THE EFFECTS OF FAT DEFICIENCY UPON ENZYME ACTIVITY IN THE RAT*

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Recent reports indicate that liver enzyme activity may be markedly altered by dietary conditions. Such enzymes as D-amino acid oxidase, arginase, xanthine oxidase, succinic oxidase, rhodanese, and adenosine pyrophosphatase have been shown to be lost or decreased in dietary protein and amino acid insufficiency (1–6).

The syndrome first reported by Burr and Burr (7) to be produced by the exclusion of fats from the diet suggested that alteration of enzyme activity might be detected in fat-deficient animals. In addition to the skin and kidney lesions and growth failure, Burr and Beeber (8) reported that rats suffering from fat deficiency have a much higher metabolic rate than the controls. In a previous study, Wesson and Burr (9) had shown also that, following a carbohydrate meal, the respiratory quotients of fat-deficient rats were well above unity. Weil and Russell (10) found that starvation decreased plasma phosphatase activity. These lowered levels were elevated following the ingestion of certain unsaturated fatty acids, while saturated fatty acids, proteins, and carbohydrates had no effect.

The recent investigations of Swanson and Artom (11) have substantiated earlier findings (12) of a high lipide content of the mitochondria. The mitochondria have been shown to carry on many of the essential oxidation processes of the cells, including the succinic oxidase and cytochrome oxidase systems (13, 14). This finding of a large concentration of lipide in association with respiratory enzymes suggests that the activity of these enzymes may be particularly altered in fat deficiency. Therefore, it was of interest to investigate the activity of certain oxidative enzyme systems in fat-deficient rats and in rats fed rations supplemented with an essential fatty acid or with corn oil. The enzyme systems which were chosen for study in this work are liver succinic oxidase, cytochrome oxidase, choline oxidase, and the endogenous respiration. By studying the relation of essential fatty acid deficiency to enzyme activity, it is hoped that some light may be thrown upon the biochemical function

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of the essential fatty acids. In addition, the feeding of tetrabromostearic acid prepared from corn oil fatty acids was investigated to see whether such a compound would amplify the effects of an essential fatty acid deficiency.

EXPERIMENTAL

Two different techniques were employed to effect the fat deficiency symptoms in rats. The mature animals were handled according to procedures described by Barki *et al.* (15). Caloric restriction causing severe emaciation was followed by feeding of the fat-free diet *ad libitum* to produce the symptoms of fat deficiency. In this phase, adult male rats of the Sprague-Dawley strain, weighing from 200 to 220 gm., were fed 3 to 4 gm. of the fat-free ration per day for a period of 21 days. At the end of this period, the animals which weighed from 105 to 119 gm. were divided randomly into four groups of five to eight animals each. These groups then were fed their respective diets *ad libitum*. One group received only the fat-free basal diet, and a second group was fed the basal diet supplemented with 100 mg. of pure methyl linoleate (Hormel Foundation) per animal per day. The two remaining groups were fed diets containing 5 per cent corn oil and 3 per cent tetrabromostearic acid, respectively.

Enzyme studies were begun at the end of 35 days of feeding *ad libitum*. The average weight increases during this *ad libitum* feeding period were 155.5 ± 6.0[1] gm. for the basal group, 156.2 ± 7.2 gm. for the group fed the 3 per cent tetrabromostearic acid diet, 182.0 ± 3.9 gm. for the linoleate-supplemented group, and 209.5 ± 5.6 gm. for the group fed the 5 per cent corn oil diet.

In the second phase, weanling male rats of the Sprague-Dawley strain, weighing from 38 to 58 gm., were randomly divided into three groups. These groups were fed respectively the fat-free basal ration, the basal ration plus 100 mg. of methyl linoleate per rat per day, and the 5 per cent corn oil *ad libitum*. Enzyme studies were begun at the end of 13 weeks. At the end of this period, the average weight was 267.9 ± 5.7[1] gm. for the fat-deficient group, 316.4 ± 7.3 gm. for the linoleate-supplemented animals, and 338.0 ± 9.6 gm. for the group receiving the 5 per cent corn oil diet.

The basal diet used in both experiments was a simplified ration consisting of 18 per cent casein, 78 per cent sucrose, 4 per cent Salts IV (16), and the following amounts of amino acids and vitamins per 100 gm. of diet: 0.25 gm. of methionine, 1.5 mg. of niacin, 2.0 mg. of calcium pantothenate, 200 γ of thiamine, 300 γ of riboflavin, 250 γ of pyridoxine, 100 mg. of choline chloride, 10 γ of biotin, 10 γ of *i*-inositol, and 20 γ of folic

[1] Standard error of the mean.

acid. Vitamins A, D, and E were supplied by a weekly supplementation of 1 drop of haliver oil containing 100 mg. of α-tocopherol per ml. The tetrabromostearic acid was prepared by bromination of corn oil fatty acids and recrystallization from a mixture of petroleum ether and ethyl ether. This was the 9,10,12,13-tetrabromostearic acid, which upon debromination with zinc yielded linoleic acid.

