4. Christian, C. L., *ibid.*, 1958, v108, 139.
5. Ishizaka, T., Ishizaka, K., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 845.
6. Christian, C. L., *J. Immunol.*, 1960, v84, 112.
7. Müller-Eberhard, H. J., Nilsson, U., *J. Exp. Med.*, 1960, v111, 217.
8. Marcus, D. M., *J. Immunol.*, 1960, v84, 273.
9. Mayer, M. M., *Progr. Allergy*, 1958, v5, 215.
10. Levine, L., Cowan, K. M., Osler, A. G., Mayer, M. M., *J. Immunol.*, 1953, v71, 367.

Received November 15, 1960. P.S.E.B.M., 1961, v106.

Studies on the Metabolism of 5-Hydroxytryptamine (Serotonin). I. Effect of Starvation and Thiamine Deficiency. (26314)

E. M. GAL AND P. A. DREWES (Introduced by R. E. Smith)
Neurochemical Research Laboratory, V.A.H. Sepulveda, and Dept. Pharmacology, University of California Medical School, Los Angeles, Calif.

In spite of the significant number of important papers related to the metabolism of 5-hydroxytryptamine (5-HT) the effect of certain stresses notably those of starvation, dietary deficiencies and of obesity, are scarcely explored. An exception is the studies on the effect of Vit. B_6-deficiency which were shown to lead to reduced levels of 5-HT in the spleen(1), possibly through impairment of 5-hydroxytryptophan decarboxylase(2), and B_2-deficiency with concomitant reduction of monoamine oxidase (MAO) activity of the liver(3). The observed metabolic disturbances which appear during starvation(4) and thiamine deficiency (5,6) were considered to evoke possible changes in metabolism of 5-HT, a substance involved in functional control of the central nervous system. This paper derives from a research program concerning the influence of dietary stresses on certain aspects of 5-HT metabolism.

Methods and materials. Sprague-Dawley male rats were divided into 3 groups. The first 2 groups were kept on thiamine sufficient and deficient diet respectively for 4½ weeks as described elsewhere(7). The third group of rats was maintained on a daily ration of 2 g of Purina standard rat chow for the first 3 days, then starved for 4 days with only 1 ml of soluble vitamin mixture ("Berocca-C", Hoffmann-LaRoche, Inc.) and water given *ad libitum*. The animals were mechanically stunned and, after withdrawal of 1 ml of cardiac blood with a heparinized needle, killed by decapitation. Blood and tissues were immediately worked up for 5-HT content according to the method of Bogdanski *et al.*(8). In the experiments aimed at determining (MAO) activity the tissues were removed immediately and, with the excess blood blotted, chilled and worked up to isolate the mitochondrial fraction by the method of Schneider *et al.*(9). Mitochondrial fraction of the spleen was obtained by pooling samples from several animals. Measurement of MAO activity was carried out manometrically(10). The main compartment of the vessels contained 0.3 ml of 0.5 M phosphate buffer pH 7.3, 1 ml of mitochondrial suspension in 0.25 M sucrose, 0.1 ml of 3×10^{-2} M KCN solution and water to bring the volume to 3 ml, while the side arm had 0.4 ml of 0.05 M 5-HT solution adjusted to pH 7.4. The inner well contained filter paper, 0.2 ml of a 1:1 mixture of 20% KOH and 1 M KCN. Each experimental flask had a control in which the side arm had 0.4 ml of water instead of 5-HT. This served to correct for any oxidation in absence of substrate. The gas phase was O_2 and temperature 37°C. After 10 minutes of oxygenation and equilibration the contents of the side arms were tipped in. At the end of each experiment the content of main compartment was withdrawn for pH determination (average varied between pH 7.5 to 7.8). Enzyme activity is expressed as $Qo_2(N)$ and was calculated from the linear period of oxidation. Mitochondrial nitrogen was determined by micro-Kjeldahl method.

Results. Effect of starvation and thiamine

TABLE I. Effect of Starvation and Thiamine Deficiency on 5-HT Levels.

