CCLXXXV. THE RELATIVE BIOLOGICAL EFFICIENCIES OF THE VITAMIN A AND CAROTENE OF BUTTER¹.

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THERE is still a great deal of uncertainty regarding the relative biological activities of vitamin A and carotene. The subject has been reviewed recently by Moore [1933], who has summarised the findings of various workers and has pointed out the very wide variations in the reported minimum doses of the two substances. On the basis of his own experiments in which he directly compared β -carotene with a vitamin A concentrate from turbot-liver oil, Moore came to the conclusion that β -carotene possesses an activity equal to that of about the same weight of vitamin A of $E_{1\text{ cm.}}^{1\%} 328 m\mu = 1600$. Carr and Jewell [1933] compared their richest vitamin A concentrate ($E_{1\text{ cm.}}^{1\%} 328 m\mu = 1600$) with the International Carotene Standard and found that the concentrate was 1.6 times as active as the Carotene Standard. As, according to Dyer *et al.* [1934], pure β -carotene has about 175 % of the vitamin A potency of the International Standard, the findings of Moore [1933] and Carr and Jewell [1933] are in good agreement.

The problem of determining the relative values of vitamin A and carotene has been approached by us from a different angle. In the course of work on typical English milk, carried out at the National Institute for Research in Dairying, we have compared the vitamin A activities of Guernsey and Shorthorn butters produced under identical conditions of feeding and management. In a preliminary paper the results of biological tests on Guernsey and Shorthorn butters, together with colorimetric determinations of their vitamin A and carotene contents, have been reported [Kon and Booth, 1933]. It was found that Shorthorn butter gives a more intense blue colour with the antimony trichloride reagent than a corresponding Guernsey butter, though, as is well known, the latter is more yellow and contains more carotene. On the other hand we found no appreciable difference in the vitamin A potencies of the butters when tested on rats. It seemed that a biological estimation of the activity of butters containing different amounts of vitamin A and carotene could be combined with

¹ Read (in part) at the Aberdeen meeting of the British Association [Kon, 1934].

a physical determination of the two latter substances to allow of a calculation of the relative biological value of vitamin A and its provitamin (carotene).

The present paper is the result of such a combined examination.

EXPERIMENTAL.

1. *Butters.* The Shorthorn butters were obtained from the typical herd of the Institute. The feeding and management of this herd has already been described [Golding *et al.*, 1933]. The method of churning and rendering the bi-weekly samples of butter has also been described in connection with other work [Booth *et al.*, 1933]. For the purpose of the present experiment the butters were collected at three seasons of the year, namely, in summer 1933, when the cows were out on grass and when 7 bi-weekly samples were collected between May 12th and June 5th; in autumn 1933, when grass was supplemented by silage and kale, 10 samples were collected between September 18th and October 23rd; and in winter 1933-4, during the period of stall feeding, when 5 samples were collected between February 12th and March 1st. At all times the feeding and management of the Guernsey cows were very similar to those of the Shorthorns, whilst during the experimental periods and for some time before, the treatment of the cows was identical.

The butters of both breeds were collected and stored under the same conditions. Aliquot portions of the butters, collected in both the summer and winter periods, were pooled for the purposes of feeding and physical tests. Owing to the fact that the experimental rats were "run out" earlier than was expected the autumn butters had to be fed as bi-weekly samples as they were available but were pooled for the spectrophotometric examination. This was rather unfortunate as the collection of the butters was started towards the end of the drought of that year. The flush of grass following the break in the drought caused a marked and progressive increase in carotene and vitamin A contents (as measured in the Lovibond tintometer) of the butters from both breeds of cows, and it is realised that the spectrophotometric and biological tests are not strictly comparable. This experiment is less reliable than the two others.

2. *Animal tests.* Rats with litters intended for vitamin A tests were transferred on the 16th day of lactation from the stock diet to the vitamin A-deficient diet of Coward *et al.* [1931]¹. The young were weaned at 21 days and "run out" in the customary way.

