VITAMINS IN MEDICINE

by

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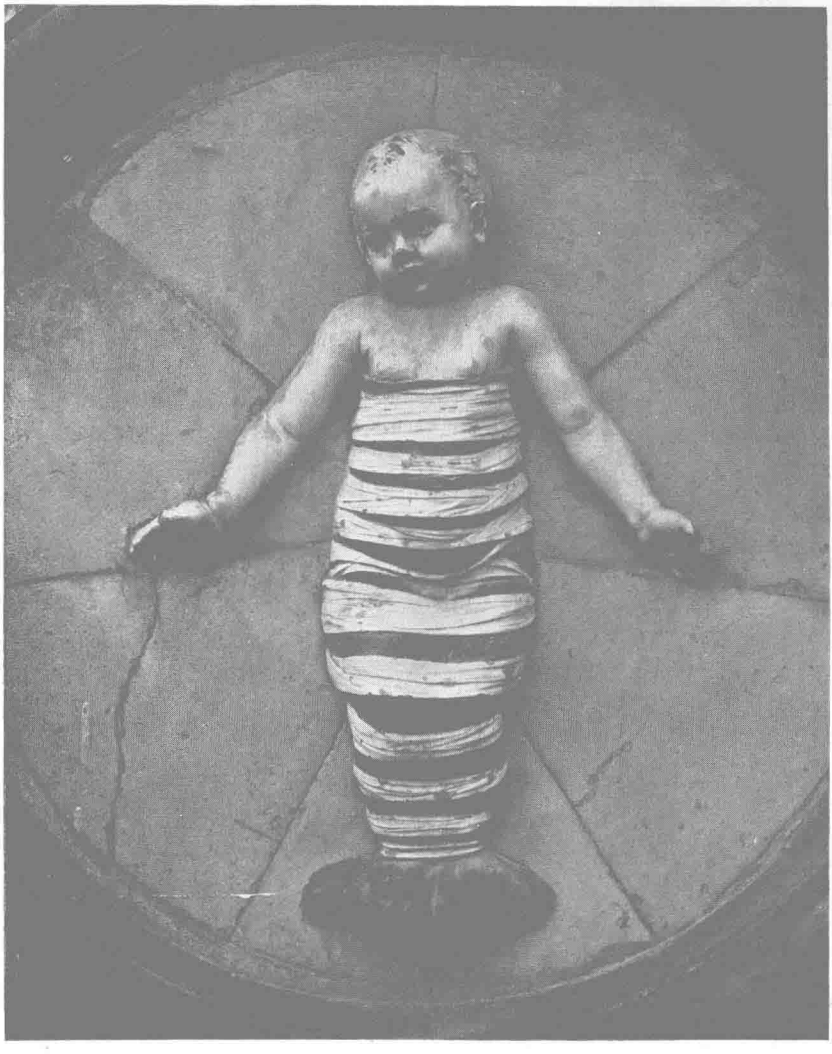

An Infant in Swaddling Clothes. This practice, which dates from antiquity, was probably done with the idea of preventing rachitic deformities. From the plaque by Andrea della Robbia (1435–1525) in the Ospedale degl' Innocenti, Florence. By kind permission of the Director, Prof. Arrigo Galeotti-Flori.

Frontispiece.

PREFACE TO THE THIRD EDITION

In the preparation of the third edition of this book most of it has been rewritten. This has been necessary owing to the changing state of our knowledge and the advances made in certain directions, notably the physiology and biochemistry of the vitamin B complex. Although the number of references has been increased from about 4,500 to 5,500 and the illustrations from 208 to 245, the size of the book remains about the same.

Our work has been greatly helped by suggestions and criticisms and also by the generosity of those who have allowed us to reproduce their original illustrations. We are especially indebted to the many workers in England, the Continent and the Americas who besides sending us reprints of their published articles have kept us informed about their current research and its results.

It is again a pleasure to acknowledge the sympathetic assistance given to us by Messrs. William Heinemann, Dr. J. Johnston Abraham, Mr. Owen R. Evans and Mr. G. F. Home, Librarian of the Royal Society of Medicine, and his staff.

