and motors, reciprocating steam engines, refrigeration, condensers, steam turbines, power plants, internal combustion engines and steam boilers. While many constructional details are given, no discussion of the mechanical problems to be met in their design is attempted. However, under each type of equipment an enumerated list of advantages and disadvantages is given which helps the reader to form a balanced opinion on current models of heat engines.

The book contains a very generous number of figures and illustrations, many of which are printed in color. These add much to the clarity of the material presented as well as to amusement. Fig. 17 for example shows a Carnot cycle complete with source, the sun with a face, and a sink, icebergs, polar bear and all. In the preface the author notes that text-books like any given item of engineering practice have an average life of only ten years. This book serves to bring similar earlier texts up to date, a purpose that is accomplished, but nothing more.

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SPECIAL ARTICLES

THE IN-VITRO DESTRUCTION OF CAROTENE BY WATER EXTRACTS OF MINCED RAT STOMACHS IN THE PRESENCE OF METHYL LINOLATE

SHERMAN[1] has demonstrated that carotene administered to vitamin A deficient rats is inactivated by the simultaneous feeding of methyl linolate. The destructive action of the methyl linolate can be prevented by small amounts of α-tocopherol. It was shown that the carotene was destroyed in the gastrointestinal tract, but the exact site and the mechanism of the destructive action was not clear. The mixing of the carotene and methyl linolate with the basal ration, *in vitro,* did not elucidate the problem, since in this case carotene destruction occurred much too slowly to explain the *in vivo* results. The same was true when the carotene and linolate were mixed with stomach or intestinal contents. Results similar to the above have been noted by Quackenbush, Cox and Steenbock[2] and by Hickman, Harris and Woodside.[3]

This paper offers a possible explanation of the above results by demonstrating the rapid *in vitro* destruction of carotene by a clear water-extract of minced rat stomach in the presence of methyl linolate.

The stomach extract is prepared by suspending the minced stomach mucosa of the freshly killed rat in ten times its weight of distilled water, allowing it to stand for 15 minutes and then centrifuging at high speed for 10 minutes. The supernatant liquid has a dry matter content of 5 mg per ml. The extract must be clear. If the minced stomach is ground in a mortar before extracting it is impossible to clarify by centrifuging and such extracts show no carotene destructive potency when tested according to the method described below.

For purposes of comparison the mode of action and potency of the so-called "carotene oxidase" or lipoxidase[4] of the soybean has been investigated. A 0.5 per cent. suspension of the ground soybean was centrifuged after standing for 30 minutes. This extract contained 2 mg of dry matter per ml.

The carotene-destroying potency of the above extracts is determined as follows. One ml. of a 0.25 per cent. solution of highly purified methyl linolate in acetone is placed in a 125 ml Erlenmeyer flask. Ten ml of water is added, followed by one ml of McIlvaine's citric acid-sodium phosphate buffer, pH 5.7, and one ml of carotene in acetone (equivalent to 68 micrograms of carotene). Finally the extract to be tested is added (usually one ml), the flask stoppered and incubated for periods up to 30 minutes at 38° C. The reaction is stopped with hydrochloric acid and the carotene is transferred to 50 ml of ethyl ether by vigorous shaking until the water phase is clear and colorless. The carotene is then determined colorimetrically by means of the Evelyn photoelectric colorimeter. For the control, a flask containing the linolate, carotene, water and buffer is incubated as above; hydrochloric acid is added and followed by the extract under test. The carotene is immediately extracted with ether. The recovery of carotene in this case is always above 95 per cent. of the amount added. The carotene destructive potency of the material under test is calculated as the percentage loss with reference to the control run at the same time.

The results of the destructive action on carotene of increments of stomach extracts of normal adult rats are shown in Table 1. The potency of the extract is greatly reduced by heating on a boiling water water bath for 15 minutes. Without methyl linolate the action of the stomach extract is insignificant.

The action of the stomach extract is very similar to that of soybean "carotene oxidase." However, some differences do exist which indicate that the active agent is not the same in both cases. From Table 2 it is evident that although the soybean enzyme is inhibited

[1] W. C. Sherman, *Proc. Soc. Exp. Biol. and Med.,* 47: 199, 1941; *Jour. Nutrition,* 22: 153, 1941.
[2] F. W. Quackenbush, R. P. Cox and H. Steenbock, *Jour. Biol. Chem.,* 145: 169, 1942.
[3] K. C. D. Hickman, P. L. Harris and M. R. Woodside, *Nature,* 150: 91, 1942.
[4] R. J. Sumner, *Jour. Biol. Chem.,* 146: 215, 1942.

