A REVIEW OF CERTAIN RESEARCHES RELATING TO THE OCCURRENCE AND CHEMICAL NATURE OF VITAMIN A*

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When Osborne and Mendel published their work on the food value of isolated proteins[68] they demonstrated more than a mere inequality in the value of various isolated proteins for growth. They demonstrated that milk or whey contained an unknown factor which was essential for normal nutrition under their experimental conditions, and what was far more important, that a small laboratory animal, such as the rat, could be used successfully in a very practical and simple manner for the determination of food values. In consequence, many investigators in the field of nutrition, shook off the shackles of conventionality, which had indicated the necessity for the use of complicated calorimetric apparatus and tedious metabolism trials over long periods of time and turned to the use of the balance with small laboratory animals kept on meticulously controlled rations. Undoubtedly, many believed this to be a shift for the moment, but now two decades have passed and tens of thousands of rats are in use for this purpose in laboratories all over the world.

As in all great achievements, the impetus given to this development by Osborne and Mendel did not come from a clear sky. Many were the previous attempts. Biological literature records the work of Lunin[47] as early as 1881. Later there followed the work of Socin[85], Röhmann[76], Henriques and Hansen[81], Falta and Nöggerath[29], Jacob[35], Willcock and Hopkins[106], Pekelharing[74], Hopkins[32], McCollum[48], and Stepp[101, 102, 103, 104]; and undoubtedly there were made many other attempts by investigators who, discouraged by not securing adequate nutrition, did not publish their results.

Outstanding among the above investigations is the work of Hopkins and of Pekelharing. Both observed independently, and at about the same time, the beneficial results accruing from the supplemental feeding of small quantities of milk which were all out of proportion to the solids contained therein. This suggested to them the necessity for the presence, in an adequate diet, of hitherto unrecognized food constituents. Unfortunately, the work of Pekel-

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haring was published in a Dutch journal where it escaped attention for many years, and Hopkins, while he obtained his results in 1906 and 1907 did not publish them until 1912. McCollum's work was notable in that he secured an increase in the weights of rats over a longer period of time than any other investigator. He, however, was much impressed with the importance of palatability. With the appearance of Osborne and Mendel's publication, he redoubled his efforts to secure normal nutrition.

In 1913, McCollum and Davis[49] and Osborne and Mendel[64] reported an inequality in the value of fats for growth. McCollum and Davis reported that growth could not be maintained on a ration which contained lard or olive-oil as the fat ingredient. Growth, however, did become possible when the lard was in part substituted by the ether extract of egg yolk or butter. They concluded that apparently fat itself was not responsible, but that certain impurities possibly of the nature of a lipoid, might be the active constituent. Osborne and Mendel, in the continuation of their protein work, found that a ration containing, among other ingredients, whole milk powder and lard, met the requirements for growth, but a similar ration containing centrifuged milk and lard, resulted in failure. When butter was substituted for the lard or a part of it, successful growth was again obtained. This led them to suggest the existence in butter of a substance having a growth-promoting effect. A little later they showed[65] that the active principle was not found in olive-oil or almond-oil, but that it was present in small quantities in beef fat and, most noteworthy of all, in abundance in cod-liver oil. Later, McCollum, Simmonds and Pitz[53] reported observations on various other fats, and found that corn-oil, cottonseed-oil, linseed-oil, olive-oil, sunflower-seed-oil, and soy-bean-oil were all practically devoid of activity. These observations constituted a challenge to investigators to determine whether fats themselves differed so notably in their nutritive value or whether the difference was caused by the presence of materials in solution. The answer was given by studies which concerned themselves with the distribution of the active principle in various plant and animal material and with determination of its stability under various conditions. Although this principle has been referred to as the fat-soluble vitamin and fat-soluble A, it is now spoken of as vitamin A.

That vitamin A occurs in plant materials other than extracted fats was shown in 1915 by McCollum and Davis[49, 50]. They demon-

strated its occurrence in corn-meal and wheat embryo. Then McCollum, Simmonds and Pitz[58] found alfalfa and cabbage leaves likewise potent in this dietary essential. Since then, vitamin A has been found in many plant materials[84] and it has come to be generally accepted that, although it is a substance indispensable for the animal, yet the animal is ultimately dependent upon plant life for its original source.

