XXI. VITAMIN A AND CAROTENE

XVI. THE EFFECT OF THE ADMINISTRATION OF LARGE AMOUNTS OF VITAMIN A ON THE VITAMIN A CONTENT OF THE HEN'S EGG

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THE growth-promoting activity of the yolk of the hen's egg has been known since the early days of vitamin research [McCollum & Davis, 1913; Osborne & Mendel, 1914]. More recently the presence of vitamin A itself, as opposed to carotene or other carotenoids capable of acting as provitamins, was established by Euler & Klusmann [1932; 1933]. This observation has been confirmed by Gillam & Heilbron [1935], who have ascribed the vitamin A activity of the egg yolk to the combined actions of vitamin A, carotene and kryptoxanthin. It may be recalled that the ability of the hen to convert carotene into vitamin A was established by Capper *et al.* [1931]. The pigments of the xanthophyll group, which normally account for the greater part of the yellow colour of the yolk, are inactive as sources of vitamin A activity [Kline *et al.* 1932].

The influence of dietary vitamin A on the vitamin A content of the egg appears to have been first established by Bethke *et al.* [1927]. They found that the addition of 2% of cod liver oil to a diet otherwise low in the vitamin increased the vitamin content of the eggs some fivefold, as estimated by biological tests. A similar increase was found when the hens were allowed access to pasture. Recently Russell & Taylor [1935] have reported that the introduction of alfalfa into the diet of hens caused an increase in the vitamin A output in the eggs, even though the basal diet itself contained 1% of cod liver oil. These workers estimated that the amount of vitamin passing into the egg varied between 11 and 32% of the amount present in the diet. Gillam & Heilbron found that the vitamin A content and the total carotenoids were higher in grass-fed than in maize-fed hens.

The present paper owes its origin to an opportunity which occurred in the course of other work to examine the effect of the intensive administration of cod liver oil on the vitamin A content of the hen's egg. The effect of the administration of a vitamin A concentrate has also been investigated. The results obtained have confirmed the view that the vitamin A content of the egg may be increased by the inclusion of large amounts of vitamin in the diet. At the same time it is clear that the amount of vitamin transferred to the egg is limited and may represent only a very small percentage of the amount made available in the diet.

EXPERIMENTAL

Collection of eggs. The eggs examined were obtained from Light Sussex hens which had been reared on free range on a normal stock ration, which had the

following composition: bran 40, yellow maize 20, sharps 20, Sussex ground oats 20, soya bean meal 7%, mineral mixture 3%, cod liver oil 1%. During the experiments the birds were kept in laboratory pens and trap-nested, so that the effect of the supplements on the eggs of individual birds could be ascertained.

Estimations of vitamin A content and of total carotenoid content of eggs. The yolks were ground up with anhydrous Na_2SO_4 and extracted in a Bolton and Revis extractor with anhydrous ether. The solvent was evaporated, and the egg fat obtained dried at 100° under partial vacuum. Portions of the fat were saponified with alcoholic KOH and the unsaponifiable residue extracted with ether. The residue after evaporation of the ether was dissolved in chloroform. Vitamin A was estimated by treating portions of this solution with the antimony trichloride reagent, the blue colour produced being matched in a Lovibond tintometer.

The total carotenoid content was measured by matching the yellow colour of the original solution in the tintometer. Since knowledge of the carotenoid content was only required to permit an estimate of the percentage of the antimony trichloride blue colour not attributable to vitamin A, it was not thought necessary to fractionate the carotenoid fraction into the individual pigments. Yellow and blue units have been calculated according to the scheme adopted uniformly throughout this series [Moore, 1930].

Vitamin A contents of the eggs and livers of hens on normal rations. For purposes of comparison with the eggs and liver reserves of birds ingesting large amounts of vitamin A, the vitamin contents of the eggs and livers of three Light Sussex birds receiving normal rations were investigated. The data obtained for the eggs are given in Table I. The birds on diet A were on free range and had been reared on the stock ration already described. The birds on diet B were reared in confinement on a ration in which 5% of dried alfalfa provided the main source of vitamin A activity.

Table I

No. of hen	Diet	Total carotenoids Y.U. per g. fat	Vitamin A in the egg			
			B.U. per g. fat		B.U. per whole egg	
			Total	Vitamin A	Total	Vitamin A
			B.U.	B.U.	B.U.	B.U.
74	A	170	80	63	440	347
7	A	350	120	85	660	468
314	B	550	170	115	935	633

Table II shows the vitamin contents of the livers of six birds receiving a normal ration, viz. the stock ration with access to grass. With the exception of hen 87 none of the birds was laying. Considerable individual variations are shown, but substantial reserves were observed in all instances.

