CCXXIX. VITAMIN A AND CAROTENE.

VIII. HIGH POTENCY VITAMIN A CONCENTRATES.

By THOMAS MOORE.

From the Dunn Nutritional Laboratory, Cambridge.

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In a previous communication [Moore, 1931, 1] experiments were described which involved the feeding of large amounts of carotene, as red palm oil, to albino rats. Since by this means the vitamin A content of the untreated liver oils could often be made to exceed that of high quality cod-liver oil concentrates it was realised that a good opportunity was afforded to carry the concentration of the vitamin beyond the limits previously attainable.

Preliminary results, communicated at a meeting of the British Association [Moore, 1931, 2], indicated that activities represented by a minimal rat dose of about 0.001 mg., or by colour values 10 times that of carotene, could be attained in concentrates prepared from the liver oils of either rats or pigs which had received liberal amounts of red palm oil. Moreover since oils of widely divergent potency were found to give final concentrates having colour values approaching a common maximum it was considered that these preparations must consist substantially of the vitamin, or alternatively that the vitamin must be associated with other substances in remarkably constant proportion.

In apparent conflict with the view expressed above Karrer, Klusmann and Euler [1931] reported the preparation of a concentrate from the liver oil of *Hippoglossus hippoglossus*, which was at first claimed to display chromogenic activity no less than 130 times that of carotene, equivalent to 10,000 "c.l.o. units." Although a correction has now been made to adjust the ratio from 130 to 13 when pure β -carotene is taken as the standard [Karrer, Morf and Schöpp, 1931; Euler and Karrer, 1931] it still might appear, from the extremely high value given in c.l.o. units, that this concentrate was considerably more potent than those prepared by the present writer¹. To throw further light on this point Prof. von Euler kindly agreed to supply samples of highly potent fish-liver oils, in order that concentrates derived from them might be compared directly with those derived from rat- and pig-liver oils. The results to be described tend to indicate that the fish oils, rat- and pig-liver oils all yield

¹ Karrer, Morf and Schöpp [1931] find that geronic acid is a common decomposition product of carotene and vitamin A concentrates when treated with ozone. From the amount of geronic acid semicarbazone derived from the *Hippoglossus* concentrate they calculate that 50-80 % of actual vitamin A was present.

preparations of approximately equal activity when the same processes of concentration are applied.

EXPERIMENTAL.

Rat-liver oils. Samples of rat-liver oil were obtained from two single animals and from a group of 34 animals. In all cases 15 % of red palm oil was administered for some months before killing, but apart from this essential feature the diets given differed in detail. Thus sample 1 was obtained from 34 rats which received a diet composed of skimmed milk powder 20 %, rice starch 60 %, red palm oil 15 %, salts 5 %; while sample 2 was derived from a single rat which received "light white casein" 20 %, rice starch 60 %, red palm oil 15 %, dried yeast (additional) 15 %. Sample 3 differed decidedly in being obtained from an animal which was exposed to deficiency of the vitamin B complex in connection with another experiment [Dann and Moore, 1931]. The liver oils were obtained by the method previously described, involving digestion of the tissues by 5 % aqueous potassium hydroxide followed by extraction with ether.

Pig-liver oils. The use of pigs was made possible through the kindness of Lever Brothers, Ltd., who undertook the feeding of the animals and also arranged for the extraction of the liver oils. The red palm oil was given as an addendum to the normal diets of the pigs in amounts up to 12 ozs. per pig per day, the feeding being commenced at the "porker" stage (live weight approximately 40 kg.) and continued until the animals reached maturity as bacon pigs. To extract the oils the livers were steamed, minced, pressed free from water, dried and solvent-extracted.

Fish-liver oils. Of the oils supplied by Prof. von Euler one was a sole-liver oil and the remaining two turbot-liver oils. The blue values determined by Miss Rydbom before dispatch were given as 347, 930 and 1200 c.l.o. units respectively.

Concentration of the vitamin.

The methods employed in the concentration of the vitamin were the same in all cases and involved no novel feature. The oils were saponified by a brief boiling with alcoholic potassium hydroxide, ether being used for the extraction of the unsaponifiable matter. The vitamin fraction was usually separated from the bulk of crystalline material by extraction with cold methyl alcohol, the last traces of sterols were then removed by digitonin. The final concentrates were compared by the antimony trichloride method applied in the way previously described. The results obtained are shown in Table I. It should be noted that the values given for the percentage of unsaponifiable matter and "final concentrate" are those actually observed, no allowance having been made for possible losses in manipulation.

SbCl₃ absorption band.

Observations by means of a pocket spectroscope revealed the prominent absorption band at 620m μ typical of vitamin A concentrates.

Biological tests.

As a biological check upon the colorimetric data rat growth tests were carried out in the case of the concentrate from the rat-liver oil No. 2. The basal diet used consisted of Glaxo caseinogen 20 %, rice starch 75 %, salts 5 %, dried yeast (additional) 7.5 %, radiostol (vitamin D) 1 drop per rat daily. The test

Table I.

Description and source	B.U. per g. liver	B.U. per mg. untreated oil	% total unsap. matter	% final con- centrate	B.U. per mg. final con- centrate	% deviation from average
Rat-liver oil No. 1 from 34 rats	1,600	90	16	3.5*	2100	12
Rat-liver oil No. 2	7,000	450	24	18.2	1900	21
Rat-liver oil No. 3 from "B free" rat	13,000	1000	47	32.6	2300	4
Pig-liver oil No. 1	—	70	17	2.8	2100	12
Pig-liver oil No. 2	—	90	13	3.3	2600	8
Sole-liver oil	—	100	7	3.3	2700	12
Turbot-liver oil No. 1	—	400	14	9.3	3000	25
Turbot-liver oil No. 2	—	450	17.5	13.7	2400	0
				Average ...	2400	

* Digitonin process omitted.