Coward and Drummond have been especially active in tracing vitamin A in its origin in the plant through various transformations of metabolism into the animal. From their work[10] it appears that there is no increase in the vitamin A content of seeds during germination, and that apparently photosynthetic activity is necessary for its actual production. In this the plant is not limited to organic foods because in the green shoots of Tradescantia, the synthesis occurs from inorganic sources. Green algae[9] were also found by Coward to synthesize the vitamin when grown in pure cultures under the influence of sunlight. On this general premise, Jameson, Drummond and Coward[86] had earlier developed the theory that a transfer of vitamin takes place from minute diatomaceous seaweeds to crustacea and the smaller fish and from them to the larger fish such as the cod. It follows from this that the potency of cod-liver oil in vitamin A is dependent among other things upon the vitamin A content of the plankton.

The wide variation in vitamin A values in different plant and animal substances caused a great deal of speculation as to its nature. McCollum and Davis[50] showed that the vitamin in butter was stable to treatment with alcoholic potash in the cold, so that it could later be taken up in solution in olive-oil. Osborne and Mendel[66] first found that when butter-fat and beef-fat were subjected to crystallization from alcohol the vitamin was concentrated in the mother-liquors or oil fraction rather than in the fraction containing the fats of high melting-point. Heating with live steam for 2½ hours did not destroy its potency, nor did storage under ordinary conditions[67]. From spinach and clover, previously dried at 60°, the vitamin was found to be readily extracted by ether[68]. Steenbock and Boutwell[90] found the vitamin not only extractable from alfalfa by means of ether or alcohol, but also resistant to saponification with alcoholic potash. Zilva[111] found it extractable by fat solvents from carrots and cabbage.

The stability of vitamin A to heat received a great deal of atten-

tion. Although at one time Drummond[11] raised the question as to whether vitamins might not be enzymic in nature, the experiments on saponification by McCollum, and on stability to live steam by Osborne and Mendel demonstrated that an enzyme need not be considered. Steenbock, Boutwell and Kent[91], however, reported that vitamin A was comparatively easily destroyed by heat, because when melted butter-fat was shaken with water it became inactive. The same result occurred when it was aerated for 12 hours at 100° C. Drummond[11] showed that the vitamin was destroyed in whale-oil by heating to 100° and also by hydrogenation at 250°. Then Hopkins[88] and Drummond and Coward[17] demonstrated that vitamin A was not labile to heat alone, but only labile to heat in the presence of oxygen. However, this did not harmonize with the observations of Steenbock and Boutwell[89] on plant materials. They found that autoclaving yellow maize, Swiss chard, carrots, sweet potatoes, and squash for three hours at approximately 15 pounds pressure and then air drying the product caused no noticeable destruction; and Sherman, Quinn, Day and Miller[82] found that 17 per cent of the vitamin A of tomato, 20 per cent of the vitamin A of an olive-oil extract of spinach leaves, and 33 per cent in butter was destroyed by heating anaerobically for 4 hours at 97° C. Heating tomatoes aerobically produced no greater destruction. Cady and Luck[3] noted a difference in the stability of the vitamin in plant as compared with animal materials. Sulfur dioxide, for example, when bubbled through cod-liver oil at 20 to 100° C. for from 15 minutes to 2 hours destroyed vitamin A completely, but similar treatment of an alcoholic extract of alfalfa was without appreciable effect. While many explanations might be suggested for this difference in stability of vitamin A, the situation was obviously a difficult one unless it were assumed that vitamin A could exist in different forms in plant as distinguished from animal tissues. Even then it was difficult to bring all the reported observations into harmony.

Drummond was apparently the first investigator who had a clue as to the possible nature of vitamin A. In an attempt to demonstrate its nature, he introduced various substances differing widely in character, among them carotin, into a diet deficient in vitamin A which he fed to rats. All his experiments yielded negative results[11]. Steenbock[86] was led to assume a certain relationship between vitamin A and yellow plant pigments. He observed that at one time, when white corn was substituted for yellow corn in the stock ration

of his rat colony, he experienced difficulty with vitamin A deficiency. He then noted that fats, which had been reported as being poor in the vitamin, were, for the most part, much less pigmented than butter known to be rich in it. Careful investigation of a large number of plant materials, such as carrots[96], sweet potatoes[96], peas[97], the leaves of plants[93, 96], and Indian corn[88, 92] showed vitamin A to be absent, or practically so, wherever yellow pigments were not present, and when butter-fat[91] was decolorized the vitamin was destroyed. In explanation, he proposed that a correlation might be permissible on a genetic basis and suggested that in the absence of close agreement between the occurrence of pigment and vitamin, there was a possibility that in such instances the vitamin might be present in a leuco form[86, 98].