Table II. *Vitamin A and total carotenoid contents of the livers of hens on a normal diet*

No. of hen	Y.U.		B.U.	
	Per g.	Whole liver	Per g.	Whole liver
13	40	1500	4200	160,000
87	110	7000	3500	230,000
—	12	1200	1000	44,000
46	23	800	5100	180,000
133	130	4000	4800	150,000
144	70	3000	2300	97,000

The effect of the intensive administration of cod liver oil on the vitamin A content of eggs and on the distribution of vitamin A in the body. During the pre-experimental period the birds received a basal ration designed to contain only small amounts of carotenoid pigments and having the following composition: ground wheat 25 parts, bran 20 parts, middlings 30 parts, Sussex ground oats 15 parts, dried skim milk 10 parts, mineral mixture 3%.

After the basal mixture had been given for 20 days the egg yolks appeared almost colourless. 10% of cod-liver oil (Engelberg's medicinal) was then added to the diet, the addition being continued for 14 days. Eggs were selected for analysis at intervals during the preparatory cod liver oil and post-cod liver oil periods.

The results obtained for the eggs of one individual (hen B 117) are given in Table III.

Table III. *Effect of the intensive feeding of cod liver oil on the vitamin A content of the egg (hen B 117)*

Date of laying of selected egg	Total carotenoids y.u. per g. fat	Vitamin A ingested as cod-liver oil B.U. per day	Vitamin A in the egg			
			B.U. per g. fat		B.U. per whole egg	
			Total B.U.	Vitamin A B.U.	Total B.U.	Vitamin A B.U.
4 March	50	0	50	45	275	247
9 March	30	0	50	47	275	258
10 March	25	0	50	47.5	275	261
					Mean	255

4 eggs in all were laid during above period. 10% cod liver oil was introduced into the diet on 11 March.

13 March	20	20,000	65	63	360	346
17 March	15	20,000	100	98	550	539
22 March	20	20,000	110	108	605	594
23 March	20	20,000	150	148	825	814
25 March	20	20,000	100	98	550	539
					Mean	566

9 eggs in all were laid during above period. Cod liver oil was discontinued on 25 March. Total vitamin A ingested as cod liver oil = 280,000 B.U. Total vitamin A included in eggs (from mean B.U. for eggs examined) = 5000 B.U.

28 March	20	0	110	108	605	594
2 April	25	0	65	62.5	358	344
5 April	10	0	20	19	110	104
7 April	20	0	40	38	220	209
9 April Egg:						
In shell gland	20	0	60	58	330	319
Ova	16	0	40	38.4	—	—
					Mean	314

There were 11 eggs between 25 March and 9 April, including that contained in the shell gland.

N.B. All values calculated from Lovibond readings have been given as the nearest round numbers. The values given for the "vitamin A B.U." have been calculated by deducting an allowance equal to 1/10 of the yellow units, and have been left without "rounding off" so that the extent of the necessary correction may be clear. In calculating the B.U. per whole egg the weight of fat per yolk has been taken as 5.5 g. The amount of vitamin A ingested as cod liver oil has been calculated on the basis of a daily food intake of 4 oz. (=114 g.).

It will be seen, as would be expected from the nature of the basal ration, that the total carotenoid content of the egg yolks fell throughout the whole experimental period. The vitamin A content rose to about double the original level during the period in which cod liver oil was given, but fell back to about this

level when the cod liver oil was discontinued. It may be noted that even in the case of eggs obtained before the commencement of the administration of the cod liver oil the band at $620\text{ m}\mu$, characteristic of the blue colour given by vitamin A in the antimony trichloride reaction, could be plainly seen. This band became more distinct when cod liver oil was given. A fainter band at about $585\text{ m}\mu$ was also present in some instances. Essentially similar results were obtained with eggs from another hen receiving similar treatment.

During the post-cod liver oil period, after 14 days on the basal mash, the bird was killed, and the organs and body fat were examined for vitamin A. The results are shown in Table IV.

Table IV. *The effect of the intensive feeding of cod liver oil on the vitamin A content of organs and body fat*

Hen B 117	B.U. per g.	Total B.U.
Liver	8730	380,000
Kidneys	90	875
Lungs	—	0
Heart	—	0
Spleen	—	0
Gizzard (without lining)	—	0
Lining of gizzard	—	0
Food from gizzard	—	0
Internal fat	22	—
Subcutaneous fat	23	—

It may be noted that the vitamin A reserve of the liver was considerably higher than that found in hens on the normal diet (Table II). Vitamin A was also found in smaller amounts in the kidneys and body fat. Traces of yellow pigmentation, insufficient to produce a blue colour in the antimony trichloride reaction, or to necessitate a correction in the amount of the blue colour of the liver or kidney attributable to vitamin A, were found in all organs. Except in the sites mentioned above, no vitamin A could be detected. It is noteworthy that the amount of vitamin A present in the body fat, viz. 22 B.U. per g., was much less than that contained in the egg fat.