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The Editors of the following journals : *American Journal of Diseases of Children*, Fig. 221 ; *American Journal of Medical Sciences*, Fig. 83 ; *American Journal of Pathology*, Figs. 225–238 ; *Annals of Internal Medicine*, Figs. 46, 47, 148–150 ; *Archives of Dermatology and Syphilology*, Figs. 19–23 ; *Archives of Diseases in Childhood*, Figs. 64–68 ; *Archives of Pathology*, Figs. 171–173 ; *Biochemical Journal*, Figs. 151–154 ; *British Heart Journal*, Figs. 82, 121 ; *British Journal of Surgery*, Figs. 129–134, 174–177 ; *British Medical Journal*, Figs. 33–38, 239, 240 ; *Canadian Medical Association Journal*, Figs. 94, 95 ; *Chinese Medical Journal*, Figs. 115, 117, 121, 122 ; *Great Ormond St. Journal*, Figs. 174–177 ; *Journal of the American Medical Association*, Figs. 144–147 ; *Journal of Clinical Medicine, Shanghai*, Figs. 85, 86, 87–93 ; *Journal of Hygiene*, Figs. 24, 211, 212, 216 ; *Journal of Hygiene and Tropical Medicine*, Fig. 107 ; *Journal of Obstetrics and Gynæcology of the British Empire*, Figs. 200, 201 ; *Journal of Pathology and Bacteriology*, Figs. 75–79 ; *Journal of Pediatrics*, Figs. 27–32, 244, 245 ; *Lancet*, Figs. 135–143 ; *New England Journal of Medicine*, Figs. 44, 45, 122–125 ; *Proceedings of the Royal Society of Medicine*, Fig. 198 ; *Quarterly Journal of Medicine*, Figs. 101–104 ; *Southern Medical Journal*, Figs. 204–209.

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CONTENTS

CHAPTER I

VITAMIN A

THE ANTIXEROPHTHALMIC VITAMIN
THE ANTI-INFECTIVE VITAMIN
AXEROPHTOL

VITAMIN A is the term used to include vitamin A_1 found in animals and sea fish, and vitamin A_2 found chiefly in fresh-water fish. Biologically and chemically they are so very similar that generally no distinction is made between them. The little that is known about vitamin A_2 is discussed on p. 85. Axerophtol was the name given by Karrer in 1938 to vitamin A_1, but it has not come into common use, except on the Continent.

Provitamin A is the name given to those plant carotenoids which can be converted by animals to vitamin A.

HISTORY

The most clear-cut effect of lack of vitamin A is night blindness, which often occurs suddenly after long exposure to a day's bright sunlight. In rural communities inability to see in the dusk is a very serious condition : fishermen, for instance, may walk off the rocks into the sea after landing in the evening. Night blindness can be cured, often in twelve hours, by eating food rich in vitamin A, such as liver. The dramatic quickness both of the onset and the cure explains why liver has been used for centuries for night blindness.

The Ebers Papyrus [1], written about 1600 B.C., probably referred to night blindness when liver was recommended for the eyes, while the Chinese in 1500 B.C. were giving liver, honey, flying fox dung and tortoiseshell, all of which would have cured night blindness [5]. Hippocrates advised the whole liver of an ox dipped in honey, and liver was known to later Roman writers. Jacob van Maerland, a Dutch poet of the fourteenth century, may be thus translated [2],

> He who cannot see at night,
> Must eat the liver of the goat
> Then he can see all right.

Guillemeau in France in the sixteenth century besides clearly describing night blindness, advised liver for its cure [3], which was also advised by other writers at that time [5].

Drummond and Wilbraham [2] find that the first mention of liver for the eyes in England was in Muffett's " Health's Improvement " (1655), though Bayly, at one time Queen Elizabeth's physician, in his book on eyes recommends " rawe herbes " among which is " eie bright " ; but the only evidence of night blindness being common at this time is references to mists and films over the eyes. " Rawe herbes " would of course provide provitamin A.

