CXXXIV. VITAMIN A-ACTIVE SUBSTANCES IN EGG-YOLK.

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Although biological experiments by numerous workers have shown that egg-yolk exhibits the growth-promoting properties usually associated with vitamin A, no wholly satisfactory explanation of the source of this activity has yet been advanced.

The presence of carotene in egg-yolk was first suggested by Willstätter and Escher [1911], being later confirmed by Kuhn and Brockmann [1932] in several varieties of hen's eggs, whilst the detection of vitamin A itself was claimed by Euler and Klussman [1932; 1933] using the antimony trichloride blue test as a criterion. As, however, these last authors were unable to detect with certainty the absorption band at 328 $m\mu$ characteristic of vitamin A, and as no details were given of any steps taken to remove xanthophylls before applying the antimony trichloride blue test, the results need confirmation. The detection of vitamin A in egg-yolk presents some difficulty on account of the smallness of the gross vitamin A activity (some of which is due to carotene), and the large amounts of lutein and zeaxanthin normally present in the yolk. As both of these latter substances absorb ultra-violet rays near 328 $m\mu$ and also give blue colours with antimony trichloride qualitatively similar to that given by vitamin A, the two main criteria for the vitamin are seriously interfered with unless a separation from the carotenoids is first obtained.

Experimental.

Vitamin A. In a preliminary experiment designed to detect the presence of vitamin A in egg-yolk the following procedure was tried. The gross unsaponifiable matter of thirty egg-yolks, after removal of sterols, was dissolved in light petroleum and about 10 mg. of a genuine vitamin A concentrate were added to one-half of the solution, whilst the other was left untouched. Both extracts were carefully treated with animal charcoal until the yellow colour of the carotenoids was just removed. Each solution was filtered and evaporated *in vacuo*, the residue being dissolved in chloroform and tested for vitamin A both by means of its ultra-violet absorption spectrum and by the antimony trichloride blue test. Whilst the genuine egg extract failed to give any positive data for the vitamin by either test, the one containing added vitamin A gave definite evidence of its presence. The conclusions to be drawn from this experiment are either that egg-yolk contains no vitamin A, or that, if present at all, the amount must be so small that a much finer method of separation is needed to detect it. With this object in view the following alternative procedure was adopted. The yolks of 60 eggs were mixed with anhydrous sodium sulphate and completely extracted with ether. After saponification of the extract with alcoholic potash the unsaponifiable portion was taken up in ether and the solvent-free residue crystallised from methyl alcohol. The sterols (18 g., m.p. 147°) were filtered off and the carotenoids transferred to light petroleum (B.P. 70–80°), the solution then being extracted 10–15 times with 90–92 % methyl alcohol. In this manner a light petroleum phase (A) containing only the carotenes [Kuhn and Brockmann, 1932] and a methyl alcoholic phase (B) containing the lutein, zeaxanthin and the major part of the vitamin A [Wolff *et al.*, 1930] were obtained. The constituents of B were again transferred to light petroleum (B.P. 40–60°), washed with water, dried and adsorbed on a column of calcium carbonate. The chromatogram was developed with petroleum-ether-benzene, a procedure whereby the xanthophylls would remain on the column while any vitamin A would pass through unadsorbed [Karrer and Schöpp, 1932]. The filtrate was evaporated *in vacuo* and the residue taken up in chloroform. On spectroscopic examination the solution exhibited a definite absorption band at 328 $m\mu$, whilst with antimony trichloride in chloroform a blue colour was obtained showing an obvious 618 $m\mu$ band together with the usual masked band at 583 $m\mu$ [Castle *et al.*, 1934]. The vitamin A in this particular batch of eggs was of the order of 0·05 mg. per 100 g. of yolk, and is probably lower than normal as the eggs were obtained from hens fed on a heavy maize ration, the yolks being paler than usual. In the yolks produced by hens on a richer grass diet approximately twice this amount of vitamin was found to be present. These experiments therefore provide definite confirmatory proof of the claim of Euler and Klussmann [1932; 1933] that vitamin A occurs in egg-yolk.