The effect of the administration of massive doses of vitamin A concentrate on the vitamin A content of eggs and on the distribution of vitamin A in the body. Before the administration of concentrate the hen (No. 7) selected for this experiment

Table V. *Effect of administration of vitamin A concentrate on the vitamin A content of the egg (hen 7)*

Date of laying of selected egg	Total carotenoids y.U. per g. fat	Vitamin A ingested as concentrate B.U. per day	Vitamin A in the egg			
			B.U. per g. fat		B.U. per whole egg	
			Total B.U.	Vitamin A B.U.	Total B.U.	Vitamin A B.U.
28 March	350	0	120	85	660	468
At the above period the bird was receiving the stock ration containing 1% cod liver oil with access to grass. Administration of concentrate commenced on 24 April.						
5 May	60	400,000	240	234	1320	1287
6 May	50	400,000	250	245	1375	1347
8 May	—	400,000	250	—	1375	—
9 May (ova)	30	400,000	230	227	—	—

Nine eggs in all were laid during the above period. Administration of concentrate was discontinued on 9 May. Total amount of vitamin A ingested as concentrate = 5,000,000 B.U. Total vitamin A included in eggs from mean blue value of eggs examined = 12,700 B.U.

was receiving the stock ration and had access to grass. The concentrate used was kindly provided by Messrs Lever Brothers, Ltd., and contained 75 B.U. per mg. It was administered orally by pipette for 14 days at the rate of about 5 g. per day. One of the eggs laid during the preparatory period and the last 3 eggs of the 9 laid during the experimental period were selected for analysis. The results obtained are shown in Table V.

The vitamin potency and the total carotenoid content of the egg of hen 7 during the pre-experimental period were considerably higher than in the case of hen B 117, presumably owing to the greater vitamin A and carotenoid contents of the ration used. During the experimental period the total carotenoid content fell very markedly, while the vitamin A content rose to approximately three times the original level, the maximum reached being more than twice that obtained under intensive cod liver oil feeding.

The distribution of vitamin A in the body of hen 7, which was killed at the end of the period of concentrate administration, is shown in Table VI. As in the case of hen B 117 traces of yellow pigmentation were found in all organs.

Table VI. *The distribution of vitamin A in the body after the administration of massive doses*

Hen 7	B.U. per g.	Total B.U.
Liver	11,000	500,000
Kidneys	340	6,300
Lungs	1.6	20
Heart	0.6	5
Spleen	1.9	50
Gall bladder	—	40
Gizzard (without lining)	1	30
Lining of gizzard	0	0
Food from gizzard	0	0
Muscle	0.6	10
Faeces	2,400	150,000 in 63 g. (one day's excretion)
Internal fat	100	—
Subcutaneous fat	110	—

The administration of massive doses of vitamin A resulted in an extremely high total reserve in the liver, viz. 500,000 B.U., which is approximately 50% more than that found in the liver under intensive cod liver oil feeding. The kidneys contained significantly more vitamin A than did those of hen B 117 and traces were found in the other organs. In the body fat the vitamin A content was five times as high, though here again the concentration was much less than that found in the egg fat.

Considerable amounts of the vitamin A ingested by hen 7 were evidently being excreted, 150,000 B.U. being present in the faeces during the last day of administration of concentrate. Assuming that similar amounts of the vitamin were being eliminated daily, a total of about 2,000,000 B.U. would have been excreted, i.e. about two-fifths of the total intake. Towards the end of the period the hen displayed a disinclination to stand. She consumed considerable amounts of water and the excreta appeared very moist. On post mortem examination the organs appeared normal, with the exception of the kidneys, the surface of which showed a considerable number of small petechiae.

DISCUSSION

The effect of dietary addition of vitamin A in increasing the vitamin A content of the egg. The above results afford confirmation of the view that the vitamin A content of the egg may be raised by the inclusion of large amounts of the vitamin in the diet. Judged by the extent of the increase above the former level the rise may be considerable, amounting to 100–300 % in the present experiments. From the results on hens given cod liver oil the increase in the vitamin A content of the egg appears to be of a temporary character, occurring only during the actual administration of the oil. The removal of the oil from the diet caused a rapid decline of the vitamin content of the egg to the level found before the administration of oil was begun, in spite of the presence in the liver of a very high reserve of vitamin. This reserve, if mobilized, would have sufficed to have maintained the vitamin A content of the egg at the raised level for about 300 ovulations.