In the enzyme studies, the rats were sacrificed by decapitation and the livers were removed immediately and chilled in cracked ice. A portion of each liver was quickly weighed, homogenized in 5 volumes of ice-cold 0.039 M sodium and potassium phosphate buffer, and strained through gauze. This homogenate was used in the choline oxidase determination.[2] The endogenous respiration was measured in the determination of choline oxidase during the first 10 minutes after temperature equilibration. Portions of the homogenate were diluted with buffer to give a 5 per cent homogenate, which was used in measuring succinic oxidase, and a 2 per cent homogenate, which was employed in the assay for cytochrome oxidase. Succinic oxidase and cytochrome oxidase were measured according to the methods of Schneider and Potter (17). All enzyme activity was assayed in a Warburg bath maintained at 37°. Aliquots of each homogenate were taken for nitrogen determinations in duplicate by using a micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

At the time of sacrifice, all rats receiving the basal diet or the diet containing 3 per cent tetrabromostearic acid exhibited the signs of essential fatty acid deficiency: scaly paws, scaly or necrotic tails, and decreased growth. In general, the symptoms were more severe in the animals placed on the experimental diets as weanlings than in the mature animals. Skin symptoms appeared somewhat earlier but were not more severe in the adults receiving the 3 per cent tetrabromostearic acid than in the animals receiving only the basal diet. The results with these two dietary treatments were generally very similar.

The experimental results concerning the effect of fat deprivation on the enzyme activity of the liver tissue are summarized in Tables I and II. Standard errors of the mean are reported only for the enzyme activity based on the moist weight of livers of the adult rats. With the rats started as weanlings, enzyme activity was studied after pooling equal portions of the livers of two animals within a group and therefore the standard errors of the mean were not calculated. Approximately the same range of results was observed within each dietary treatment of both the adult (Table I) and the weanling rats (Table II). The enzyme ac-

[2] Williams, J. N., Jr., Litwack, G., and Elvehjem, C. A., to be published.

TABLE I

Effects of Fat Deficiency on Liver Enzymes of Adult Rats

Enzyme system	Ration	No. of rats	Activity based on fresh liver	Activity based on liver N
			μl. O_2 per hr. per mg.	*μl. O_2 per hr. per mg.*
Succinic oxidase	Basal	6	25.4 ± 1.6*	910
	3% tetrabromostearic acid	5	26.3 ± 1.0	1050
	Basal + methyl linoleate	6	26.1 ± 1.0	990
	5% corn oil	6	24.5 ± 1.6	980
Cytochrome oxidase	Basal	5	82.8 ± 1.2	2970
	3% tetrabromostearic acid	4	63.6 ± 4.8	2480
	Basal + methyl linoleate	4	60.0 ± 3.0	2270
	5% corn oil	5	55.3 ± 4.8	2200
Choline oxidase	Basal	6	3.12 ± 0.20	112
	3% tetrabromostearic acid	5	3.05 ± 0.39	119
	Basal + methyl linoleate	6	2.91 ± 0.19	110
	5% corn oil	6	2.51 ± 0.15	100
Endogenous respiration	Basal	7	0.61 ± 0.08	22
	3% tetrabromostearic acid	5	0.67 ± 0.01	26
	Basal + methyl linoleate	6	0.80 ± 0.09	30
	5% corn oil	6	1.21 ± 0.15	48

* Standard error of the mean.

TABLE II

Effects of Fat Deficiency on Liver Enzymes of Weanling Rats

Enzyme system	Ration	No. of rats	Activity based on fresh liver	Activity based on liver N
			μl. O_2 per hr. per mg.	*μl. O_2 per hr. per mg*
Succinic oxidase	Basal	7	25.7	970
	" + methyl linoleate	6	27.5	990
	5% corn oil	6	26.9	990
Cytochrome oxidase	Basal	7	82.3	3110
	" + methyl linoleate	6	60.0	2170
	5% corn oil	6	64.1	2350
Choline oxidase	Basal	7	3.42	129
	" + methyl linoleate	6	3.37	122
	5% corn oil	6	2.66	98
Endogenous respiration	Basal	7	0.65	26
	" + methyl linoleate	6	0.65	24
	5% corn oil	6	1.36	50

tivities are reported in terms of liver nitrogen, as well as the fresh weight of the livers, since dilution of the liver by lipide or glycogen might cause erroneous conclusions to be drawn from the enzyme results. Enzyme ac-

tivity in terms of liver nitrogen negates those effects. In all cases, however, the dietary treatment had no significant effect on liver nitrogen. These values ranged from 25.1 to 27.9 mg. of nitrogen per gm. of moist liver.