Aykroyd [1] in his accounts of Newfoundland and Labrador fishermen says they not only recognize how bright sunlight may bring on night blindness, but also use liver, preferably the raw liver of a gull or puffin, for a cure.

The beginning of the present century saw the realization that more

serious eye affections—especially "conjunctivitis" in children—were due to lack of some food factor. Mori [4] in Japan in 1904 treated juvenile conjunctivitis with cod-liver oil, believing the diet was inadequate in fats, while Monrad in 1917 thought that the outbreak of conjunctivitis which occurred in Danish children at that time was due to a deficiency of a fat soluble factor, caused by the export of the country's animal fats to England and Germany.

Animal experiments as early as 1909 had shown that rats on deficient diets developed conjunctivitis [6] ; that a fat soluble factor was involved was proved by Osborne and Mendel [7] in 1913, and McCollum and Simmonds [8] in 1917. The latter workers called the factor "fat soluble A" and pointed out the similarity between xerophthalmia in rats and the conjunctivitis found in children on fat-deficient diets.

Conjunctivitis and xerophthalmia are, however, only the most noticeable examples of the change in the epithelial surfaces of the body brought about by lack of vitamin A. Wolbach and Howe [9] in 1925 found that "the specific tissue change due to deprivation of fat soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium." This replacement of a specialized epithelium by a primitive type leads to a lowered local resistance to infection.

The name "anti-infective" vitamin was first given to vitamin A by Green and Mellanby [10] in 1928 because they found that animals killed by lack of vitamin A showed multiple foci of infection in those areas where the epithelium had altered. At this time the infection was regarded as the direct, and not secondary, effect of lack of vitamin A.

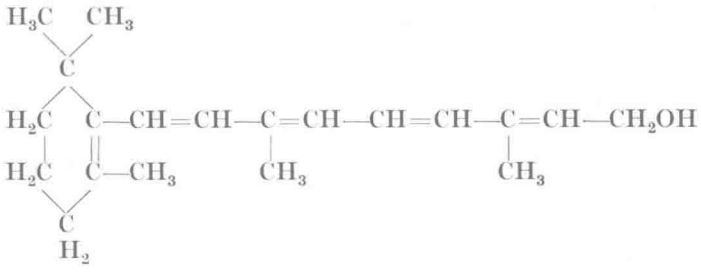
The separation of vitamin D from vitamin A was not complete before 1925. As early as 1909 Stepp [11] had found that there was some unrecognized factor in fats necessary for growth, and in 1913 McCollum and Davies [12] and also Osborne and Mendel [7] confirmed this, the latter workers also stressing that different fats varied in their value for growth. Mellanby in his work on rickets from 1918 onwards originally believed that the antirachitic factor, whose existence he discovered, was the same as the fat soluble A factor of McCollum and Davis. But in 1922 and the following years several very important papers appeared, all showing that there were two separate factors in fats—the growth promoting or anti-xerophthalmic factor and the anti-rachitic factor. Thus Hume [13] and also Goldblatt and Soames [14] found that ultra-violet irradiation, while it cured rickets, would not prevent xerophthalmia or maintain growth in animals on fat deficient diets. A year later in 1923 Goldblatt and Zilva [15] found that the growth-promoting and antirachitic functions of cod-liver oil were destroyed by heat and oxidation at different rates, and they also observed that spinach was excellent for growth but not for preventing rickets. Mellanby [16] in 1926, comparing the diets of a series of puppies which had died or survived an epidemic of bronchopneumonia, reported that the protective value of the diet against infection was not related to its protective value against rickets.

The carotene content of plants was found by Rosenheim and Drummond [17] in 1920 and by Coward [20] in 1923 to vary with their vitamin A potency, a relationship which was further emphasized by Rosenheim and Drummond's [18] observations in 1925 on the similarity of the colour reactions of the two [19]. Between 1929 and 1930 Moore [21, 22, 23] and Capper [24] were largely responsible for showing that carotene could be used by animals as a source of vitamin A, into which it is converted in the body.