Drummond and Coward[16] subjected the pigment theory to test by comparing a large number of animal and plant oils. In the examination of 24 samples they found no positive correlation. They concluded that unless it were assumed that the vitamin did exist in a leuco form, the frequent association of pigment with vitamin must be regarded as an accident. This fell in line with the criticism of Palmer[70], who regarded the simultaneous occurrence of vitamin A and carotinoids as fortuitous. Palmer and Kempster[71] had already observed that it was possible to raise a flock of chickens from hatching through a second generation on a diet practically free from carotinoids, and that there were no carotinoid pigments in the blood of a number of species of animals such as sheep, swine, dogs, cats, rabbits and guinea pigs[69]. Palmer and Kennedy[72] later reported growth and reproduction in rats on carotinoid-free egg yolk and on ewe's milk containing at the most 0.00014 per cent of carotin.

In spite of the work of Palmer and his co-workers, and of negative work of their own, Steenbock, Sell and Buell[98] maintained that, although vitamin A need not necessarily be a pigment, no doubt remained that chemically and physiologically it and carotin were related. Subjecting an alcoholic extract of alfalfa to diphasic treatment with petroleum ether, Steenbock and Boutwell[90] found the vitamin to go into the petroleum ether fraction leaving xanthophyl in the alcohol solution. Steenbock, Sell, Nelson and Buell[100] found that carotin, constant in melting-point, was able to induce growth in the rat, but that attempts to acetylate possible impurities in order to facilitate their removal did not lead to success. They experienced general difficulty in getting satisfactory growth, although ophthalmia

was prevented. This led Steenbock and co-workers[87, 95] to a more detailed study of the requirements for growth, with resultant recognition of vitamin D as a growth essential.

While the identity of vitamin A with plant pigments was being discussed pro and con, various developments in technic, which facilitated progress, were taking place. These concerned themselves with improvements in biological methods of assaying, chemical and physical tests, and the actual isolation of the vitamin.

In the technic of assaying, recognition was given to the fact that simple growth comparisons of young rats of equal weight did not give entirely reliable results. In the first place, individuals might be so abundantly supplied with vitamin A reserves from their stock diet that their growth would be prolonged for many weeks and even months beyond expectation[28, 64, 80, 81, 83, 99]. And in the second place, it was found that vitamin D must be supplied in the diet or provided out of storage reserves if the animal was to respond to vitamin A additions. The first difficulty was taken care of, either by restricting the vitamin A content of the stock ration of the experimental animals, thereby reducing their reserves, or by using a curative instead of a prophylactic technic. The second difficulty was met by incorporating in the diets preparations which had been treated with ultraviolet radiations. In addition, investigators checked up conclusions based on growth by noting variations in the occurrence of ophthalmia, infections of the respiratory tract, salivary glands, and the presence of urinary calculi. Evans[27] also reported the reliability of using the persistence of cornified cells in the vagina as an index. This, however, has not been widely used.

While improvements in biological technic were being made, it soon became obvious that progress would be expedited if a suitable chemical and physical test for vitamin A were made available. Drummond and Watson[18] suggested the possibility of using the H_2SO_4 test which has long been recognized in the British and German Pharmacopœias as a test for fish-liver oils. They observed a parallelism between the purple color produced and vitamin A values, but later Rosenheim and Drummond[77] recommended a more sensitive arsenic trichloride reaction. This gave fair agreement with the growth-promoting values of 30 different oils. But Carr and Price[6] found antimony trichloride a still more sensitive reagent which also gave a more lasting color. The acceptability of this test for further experimental purposes was announced by Willimott and Moore[107]

and Willimott and Wokes[108], but at present there obtains much confusion. Euler, Karrer and Rydbom[26], for example, have found that lycopin, bixin, capsanthin, alpha crocetin, dihydro-alpha-crocetin, xanthophyl and fucoxanthin all give antimony trichloride reactions.