Fat deficiency appeared to affect differently each of the four enzyme systems studied. The succinic oxidase activity did not appear to be altered by any of the dietary treatments. Although succinic dehydrogenase activity has recently been reported to be dependent upon an intact lecithin portion (18), it appears that the maintenance of this enzyme is not dependent upon a dietary source of an essential fatty acid.

The activity of the cytochrome oxidase, however, is markedly increased in fat deficiency. Results obtained with the adult animals (Table I) and with the weanling animals (Table II) are identical. In each case the activity of livers from rats fed the basal diet was 38 per cent greater than from the linoleate-supplemented animals or from the animals receiving corn oil. This is particularly interesting in view of the observation of Burr and Beeber (8) and Wesson and Burr (9) that fat-deficient rats had a markedly increased metabolic rate. The latter authors reported that the basal and assimilatory metabolic rates of fat-deficient animals were 25 per cent greater than the rates of the control animals. Thus the liver cytochrome oxidase activity appears to parallel the metabolic rate in fat deficiency. This increased cytochrome oxidase activity in liver and perhaps other tissues may account in a large part for the increased metabolic rate. This phenomenon also may reflect a specific metabolic function of the essential fatty acids. A methyl linoleate supplement maintained a level of activity of cytochrome oxidase which appears to be normal, while it had little or no effect on the other enzyme systems studied here.

Although the animals fed the tetrabromostearic acid (Table I) showed symptoms of fat deficiency, these animals had only a slight increase in cytochrome oxidase activity. This increase becomes evident when expressed on the basis of liver nitrogen, but the low number of observations does not allow the assignment of any significance to this oxidase activity. With this exception, however, liver enzyme activity in the animals fed the tetrabromostearic acid was almost exactly the same as that in animals fed the basal diet

The choline oxidase appears to be elevated somewhat in all animals except those fed the corn oil diet. The activity of the liver choline oxidase of the rats fed the corn oil is approximately the same as that found for rats fed a commercial stock diet.[2] Like succinic oxidase, choline oxidase activity does not appear to be dependent upon a dietary source of the essential fatty acids, but, unlike succinic oxidase, it appears to be regu-

lated by the dietary inclusion of a fat or oil. Bernheim (19) has shown that choline oxidase is inhibited by fatty acids. Handler and Bernheim (20) have demonstrated a greatly depressed activity in fatty livers. The slight reduction in activity in livers of animals fed the corn oil probably reflects the same phenomenon.

The endogenous respiration of the livers of the fat-deficient animals is sharply decreased. Also this does not appear to be the effect of a lack of a dietary source of an essential fatty acid, since the supplementation with methyl linoleate did not appear to increase the endogenous respiration. The daily supplement of 100 mg. of methyl linoleate per animal per day may not have been sufficient to maintain this activity. On the other hand, feeding the diet containing corn oil appeared to double the average rates of endogenous respiration. Only in the case of tryptophan has it been shown that the inclusion of a specific nutrient in the diet markedly increased the endogenous respiration of the liver (5). Methionine deficiency (4) and various vitamin deficiencies (21, 22) have little effect on endogenous respiration. Therefore it appears that fat deficiency may affect enzyme systems other than the specific systems studied here. It is also possible that dietary fat supplies some of the substrates for endogenous respiration.

The high lipide content of the mitochondria (11, 12) and the action of lecithinase on cytochrome oxidase and on succinic dehydrogenase activity (18) suggest that certain of the respiratory enzymes may be lipoproteins. The enzyme systems, succinic oxidase, cytochrome oxidase, and choline oxidase, do not appear to be lost or decreased in activity when the dietary source of essential fatty acids is withheld. If these systems require the essential fatty acids, the results indicate that they are held tenaciously through long periods of deprivation or that enough of these fatty acids are supplied by the limited synthetic powers of the rat or by the carrier of the vitamins A and D. The results of these experiments do suggest that the imposition of a fat deficiency alters the normal balance of enzyme activity of tissues. The cytochrome oxidase activity is increased, and that there may be a concomitant decrease in the activity of another enzyme system is suggested by the decrease in endogenous respiration. Further investigation is required to elucidate these points.

SUMMARY

A fat deficiency in the rat causes a marked increase in liver cytochrome oxidase activity, a slight increase in choline oxidase activity, and a marked decrease in endogenous respiration. The activity of the succinic oxidase system is not altered by this deficiency condition.

Supplementation with 100 mg. of methyl linoleate per rat per day re-

duced the cytochrome oxidase to the level of that produced by a 5 per cent corn oil diet. The methyl linoleate at the level employed had little or no effect on the choline oxidase activity or on the endogenous respiration.

The addition of 9,10,12,13-tetrabromostearic acid to the basal diet appears to have little effect on the development of fat deficiency, except possibly to reduce cytochrome oxidase activity.

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