The chemistry and isolation of vitamin A and its relationship to carotene was settled chiefly by the work of Karrer [25, 26 27] and Heilbron [28, 29] and their co-workers and of Holmes and Corbett [30] between 1930 and 1937. The final synthesis [31] was the result of English, Dutch, Swiss and American research which, stimulated by the threat of a shortage of vitamin A during the Second World War, came to a successful conclusion in 1947.

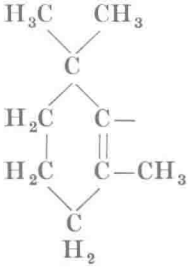
CHEMISTRY OF VITAMIN A AND CAROTENE

Karrer and his collaborators in Switzerland [25, 26, 27, 33, 34] first suggested the now accepted formula for vitamin A, which was confirmed by Heilbron and others [28, 35] in England. Vitamin A has the formula,

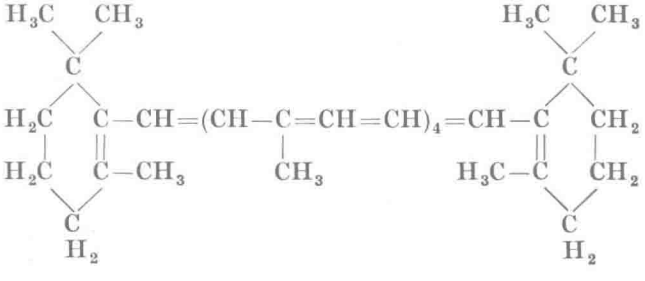


being formed in the body from one of its carotenoid provitamins, alpha-, beta-, and gamma-carotene and cryptoxanthine [32], and a few other rare carotenoids (p. 10).

All the carotenoid provitamins have the same fundamental structure as vitamin A; they possess the same essential unsubstituted beta-ionone nucleus [34, 36]

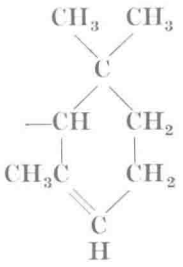


with a similar aliphatic side chain, though the latter is twice as long with a terminal group which gives to each carotenoid its own particular properties. For instance, in beta-carotene [25, 33]

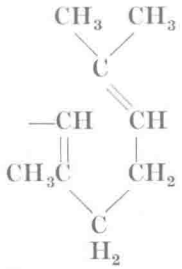


this terminal group is a second unsubstituted beta-ionone nucleus, and so the whole molecule could be in theory, and in the living animal most probably is, split by hydrolysis at the middle of the aliphatic chain into two complete molecules of vitamin A (p. 13), even though the chemical conversion of beta-carotene to vitamin A is extremely difficult and gives very small yields [37]. The Russian statement that vitamin A is formed when carotene is treated with iodinated casein is incorrect [38].

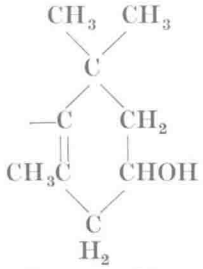
The other carotenoids not having a second unsubstituted beta-ionone nucleus as their terminal group,



Alpha-carotene



Gamma-carotene



Cryptoxanthin

but in its place the various terminal groups [39] shown above, cannot at best form more than one molecule of vitamin A.

Stereomeric isomers of both vitamin A and its carotenoid precursors occur naturally. In theory vitamin A—since it has but two " stereochemically effective " double bonds [41]—can have only four different spatial configurations : *trans-trans*, *trans-cis*, *cis-trans* and *cis-cis*. Vitamin A is believed to be the *trans-trans* form, and neovitamin A (p. 6), the only other form of vitamin A so far known to occur naturally, the *trans-cis* form.