Tissue	5-HT levels, $\mu g/g$ or ml		
	Starved	Thiamine-sufficient (control)	Thiamine-deficient
Brain	.47 ± .03* (8)	.45 ± .04 (8)	.50 ± .04 (12)
Spleen	4.60 ± .53 (8)	1.75 ± .19 (8)	3.70 ± .38 (12)
		$p<0.01$	$p<0.01$
Small intestine	4.38 ± .50 (8)	4.18 ± .28 (8)	2.91 ± .23 (12)
			$p<0.01$
Blood	1.02 ± .19 (10)	1.04 ± .11 (11)	.73 ± .06 (13)
			$p=0.01$
Initial body wt (g)	143 ± 7	140 ± 6	141 ± 7
Final (% change)	−27	+33	−16

* Stand. error. No. of animals is given in parentheses.

deficiency on in vivo *levels of 5-HT.* Concentration of 5-HT recovered in the blood, brain, small intestine and spleen of rats under the specified dietary conditions is represented in Table I. It is of interest that neither starvation nor thiamine deficiency affected levels of 5-HT of the brain while there was a statistically significant increase of 5-HT concentration in the spleen of stressed animals. Among the organs analyzed, only the weight of the spleen of the deficient and starved animals displayed marked difference from that of control animals. Although weight of the spleen of these animals was 40% less per hundred g of body weight than the splenic weight of controls, the absolute amount of 5-HT was nevertheless twice as much in the spleen of the stressed animals. Degree of decrease in 5-HT concentration of the blood and small intestine was significant ($p<.01$) only in thiamine deficient animals. It should be pointed out that 5-HT values of rat blood indicated here are 3 to 4 times of those previously reported by others(8). Recent analyses of others have confirmed the blood values reported in these studies (private communication by H. Weisbach).

Effect of starvation and thiamine deficiency on 5-HT oxidase activity of rat tissues. The oxidation 5-HT by the mitochondria of liver, kidney and brain of normal rats as measured by the colorimetric method(11) was earlier demonstrated by Sjoerdsma *et al.*(12). Results of the manometric assays reported here seem to be in good agreement with respect to relative rate of oxidation of 5-HT found in those tissues of normal rats by the colorimetric method. There is significant difference in MAO activity of the brain and small intestine between control and the starved as well as the thiamine deficient animals (Table II). In the other tissues studied, notably liver, kidneys and spleen, no difference in MAO activity between control and experimental groups was detectable. In spite of the more than 40% loss of liver protein during starvation(13) the mitochondrial enzyme protein responsible for MAO activity does not seem to be impaired either in starvation or in thiamine deficiency. Earlier Sourkes (14) using 5-HT as substrate demonstrated the MAO activity of the liver of B_6-deficient rats was normal or occasionally even elevated. The results were qualitatively similar with tyramine as substrate. The comparison of the MAO values based on mg of mitochondrial nitrogen rather than wet weight of tissue increases their validity as a true measure of difference between the 3 groups.

TABLE II. Mitochondrial Monoamine-Oxidase Activity of Rat Tissues with 5-HT as Substrate.

Tissue	Qo_2 (N)*		
	Starved	Thiamine-sufficient (control)	Thiamine-deficient
Brain	45 ± 3.7† (9)	31 ± 3.1 (9)	46 ± 3.8 (9)
		$p<0.01$	$p<0.01$
Small intestine	77 ± 11 (7)	28 ± 2.3 (8)	91 ±14 (8)
		$p<0.01$	$p<0.01$
Kidney	37 ± 11 (6)	36 ± 2.9 (9)	29 ± 5.6 (9)
Liver	70 ± 7.1 (8)	54 ± 7.8 (9)	55 ± 3.7 (9)
Spleen‡	26 ± 12 (4)	23 ± 5.6 (4)	32 ± 6.5 (4)

* Expressed as μl of O_2 consumed/mg mitochondrial nitrogen/hr.

† Stand. error.

‡ Each assay represents pooled tissues of 2 animals. No. of assays is given in parentheses.

