XXXIII. CAROTENE AND VITAMIN A.
THE CONVERSION OF CAROTENE INTO VITAMIN A BY FOWL.

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It has now been established with a fair degree of certainty that carotene possesses vitamin A activity [Euler, Euler and Hellström, 1928; Moore, 1929, 1, 2, 1930; Collison, Hume, Smedley-MacLean and Smith, 1929; Kawakami and Kimm, 1929; Hume and Smedley-MacLean, 1930; Karrer, Euler and Rydbom, 1930]. It has also been shown that, in the rat, carotene is converted into the "classical" vitamin A of liver oils, characterised by the blue colour given with the antimony trichloride reagent (absorption band 610–630 $\mu\mu$) and by the presence of an absorption band in the region of 325 $\mu\mu$ [Capper, 1930; Moore, 1930]. Before any generalisations could be made it seemed desirable that similar experiments should be carried out with animals differing widely from the rat. For this reason, as well as for the fact that in the fowl the problem presents points of peculiar interest, the work to be described was undertaken.

It will be shown that the fowl as well as the rat can convert carotene into vitamin A; that the vitamin A requirements of the fowl are higher than those of the rat weight for weight, and that the liver oil is normally very much richer in vitamin A than is cod-liver oil.

Experimental.

Exp. 1. The pullets used were White Wyandottes which had originally been intended for another experiment, and a deficiency of vitamin A in the diet was in the nature of an unexpected accident. The basal diet consisted of:

Cereals	
Bran 2 parts, pollards 1 part, yellow maize meal 1 part, Sussex ground oats 1 part by weight...	78 %
Soya bean meal	18 %

Mineral mixture

Steamed bone flour	10 parts by wt.		
Commercial potassium chloride ("muriate of potash")	2	,,	
Common salt	1·5	,,	
Sulphur	0·25	,,	
Iron oxide	0·1	,,	
Potassium iodide	0·01	,,	4 %

A scratch grain consisting of whole wheat was fed at the rate of approximately 50 % of the total food consumed and oyster shell was supplied *ad lib.* as a source of lime.

Group A had been kept indoors in a hut glazed with vita glass and had received the basal diet only.

Group B had received the basal diet but in addition had had free access to an open air grass run.

Group C had been kept indoors in a hut glazed with ordinary glass and in addition to the basal diet had received natural cod-liver oil at the rate of about 2 % of their diet.

The birds had been put on these diets as day-old chicks, except that for the first 9 weeks separated milk replaced the mineral mixture. At the age of approximately 16 weeks while the birds of Groups B and C remained healthy and normal the majority of those of Group A began to decline in weight and were obviously unwell. Their sense of balance appeared to be disturbed and they walked with difficulty and with a staggering gait. Rachitic trouble was at first suspected but normal calcium and phosphorus figures were obtained by Mr R. H. Common for samples of their blood-serum, and when two of the birds were killed autopsy revealed normal ossification. On examination of their livers for the presence of vitamin A negative results were obtained both by the $SbCl_3$ test, and spectroscopically by the absence of any selective absorption in the region of $325\mu\mu$. The liver oils of birds of Groups B and C and of birds procured in the ordinary market all gave an intense blue colour with $SbCl_3$ and a well marked absorption band in the region of $325\mu\mu$.

In testing the livers for vitamin A the technique described by Moore [1930] was followed in the main. The liver was minced, mixed with about twice its bulk of 5 % KOH and left for several days at least. It was then extracted four times with ether, the ethereal extract washed three times with water, dried over anhydrous sodium sulphate and the ether evaporated off by slight warming under reduced pressure. The oil thus obtained was then dissolved in chloroform in such dilution that 0·2 cc. solution + 2 cc. $SbCl_3$ reagent gave between 3 and 10 blue units on a Lovibond tintometer. The "blue units" and "yellow units" were then calculated as described by Moore [1930].

The apparatus used for the determination of the absorption spectra

was that previously described [Capper, 1930]. The results are shown in Table I.

Table I.

No. of bird	Diet	Wt. of liver (g.)	Wt. of liver oil obtained (g.)	Total "blue units" found in liver	"Blue units" per g. of liver	Total "yellow units"
A 1	Basal only	11	0·15	0	0	—
A 2	„	18	0·10	0	0	15
B 1	Basal and greenstuff	16	0·22	2,200	140	150
B 2	„	24	0·19	48,100	2,000	6500
C 1	Basal and cod-liver oil	24	0·13	24,800	1,030	80
*C 2	„	23	0·13	5,500	240	25
M 1	Birds procured in the open market	43	1·05	103,000	2,370	1060
M 2		33	0·90	41,200	1,250	375
M 3		27	0·50	375,000	10,100	—

* This value is probably too low as there was some loss due to emulsification in certain stages of the separation.

