CCV. THE VITAMIN A CONTENT OF "LIGHT WHITE" CASEIN

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(Received 20 August 1939)

THE presence of vitamin A in specimens of casein not subjected to treatment for effecting its removal or destruction has been assumed from the early days of research on vitamins. "Light white" casein (sodium caseinate), a form of casein much used in nutritional experiments, was examined as a source of vitamin A for rats by Culhane [1933]. She found that its use as the protein constituent of a basal diet for rats in place of inactivated casein prevented or delayed xerophthalmia and prolonged the survival period. Without further supplements of vitamin A, however, the rats eventually stopped growing and showed signs of deficiency. Culhane calculated that "light white" casein possesses 1/200th of the activity of cod liver oil, and that its inclusion in the diet at the level of 20 % was equivalent to a daily dose of 0.05 mg. of carotene. These conclusions would appear to be based on unduly low estimates of the activities of carotene and cod liver oil.

As far as we are aware vitamin A has not hitherto been detected in casein by chemical means. The object of the experiments described below has been to confirm the presence of vitamin A by this means, to arrive at a rough approximation of the amount present and to observe whether or not the vitamin is present in any unusual form or state of combination.

EXPERIMENTAL

Specimens of "light white" casein (40–300 g.) were extracted under different conditions as shown in Table I. The solvents were usually employed at the rate of 5 ml. per g. of caseinogen. Ether and ethylene dichloride were used cold. Ethyl alcohol was used at boiling point by refluxing, usually for periods of 2 hr.

Amounts of fat obtained. With cold ether or ethylene dichloride the amounts of fat obtained were much smaller than with hot alcohol. Moreover, the fat appeared to dissolve in two distinct fractions. In experiments in which successive extractions were made a yield of about 0.4 % of fat was usually obtained in the first extraction in the cold. In a second extraction in the cold the yield was very small. A further yield of more than 1 % was, however, obtainable by using hot alcohol.

Colorimetric estimations. The fatty extracts were saponified and the non-saponifiable residues extracted in the usual way. The determination of yellow coloration in the untreated extract and of the blue coloration after the adding of SbCl_3 was made with a Lovibond tintometer. With the small amounts of fatty extract available, and with only a low concentration of vitamin A present, highly accurate results were not to be expected.

With the extracts obtained with ether and ethylene dichloride blue colours were never given in the SbCl_3 tests. In view of the very small amounts of material available it is doubtful whether a complete absence of vitamin A from these fractions is to be inferred. With the extracts obtained with hot alcohol blue colours were always given. In agreement with experience with similar extracts from butter fat the blue colours faded very rapidly; accurate matching in the tintometer was therefore difficult. With one of the extracts richest in vitamin A a faint absorption band at $620\text{ m}\mu$ was observed.

Table I. *The colorimetric examination of fat extracted from specimens of "light white" casein*

Specimen	No. of exp.	Solvent	% fat obtained	Lovibond units per 100 g. casein		Remarks
				Y.U.	B.U.	
A	1	Ethylene dichloride, 2 extractions	0.5	12.5	0	Casein, after 2 more extractions, + in biological tests
	2	Ethyl alcohol, hot, 2 extractions	2.0	50	50	
B	3	Ether	0.45	28	0	Extracted casein, + in biological tests
	4	Ether, 1st extraction	0.4	10	0	
		Ether, 2nd extraction	0.09	2	0	
		Total	0.49	12	0	
	5	Alcohol, hot, 1st extraction	1.7	82	27	Extracted casein, - in biological tests
		Alcohol, hot, 2nd extraction	0.17	3	2	
		Total	1.87	85	—	
	6	Ether, 1st extraction	0.39			
		Ether, 2nd extraction	0.025			
		Alcohol, hot, 3rd extraction	1.34			
C	8	Total	1.76			620 $\text{m}\mu$ absorption band observed
		Ether, 1st extraction	0.43			
		Ether, 2nd extraction	0.028			
		Alcohol, hot, 3rd extraction	1.14	50	23	
		Total	1.60			
C	8	Alcohol, hot	—	90	100	620 $\text{m}\mu$ absorption band observed

All the extracts were coloured yellow. The yellow/blue ratio was so high in some instances as to suggest that the blue colour might be mainly derived from carotene, but in others (Exps. 2 and 8, Table I) lower ratios indicated the presence of vitamin A. In view of the possibility of the presence of yellow pigments other than carotenoids it does not seem safe to assume the absence of vitamin A, even from the extracts with high yellow/blue ratios.