Discussion. Acute starvation and thiamine deficiency, while non-specific stresses, provoke in the adrenals and thymus a definite response which is quite different from the effects of riboflavin and B_6-deficiencies. These latter fail to produce adaptation syndrome although in B_6-deficiency the atrophy of thymus is observable as a possible consequence of inadequate protein synthesis(5). With this in mind it was expected that the effects of starvation and thiamine deficiency through adrenal involvement would be reflected in changes in the metabolism of 5-HT. Furthermore, thiamine deficiency causes anemia by affecting the haemopoietic system (15) while starvation in rats produces a concentrating effect through body weight loss and dehydration(16) without apparent effect on haemopoesis. Of course such effect would also be operative in the ensuing inanition during the final stages of thiamine deficiency. The increased 5-HT levels of the spleen in the starved and deficient animals is therefore suggestive of platelet destruction and consequent release of serotonin as a result of inanition and concentrating effect of the spleen. However, the lowered 5-HT values of the blood and small intestine in the thiamine deficient animals might be related both to an increased release of 5-HT from the intestine to other organs only during chronic inanition like B_1-deficiency and to an increased MAO activity of the small intestine as shown in Table II.

The reason for the increased MAO activity of the brain and small intestine is difficult to interpret, particularly with respect to the starved animals. The loss of considerable protein from the intestines during starvation (13) and the significant decrease in number of cells in the duodenal mucosa(17) would indicate an imbalance in favor of protein catabolism which should tend to decrease enzymatic activity. On the contrary, it is obvious from the results that the enzyme protein of MAO was not adversely affected by either acute starvation or a 4 week period of thiamine deficiency in any of the tissues studied.

Summary. Blood and small intestine of thiamine deficient rats showed reduced levels of 5-HT while 5-HT content of the spleen of both starved and deficient animals was elevated. Mitochondrial monoamine oxidase activity was increased in the brain and small intestine during starvation and B_1-deficiency without any detectable change in that of the liver, spleen and kidney.

1. Yeh, S. D. J., Solomon, J. D., Chow, B. F., *Fed. Proc.,* 1959, v18, 357.

2. Weissbach, H., Bodanski, D. F., Redfield, B. G., Udenfriend, S., *J. Biol. Chem.,* 1957, v227, 617.

3. Hawkins, J., *Biochem. J.,* 1952, v51, 399.

4. Cameron, A. T., Carmichael, J., *Canad. J. Res. Sect. E.,* 1946, v24, 37.

5. Deane, H. W., Shaw, J. H., *J. Nutrition,* 1947, v34, 1.

6. Grana, E., Capelli, V., Ceriani, T., *Boll. Soc. Ital. Sper.,* 1960, v36, 284.

7. Gal, E. M., Razevska, D. E., *Arch. Bioch. Biophys.,* 1960, v89, 253.

8. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharmacol. Exp. Therap.,* 1956, v117, 82.

9. Schneider, W. C., Hogeboom, G. H., *J. Biol. Chem.,* 1950, v183, 123.

10. Creasy, N. H., *Biochem J.,* 1956, v64, 178.

11. Udenfriend, S., Weissbach, H., Clark, C. T., *J. Biol. Chem.,* 1955, v215, 337.

12. Sjoerdsma, A., Smith, T. E., Stevenson, T. D., Udenfriend, S., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 36.

13. Addis, T., Poo, L. J., Lew, W., *J. Biol. Chem.,* 1936, v115, 111.

14. Sourkes, Th. L., *Rev. Canad. Biol.,* 1958, v17, 328.

15. Rinehart, J. F., Greenberg, L. D., Gizton, L. L., *Blood,* 1948, v3, 1453.

16. Lasher, E. P., Simmons, B. S., Everett, N. B., *J. Nutrition,* 1958, v65, 317.

17. Hooper, C. S., Blair, M., *Exp. Cell Research,* 1958, v14, 175.

Received November 16, 1960. P.S.E.B.M., 1961, v106.