The large amount of vitamin A that may sometimes be found in the livers of fowls is shown by the values recorded for the liver of hen No. M 3 where the natural liver oil appears to be richer in vitamin A than the unsaponifiable residue of cod-liver oil, which generally gives about 300,000 blue units per g. of concentrate as compared with 750,000 blue units per g. of this liver oil. The high vitamin A content indicated by the $SbCl_3$ test was confirmed by the persistence of the absorption band in the region of 325$\mu\mu$ in the absorption spectrum and for M 1 by feeding tests on rats carried out at Cambridge by Dr Moore, who found the liver oil active as vitamin A at about the level indicated by these figures (see Fig. 5).

Nine birds of Group A were then selected for further experiment. All were confined in the same wooden house glazed with vita glass; three continued to receive the basal diet only, three received in addition cod-liver oil concentrate, while the remaining three received the basal diet + carotene dissolved in arachis oil. The cod-liver oil and carotene solutions were administered orally by means of a fountain pen filler. The growth curves and daily doses of these birds are shown in Fig. 1.

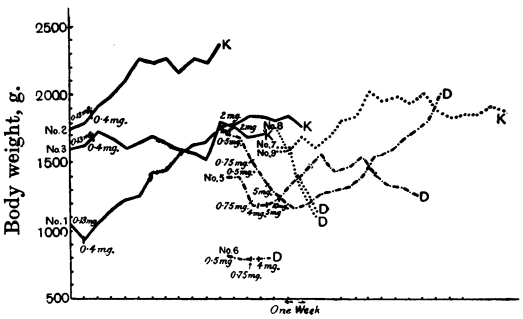

Fig. 1. ——— Birds receiving carotene.
—·—·— „ cod-liver oil concentrate.
..... „ neither carotene nor cod-liver oil.
The figures indicate the daily doses. D = died. K = killed.

The large vitamin A requirement of fowls was not realised at first and the birds continued to decline in weight and to become more helpless until the daily dose of cod-liver oil concentrate had been increased to about 10 mg. and of carotene to 0·5 mg. (The carotene used was prepared by Dr Moore from red palm oil, M.P. 168° (uncorr.).) Before the dose of carotene had been increased to 0·5 mg. No. 1 had so declined as to be unable to stand upright and lay helpless on its side; with the increased dose of carotene, however, recovery was most rapid, walking became possible within 24 hours and weight steadily increased. No. 4 had reached almost the same state before the dose of cod-liver oil concentrate had been increased to 10 mg. daily, after which recovery and growth were continuous and death after 16 weeks was due, not to any dietary deficiency, but to prolapsus of the oviduct. Nos. 5 and 1 eventually became broody and for this reason lost weight during the final weeks of the experiment. Of the control birds which continued to receive the basal diet only, Nos. 7 and 8 rapidly declined and died, No. 7 developing eye trouble resembling xerophthalmia. No. 9, which was the strongest bird at the beginning of the experiment, continued to live and at no time exhibited any pathological condition. Since the birds were confined together it may conceivably have received some vitamin A from the excreta, etc. of the birds receiving cod-liver oil concentrate or carotene or it is possible that it possessed lower requirements and utilised what it received more efficiently.

On examination the liver oils of all the birds that had received either cod-liver oil concentrate or carotene gave positive reactions for vitamin A with $SbCl_3$ and showed absorption bands at about 325$\mu\mu$, while the oils of the birds that had received the basal diet only invariably gave negative results both colorimetrically with $SbCl_3$ and spectroscopically. The results of the examination of the livers are shown in Table II.

Table II.

No. of bird	Daily dose during final weeks of exp.	Wt. of liver (g.)	Wt. of liver oil (g.)	Total "blue units" with $SbCl_3$ found in liver	"Blue units" per g. of liver	Total "yellow units" in $CHCl_3$
1	2 mg. carotene	32	0·24	8,350	265	20
2	1 mg. ,,	56	1·63	330	6	150
3	2 mg. ,,	23	0·28	3,700	160	150
4	10 mg. C.L.O. conc.	36	0·46	8,250	225	25
5	10 mg. ,,	20	0·18	10,500	525	25
6	4 mg. ,,	32	0·16	165	5	10
7	0	34	0·14	0	0	5
8	0	26	0·14	0	0	10
9	0	41	0·35	0	0	—

This experiment suggests strongly that the fowl as well as the rat can transmute carotene into vitamin A. At the same time it is in some respects open to criticism, and not completely satisfactory. Since the fowls used were approaching maturity, regular growth curves could not be expected; the diet was not sufficiently synthetic and birds receiving different diets were confined

in the same house. For these reasons a second experiment was carried out on more rigid lines.