The carotenoid stereoisomers were first described by Gillam and El Ridi [44] in 1936, when they found that, during adsorption on alumina, beta-carotene was spontaneously transformed into " pseudo alpha-carotene " —the neo-beta-carotene B of later workers. Since 1936 much research has been devoted to the carotene isomers, but they are mostly artificial products, almost all the naturally occurring carotene fortunately being in the all-trans form, so that, for instance, the thirty-two possible alpha-carotenes can remain a fascinating academic study without being a nightmare in practical nutrition. The physiology of the carotenoids being discussed on p. 10, it is only necessary here to emphasize that the tendency to spontaneous isomerization may complicate analyses involving carotenoids.

Vitamin A and its carotenoid precursors being fat soluble and unsaponifiable are found concentrated in the unsaponifiable extracts of fats. Separation of vitamin A from the carotenoids can be carried out by dissolving both in petroleum ether and adding alcohol, when the latter will dissolve the vitamin A but not the carotenoids. Since alcohol and petroleum ether are not miscible, the alcohol layer containing the vitamin can be easily separated. Vitamin A esters, however, remain with the carotenoids. Chromatographic adsorption is extensively used for the separation of vitamin A from other substances such as kitol [50], for the separation of the alcohol from the ester [77] or for the separation of the carotenoids from each other [78].

Pure crystalline vitamin A alcohol was first isolated—from fish-liver oil—by Holmes and Corbett [30] in 1937, though it was subsequently shown that the pale yellow crystals were solvated, their true melting point not being 6° C. but 60° C. The blue value (p. 6) was 100,000 and E $\frac{1\%}{1 \text{ cm.}}$ 328 equalled 2,000. The biological activity was reported to be 3,000,000 I.U. per gram, which gave a conversion factor nearer 1,500 than 1,600 (p. 9). Mead, Underhill, and Coward [45] in 1939, using two crystalline esters of vitamin A, reported that the biological activity was 3,181,000 and 3,424,000 I.U. per gram, and since E $\frac{1\%}{1 \text{ cm.}}$ 328 equalled 1,600–1,800, the conversion factor was about 2,000. Many subsequent workers have confirmed these findings [39, 46], apart from very minor differences, and the crystalline vitamin A acetate is now used in the U.S.A. as a Reference Standard (p. 10). Some American reports [46] about abnormally high biological values and conversion factors for the crystalline alcohol and ester were due to one of the older undervalued U.S.A. Reference Oils having been used for the assays (p. 10).

Three other forms of vitamin A besides vitamin A_2, which is discussed on p. 85, may occur in fish-liver and other oils : anhydro or " cyclized "

FIG. 1. Vitamin A—Alcohol crystals. Magnification : 10×.

vitamin A, kitol and neovitamin A. The first of these [47] is formed spontaneously in fish-liver oils which have been " maltreated " ; it is not a

FIG. 2. Neovitamin A—Alcohol crystals. Magnification : 15×.

cyclized vitamin A alcohol as was at one time thought, but is a hydrocarbon formed by dehydration which has no biological activity. Its only practical

importance is that it robs oils of some of their value and its absorption bands with the Carr-Price reaction may complicate assays by overlapping those due to vitamin A.

Kitol [48, 49, 50], on the other hand, is a di(vitamin A) normally present in large amounts in fresh whale-liver oil and has been reported in that of sharks, dogfish and lambs, while in that of pike is a substance similar to kitol but related to vitamin A_2. Kitol has no biological activity and causes considerable difficulty when estimating vitamin A in whole liver oils owing to its colour reactions, though it can be removed by chromatography [50]. Barua and Morton [50] have suggested that it may be formed to detoxify excessive accumulations of vitamin A. When heated it forms vitamin A and degradation products, a reaction of great value in increasing the yield of the vitamin.

Neovitamin A [51, 54] forms about thirty-five per cent. of the vitamin A in many common fish-liver oils. It is a stereoisomer of vitamin A, probably being in the *trans-cis* form, and it has the same biological potency for rats, being converted into the usual *trans-trans* form and stored as this in the liver after feeding. It has maximum absorption in the ultra-violet region of 328 millimicrons, a conversion factor of 1645 and it reacts more slowly than vitamin A with maleic anhydride, this forming the basis of its assay in fish-liver oils.

