to work which has been carried out on the relationship between the blue value of an oil and its biological potency. Lathbury (ibid.), for example, found that the blue value, determined on the unsaponifiable fraction, should be multiplied by 40 to obtain the biological activity in units per gram in the case of crude oils, whilst for concentrates the blue value should be multiplied by 20. Assuming the conversion factor of 1600 for converting the extinction coefficient to biological units, the potency of the purest preparation of vitamin A hitherto made[3], which had a coefficient of 1600, is 2½ million units per gram. This concentrate had a blue value of 80,000, from which the conversion factor for blue value to biological units becomes 30. The determination of blue value is subject to greater errors than the spectrophotometric test but may be a very useful indication of the potency of an oil or concentrate when carried out under well-defined conditions.

The report of the vitamin A sub-committee concludes with a section upon the possible use of cod liver oil as a subsidiary standard. Provided its potency is maintained, a standard oil would be a convenience owing to the fact that the quantities of β-carotene available as international standard are somewhat limited. The report of the 1934 International Conference is also reprinted in an appendix.

Only further experience can decide whether the different methods of estimating vitamin A will always give the same results. A recent letter in our columns[4] suggests that more work is required before the relationship between the extinction coefficient and the biological activity can be considered to be quantitatively established.

[1] *Biochem. J.*, **28**, 1665 (1934).
[2] *Biochem. J.*, **28**, 2254 (1934).
[3] Carr and Jewell, NATURE, **131**, 92 (1933).
[4] Bacharach, Drummond, and Morton, NATURE, **137**, 148 (1936).

Sign Language as a Form of Speech*

By Sir Richard Paget, Bt.

THE relationship between mouth gestures (which produce speech) and bodily gesture was indicated by Darwin ("Expression of the Emotions", 1872): it had been noticed thirty years earlier by Charles Dickens in the "Pickwick Papers", where he shows Sam Weller, junior, forming with his tongue "imaginary characters to correspond" with the letters of his valentine to Mary. This hand and mouth association deserves to be systematically studied. It lies at the very root of human speech: its rudiments are found in the behaviour of chimpanzees.

The present object is to give an account of gesture as an alternative to speech, for the communication of ideas between people of different races. I will deal first with the natural sign language of deaf mutes. As to this, I have had the fact questioned—by a responsible authority—whether the sign language of deaf mutes *was* 'natural' at all. The fact is that in all countries, deaf mutes develop a natural and mutually understandable pantomime. On August 18, 1935, a deaf mute service was held in St. Paul's Cathedral; it was attended by deaf mutes of fourteen different countries. The service was conducted in pure sign language (though *The Times* unfortunately described it as "international signs alphabet"), and all present could understand. Of course, separate communities develop their distinctive slang; thus, the present English deaf mute sign for 'Prime Minister' might be 'pipe-in-mouth'!

The Red Indians of North America also had a universal sign language by which all the different tribes, speaking more than a hundred different languages, could communicate.

Literature on sign language is very scarce: the British Museum Library appears to have no book on the deaf mute sign language, and only one on Red Indian signs—that of Garrick Mallery—"Sign Language among the North American Indians"

* Substance of a Friday evening discourse delivered at the Royal Institution on December 13, 1935.