Exp. 2. The basal diet used had the following composition:

Caseinogen (Glaxo physiological AB) ...	18 %
Agar	2
Yeast (dried)	15
Dextrin	60
Salt mixture	5

The salt mixture had the composition given by Hart, Halpin and Steenbock [1920].

Vitamin D was supplied by administering 2 drops (afterwards increased to 10 drops) of radiostol or an equivalent amount of ostelin daily. The food was supplied dry and drinking water was available *ad lib.* No litter was used but sand covered the floor of the houses.

Twelve White Wyandotte[1] chickens (Group A) were placed on this diet at the age of 6 weeks and confined in a wooden poultry house. Six more chickens (Group B) from the same batch were placed in a similar house and received the same basal diet with the addition of 0·5 mg. cod-liver oil concentrate daily.

For some weeks the Group B birds gained weight considerably faster than the others, as may be seen from the growth curves in Figs. 2 and 3, but after about 6 weeks both groups declined in weight and four of Group A and three of Group B died. (One bird in Group A died soon after being placed on the diet and has been omitted from consideration.) When examined for the presence of vitamin A the livers of all six gave negative results. It was then realised that the doses of cod-liver oil concentrate were insufficient and these were increased as shown in Fig. 2. It is interesting to note that only No. 7 exhibited eye trouble in any way resembling xerophthalmia, nor did any of them display the curious staggering walk shown by the birds in Exp. 1. The "third eyelids" of all the birds were partially closed and they had a generally dejected appearance. *Post mortem* examination of those that died revealed powdery white deposits which gave the murexide test for urates round the heart, liver and other organs, and the disease was diagnosed in the Animal Diseases Laboratories of the Northern Ireland Ministry of Agriculture as visceral gout. This condition would appear to be similar to, but more severe than, that found by Hart *et al.* [1924] in their work on the nutritional requirements of chicks.

The remaining birds of Group A were now given carotene dissolved in arachis oil. The daily doses and growth curves are shown in Fig. 3.

With Nos. 7, 10 and 13 the disease had progressed too far and they succumbed within 2 days. All the remaining birds in both groups now rapidly

[1] In a previous communication these chickens were by a slip reported as White Leghorns (Capper, *Nature*, 1930, **126**, 685). The opportunity is now taken to correct the error.

improved in appearance and at once began to increase in weight. Eventually Nos. 0, 2, 6 and 17 became fully mature birds, and when killed ossification was found to be good and, except for a certain paleness, the organs appeared

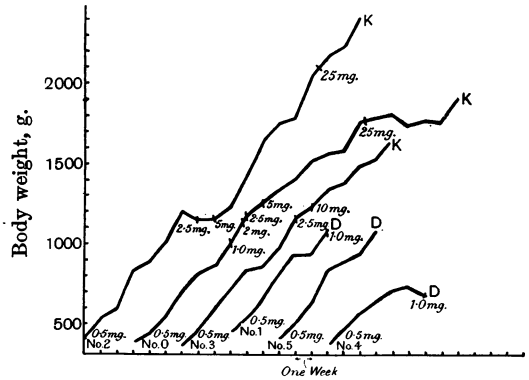

Fig. 2. Growth curves of birds receiving cod-liver oil concentrate. The figures indicate the daily doses. D = died. K = killed.

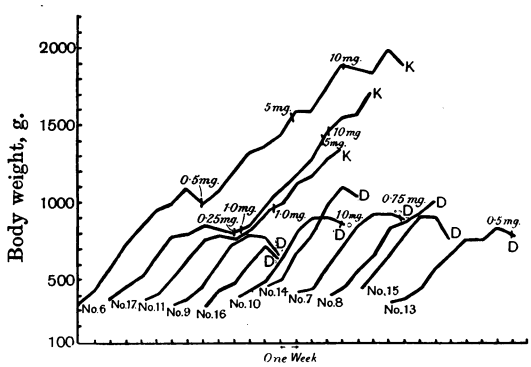

Fig. 3. Growth curves of birds receiving carotene and controls. The figures indicate the daily doses. D = died. K = killed.

normal and fully developed. No. 6 commenced to lay at the age of 23 weeks, having then been fed for 17 weeks on the synthetic diet and in the next month which followed before it was killed laid 20 eggs ranging in weight from 34·5 g. to 59 g. with an average weight of about 40 g. The shells of these eggs were brown in colour but the yolks were almost colourless, possessing only a faint yellow tinge when hard boiled.

