CCXCIII. CAROTENOIDS AND VITAMIN A IN COW'S BLOOD SERUM.

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It has been shown that cow's blood serum [Fine, 1933] possesses the power of promoting growth when fed to rats on a vitamin A-free diet. Part at least of this activity must be due to carotene, which can act as a provitamin A [Euler and Hellström, 1928; Euler *et al.*, 1929] and, together with bilirubin, is mainly responsible for the golden yellow colour of blood serum [Palmer and Eccles, 1914; Palmer, 1922].

In addition to carotene the presence of vitamin A in blood serum has been demonstrated by means of the antimony trichloride blue test [Rösio, 1929; Eekelen, 1931; Euler and Virgin, 1932; Lundborg, 1933; Menken, 1934; Eekelen and Emmerie, 1935]. Rosenthal and Szilard [1935] have confirmed this by a test which is specific for the vitamin even in the presence of carotene [Rosenthal and Erdélyi, 1934, 1, 2]. Still further confirmation is supplied by the observation that an alcoholic extract of blood serum exhibits selective light absorption near $325m\mu$ [Chevallier and Choron, 1934].

Since the blood serum of the cow must carry the carotenoids of the food (converted partially into vitamin A at some point on the journey) to the udder, and hence to the milk, we decided to determine the carotene and vitamin A in the blood sera of cattle for comparison with the values previously found for milk fat [Gillam *et al.*, 1933; Watson *et al.*, 1933; 1934].

The present paper records the results of a preliminary examination of a number of cow and bull sera using spectrophotometric methods for determination of both carotene and vitamin A.

Extraction of the unsaponifiable matter.

100 ml. of the blood serum are heated on a water-bath (1 hour) with aqueous KOH (10 ml. 60%) [cf. Davis, 1933]. The mixture is cooled, treated with alcohol (30 ml.) and extracted with ether (50 ml.) four times. The combined ether extracts are washed with water and dried over sodium sulphate and the ether is removed in a stream of nitrogen. The residue is dissolved in chloroform and made up to 100 ml. When the quantity of serum available is limited, much smaller portions can be used if necessary.

Determination of the carotene and vitamin A.

The absorption spectrum of the solution is now determined in the visible and ultraviolet (Spekker photometer and Hilger E 3 quartz spectrograph), the procedure being identical with that already described in detail in the case of butter [Gillam, 1934]. From the observed intensity of absorption of light at $460m\mu$ the carotenoids present in the serum can be calculated as carotene, using the standard value of $E_{1\text{ cm.}}^{1\%} = 2200$ [Gillam, 1935]. ($E_{1\text{ cm.}}^{1\%} = \log \frac{I_0}{I}$ for a 1 cm. layer of 1% solution, I_0 = intensity of incident light, I = intensity of transmitted light).

The light absorption at $328m\mu$ is due to vitamin A *plus* carotene, but it has been shown that the value due to carotene at this wave-length can be obtained by dividing the observed intensity at $460m\mu$ by the factor 6.5 [Gillam, 1934]. After this correction is applied to the gross absorption at $328m\mu$ the vitamin A present is calculated in mg. per 100 ml. of serum from the equation $\frac{E \times 1000}{16}$, where $E = E_{1\text{cm.}}^{1\%}$, mentioned above. The resulting value is based on the assumption that pure vitamin A has a value of $E_{1\text{cm.}}^{1\%} = 1600$ [Carr and Jewell, 1933; *cf.* also Karrer and Morf, 1933].

In butter the amount of vitamin A present is such that its absorption band is often seen clearly in the absorption curve of the unsaponifiable matter. In blood serum, however, the vitamin A is commonly present in much smaller amounts and the absorption due to the vitamin, although obtainable by subtraction, is not readily seen. In order to test for vitamin A more definitely the light petroleum solution of the carotenoids obtained from 90 ml. of serum was extracted with 90 % methyl alcohol until no more colour was removed. By this means the vitamin A was freed from carotene and the absorption spectrum of the methyl alcohol solution now showed the characteristic vitamin A band at $328m\mu$, together with weakly defined absorption bands at 477 and $447m\mu$, associated with the yellow colour (see below).

The presence of vitamin A was further confirmed by the fact that, when transferred to chloroform and concentrated, the solution gave a blue colour with antimony trichloride, showing a clear absorption band at $617m\mu$ and a subsidiary maximum at $583m\mu$, the intensity being much greater than could possibly be due to the small amount of carotenoid present.

The pigment responsible for the weak yellow colour of the methyl alcohol phase had the following properties:

- (a) hypophasic in 90 % methyl alcohol to light petroleum;
- (b) absorption maxima in chloroform at 488 and $456m\mu$;
- (c) when mixed with carotene and adsorbed on alumina it showed itself as a lemon-yellow ring well above that of the carotene.