The synthesis of vitamin A became of practical importance during the Second World War because of the risk that supplies from fish-liver oils could not be maintained. The consequent wave of research, besides showing that all earlier reported syntheses—such as that of Kuhn and Morris [40] in 1937—could not be confirmed, did also produce, in amounts too small to have any dietetic value, a number of substances with vitamin A activity ; but Milas [41], writing in 1947, ended his very detailed review of the highly complicated chemistry of this subject by pointing out that " in no case has a synthetic product been shown to be identical in *every respect* with the natural product." However, by 1948 Sir Ian Heilbron [31], in a very lucid summary of current research, was able to talk of " the large-scale production of the synthetic vitamin." For further progress the reader should consult the papers by Isler, Wendler or Milas and their collaborators [42], and that of Sobotka and Chanley [43], who give a preliminary account of research designed for the total synthesis of vitamin A without starting from the natural beta-ionone, mostly obtained from lemon-grass oil, which all other workers have used.

Vitamin A gives a band of maximum absorption in the ultra-violet region at 325 millimicrons [39], but as it was originally thought [52] that the maximum absorption was at 328 millimicrons—which is now known to be due to the neovitamin A of fish-liver oils discussed above—this is still generally used when determining vitamin A in fish-liver oils by spectrophotometry [53].

The colour reactions of vitamin A are of great practical importance. They occur with sulphuric acid [17] and with the chlorides of polyvalent metals [18, 19] ; the most satisfactory reagent was found by Carr and Price [19] to be antimony trichloride dissolved in chloroform. This test is often called the Carr-Price colour test. With vitamin A a blue colour develops rapidly and then fades. The blue colour, however, is not only given by vitamin A but also by carotenoids [18] and " oxycholesterol " [54]. Therefore to confirm the presence of vitamin A the absorption spectrum of the Carr-Price reaction must be examined, when, if vitamin A is present, specific absorption bands will be found. Activated glycerol dichlorohydrin has been used instead of antimony trichloride [55], but it would appear to have no advantages [56].

Vitamin A exhibits fluorescence when irradiated by a mercury vapour lamp [57] : this has been used by Popper to demonstrate vitamin A in the tissues (Figs. 3, 4, 5) by the technique which he describes in the *Archives of Pathology* [58]. The differences in the fluorescence of the alcohol and the

ester have been used to determine the amounts of each in preparations of vitamin A [57.]

Biological activity is lost when vitamin A takes part in chemical reactions which affect double bonds, such as oxidation, hydrogenation and bromination [59]. Heat does not destroy vitamin A in butter at temperatures below about 120° C. in the absence of oxygen [60] ; when oxygen is present slow destruction occurs in oils even at room temperatures [61] and rancidity accelerates this, though it probably causes no appreciable loss in human food [64]. Many fats when heated develop an " anti-vitamin A " factor which destroys the biological activity of vitamin A ; whether this is a chemical or biological effect is unknown [65, 66]. Exposure of cod-liver oil to light reduces its vitamin A content, so the oil should be stored in dark bottles [62, 63]. Vitamin A in " cod-liver oils " prepared from concentrated fish-liver oils diluted with cottonseed, peanut or maize oil is less stable than vitamin A in genuine cod-liver oil, nearly twice as much being destroyed after exposure to winter sunshine for eleven days [67].

ESTIMATION OF VITAMIN A AND CAROTENE

Vitamin A can be estimated biologically ; physically, by absorption spectra estimations ; and chemically, by colour reactions. Since essentially the same methods are used in the estimation of carotene, these will not be fully described.