The following table shows the amount of "pure" vitamin A, as characterized by the data of Carr & Jewell [1933], found in eggs from hens on various rations. The values have been calculated on the assumption that pure vitamin A has 4300 B.U. per mg., and allowance has been made for the proportion of the blue colour due to carotenoid pigments in each instance.

Table VII. *Vitamin A content of eggs from hens on different diets*

No. of hen	Diet	"Pure" vitamin A in mg. per 100 g. fresh yolk (containing 30 % of fat)
B 117	Basal	0.33
B 141	Basal	0.35
7	1 % cod liver oil + grass	0.6
74	1 % cod liver oil + grass	0.45
314	5 % alfalfa	0.8
B 117	10 % cod liver oil	0.65
B 141	10 % cod liver oil	0.60
7	100 mg. "pure" vitamin A daily as concentrate	1.7

If the level of vitamin A found in the eggs of hens receiving only the basal diet is taken as a basis of comparison it will be seen that the addition of 10 % of cod liver oil, or of 5 % of alfalfa to the diet, resulted in the vitamin A content of the eggs being doubled. The administration of massive doses of concentrate caused a fivefold increase.

The amounts of vitamin A found in the present work, even in eggs from hens receiving the basal diet alone, were much higher than those reported by Gillam & Heilbron [1935], who obtained values of 0.05 and about 0.1 mg. of "pure" vitamin A per 100 g. of fresh yolk for maize-fed and grass-fed hens respectively. This difference may be due in part to differences in dietary history, since although the birds used in the present work were ingesting little or no vitamin A or carotene while on the basal ration, they had previously received the normal stock diet, with 1 % of cod liver oil and access to grass. The liver reserves if available must therefore have been sufficient to maintain the vitamin A content of the eggs at a fairly high level.¹ It may be noted however that the methods adopted

¹ As shown above a high liver reserve will not maintain the vitamin A reserve at the raised level found during the intensive vitamin A administration. It does not follow, however, that the reserve will not be mobilized for transference to the egg during dietary deficiency of the vitamin.

for estimating the vitamin by ourselves and by Gillam & Heilbron were essentially different. The procedure adopted by the latter workers in fractionating their unsaponifiable matter on an adsorption column is possibly open to the criticism that the recovery of the vitamin A fraction may have been incomplete.

A rough approximation of the vitamin A content of eggs from hens receiving ordinary dietaries may be deduced from the data on eggs laid by hens 74 and 7 (Table I) which received a normal ration supplemented with 1% of cod liver oil and access to grass. Taking the combined carotene and kryptoxanthin contents as 5% of the total carotenoids, and the international unit of vitamin A as equivalent to 1.2 Y.U. of carotene, and to 1.67 B.U. of vitamin A, the total vitamin A contents of these eggs may be calculated as 45 and 65 I.U. per g., or 250 and 350 I.U. per whole egg for the eggs laid by hens 74 and 7 respectively. The vitamin A content of egg yolk fat therefore appears to be equal to or perhaps slightly superior to that of good butter fat.

The proportion of the dietary vitamin A transferred to the egg. Whilst it is evident that the intensive administration of vitamin A greatly increases the vitamin content of the egg it must be realized that the amount transferred to the egg under these conditions represents only a small percentage of the dietary intake. Thus in the case of hen B 117 (Table III) cod liver oil equivalent to 280,000 B.U. was administered over 14 days. During this period 9 eggs were laid, calculated to contain a total of 5000 B.U. of vitamin A. The proportion of vitamin A transferred to the egg was therefore only some 2%. In the case of hen 7, which received concentrate equivalent to about 5,000,000 B.U. during the experimental period, 9 eggs containing in all 13,000 B.U. of vitamin A were laid. The proportion secreted in this instance was thus only 0.2%. Since in this experiment a considerable proportion of the ingested vitamin was recovered in the faeces the amount of vitamin A transferred to the egg, when calculated in reference to the amount of vitamin absorbed from the alimentary tract, would have represented a somewhat higher percentage.

These percentages are much smaller than those reported by Russell & Taylor [1935], who found that the output of vitamin A in the eggs of hens on normal rations containing 1% of cod liver oil varied between 11 and 32% of the dietary intake. It is obvious therefore that the proportion passing to the egg decreases rapidly as the dietary intake increases, and that there is no fixed proportionality which can be regarded as applicable under all conditions of nutrition.