The antimony trichloride color has also been studied spectrometrically in an attempt to secure greater reliability. Morton, Heilbron and Thompson[61] found that the blue color produced actually consists of two bands with maxima at 572 and 606 mμ, but these shifted to 583 and 620 in concentrates, and sometimes the 572 line masked the 606 line almost completely. In the opinion of Heilbron and Morton[30], the 606 line produced by liver oils and the 620 line produced by concentrates must be caused by separate entities. Moore[55] believed the antimony trichloride reaction valid as a measure of vitamin A in cod-liver oil but not in plant extracts because of a reaction given by carotin. He[57] has, however, made extensive use of a combined colorimetric and spectrophotometric technic based on the fact that the natural yellow color of carotin in chloroform solution, compared with the blue produced by antimony trichloride in the same volume, has a ratio of about 11 Lovibund units of the former to 1 of the latter, while vitamin A concentrates of cod-liver oil show a yellow to blue ratio of 1 to 100. This method has been especially valuable in studying the transformation of carotin into vitamin A.

Takahashi and co-workers[105] announced that oils rich in vitamin A examined *directly* showed absorption in the ultraviolet at 328 mμ, which Morton and Heilbron[59, 60] confirmed. Objections have been raised against this also because this reaction is given by the biologically inactive dehydroergosterol[78], but Heilbron and Morton[30] believe that for fish oils, or their concentrates, it is, nevertheless, a reliable index. Furthermore, dehydroergosterol does not give the antimony trichloride reaction.

When it is realized that in the chemical and physical experiments, investigators used various oils, concentrates, plant extracts and often employed different technic in relation to nature of diluent, time of reaction and concentration, as well as biological controls, it is scarcely surprising that the early physical and chemical tests were difficult of correlation. On the whole, however, they have expedited progress tremendously.

Attempts at direct isolation of the vitamin at this stage were also not successful. Drummond and co-workers in 1924 made the first

attempt[12, 15]. Cod-liver oil was saponified, extracted with ether, cholesterol was removed by freezing from methyl alcohol solution, and then with digitonin. The residue was distilled with superheated steam and nitrogen, and the distillate was fractionated at 3 mm. pressure. The fraction which was most active biologically came over at 180-230° C., but it contained at least three substances which could not be separated by redistillation, crystallization or differential solution. The preparation was a thousand-fold as active as cod-liver oil. In a later paper[14] they stated that they considered further attempts to isolate vitamin A from cod-liver oil scarcely worth while.

Takahashi and co-workers[105] made a preparation from 2000 kg. of cod-liver oil which they called biosterin because they believed the vitamin to be a sterol. In general, it was obtained by extraction of the Ca soaps, and removal of impurities by absorbing them on calcium carbonate, crystallizing them out at 0° C., precipitating them with digitonin, and adsorbing them on charcoal. Then diphasic separation in petroleum ether and methyl alcohol gave the final product of which 0.001 of a milligram daily prevented loss of weight in a rat, and 0.015 mg. restored its health. As the preparation of Drummond, Channon and Coward had approximately the same potency, the Japanese preparation apparently was not a pure substance[15].

After Steenbock and co-workers[87, 94] had pointed out that many of the conclusions regarding the occurrence of vitamin A were unwarranted, because in the absence of vitamin D, either in the diet or in the storage reserves of the animal, vitamin A could not exercise its growth-promoting power, Euler and co-workers[22] took up the carotin problem. They suggested that the failure to demonstrate a relation between carotinoid and vitamin A was probably due to a lack of availability of the carotinoids in the form in which they were administered, or,—and this they considered the more probable—to the fact that vitamin D had not been furnished in the diet.

They prepared carotin from carrots according to Escher's method and found that administered to rats, which were receiving plenty of vitamin D, it restored growth promptly in daily doses of 0.005 mg. However, spectrophotometric studies of the color produced with carotin and cod-liver oil on the addition of antimony trichloride in chloroform solution showed a maximum absorption band farther removed to the green with carotin than with cod-liver oil. They

ruled out the effect of oil on the reaction and suggested that the antimony trichloride reaction possibly was produced by a different substance but one closely related to carotin or that different vitamin A's of the carotinoid type gave an $SbCl_3$ reaction of different intensities. Duliere, Morton and Drummond[19] attempted to repeat the Swedish feeding experiments and obtained negative results even with 0.5 mg. doses of a specially purified carotin melting at 184.9°. They concluded that Euler's preparation had been contaminated with vitamin A.