TABLE 1

THE DESTRUCTION OF CAROTENE BY WATER EXTRACTS OF MINCED RAT STOMACH MUCOSA AS COMPARED WITH SOYBEAN "CAROTENE OXIDASE"

Volume extract	Rat stomach mucosa extract*			Sobyean extract		
	Number of determinations	Destruction of carotene		Number of determinations	Destruction of carotene	
		Range	Average		Range	Average
ml		*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*
1.0	6	57.8–86.2	74.2	6	70.0–78.5	74.0
0.5	6	61.0–79.7	70.8	4	48.8–72.8	61.9
0.25	2	58.8–69.1	64.0	..	.....	...
0.20	4	51.0–55.8	54.1	2	40.0–44.4	42.2
0.10	1	.....	45.3	2	28.7–32.5	30.6
0.05	1	.....	28.0	..	.....	...
1.0 (no Linolate)	5	0–28.0	12.2	3	0–35.0	15.0
1.0 (heated) †	1	.....	36.8	..	.....	...
0.5 "	1	.....	32.8	..	.....	...
0.1 "	1	.....	20.0	..	.....	...

* Rats were on a stock diet: Average weight of the six rats used was 215 grams.
† The extract was heated on a boiling water bath for 15 minutes.

by a water extract of a low-grade wheat flour, the active agent in the stomach extract is not. On the other hand, the stomach extract appears to be more susceptible to inhibition by α-tocopherol than does the soybean extract. A further point of difference between the two extracts became evident in a study of the pH activity curves. Over a pH range of 3.0 to 6.6 using the citric acid-sodium phosphate buffer system the soybean lipoxidase was inactive below pH 4.0 and showed its maximum at about pH 5.5; the falling off was gradual on the alkaline side of the optimum. However, the rat stomach showed considerable activity even at pH 3.0. This may have a relation to a natural resistance to the acidity of the normal stomach possessed by this active carotene-destroying agent. In other respects the pH activity curve of the stomach extract was very similar to that of the soybean enzyme. The pH optimum was 5.7 and the falling off on the alkaline side was gradual.

TABLE 2

COMPARATIVE INHIBITION OF RAT STOMACH AND SOYBEAN "CAROTENE OXIDASE" BY TOCOPHEROL AND CENTRIFUGED 5 PER CENT. WATER EXTRACT OF SECOND CLEAR WHEAT FLOUR

Incubation time	Rat stomach extracts* †			Soybean extracts†		
	Number of determinations	Destruction of carotene		Number of determinations	Destruction of carotene	
		Range	Average		Range	Average
Minutes		*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*
15	5	35.5–65.9	52.6	3	65.1–70.5	67.6
30	14	56.8–81.9	70.1	3	71.3–80.8	76.0
30 (plus flour extract)	5	51.5–78.9	66.8	5	23.6–43.5	37.1
15 (1 mg) (Tocopherol)	1	.....	5.0	1	.....	42.0
30 (1 mg) (Tocopherol)	1	.....	25.0	1	.....	45.5

* Rats had been on a synthetic ration composed of 30 per cent. lard, 5.5 per cent. salts, 25 per cent. casein, sucrose and synthetic vitamins. Average weight was 178 grams.
† One ml of extracts used.

Other organs have been tested for a carotene-destroying agent in their water extracts. Preliminary results have shown that liver is quite potent while muscle and small intestine appear to have little or no potency. However, it should be mentioned that the extracts of small intestines were not clear and contained a large amount of extraneous material. As mentioned above, lack of clarity in stomach extracts indicates a resistance to destruction of carotene, and the same may be the case with the intestinal extracts. The fact that muscle extract shows no potency appears to exclude the possibility that the destructive action of the tissue extracts on carotene is an artifact resulting from iron-porphyrins or similar compounds extracted from the blood contained within the organs.[5]

Some time after the above results were obtained an attempt was made to repeat certain phases but with slightly anomalous results. No apparent change in the procedure was made, but a new supply of methyl linolate was employed, with the result that a lag phase of from one to two hours incubation time was noted during which little or no destruction of carotene occurred. However, after this time interval carotene destruction began and proceeded rapidly to near completion within 30 minutes. The general results were the same as previously noted, in that methyl linolate was required in conjunction with the stomach extract, neither being effective by itself. This peculiarity may have been caused by a small amount of an antioxidant in the new batch of linolate, or it may have been related to a variation in the efficiency of extraction of the active agent from the minced stomach.

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AN UNIDENTIFIED NUTRIENT REQUIRED FOR PROPER UTILIZATION OF DL-ALPHA-TOCOPHEROL BY THE CHICK[1]

DURING attempts to study the rôle of vitamin E in chick nutrition with a simplified ration, the chick was not protected from a vitamin E and A deficiency when

[5] F. Haurowitz, P. Schwerin and M. M. Yenson, *Jour. Biol. Chem.*, 140: 353, 1941.

[1] Technical Contribution No. 107, South Carolina Agricultural Experiment Station. Published by permission of the director of the South Carolina Agricultural Experiment Station. We are indebted to Merck and Company, Inc., Rahway, N. J., for dl-alpha-tocopherol; Anheuser-Busch and Company, St. Louis, Mo., for dried brewers' yeast; Distillation Products, Inc., Rochester, N. Y., for vitamin A; Central Soya Company, Inc., Decatur, Indiana, for soybean phosphatides; and E. I. du Pont de Nemours and Company, New Brunswick, N. J., for vitamin D_3.