Note. Values given in B.U. per mg. can be converted to the scale of blue values advised by the Pharmacopœia Commission by multiplying by 18.2.

Fig. 1. Biological check of vitamin A activity carried out on concentrate from rat-liver oil (R.L.O.) No. 2.

doses were made up in coconut oil, which possesses a great advantage over many natural oils in not interfering with the antimony trichloride reaction when it is desired to check the stability of the solutions [Norris and Church, 1930]. From Fig. 1 it will be seen that slow growth was restored by doses of

0.001 mg. of concentrate. Of two animals receiving 0.0005 mg. one showed very slow growth while the other died.

DISCUSSION.

Constancy of the final concentration. The outstanding feature of the above results lies in the constancy of the vitamin A activity, as judged by the SbCl_3 reaction, attained in the various concentrates. Not only did the values given by the rat- and pig-liver oil preparations as a class approximate closely to those given by the fish-liver oil preparations, but in each class oils of widely different potencies gave final concentrates of almost equal activities. As an extreme illustration of this point attention may be directed to rat-liver concentrates Nos. 1 and 3 which from original colour values of 90 and 1000 B.U. per mg. respectively gave concentrates of almost equal activity. The original blue values of any oil, indeed, could be taken as an indication of the quantity of concentrate that could be obtained, and not of the final strength of the concentrate¹.

Degree of concentration attained. The average SbCl_3 value of 2400 B.U. per mg. (Pharmacopœia blue value 45,000) given by the concentrates was about 12 times that given by the specimen of carotene recently supplied for experiments on the international standardisation of vitamin A and 2400 times greater than the specimen of cod-liver oil supplied on the same occasion. In view of the possibility of errors arising through small modifications in technique, personal factors in matching shades, and above all through variations and misunderstandings in the mode of expression of results, it would perhaps be inadvisable to attempt a comprehensive comparison of these figures with those given by Euler and Karrer for their *Hippoglossus* concentrate. A thorough study of such data as are available, however, suggests that this preparation was of the same order of activity as those at present under investigation, and this conclusion is borne out by the closely concordant results given by the *Hippoglossus* and rat-liver oil concentrates in biological tests. It seems possible, from a consideration both of the colour data and of the additional stages of freezing and adsorption adopted in preparing the *Hippoglossus* concentrate, that an activity some 70 % greater than the average value found in the present experiments was attained. Such a superiority would imply that the simplest explanation of the constant activity of the final concentrates obtained in the present experiments—that they contained a predominant proportion of vitamin A—could not hold. It would become necessary to assume that the vitamin in its most active sources is associated with a remarkably constant proportion of non-chromogenic material possessing similar solubilities.

The conversion of carotene. The above work permits further light to be shed on the phenomena accompanying the conversion of carotene to vitamin A.

¹ This statement must for the present be confined to oils similar to those under investigation. With oils of lower colour value, such as ordinary cod-liver oils, it does not seem possible to attain concentrates of such high activity, at least by the simple methods employed in the present work.

In view of the blue values given by the concentrates it must be implied that conversion is accompanied by at least a 12-fold increase in chromogenic activity. At the same time it is not necessary to infer that these spectroscopically different colorations bear a common relation to biological activity. Thus, although Karrer, Morf and Schöpp [1931] state that their *Hippoglossus* concentrate possessed 10 times the biological activity of carotene, this conclusion is not clear from the evidence presented by Euler and Karrer [1931]. A careful comparison of the biological activity of the most active concentrates against carotene in parallel experiments seems to be demanded, and is now in progress in this laboratory.

Another point of interest lies in the extreme change in the solubility in methyl alcohol from the insoluble carotene to the almost miscible vitamin A. This matter has received previous mention [Moore, 1930] but deserves emphasis in view of the recent work of Angus *et al.* [1931] who find that vitamin D is much more freely soluble in this solvent than the pro-vitamin ergosterol. When it is also recalled that vitamin E is likewise readily soluble in methyl alcohol [Olcott and Mattill, 1931] it becomes apparent that the fat-soluble vitamins as a class are sharply characterised in this respect.

SUMMARY.

Vitamin A concentrates prepared from the liver oils of rats and pigs which had received diets containing large amounts of carotene, as red palm oil, were compared with concentrates derived from turbot- and sole-liver oils. Although the initial blue values of the oils varied widely little difference could be detected in the activities of the final concentrates, which approached an average value of 2400 B.U. per mg. (Pharmacopœia colour value 45,000) in the antimony trichloride test, corresponding to a minimal dose of about 0.001 mg. in rat growth experiments. These results suggest either that the concentrates must have consisted substantially of actual vitamin A, or alternatively that the vitamin must have been associated with other substances of similar solubility properties in remarkably constant proportion.

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REFERENCES.

- Angus, Askew, Bourdillon, Bruce, Callow, Fischmann, Philpot and Webster (1931). *Proc. Roy. Soc. Lond. B* **108**, 340.
Dann and Moore (1931). *Biochem. J.* **25**, 914.
Euler and Karrer (1931). *Helv. Chim. Acta*, **14**, 1040.
Karrer, Klusmann and Euler (1931). *Arkiv. Kem., Min., Geol.* **10**, No. 16.
— Morf and Schöpp (1931). *Helv. Chim. Acta*, **14**, 1036.
Moore (1930). *Biochem. J.* **24**, 692.
— (1931, 1). *Biochem. J.* **25**, 275.
— (1931, 2). Report of the British Association.
Norris and Church (1930). *J. Biol. Chem.* **85**, 477.
Olcott and Mattill (1931). *J. Biol. Chem.* **93**, 59.