The carotene fraction. The dried light petroleum solution (A) obtained in the phase separation was passed through an alumina column and washed down with light petroleum. The resulting chromatogram indicated the presence of more than one carotenoid, usually as a broad yellow-brown zone near the top, a strong yellowish ring below and a weak pink-brown ring lower still. Each ring was separately eluted with methyl alcohol and spectroscopically examined, with the result that they were found to exhibit absorption maxima identical with each other and with those of authentic β-carotene. The slight difference in their adsorption behaviour suggests that the upper yellow-brown ring is due to kryptoxanthin [Kuhn and Grundmann, 1933] whilst the lower one is due to the typical

Table I. *Alumina adsorption of "carotene" fractions from the egg-yolks of maize-fed hens.*

	Zone 1	Zone 2	Zone 3
Colour of adsorbed pigment	Brownish yellow	Strong yellow-brown with bright yellow background	Several weak pink bands
Location in column	Very near the top	Lower down than 1	Below 2
Absorption spectrum of the pigment in light petroleum (B.P. 70–80°)	Indefinite	Bands at 478 and 450 $m\mu$	Bands at 480 and 451 $m\mu$
Phase tests:			
1. Petroleum and 90 % CH_3OH	Mainly alcohol-phasic	Wholly petroleum-phasic	Wholly petroleum-phasic
2. Petroleum and 95 % CH_3OH	Mainly alcohol-phasic	Partly alcohol-phasic	
Adsorption on $CaCO_3$	Held strongly	Weakly adsorbed as a yellow band	Unadsorbed
Nature of pigment	Xanthophylls *plus* oxidation products	Kryptoxanthin	Carotene
Relative amounts present	—	13	1

β-carotene. The presence of the former carotenoid in egg-yolk is almost certainly attributable to the ingestion of maize, which contains kryptoxanthin [Kuhn and Grundmann, 1934] in addition to zeaxanthin [Karrer *et al.*, 1929]. The presence of kryptoxanthin in egg-yolk was suggested as a possibility (although not submitted to test) by Brockmann and Völker [1934] to explain the relatively high values obtained by Euler and Klussmann [1933] for the carotene content of of egg-yolk.

In order to confirm, if possible, the occurrence of kryptoxanthin in egg-yolk we obtained eggs from hens specially fed on a rich maize diet, and extracted 60 yolks according to the above scheme. In this case the adsorption column exhibited three main coloured zones, the characteristics of which are given in Table I. These results and a comparison with authentic kryptoxanthin extracted from *Physalis* by the method of Kuhn and Grundmann [1933] confirm the presence of this pigment. A further comparison of the kryptoxanthin with lutein and β-carotene shows that its adsorption affinity is intermediate between those of these two substances, as its constitution and observed properties would lead one to expect.

Quantitative evaluation of the pigments in several batches of eggs gave the results shown in Table II.

Table II.

Batch no. ...	1	2	3	4	
No. of eggs	60	17	24	24	
Diet of hens	Heavy maize ration	Grass	No grass	Grass	
Total carotenoids	2·0	4·2	4·4	11·0	mg. per 100 g. yolk
Kryptoxanthin	0·19	0·14	0·013	0·17	mg. per 100 g. yolk
Carotene	0·015	0·02			mg. per 100 g. yolk
Vitamin A	Order 0·05	Order 0·10	—	—	mg. per 100 g. yolk
% kryptoxanthin in total carotenoids	9·5	3·3	—	—	

It is thus evident that the "carotene" fraction of egg-yolk consists largely of kryptoxanthin, which is remarkably similar in properties to β-carotene and easily mistaken for it.

Summary.

Using chromatographic methods with spectroscopic control an examination has been made of the unsaponifiable matter of egg-yolk with a view to elucidating the cause of its growth-promoting activity. After a phase test separation of the carotene from the xanthophyll, followed by adsorption of the latter on calcium carbonate, it has been possible to obtain the vitamin A free from carotenoids and to confirm its presence both by the antimony trichloride blue test and by the characteristic absorption band at 328 $m\mu$. The petroleum-phasic carotenoids have also been examined and found to consist of kryptoxanthin and β-carotene. By feeding fowls on a diet rich in maize it has been found possible to increase the kryptoxanthin content of the resulting egg-yolks, although the final value for the content of this vitamin A-active carotenoid in egg-yolk is still very small (order 0·2 mg. per 100 g. yolk).

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