The distribution of vitamin A throughout the body after the intensive administration of vitamin A. From Tables IV and VI it will be seen that the administration of both cod liver oil and of vitamin A concentrate resulted in accumulation of very high vitamin A reserves in the liver, and of a smaller amount of vitamin in the kidneys. In the case of the bird given cod liver oil a small concentration of the vitamin was detected in the stored fat deposits, but not in other sites. In the bird given concentrate traces of vitamin were found in many organs. These results are in close agreement with those obtained by Moore [1931] and by Davies & Moore [1934] in similar experiments on the rat, and with general experience on other mammals. It is noteworthy that in both the above hens the vitamin A concentration in the egg fat was decidedly greater than in either the interperitoneal or subcutaneous fat deposits.

The role of the liver in the vitamin A economy of the hen. The predominance of the liver as the main site of storage of vitamin A in the hen, reviewed in conjunction with the decreasingly small proportion of vitamin A passing into the egg with increasing dietary intake, recalls the similar predominance of the role

of the liver in the mammal and the limitation of the amount of vitamin A passed to the young animal, both prenatally and during lactation. Thus Moore [1932], Fraps and Treichler [1932] and Watson *et al.* [1934] have shown that the amount of vitamin A secreted in the milk of the cow does not exceed a restricted upper limit, which represents only a small fraction of the potential vitamin A ingested by the cow as carotene. In the case of the rat Dann [1932; 1934, 1, 2] has concluded that both the vitamin stores of the embryo and the amount of vitamin A secreted in the milk are increased only to a limited extent when large amounts of carotene or vitamin A are ingested by the mother. It appears therefore that the restriction of the amount of vitamin A made available to the chick during its early stages of development is in accordance with the general rule applying to mammals.

If it is agreed, as the work of Russell and Taylor strongly suggests, that the liver reserves may be mobilized for transference to the egg during dietary deficiency of the vitamin, we may picture the liver as a buffer between the maternal food supply on the one hand, and the rest of the maternal organism with the developing egg on the other. Thus it is capable not only of effecting the transfer of vitamin A to the egg but of restraining the free passage of the vitamin to the egg in face of dietary excess. This finding may be contrasted with results obtained by Branion *et al.* [1933] who have found that the passage of vitamin D to the egg is much less restricted. Thus a fivefold increase in the cod liver oil content of the diet doubled the vitamin D content of the egg, while a hundredfold increase of dietary vitamin D in the form of calciferol increased the content in the egg thirty times. By the intensive feeding of calciferol the vitamin D content of the egg was raised to 18,000 Steenbock units, with loss of hatchability. These results serve to emphasize the particular affinity of the liver for vitamin A, and its role in conserving superfluous supplies.

The vitamin A reserves of hens kept on an ordinary farm dietary, with 1% of cod liver oil and access to grass (Table II), are of interest in view of their high order of magnitude (median value *ca.* 4000 B.U. per g.). This may be appreciated by comparison with the 400 B.U. per g. taken as the typical reserve in human beings. These values are similar to those found by Capper *et al.* [1931] for fowls on normal rations, but are considerably higher than those reported by Russell & Taylor [1935]. The birds used by the latter workers were kept in confinement but were given diets containing 1% of cod liver oil and sometimes 5% of alfalfa.

SUMMARY

1. The administration of cod liver oil to a laying hen at the rate of 10% of the diet, which was otherwise low in vitamin A and carotene, caused the vitamin A content of the eggs, as determined by the antimony trichloride method, to be doubled. The vitamin A content of the egg resumed its former level soon after the withdrawal of the cod liver oil from the diet.

2. The intensive administration of a vitamin A concentrate raised the concentration of vitamin A in the egg to about five times the level found on the basal diet alone. In this experiment the limits of absorption by the intestine appeared to have been reached, since a considerable proportion of the ingested vitamin was recovered from the excreta.

3. In spite of these considerable increases in the vitamin A content of the eggs the proportion of the vitamin A ingested which appeared in the eggs amounted to only 2 and 0.2% for cod liver oil and concentrate feeding respectively. The transference of vitamin A to the egg is therefore limited,

and decreases as the intake increases. This limited transference of vitamin A to the egg recalls the similar limitation which is usually found in the amounts of vitamin A made available to the young mammal during pregnancy and lactation.

4. At the conclusion of the egg-laying experiments the hens were killed and the distribution of vitamin A in the various organs determined. High concentrations of vitamin A were found in the liver. Much lower, but still considerable, concentrations were found in the kidneys. In the other organs examined negative results were obtained in the case of the bird given cod liver oil, while small traces were found in some organs of the bird given concentrate. These results suggest that the role of the liver in the vitamin A economy of the bird is essentially the same as in the mammal.

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