6287

Presence of Carotenase in the Liver of the Dog.

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That carotene is converted to vitamin A *in vivo* has been shown by Moore,[1] Capper and his co-workers,[2, 3] and by Drummond, Ahmad, and Morton.[4] Moore[5] postulated that this conversion takes place in the liver. Olcott and McCann[6] made a toluene water extract of the livers of A-deficient rats, and with this extract were able to convert colloidal carotene to vitamin A *in vitro*. They believed the activity of the extract to be due to an enzyme which they named carotenase.

We attempted to prepare carotenase from the livers of normal dogs and of dogs on A-deficient diets. We succeeded only once out of 4 trials. In the successful attempt, we noted that in preparing the carotenase, much of the blood had remained in the liver. It occurred to us that the blood buffers present might have been responsible for the preservation of the enzyme. We therefore made a liver extract, using a phosphate buffering solution of pH 7.4 instead of distilled water. The buffer was made by taking 39.5 cc. of 0.2 N (carbonate free) NaOH, 50 cc. of 0.2 M acid potassium phosphate, and this was made up to 200 cc. with distilled water. We found that such a solution always converted colloidal carotene to vitamin A as shown by a positive antimony trichloride reaction, even when watery extracts of the same liver did not. Colloidal carotene itself failed to give a positive antimony trichloride reaction. The liver extract solution also was tested but gave no reaction.

Olcott and McCann showed that the enzyme was inactive after boiling. We have noted that it is also destroyed by exposure to cold. The enzyme can be kept active by keeping it at 37°C.

Method. 30 gm. of liver was thoroughly ground with sand and

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[1] Moore, T., *Biochem. J.*, 1930, **24**, 692.

[2] Capper, N. S., *Biochem. J.*, 1930, **24**, 980.

[3] Capper, N. S., McKibbin, I. M. W., and Prentice, J. H., *Biochem. J.*, 1931, **25**, 265.

[4] Drummond, J. C., Ahmad, B., and Morton, R. A., *J. Soc. Chem. Ind.*, 1930, **49**, 291 T.

[5] Moore, T., *Biochem. J.*, 1931, **25**, 275.

[6] Olcott, H. S., and McCann, D. C., *J. Biol. Chem.*, 1931, **94**, 185.

Presence of Enzyme Carotenase in Buffered and Unbuffered Liver Extract Solutions.

Dog	Previous Diet	Carotenase	
		Unbuffered	Buffered
40	Normal	negative	
61	A-deficient diet	negative	
51	A-deficient diet	negative	
41	Normal	positive	
12	A-deficient diet plus carotene	negative	positive
65	A-deficient diet	positive	positive
	after 4 days' incubation at 37°C.	faintly positive	positive
42	Normal	negative	positive
	after 4 days' incubation at 37°C.	negative	positive

75 cc. of the above buffering solution saturated with toluene was added. The mixture was allowed to incubate at 37°C. for 24 hours and then filtered. Two cc. of the filtrate were added to 6 cc. of a colloidal solution of carotene* containing approximately 0.02 mg. of carotene per cc. The latter was prepared according to the method of Fodor and Schoenfeld.[7] This mixture was then incubated at 37°C. for 36 hours. It was then extracted by shaking with ether, the ether extract was dried with anhydrous sodium sulfate and metallic sodium, and evaporated to dryness by vacuum distillation in a stream of nitrogen. The residue was taken up in 5 cc. of anhydrous chloroform. To 1 cc. of this solution was added 1 cc. of the antimony trichloride reagent. The appearance of a blue color is interpreted as being due to the presence of vitamin A.

Conclusions. 1. Carotenase is active in solutions of pH 7.4 and inactive in solutions on unfavorable acidity or alkalinity.

2. Carotenase is destroyed by cold.

3. In solutions of pH 7.4 carotenase maintains its activity for a considerable period of time if kept at 37°C.

* The crystalline carotene was obtained from the Mead Johnson Laboratories.

[7] Fodor, A., and Schoenfeld, M., *Biochem. Z.*, 1931, **233**, 243.