If we make the assumption that all the yellow pigment of the extracts was carotene the total vitamin A activity of the two richest extracts (Exps. 5 and 8, Table I) may be calculated as about 80 and 120 I.U. per 100 g. respectively. The value of 1 I.U. per g. would seem a reasonable approximation for a typical casein. Taking the fat content at the round figure of 2%, the vitamin A of the fat amounts to 50 I.U. per g. This value lies within the range given by Booth *et al.* [1934] for butter fat. A rat eating daily 10 g. of a basal diet containing 20% of caseinogen would receive 2 I.U. of vitamin A activity.

Biological tests. In our main biological experiments young rats were given a diet deficient in vitamin A (caseinogen 20%, sugar 60%, arachis oil 15%,

salt mixture 5% and dried yeast 10%, radiostol (vitamin D) 1 drop per rat per week. The caseinogen was supplied by Glaxo Laboratories, Ltd. It was of the "acid type" and had been extracted with hot alcohol. After about 6 weeks a growth plateau was attained. Groups of rats were then given the same basal diet but with the Glaxo caseinogen replaced by (a) untreated "light white" casein, (b) "light white" casein which had been extracted twice with ether (Exp. 4, Table I), (c) "light white" casein which had been extracted twice with hot alcohol (Exp. 5, Table I). The growth responses observed over a period of 6 weeks are given in Table II.

Table II. *Biological tests on untreated and extracted "light white" casein*

	Rat no. and sex	Caseinogen used	Wt. increase and condition after 6 weeks
Group A	1 ♂	Untreated "light white" caseinogen	In good condition. Gained 33 g.
	2 ♂	" " "	In fair condition. Wt. increased by 3 g.
	3 ♀	" " "	In good condition. Wt. increased by 19 g.
	4 ♀	" " "	In good condition. Wt. increased by 19 g.
Group B	5 ♂	"Light white" caseinogen extracted with cold ether	In good condition. Gained 24 g.
	6 ♂	" " "	Died after 17 days. Lost wt. rapidly during the last week
	7 ♀	" " "	In fair condition. Wt. maintained
	8 ♀	" " "	In good condition. Wt. increased by 16 g.
Group C	9 ♂	"Light white" caseinogen extracted with hot alcohol	Died after 14 days. Lost 47 g.
	10 ♂	" " "	Died after 10 days. Lost 21 g.
	11 ♀	" " "	Died after 38 days. Lost 58 g., xerophthalmia
	12 ♀	" " "	Died after 20 days. Lost 44 g., slight xerophthalmia
	13 ♀	" " "	Died after 13 days. Lost 29 g.

It will be seen that all the rats survived, with slow growth, when untreated "light white" casein was included. With "light white" casein extracted with ether 1 out of the 4 rats died, 1 maintained its weight and 2 showed slow growth, indicating a partial loss of activity from the caseinogen. With "light white" casein extracted with hot alcohol all 5 rats in the group lost weight rapidly and died.

In preliminary experiments another sample of untreated "light white" casein promoted slow growth in 2 rats over periods of several weeks, after which growth stopped, with decline and death in one animal. The same casein after 4 extractions with ethylene dichloride (Exp. 1, Table I) caused restoration of growth for about 4 weeks, after which constant weight was maintained.

In our experience about 1–2 i.u. of vitamin A are usually necessary to promote slow growth in rats. The results of these biological experiments indicate, therefore, that "light white" casein before extraction has a biological activity in good agreement with the results of the colorimetric determinations, and with the general inference that the fat present has an activity approximately equal to that of butter fat.

SUMMARY

1. "Light white" casein contained enough vitamin A to promote slow growth in rats depleted of the vitamin when included in the diet at the level of 20 %.

2. When specimens of the casein were extracted with hot alcohol the extracts were coloured yellow and gave blue colorations when treated with the SbCl_3 reagent, indicating that carotene and vitamin A were present in amounts consistent with the biological activity. The value of 1 i.u. per g. may be taken as the approximate vitamin A content of typical casein.

3. Ether and ethylene dichloride, used in the cold, extracted only a small fraction of the fat present in "light white" casein, and the SbCl_3 reaction could not be demonstrated in the extracts. The vitamin A activity of the casein was at least partially retained.

Our thanks are due to Dr L. J. Harris for his valuable criticism.

REFERENCES

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