The Biological Estimation. In biological estimations—which have been fully discussed by Coward [68] in her classical book on the subject—either the curative or the prophylactic method can be used ; that is the potency of the substance being tested is estimated either by its capacity to cure the symptoms of vitamin A deficiency or by its capacity to prevent their onset. Of the two methods, the curative is generally considered more satisfactory, though it has been criticized firstly on the grounds that the ill-health of the vitamin A depleted animals at the beginning of the estimation varies, this variation altering their subsequent response to vitamin A, and secondly on the grounds that the " depletion period " during which a vitamin A deficient diet is given is avoided in prophylactic tests. Coward [68] has pointed out that neither criticism is valid because firstly deficient rats show no greater individual variations than do normal rats in their response to vitamin A, and secondly in the prophylactic test the animals at the beginning have different amounts of vitamin A already stored, thus introducing a variation in response which can only be ignored if the average results from a large number of rats are used. Various symptoms may be taken for indicating the onset of cure of the deficient state. Of these change in weight has been the most widely used, and has the great advantage that it is easy to measure. Against it has been urged that it is not specific for vitamin A—even though vitamin A has a specific action on growth [69]—since any other deficiency has the same effect. Xerophthalmia (p. 33) and the changes in the desquamated epithelial cells of the vagina (p. 36) are sometimes used, being only caused by lack of vitamin A. Deciding, however, when early xerophthalmia is present or when it is cured depends on what criteria each worker adopts, and so is liable to great variation. The changes in the vaginal epithelium are difficult to interpret [70] unless the animals are previously spayed to prevent the changes due to œstrus [71].

Irving and Richards [72] in 1940 found that after seven weeks on a vitamin A deficient diet young rats had a constant degeneration of nervous tracts in the medulla. There was only a very small difference between the amounts of vitamin A which could and could not protect the rats against this degeneration. This method of assay was very carefully checked by Coetzee [73] in 1949, who reported that it was possibly more accurate than the standard curative method and took two weeks less to perform.

Guggenheim and Koch's method of assay [74] is based on the amount of vitamin A stored in the livers of rats which, after the usual depletion period, are dosed with the test substances for two days and are killed on the fourth day. This again is a much quicker method than the curative method and is claimed to give as accurate results.

Since the most widely used method of biological estimation is the curative based on the growth response this will be discussed fully ; the same principles apply to the other methods [68]. But whatever biological method is used it cannot be too strongly emphasized that when comparing the relative potencies of two substances the diet must contain ample of everything necessary for the absorption and metabolism of *both*, and further that both must not only be dissolved in the *same* solvent but also that this solvent must not favour the absorption of one more than the other. Failure to recognize these principles has greatly decreased the value of many assays ; the subject is fully discussed on p. 13.

The young rats used for estimations have to be first given a diet deficient in vitamin A until they stop growing or start to lose weight. This is called the depletion period ; it can be shortened by having given the suckling mothers a diet lacking in vitamin A. At the beginning of the estimation the rats should weigh about 70 to 90 grams, and should not have severe xerophthalmia. Their diet must of course contain all essentials apart from vitamin A so that their lack of growth and its resumption can only be caused by it. The particular importance of ample vitamin E in the diet is discussed on p. 23. When the rats have been depleted of their stores of vitamin A they are divided into two groups, each containing similar proportions of males and females. The most accurate results are obtained by dividing pairs of littermate rats between the two groups. One group is fed the substance under investigation and one the standard preparation of vitamin A. By comparing the growth of the two the vitamin A content of the first can be determined. The method is accurate to within thirty-three per cent. The period of the test feeding should last four weeks or longer, the vitamin A preparations being given daily or twice weekly. The mouse [611] may prove a satisfactory alternative to the rat for biological assays.

Gain in weight, however, is not directly proportional to the amount of vitamin A given ; doubling the vitamin A does not double the growth. Therefore if 1 gram of an oil of unknown potency causes a gain of weight of 8 grams and 1 gram of the standard oil causes a gain of 4 grams, the unknown oil is not twice as potent as the standard oil, but possibly only about one and a half times as potent. To enable a comparison to be made between substances causing different gains in weight Coward has made a curve of reference (by feeding groups of rats on varying amounts of the same oil) which shows how weight increases in proportion to the intake of vitamin A. By referring the gain of weight on two different oils to this curve, the relative values of the two to each other can be decided.

