ity equivalent to 14 μM fatty acids/g/hr in a patient primed with glucose and insulin, using a direct incubation technic as well as an acetone-powder extraction method, lends support to the presence of lipoprotein lipase in human adipose tissue. Although insulin, long known to decrease the free fatty acid release from adipose tissue(11), was being given in large amounts, appreciable lipoprotein lipase activity was found in the adipose tissue of the patient.

Recently there have been two reports of virtual absence of lipoprotein lipase in human adipose tissue. Angervall(9) reported lack of activity in 3 of 4 samples and insignificant activity in the fourth. Macroscopic blood, which is known to contain inhibitors of lipoprotein lipase, was present on the tissue and might account for his findings. In the other report(8), in which acetone powder extracts from 7 human beings showed no lipoprotein lipase activity, serum was not present in 3 of the incubation mixtures. Two others showed only slight activity. The importance of serum albumin as a fatty acid acceptor in this type system was well demonstrated by Reshef, *et al.*(12). Furthermore, the apparent advantage of using whole tissue over acetone extracts, as suggested by Angervall(9), may also be responsible for his negative results.

If one accepts the importance of the role of adipose tissue as a potent repository source of energy *via* release of FFA, the demonstration in adipose tissue of an enzyme capable of hydrolyzing triglyceride and freeing fatty acid radicals is not surprising. Its exact physiological role, and especially the part played by heparin *in vivo,* are not completely clear. It seems clear, however, from our data that such an enzyme is present in human adipose tissue.

Summary. Subcutaneous adipose tissue was obtained from 8 patients undergoing various surgical procedures. All were in good nutritional balance. In each instance it was possible to demonstrate considerable lipolytic activity *in vitro* in the fatty tissue. It was further possible to identify this hydrolytic enzyme as lipoprotein lipase, similar in its characteristics to that previously reported in animal blood and tissue and human blood.

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Studies on the Metabolism of 5-Hydroxytryptamine (Serotonin) II. Effect of Tryptophan Deficiency in Rats. (27520)

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During studies(1,2) on the effect of reserpine on 5-hydroxytryptamine (5-HT) metabolism in tryptophan deficient rats enough data accumulated to permit a separate presen-

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tation of the relation of tryptophan deficiency to 5-HT metabolism. The role of tryptophan, an essential amino acid, through its hydroxylation as an indispensable precursor of 5-HT synthesis is well established(3). It was observed that feeding of tryptophan to rats led to increase of intestinal 5-HT(4), while Zbinden *et al.*(5) observed that in tryptophan deficiency the 5-HT content of the intestine was lowered. These authors, however, found no reliable change of 5-HT level in the intestine of tryptophan deficient rats and only 37% decrease of the normal 5-HT level in the brain of rats after 31 days on deficient diet.

These observations are at variance with our results obtained from tryptophan deficient rats. A record of 5-HT values of other tissues of tryptophan deficient rats is also presented. Results on the effect of phenylisopropylhydrazine administration and of sulfasuxidine feeding on 5-HT levels are also included.

Methods and materials. Sprague-Dawley male rats of 130 g average weight were divided into 4 groups. The first and second groups were kept on tryptophan sufficient diet with and without sulfasuxidine (10 g/kg) added, while the third and fourth groups were on tryptophan deficient diet with and without sulfasuxidine. The animals were fed these diets for a maximum of 6 weeks. Each animal was kept in a separate metabolic cage and was given a daily ration of 7 g of diet made up in a pellet and water *ad libitum.* The tryptophan deficient diet was prepared according to our specifications by Nutritional Biochemicals Corp. as follows: vitamin-free casein acid hydrolysate salt-free, 156 g; white dextrin, 415 g; sucrose, 156 g; lard, 197 g; cod liver oil, 52 g; agar, 21 g; L-cystine, 2.9 g per kg of diet. Each kg of this basic diet was thoroughly mixed with 5 g of niacin‡ and 20 g of salt mixture W (Nutritional Biochemicals Corp.) and supplemented by the following vitamins: choline chloride, 50 mg; thiamine hydrochloride, 1 mg; riboflavin, 1 mg; pyridoxine hydrochloride, 1.25 mg; calcium d-pantothenate, 10 mg; inositol, 50 mg; biotin, 0.01 mg; folic acid, 0.25 mg; menadione, 5 mg. The tryptophan sufficient diet was prepared by supplementing the above diet with 10 g of DL-tryptophan/kg of mixture.