For the feeding tests the summer and autumn butters were mixed with coconut oil (B.D.H.) and fed six times a week from calibrated dropping pipettes delivering 17 mg. of the mixture per drop. The amount of coconut oil added (28 g. to each 40 g. of butter) was such that one drop of the mixture contained 10 mg. of butter. The winter butter was fed undiluted. In the case of the pooled butters the bulk was stored in the refrigerator at -2° , fresh samples being withdrawn every 3 days. The same applied to the unpooled bi-weekly samples of autumn butter. The International Standard of vitamin A dissolved in coconut oil was fed at the same time as the butters. The feeding experiment lasted 5 weeks, but the results are given for a 3-week period. It has been shown by Coward [1933] that a 3-week period gives results statistically as reliable as those given in the customary 5-week period, and in our experience the shorter experimental period is, at low levels of vitamin A feeding, much less complicated by the death of animals than is the longer period. As far as possible, litter-mates

¹ Dextrinised potato starch was used instead of dextrinised rice starch.

were allotted to corresponding levels of the butters, and in the three experiments the percentage of litter-mates on corresponding levels did not fall below 75. Very much the same percentage applies to the comparisons of the carotene (International Standard of vitamin A) with the butters. The results of the biological tests are given in Table I.

Table I. *Biological tests of the vitamin A activities of Shorthorn and Guernsey butters at different seasons of the year. 3-week test period.*

SUMMER.												
International Standard				Shorthorn butter				Guernsey butter				
1 γ		2 γ		0.02 g.		0.04 g.		0.01 g.		0.02 g.		
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
Initial no. of rats	7	7	6	6	7	7	6	6	6	7	6	
Final no. of rats	6	5	6	6	7	6	6	5	6	4	6	
Average gain or loss (g.)	-1.7	-8.3	+10.7	+3.8	+6.3	+1.3	+22.3	+14.8	-6.0	-11.8	+10.0	+0.3
AUTUMN.												
1 γ		2 γ		0.02 g.		0.04 g.		0.02 g.		0.04 g.		
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
Initial no. of rats	8	8	8	8	8	8	8	8	8	8	8	
Final no. of rats	8	7	7	7	6	8	8	7	7	7	8	
Average gain or loss (g.)	-1.3	-14.1	+5.4	+1.9	-1.5	-6.5	+14.3	+7.8	-6.1	-7.6	+1.7	-1.3
WINTER.												
1.5 γ		3 γ		0.05 g.		0.1 g.		0.05 g.		0.1 g.		
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
Initial no. of rats	8	8	8	8	8	8	8	8	8	8	8	
Final no. of rats	8	8	8	8	8	8	8	8	8	8	8	
Average gain or loss (g.)	+13.3	+7.0	+13.6	+11.7	+15.7	+12.3	+16.0	+20.5	+11.2	+6.6	+16.6	+16.3

An approximate estimate of the number of units of vitamin A present in the experimental butters may be derived from the data of Table I. By plotting for each experiment separately the response against logarithm of dose of standard and interpolating for the butters, values of 85 and 65 units per g. of butter fat can be calculated for the summer and autumn samples of each of the two breeds respectively. The calculation for winter butter is made difficult by the anomalous behaviour of the rats receiving the two levels of International Standard, but a value of about 40 units seems not far from the truth.

Table II. *The carotene and vitamin A values of the butters (spectrophotometric results).*

Sample ...	Shorthorn butter mg. per 100 g. of fat		Guernsey butter mg. per 100 g. of fat	
	Carotene*	Vitamin A†	Carotene	Vitamin A
Summer (May 12-June 5) 1933	0.78	1.10	2.24	0.89
Autumn (Sept. 18-Oct. 23) 1933	0.67	0.93	1.80	0.70
Winter (Feb. 12-Mar. 1) 1934	0.19	0.39	0.50	0.34

* Basis $E_{1\text{ cm.}}^{1\%}$ 460 $m\mu$ = 1900 for pure carotene in chloroform.

† Basis $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ = 1600 for vitamin A [Carr and Jewell, 1933]. Taken provisionally as the pure substance.

3. *Spectrophotometric determination of carotene and vitamin A.* For this purpose each butter sample was saponified and the unsaponifiable matter extracted as previously described [Booth *et al.*, 1933]. It was dissolved in "anaesthetic" ether and transferred without loss to a brown glass ampoule where the solvent was removed by warming in a stream of nitrogen. The ampoule was finally filled with nitrogen and sealed up to await spectroscopic examination. The carotene content of the butter was calculated from the intensity of absorption of the chloroform solution of the unsaponifiable matter at $455\text{--}460m\mu$ as measured on a Hilger Nutting spectrophotometer. The intensity reading was corrected for xanthophyll by subtracting 6 % of the gross value [cf. Gillam, 1934].