Moore[54] obtained carotin from Morton and Euler and found it growth-promoting in 0.01 mg. daily doses, but, impressed by the small amounts of vitamin D which had been found necessary to cure rickets, he stated that the growth observed by them might still be caused by an impurity, as it would take only 1 per cent of a contaminant as active as vitamin D to produce the effect. Later, Moore[56] reported that all samples of purified carotin were active in curing ophthalmia and restoring growth, and that this could hardly be due to an impurity because carrot fat was less active than the carotin it contained. He finally drew the conclusion[57] that carotin or some precursor of it was vitamin A. As evidence he cited that: the liver oil of a rat depleted in vitamin A would not give an $SbCl_3$ reaction. However, after feeding fresh carrots or carotin recrystallized 12 times, the symptoms of vitamin A deficiency were cleared up and there appeared in the liver oil an absorption band at 328 mμ, a trace of yellow color and an intense blue reaction with $SbCl_3$, which showed an absorption band at 610-630mμ. Carotin gave no band at 328 and absorption in the greenish-blue at 590 instead of at 610-630. From this it appeared that carotin had been transformed into vitamin A,—a faintly yellow-colored substance. Capper[4] examined rat-liver oils obtained from Moore and found no absorption in the region of 328mμ (325) when produced on an A deficient diet unless carotin was fed.

Capper, McKibbin and Prentice[5] secured growth with chickens on a diet free of vitamin A by including carotin in their ration. After its administration, carotin could not be detected in the beaks, shanks, or livers, but oil from the latter showed an absorption band at 328, and also at 610-630 after treatment with $SbCl_3$. Carotin had apparently been changed to vitamin A. Collison, Hume, Smedley-MacLean and Smith[7] prepared carotin from spinach, cabbage, and carrots, and, while they made no claims for purity of their prepara-

tions, they found close correlation of biological activity with the carotin crystals. At any rate, there resulted no reduction in physiological activity with their purification.

Work from Euler's laboratory[23, 26] strengthened the idea of specificity of action of carotin when it revealed that other carotinoids and derivatives, viz.: xanthophyl, lycopin, and its carboxylic acid, alpha-crocetin, bixin and capsanthin, norbixin, dehydronorbixin, fucoxanthin and dihydro-alpha-crocetin-methylester all gave negative biological tests. Positive results were obtained with dihydro-alpha-crocetin but this admittedly needed confirmation, which Drummond and co-workers found impossible[13]. Euler and co-workers pointed out that if growth promotion by carotin was caused by contamination, then the impurity must be effective in doses of 0.00001 mg. Euler, Demole, Karrer and Walker[20] obtained growth with lettuce, spinach, beech leaves, grass, nettle, and yellow corn parallel to their carotin content, and this in spite of a big difference in the content of unsaponifiable matter and variation in the carotin and xanthophyl ratio. They concluded: (a) that the activity could not be due to a substance other than carotin, (b) that vitamin A is not carotin, and (c) that the animal may either transform carotin to vitamin A or make its synthesis possible. Then Euler, Demole, Weinhagen and Karrer[21] found that the carotin content of leaves decreases as they turn yellow in the fall, and so does their power to promote growth in the rat. Later Karrer, Hellström and Rydbom[41] purified carotin by decomposing the triiodide with $Na_2S_2O_3$. They found that the recovered carotin had the same biological activity, melting-point and absorption bands as the original sample. Javillier and Emerique[37] also found a sample of highly purified carotin, melting at 184-185°, very active, and one preparation from spinach which had been kept in the laboratory in diffuse light and sealed in hydrogen for 40 years still was possessed of the power to promote growth.