The animals were stunned and after withdrawal of 1 ml of cardiac blood into heparinized centrifuge tubes, killed by decapitation.

Blood and the weighed tissue samples were analyzed for 5-HT content according to a suitably modified method of Bogdanski *et al.* (6). In some experiments the animals were given a single intraperitoneal injection of 1.86 mg/kg of β-phenylisopropylhydrazine HCl (PIH) ("Catrone" Lakeside Laboratories, Inc.) a powerful monoamine oxidase inhibitor(7).

Results. Effect of tryptophan deficiency on in vivo *levels of 5-HT.* The results given in Table I indicate that the concentration of 5-HT in the blood, brain, and small intestine of tryptophan-deficient rats is lowered by the fifth week of dieting. These differences were significant ($P<0.01$), except in the case of the spleen, where in spite of an apparent lowering of 5-HT, the values did not differ significantly at the 5% level from those of the non-deficient animals. Addition of sulfasuxidine to the diet did not appreciably influence 5-HT levels in either group when compared to 5-HT values obtained from rats kept on diet without sulfasuxidine. There was an increase of 5-HT in the small intestine of tryptophan-deficient rats given sulfasuxidine against that of deficient rats that were given none. Furthermore, the 5-HT values of the spleen of deficient rats of the sulfasuxidine group when compared to their tryptophan-sufficient controls displayed a marked lowering of 5-HT ($P<0.01$). This was in contrast to the values observed in rats not given sulfasuxidine. Analysis of the change in body weight revealed an average of 10% gain in the sufficient and 22% loss in the deficient animals. There was some indication that

‡ Tryptophan conversion to nicotinate constitutes the major pathway of L-tryptophan metabolism. The unusually high level of niacin (5g/kg) in the diet therefore was to compensate for the impairment and gradual loss of nicotinate production due to tryptophan-deficiency, thereby restricting the effects of deficiency as much as possible to the tryptophan to 5-HT pathway.

TABLE I. Effect of Tryptophan Deficiency on 5-HT Levels.

Tissue	5-HT levels, μg/g or ml			
	Sufficient		Deficient	
	None	S*	None	S*
Brain	.47 ± .03† (44)	.49 ± .04 (24)	.16 ± .03 (30)	.20 ± .02 (23)
Blood	.86 ± .10 (22)	.60 ± .09 (14)	.12 ± .06 (9)	.10 ± .04 (12)
Small intestine	3.85 ± .39 (20)	4.63 ± .23 (14)	1.21 ± .12 (10)	1.71 ± .16 (18)
			p < .02	
Spleen	6.71 ± 1.24 (20)	7.53 ± .57 (14)	4.31 ± .81 (10)	3.44 ± .76 (18)
			p < .01	

* S = Sulfasuxidine added to diet.
† Stand. error.
No. of animals given in parentheses.
Animals sacrificed after 5 wk on diet.

feeding of sulfasuxidine decreased the mortality rate among the deficient animals within the period of 6 weeks. Further evidence however is required to permit causal assignment of this observation to an effect of sulfasuxidine.

In all, these results reveal a greater than 65-70% decrease in serotonin level of the small intestine and the brain of Sprague-Dawley male rats within 5 to 6 weeks on tryptophan-deficient diet. Examination of the absolute frequency distribution of the cerebral 5-HT of the population of 30 deficient rats represented in Fig. 1 indicates a 0.266 relative frequency of samples with only 30% lower 5-HT values than that of the brain of tryptophan-sufficient animals on the 35th day of dieting. Although there was 0.25 relative frequency of the controls in the 0.30-0.40 μg/g serotonin class interval also, one can be reasonably confident that the chances of having greater frequency of this class among tryptophan-deficient animals at 5% level of significance would be unlikely. Again it should be noted that some of the highest 5-HT values (*i.e.*, 0.65 μg/g) found among the tryptophan-sufficient rats were distributed below the 0.76 μg/g average observed by Zbinden *et al.*(5), a value improbably high even for the whole brain of 7-months-old rats(8). Although in earlier experiments(2) a 40-50% increase of 5-HT was observed in the brain of tryptophan-sufficient rats subsequent analyses reduced that to an average 20-25% increase within 5 weeks of dieting. Reasons for the discrepancy between the observations of others(5) and those reported here are not immediately obvious.