The vitamin A was determined from the intensity of absorption of the chloroform solution at $328m\mu$, due allowance being made for the absorption of carotene and xanthophyll at this wave length [Gillam, 1934]. The results are shown in Table II.

DISCUSSION.

The results of the biological tests reported above show that no appreciable difference exists between the vitamin A activities of Shorthorn and Guernsey butters, and that this relationship persists throughout the year. Thus the following equations can be written:

- (1) Summer experiment. 0.78 part of carotene + 1.10 parts vitamin A = 2.24 parts carotene + 0.89 part vitamin A. Hence 1 part vitamin A = 7 parts of carotene.
- (2) Autumn experiment. 0.67 part of carotene + 0.93 part vitamin A = 1.80 parts of carotene + 0.70 part of vitamin A. Hence 1 part of vitamin A = 5 parts of carotene.
- (3) Winter experiment. 0.19 part of carotene + 0.39 part of vitamin A = 0.50 part of carotene + 0.34 part of vitamin A. Hence 1 part of vitamin A = 6.2 parts of carotene.

These figures yield an average ratio of about 6:1 for the relative biological activities of equal weights of vitamin A and carotene as present in butter.

This ratio is markedly different from those obtained by Moore [1933] and Carr and Jewell [1933] by direct comparison of crystalline carotene with potent concentrates of fish-liver oil vitamin A.

In view of this difference from previous comparisons of the potency of the two active substances it is necessary to be critical of the various factors on which our calculations depend.

(a) *Animal assay.* As is the case in all biological tests, the estimation of vitamin A is subject to appreciable experimental errors. We used fairly large groups of rats from a carefully standardised stock for our tests and therefore have no reason to believe that our errors in this respect are any greater than in previous comparisons.

(b) *Spectroscopic determinations.* The errors here are, comparatively speaking, very small. The determination of carotene is quite straightforward and the probable error is at most $\pm 3\%$. The determination of vitamin A depends on the light absorption of the solution of the unsaponifiable matter at $328m\mu$. It assumes that the absorption at this wave length (after correction for carotenoids) is due solely to vitamin A. If this condition is not fulfilled, the vitamin A value would be too high. This would have the effect of reducing the relative potency of the vitamin A in the subsequent calculation. On this basis the claim that the true vitamin A of butter is some six times as potent as the carotene fraction is a conservative one.

(c) *Method of calculation.* The indirect method of calculating the relative biological activities of vitamin A and carotene by solving simultaneous equations is obviously much more liable to error than a direct comparison of the activities of the two substances as measured in separate biological tests. Even a small change in the figures caused by errors of the biological tests would involve a marked alteration in the calculated ratio. The method is admittedly inaccurate and one must be very cautious in the interpretation of the results though the constancy of the ratio calculated from three independent experiments on fairly large groups of rats is certainly highly satisfactory and suggestive.

We realise that our observations apply only to vitamin A and carotene as present in butter. Whether the same relationship exists between fish-liver vitamin A and crystalline carotene cannot be decided on the basis of these results.

In Table III we have compared the biological values of Shorthorn and Guernsey butters as actually determined with those to be expected on the basis of different vitamin A/carotene activity ratios and calculated from the data of Table II.

Table III. *Comparison of the vitamin A activities of Shorthorn and Guernsey butters found in biological experiments with values calculated from the vitamin A and carotene contents of the butters for different activity ratios.*

Season	Units per g. of butter fat.					
	Biological values calculated. β -carotene units				Biological values found	
	6 : 1 basis		1 : 1 basis		International units S and G	β -Carotene units S and G
	S	G	S	G		
Summer	74	76	19	31	85	49
Autumn	62	60	16	25	65	37
Winter	25	25	6	8	40	23

SUMMARY.

1. The vitamin A activities of Shorthorn and Guernsey butters representative of summer, autumn and winter feeding have been determined biologically. No difference was found in this respect between the butters of the two breeds in each of the three seasons.

2. The carotene and vitamin A contents of the butters have also been determined by spectrophotometric methods. It was found that although the total biological activity is the same for butters of each breed (on the same diet and at the same season) the relative contributions of the two factors, carotene and vitamin A, vary considerably in the two breeds.

3. Calculations from these combined results suggest that the vitamin A of butter possesses more growth-promoting activity than does the carotene, being probably about six times as potent.

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