With all this evidence available there was little reason to doubt the physiological activity of carotin, but the negative findings of Drummond still required explanation. Collison, Hume, Smedley-MacLean and Smith[8] suggested that Drummond might have obtained negative results because he alone used fat-free diets. But later, Hume and Smedley-MacLean[34] pointed out that his difficulty might have been caused by the use of ethyl oleate as a solvent, which in their experience caused rapid decolorization of carotin and negative biological tests. This was admitted later by Drummond, Ahmad and Morton[13], who then recommended ethyl laurate instead.

Discarding the use of ethyl oleate they found 0.005 mg. of carotin daily sufficient to promote growth. Thus all findings relating to the biological activity of carotin were brought into harmony; and it was henceforth necessary to recognize that in earlier work investigators had confused the activity of carotin with that of vitamin A itself.

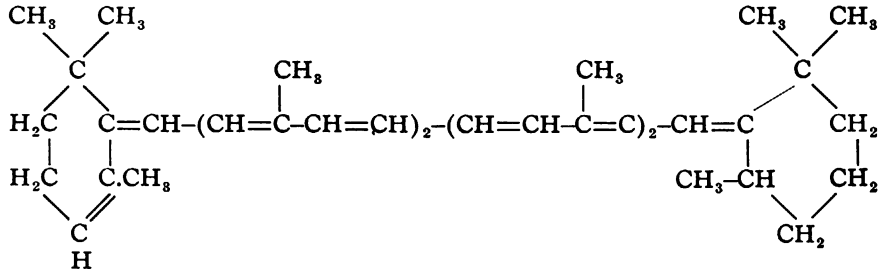
But at this stage of development an unexpected complication arose. Karrer, Helfenstein, Wehrli, Pieper and Morf[39] recrystallized 140 gms. of carotin and observed an increase in its melting-point from 167.5 to 182° with no change in ultimate composition. The more soluble fractions were strongly dextrorotatory and the more insoluble were optically inactive. This suggested the existence of isomeric forms. Kuhn and Lederer[46] independently came to the same conclusions as a result of measurements of the optical activity of carotin obtained from different sources. Carotin from carrots showed a specific rotation ranging from +25° to +185°, and carotin from nettle leaves a rotation of +5°. They named the more soluble carotin, alpha carotin and the other, beta carotin. Both isomers, so far as they have been investigated, show about the same biological activity[25]. In other respects they differed as follows:

Melting point	*Absorption bands in CS_2*	[*a*] D^{20} *in benzol*
a-carotin 174-175°C.	511mμ 478mμ	+ 380°
B-carotin 181-182°C.	521mμ 483mμ	± 0

Needless to say, all these investigations aroused a tremendous interest in the chemical constitution of carotin about which practically nothing was known 5 years ago. Lately Karrer, Kuhn and Zechmeister have interested themselves actively in this problem, and in the resultant investigations have drawn freely upon existent knowledge of the chemistry of synthetic polyenes, lycopin, phytol, squalene and other substances.

Zechmeister, Cholnoky and Wrabley[110] found that under certain conditions 1 molecule of carotin added on 22 hydrogen atoms. This, to them, constituted evidence that carotin is, for the most part, an aliphatic compound but must contain 2 rings in its empirical formula of $C_{40}H_{56}$. Zechmeister and Cholnoky[109] concluded from the rate of hydrogenation that, beside 8 olefine double bonds there must be present 3 other double linkages. Karrer and Helfenstein[38] pointed out that a formula for carotin must meet the requirements of 2 ring structures, 11 double bonds, the production of ionone on moderate oxidation, and on strong oxidation the production of dimethyl malonic acid, dimethyl succinic acid, dimethyl glutaric acid, and 4

molecules of acetic acid for each molecule of carotin. They were successful in isolating these substances and called attention to the fact that the odor of ionone or violets is always noticeable in carotin preparations after they have been exposed to the air, and that beta ionone yields the same dicarboxylic acids as carotin when oxidized. Karrer, Helfenstein, Wehrli and Wettstein[40], and Pommerer, Rebmann and Reindel[75] obtained geronic acid, after reducing carotin with aluminum amalgam and then oxidizing it with ozone. This accounted for the sixth carbon atom in the rings,—the previous isolation of dimethyl malonic, succinic and glutaric acids, having already accounted for 3, 4 and 5 carbon atoms of the ring respectively. Of three formulae previously suggested for alpha carotin[42], Karrer and Morf[43] consider the following the most acceptable. The structure of the second ring was, however, left in doubt.