These properties agree with those of lutein and the amount present is of the same order as that found in butter, *i.e.* about 5 % of the total carotenoids [Gillam *et al.*, 1933; Gillam, 1934]. This is in agreement with the previously reported occurrence of small quantities of "xanthophyll" in blood serum [Palmer and Eccles, 1914].

In view of the fact that the lutein content of grass is twice that of the carotene, whereas in blood serum the latter constitutes nearly the whole of the carotenoids, it is clear that in the metabolism of the cow carotene is absorbed preferentially [*cf.* Zechmeister and Tuzson, 1934]. In the horse this selective absorption of carotene appears to be more nearly complete than in the cow and is probably effected by the walls of the gut [Zechmeister *et al.*, 1935].

By concentrating the carotene fractions from several litres of cow serum and using the adsorption method already applied to butter [Gillam and Heilbron, 1935] we have found that small amounts of kryptoxanthin occur occasionally in blood serum exactly as in the case of butter. The adsorption of blood serum carotene on alumina also shows the separation of pigment into red and yellow zones previously observed with butter [Gillam and Heilbron, 1935], these two zones exhibiting absorption maxima closely simulating those of β - and α -carotene respectively.

Twenty-one samples of bovine blood sera falling approximately into two groups, which can be regarded as winter and summer samples respectively, were

examined. The results show that the average carotene and vitamin A contents of the sera of cows are consistently higher than those of bulls in both winter and summer, whilst the average values for the sera of cattle in winter are definitely lower than the corresponding ones obtained during the spring and summer.

These results, obtained by the more precise spectrophotometric methods, are in agreement with the findings of Rösio [1929] who, using the Lovibond blue test, has shown that species, age, sex and the presence or absence of gestation are important factors controlling the vitamin A and carotene contents of the sera of animals. Similarly, Lundborg [1933] has shown that the blood serum of the cow is several times richer in vitamin A than that of the ox.

It has been shown that access to carotene-rich fodder increases the carotene content of cow's blood serum, previously at a low level through poor feeding [Semb *et al.*, 1934], just as it does in the case of milk fat. These variations in the quality of the food eaten are undoubtedly the cause of the definitely higher values of the summer sera over those of the winter samples, and it is equally certain that the explanation of the exceptional cases in both the winter and summer groups (*cf.* Tables) is to be found in differences in diet. As, however, the samples examined here were only casual and nothing was known of the nutritional history of the cattle this is necessarily only conjectural until more carefully controlled experiments can be carried out.

Table I. *Carotene and vitamin A values of bovine blood sera*
(“Winter samples”).

Cows				Bulls			
Date	Sample no.	Carotene mg./100 ml.	Vitamin A mg./100 ml.	Date	Sample no.	Carotene mg./100 ml.	Vitamin A mg./100 ml.
22. ii. 35	2	0.17	—	4. iii. 35	5	0.052	0.04
25. ii. 35	3	0.41	0.15	6. iii. 35	6	0.091	0.053
28. ii. 35	4	0.30	0.13	14. iii. 35	8	0.122	0.063
8. iii. 35	7	0.83	0.16	18. iii. 35	9	0.056	0.049
21. iii. 35	10	0.28	0.077	—	—	—	—
Averages		0.40	0.13			0.08	0.051

Table II. *Carotene and vitamin A values of bovine blood sera*
(“Summer samples”).

Cows				Bulls			
Date	Sample no.	Carotene mg./100 ml.	Vitamin A mg./100 ml.	Date	Sample no.	Carotene mg./100 ml.	Vitamin A mg./100 ml.
26. iii. 35	11	0.87	0.28	27. iii. 35	12	0.86	0.17
13. v. 35	13	0.68	0.20	22. v. 35	14	0.16	0.13
14. vi. 35	16	1.22	0.53	14. vi. 35	15	0.94	0.19
14. vi. 35	18	1.05	0.102	28. v. 35	17	0.091	0.056
17. vi. 35	19	1.46	0.31	17. vi. 35	20	0.104	0.08
17. vi. 35	22	1.39	0.34	17. vi. 35	21	0.38	0.23
Averages		1.11	0.29			0.42	0.143

SUMMARY.

An examination of the unsaponifiable matter of cow's blood serum shows that, in addition to the main pigment (carotene), there are also present a small amount of lutein and, as in the case of butter, casual traces of kryptoxanthin. Vitamin A is also present.

The carotene and vitamin A values of a number of samples of cow and bull sera have been determined spectrophotometrically. The results show that the cow sera are on the average several times richer in carotene and vitamin A than are those of bulls, and that both cow and bull sera usually contain more of these two constituents in summer than in winter.

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