Even in rats from the same colony under the same conditions the rate of growth or other response [73] for the same amount of vitamin A may differ at different times; so the response to the standard vitamin A preparation must be worked out afresh for every fresh group of biological estimations. A laboratory cannot once work out the response of its animals to the standard, and use the results for reference in all subsequent work ; Coward, for instance, found that had she done this, one sample of oil would have appeared to contain five times the amount of vitamin A it did nine months earlier.

Physical Estimation. Absorption spectrum estimations of vitamin A have been found to be reliable, giving results in harmony with biological estimations as long as the unsaponifiable fraction is used when the substance being tested contains less than 10,000 I.U. per gram of vitamin A. The reason for using the unsaponifiable fraction is that in oils there are substances other than vitamin A which increase absorption in the region of 328 millimicrons.

This irrelevant absorption has been investigated by Morton and Stubbs [53], who have explained in their outstandingly important papers how it may be measured and discounted.

It is necessary to be able to convert the results of absorption spectrum estimations into International Units. "It has been found that, within certain defined conditions, measurement of the coefficient of absorption (E) at 328 millimicrons affords a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of converting values obtained for $E\ \frac{1\%}{1\ cm.}$ 328 millimicrons into a figure representing the International Units of vitamin A per gram of the material examined, the factor 1,600 is recommended for adoption" and "the intensity of absorption at 328 millimicrons may be determined to within $\pm$ 2·5 per cent. by any of the recognized methods of spectrophotometry" (Report of the Second International Conference, 1934, on Vitamin Standardisation of the League of Nations). But the vitamin A sub-committee of the Accessory Food Factors Committee of the Medical Research Council [337] reported in 1943 that the conversion factor should be 1,740, a figure in agreement with that of Irwin [75], who in 1944 analysed statistically the results of vitamin A assays, carried out to determine the conversion factor, by nine or ten different laboratories on halibut-liver oil, the U.S.P. reference oil and vitamin A naphthoate. The average for the halibut-liver oil was 1,570, for the U.S.P. reference oil 1,820, and for the naphthoate 1,770. These values, from their logarithms and their standard errors, were consistent with the same conversion factors for all three substances. Pooling these results gave the conversion factor, mentioned above, of 1,740 with limits of error of $\pm$ 120. The problem has been further clarified by Morton and Stubbs [53], who in 1947 showed that when corrections were made for irrelevant absorptions in oils 1,800 was the correct factor, but that when no such corrections were made 1,600 gave the correct result for the average oil, though of course this introduces an error for those oils which have more or less irrelevant absorption than the average. In spite of early German claims [76] that the natural vitamin A ester was twice as active as the alcohol, it is now certain that both have the same activity [36, 46] when assayed correctly (p. 8), and so have the same conversion factor. The conversion factor of 2,000 which has been used in America in the past was due to the U.S.P. reference cod-liver oil being over-valued (p. 10).

Chemical Estimation. Colour reactions (p. 6) for the estimation of vitamin A are chiefly used for oils and concentrates and, with an instrument such as the photoelectric spectrophotometer described by Thompson [79], can give accurate measurements of the amounts of vitamin A and of carotene when these occur separately or together. With the Carr-Price reaction two bands of maximum absorption due to vitamin A are found at 572 and 606 millimicrons, which in concentrated solutions are displaced to 583 and 620; the latter is that used when estimating vitamin A [68, 79]. For carotene, when dissolved in chloroform, the band at 463 is used.

UNITS OF VITAMIN A

The International Unit of vitamin A—generally abbreviated to I.U. or i.u.—is that amount which has the same vitamin A activity as 0·6 microgram of the international standard beta-carotene, when both are assayed biologically under standard conditions (pp. 8, 12).

The reason for this absurd unit is that in 1931, when the International Unit was defined for the first time, no pure preparation of vitamin A had yet been made though supposedly pure beta-carotene was available. Since carotene is converted into vitamin A in the body the former was chosen as the yardstick for the biological assay of the latter, and the International Unit was defined as the vitamin A activity of 1 microgram of a special sample of