The livers of all the birds were examined for the presence of vitamin A both colorimetrically by $SbCl_3$ and spectroscopically. In addition, representative liver oils were despatched to Cambridge in evacuated tubes and tested biologically for vitamin A on rats by Dr Moore. The results of the examination of the liver oils are shown in Table III.

Table III.

No. of chicken	Diet during final weeks	Wt. of liver (g.)	Wt. of liver oil (g.)	Total "blue units" found in liver	"Blue units" per g. of liver	Total "yellow units" in liver	Absorption spectrum
0	Basal + 25 mg. C.L.O. conc.	42	0·77	8,250	190	50	Band at 325 μμ
1	Basal + 0·5 mg. C.L.O. conc.	30	0·22	0	0	8	No band near 325 μμ
2	Basal + 25 mg. C.L.O. conc.	63	0·59	15,100	240	30	Band at 325 μμ
3	Basal + 10 mg. C.L.O. conc.	34	0·23	11,000	320	15	—
4	Basal + 0·5 mg. C.L.O. conc.	22	0·16	0	0	8	No band near 325 μμ
5	Basal + 0·5 mg. C.L.O. conc.	25	0·19	0	0	15	—
6	Basal + 10 mg. carotene	41	0·59	8,200	200	100	Band at 325 μμ
7	Basal only	20	0·12	0	0	10	No band near 325 μμ
8	,,	29	0·16	0	0	10	,,
10	,,	25	0·13	0	0	10	No band near 325 μμ
11	Basal + 1 mg. carotene	28	0·24	250	9	29	—
13	Basal only	22	0·14	0	0	8	No band near 325 μμ
14	,,	24	0·09	0	0	15	,,
15	,,	22	0·21	0	0	—	—
16	,,	17	0·08	0	0	10	No band near 325 μμ
17	Basal + 10 mg. carotene	44	0·19	1,300	30	35	Band at 325 μμ

The liver oils of chickens which had received the same diet gave similar absorption spectra. Typical examples of the absorption spectra of the liver oils are shown in Fig. 4, and in Fig. 5 are shown the growth curves and daily doses of the rats which were given the chicken-liver oils as a source of vitamin A.

It may be pointed out here that when the chickens were first placed on the synthetic diet, after having received a normal diet containing greenstuff, their beaks and shanks were highly pigmented, but after a few weeks on the carotenoid-free synthetic diet the yellow colour faded completely and even after receiving large doses of carotene daily for periods of up to 9 weeks no increase in pigmentation was noted, which is in agreement with the results obtained by Palmer and Kempster [1919] who found that xanthophyll was necessary for the pigmentation of the beak and shanks. In addition, while the liver oil of a hen on a normal diet was found to give deeply pigmented solutions in which absorption bands corresponding to those recorded for xanthophyll were observed, the liver oils of the birds which had received the synthetic diet + carotene gave solutions which were only slightly yellow in colour.

If we take 0·5 mg. carotene as the minimum daily requirement for a hen weighing 2000 g. and compare it with the minimum daily requirement of carotene (0·002 mg.) found by Moore [1930] for a rat weighing 100 g. it is

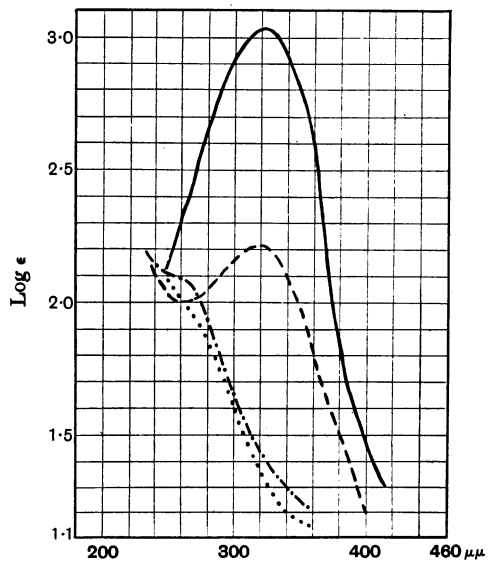

Fig. 4. ——— Liver oil of chicken No. 3 in chloroform.
— — — „ „ 6 „
—·—·— „ „ 8 „
..... „ „ 1 „

ϵ is defined by $\log I_0/I = \epsilon cd$, where c is the concentration of the liver oil in g. per cc., d is the cell thickness in centimetres.