Later they proposed[44] a similar but symmetrical formula for beta carotin. They estimated the amount of geronic acid which it could produce by forming the semicarbozone of the oxidation products and found that one molecule of beta carotin gave about the same amount of this substance as two molecules of ionone oxidized in the same manner. This suggested that the second ring in beta carotin is identical with the first.

Apparently the elucidation of the formula for carotin is well under way, but how it functions as vitamin A is not so clear. It appears that it is absorbed with some difficulty, at least in certain species such as the rat with which most of the experiments have been carried out[1]. Also, when injected intramuscularly it can be recovered in quantity post mortem from the site of injection. When it is absorbed it does not appear extensively, if at all, in the liver[13, 55, 73].

Euler and co-workers[24] did not find it in the liver of rats when fed in amounts less than 0.029 gms. daily, but Buckley and co-workers[2] found large deposits of it in cattle during parenchy-

matous hepatic degeneration. In this case its metabolism had evidently been interfered with.

Moore[58] is of the opinion that the transformation of carotin into vitamin A takes place in the liver. He confirmed colorimetrically the observations of Sherman and Boynton[79], who used feeding tests, that the liver, in contrast with other tissues, contains large amounts of vitamin A, and, as previously stated, he also found that this storage was increased tremendously after feeding carotin. In one instance, after giving carotin to a rat, which had been depleted in vitamin A, he found sufficient vitamin A in its liver to meet its requirements for 35,000 days. Kidney and lung tissue were next in the degree to which they stored the vitamin. But the intestine still contained unchanged carotin. This, in spite of the fact that the liver fat gave an antimony trichloride reaction with a band at 610 to 630, which is characteristic of vitamin A, instead of one at 590, which is characteristic of carotin, led him to believe that carotin was absorbed as such and then converted into the vitamin.

Ahmad and Drummond[1] observed, on the other hand, that in the ceca of rats which had been given carotin after vitamin A depletion, the antimony trichloride reaction did not harmonize with that produced by carotin. They suggested that vitamin A was produced by bacterial action or else by body tissues and subsequently excreted with the bile into the intestine. However, incubation of carotin with mixed bacterial cultures from the intestine failed to produce decisive results, and the perfusion of a cat's liver with colloidal carotin likewise failed to demonstrate the expected conversion. Lately, Olcott and McCann[62] have claimed the conversion of carotin to vitamin A *in vitro* by a carotinase in liver tissue. Their conclusions are based on spectroscopic evidence alone which requires confirmation using more animals and additional criteria.

A recent publication[45] from Karrer's laboratory on the properties of a vitamin A concentrate obtained from the liver-oils of Hippoglossus supports, in a manner that is almost conclusive, the theory of the transformation of carotin into vitamin A. In its preparation no radically new methods were used, but the liver-oil of this species of fish apparently constituted an excellent source of raw material. Biologically, the preparation was 10 times as potent as carotin; it gave an antimony trichloride reaction which was 13-15 times stronger than that given by carotin and 10,000 times stronger than that produced by cod-liver oil, and like carotin, it contained an

isoprene group and an ionone ring as revealed by the production of acetic and geronic acids on oxidation. The authors admit that they did not have at hand a pure preparation of the vitamin, but one which apparently contained 50-80 per cent of this substance. In appearance it had a light-yellow color resembling dihydro carotin which may be of interest because Euler, Karrer, Hellström and Rydbom[25] reported that dihydro carotin was found to be growth-promoting at all levels fed, which were as low as 0.03 mg. daily. On further hydrogenation of carotin there resulted products which showed selective absorption at 328-330 mμ, which is assumed to be characteristic of the vitamin A of fish-liver oils. However, Ahmad and Drummond[1] failed to obtain conclusive results on the production of growth with reduced carotin. More experiments are evidently necessary to settle this point.

It is evident that the net of evidence is drawing closer around carotin and the attempts of synthetic organic chemistry to do its part, which now seems in order[45], will be awaited with the greatest interest. The fact that a widely-distributed plant pigment such as carotin has an important biological function in the maintenance of animal life is in itself a most noteworthy finding. It can be safely prophesied that further elucidation of its rôle in the pathology and physiology of the cell and higher organisms will occupy the attention of a host of investigators for years to come.

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