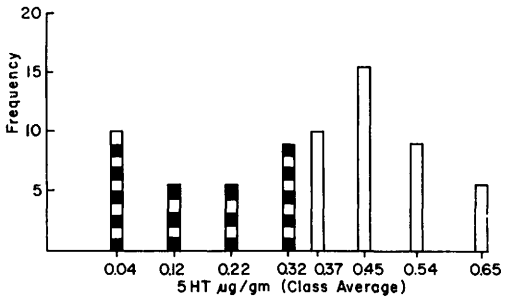

FIG. 1. Frequency distribution of cerebral 5-HT values in tryptophan-sufficient (N=40, □) and deficient (N=30, ■) rats on fifth week of dieting.

Effect of in vivo *administration of PIH on 5-HT levels in tryptophan-deficient rats.* The results of this preliminary experiment given in Table II point to a rise in 5-HT in both blood and brain of the tryptophan-sufficient and deficient animals concomitant to the expected inhibition of monoamine oxidase activity. The 5-HT increase in the tryptophan-sufficient animals was consistent with unimpaired conversion of tryptophan to serotonin. In the tryptophan-deficient animals only the blood showed reliable increase of 5-HT following PIH administration while the difference in the cerebral serotonin levels is not proved. A reliable increase of 5-HT in the brain of tryptophan-deficient rats in the presence of monoamine oxidase inhibitors would imply a continued synthesis of 5-hydroxytryptophan from tryptophan released by catabolized protein. On the other hand, the probability that in tryptophan-deficient rats

TABLE II. Effect of PIH on 5-HT Levels of Rat Tissues.*

	5-HT levels, μg/g or ml			
	Sufficient		Deficient	
Tissue	None	PIH	None	PIH
Blood	.80 ±.10† (8)	1.48 ±.21 (4)	.12 ±.06 (9)	.37 ±.07 (4)
		p <.02		p <.01
Brain	.42 ±.05 (8)	.76 ±.11 (9)	.14 ±.04 (12)	.30 ±.11 (4)
		p <.02		
Liver	.29 ±.03 (8)	.28 ±.04 (9)	—	.24 ±.09 (4)

* Animals after 6 wk on diet received I.P. inj. of PIH (1×10^{-6}M/kg) and were sacrificed after 3 hr.

† Stand. error.

the haemocephalic barrier becomes permeable to serotonin remains to be tested.

Discussion. These results permit the deduction that there is a marked lowering of 5-HT in all tissues of tryptophan-deficient rats. The decrease of 5-HT was gradual and that rate of diminution varied according to the tissue. Feeding of sulfasuxidine did not appreciably alter 5-HT levels in the tissues of animals of either group with the exception of the small intestine of tryptophan-deficient animals, where the noticeable increase of serotonin could reasonably be ascribed to an inhibition of the bacterial catabolism, among others, of serotonin. Also, the uncommonly high mortality rate due to the decreased resistance of the tryptophan-deficient animals to bacterial infection of the intestine was reduced by the antibacterial action of sulfasuxidine. However, the diet was strongly supplemented by niacin, biotin, and folic acid not only to compensate for the impaired biological synthesis of nicotinic acid but to prevent any dyscrasia which sometimes develops in animals given oral sulfasuxidine. During the experiment deficient animals usually develop anorexia. However, earlier studies(9) indicate that the effect of anorexia or starvation on 5-HT levels in Sprague-Dawley rats is quantitatively different from that produced by tryptophan-deficiency. None of the tryptophan-deficient animals even at an advanced stage of deficiency showed any sign of sedation or any appreciable change of the norepinephrine level of the central nervous system, an indication supporting earlier observations(2,5).

Summary. Tryptophan-deficient rats showed significant decrease of 5-HT in all their tissues analyzed. Feeding of sulfasuxidine, presumably through its antibacterial action, led to greater recovery of intestinal serotonin in tryptophan-deficient rats. Injection of PIH resulted in a statistically significant increase of 5-HT in the blood but not in the brain of deficient animals.

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