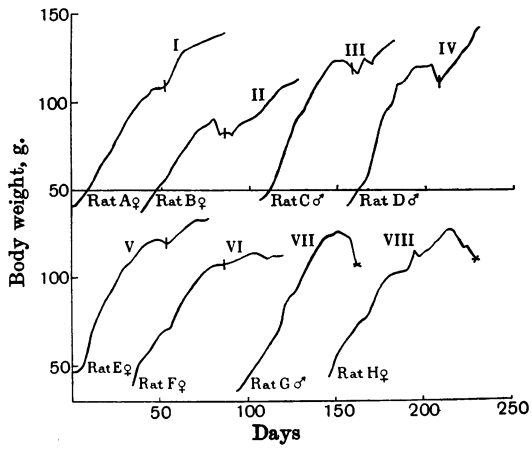

Fig. 5. Confirmation of the vitamin A activity of chicken-liver oils by rat-growth tests.
I and II. Liver oil of bird M 1 (Table I) 0·013 mg. daily = 1 B.U.
III. Liver oil of bird 3 (Table II) 0·2 mg. daily = 2 B.U.
IV. „ „ 0·4 „ = 4 B.U.
V. „ bird 6 (Table (III) 0·4 „ = 5 B.U.
VI. „ bird 11 (Table III) 2·0 „ = 2 B.U.
VII and VIII. Negative controls.

Rats *A*, *B*, *E*, *G* received a basal diet containing Glaxo caseinogen 20 %, rice starch 60 %, palm kernel oil 15 %, salt mixture 5 %, supplemented by 10 % of dried yeast and one drop of radiostol daily. The basal diets of rats *C* and *D* differed in containing a similar amount of arachis oil in place of palm kernel oil and 7·5 % of marmite extract in place of dried yeast, the basal diets of rats *F* and *H* in containing additional starch in place of the fat component. The test doses of chicken-liver oils were diluted in arachis oil and administered at the points indicated in the figure by a vertical line.

seen that the vitamin requirements of the fowl appear to be greater than those of the rat weight for weight.

As misleading results have been obtained in the past by other workers through the use of a solvent in which the carotene altered, it may be stated that the solution of carotene in arachis oil used in the experiments described was kept in a cool dark place when not in use and no decomposition of the carotene was detected when the depth of colour of the solution after some months was measured in a Lovibond tintometer and compared with the colour of a freshly prepared solution.

Discussion.

These experiments support the general theory that in animals carotene behaves as a precursor of vitamin A as suggested originally by Moore [1929, 3]. Even with large doses of carotene the liver oils were only slightly more pigmented than when no carotene was given, but while the liver oils of the fowls fed on the vitamin A-free diet gave no blue colour with the antimony trichloride reagent, no absorption band at 325 $\mu\mu$ and were biologically inactive, those of fowls given carotene in addition gave an intense blue colour (absorption band 610–630 $\mu\mu$) with $SbCl_3$, an absorption band at 325 $\mu\mu$ and proved adequate as a source of vitamin A when fed to rats.

The result of recent work on the relation of carotene to vitamin A makes it clear that biological tests alone cannot distinguish between carotene and the "classical" vitamin A and it would seem probable that vitamin A is a product of animal synthesis and ultimately owes its origin entirely to carotene. Land animals can obtain carotene from vegetable matter, while Ahmad [1930] has shown that carotene in diatoms is probably the source of the vitamin A of fish-liver oils.

Summary.

Chickens were successfully reared to maturity on a synthetic vitamin A-free diet to which either carotene or cod-liver oil concentrate was added. The carotene was not stored in the liver unchanged but was converted into vitamin A characterised by the blue colour given with $SbCl_3$ (absorption band 610–630 $\mu\mu$) and the presence of an absorption band at 325 $\mu\mu$.

The beaks and shanks of chickens, which had become colourless through the absence of carotenoids from the diet, did not become more yellow when carotene was added to it.

The poultry disease known as visceral gout would appear to be related to vitamin A deficiency and to be curable by the administration either of carotene or of cod-liver oil.

The vitamin A content of hen-liver oils is shown to be very high and the vitamin A requirements of the fowl large.

We have pleasure in expressing our thanks to Dr Moore for supplying the carotene used in the experiments, for carrying out the biological tests recorded

and for his unceasing interest in the work; to Imperial Chemical Industries, Ltd., for a grant which helped to defray the cost of the experiments; to Messrs Joseph Nathan, Ltd., for generously supplying the ostelin and cod-liver oil concentrate used, and to Miss A. C. Woods and Miss H. Kennedy of the Agricultural Research Institute of Northern Ireland at Hillsborough, without whose skilful care of the fowl the experiments would have been impossible.

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