STRUCTURAL CHANGES IN ISOLATED LIVER MITOCHONDRIA OF RATS DURING ESSENTIAL FATTY ACID DEFICIENCY

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ABSTRACT

Liver mitochondria isolated in 0.44 M sucrose from rats deficient in essential fatty acids (EFA) oxidized citrate, succinate, α-ketoglutarate, glutamate, and pyruvate at a faster rate than did mitochondria isolated from normal rats; however, the oxidation of malate, caprylate, and β-hydroxybutyrate was not significantly increased. The mitochondria from deficient rats exhibited an increased ATPase activity and extensive structural damage as revealed by electron microscope examination of thin sections. An increase in citrate oxidation and ATPase activity, together with some structural damage, could be demonstrated as early as the 4^{th} week in rats on a fat-free diet. Saturated fat in the diet did not prevent the change in mitochondrial structure but accelerated its appearance. Both the biochemical and structural defects could be reversed within three weeks after feeding deficient rats a source of EFA. In the presence of a phosphate acceptor the effect of EFA deficiency on substrate oxidation was largely eliminated. A trend toward a reduced efficiency of oxidative phosphorylation was noted in mitochondria from EFA-deficient rats, but significant uncoupling was found only in the case of citrate, β-hydroxybutyrate, and glutamate in the presence of malonate. Together with the increased ATPase activity, the uncoupling of phosphorylation could account for the poor respiratory control found with the deficient preparation. However, EFA deficiency was without effect on the respiration of liver slices, which supports the belief that the observed changes in oxidation and phosphorylation are an artifact resulting from damage sustained by the deficient mitochondria during their isolation.

INTRODUCTION

An increased metabolic rate associated with deficiency in essential fatty acids (EFA) in rats was originally observed by Wesson and Burr (1). Subsequently, numerous metabolic defects have been reported in liver homogenates and subcellular fractions, often with conflicting results (2–5). It was first suggested by Levin *et al.* (5) that the biochemical changes in mitochondria isolated from EFA-deficient rats are due to an abnormally fragile membrane structure which becomes extensively damaged during the process of isolation. More recently, Richardson *et al.* (6) suggested, on the basis of conflicting reports concerning the site of uncoupled oxidation from phosphorylation, that this phenomenon was associated with a nonspecific change such as defective mitochondrial membrane structure rather than with a definite role of the EFA in the oxidative phosphorylation process.

Since a significant part of mitochondrial mem-

brane is composed of phospholipids which, in turn, contain a high percentage of EFA, the simultaneous loss of EFA and of structural integrity might be expected. Hayashida and Portman (7) observed a 79 per cent decrease in the EFA content of mitochondria isolated from rats fed a fat-free diet. The greater part of the loss has been found to occur within the first 6 weeks after feeding an EFA-deficient diet (8). That mitochondria from EFA-deficient rats are structurally different from mitochondria from normal rats is indicated by their more rapid rate of swelling (7, 9). Furthermore, microscopic examination of isolated mitochondria revealed many abnormal appearing forms (5). More recently, Wilson and Leduc (10) have reported that liver mitochondria from EFA-deficient mice are altered *in situ*.

In view of the early biochemical lesions which occur in liver homogenates and subcellular fractions during the course of development of EFA deficiency (11), it becomes of interest to examine more closely the morphological changes in mitochondria isolated from EFA-deficient rats. It is the purpose of this paper to demonstrate that the biochemical lesions found in mitochondria isolated from EFA-deficient rats are directly associated with alterations in mitochondrial structure. Evidence will also be presented that these changes are probably an artifact caused by damage sustained by the deficient mitochondria during their isolation.

METHODS

Weanling male albino rats from the Holtzman Company, Madison, Wisconsin, were housed individually in hanging wire cages and given food and water *ad libitum*. EFA deficiency was produced by feeding either a fat-free diet or one containing 25 per cent Hydrol (a fully hydrogenated vegetable oil), as described previously (11). Corn oil was used as the source of EFA in the control diets.

At the desired time the rats were killed by stunning followed by decapitation. The liver was quickly removed and placed in either 0.44 M or 0.25 M sucrose. Mitochondria were isolated from a 10 per cent homogenate in either 0.25 M or 0.44 M sucrose by differential centrifugation in an International refrigerated centrifuge, model PR-2, by a slight modification of the method of Schneider and Hogeboom (12), as described previously (11). The mitochondria were washed once, resuspended in either 0.25 M or 0.44 M sucrose, and their oxidative and adenosine triphosphatase (ATPase) activities were determined. This entire operation, from the death of the animal to the completion of the incubation, required 2 hours.

The incubation medium used in the manometric determination of mitochondrial oxygen consumption contained the following substances per flask: 40 μmoles potassium phosphate buffer (pH 7.4), 6 μmoles ATP, 20 μmoles magnesium chloride, 0.08 μmoles cytochrome c (horse heart, Sigma Chemical Company, St. Louis, Missouri), either 296 or 330 μmoles sucrose, and mitochondria from 200 mg of tissue (*ca.* 0.8 mg N). To determine oxidative phosphorylation, 50 μmoles glucose and 0.5 mg hexokinase (Sigma, type III) were added from the side arm at 0 time. The substrates were of the following concentrations: citrate, 45 μmoles; succinate, 45 μmoles; caprylate, 30 μmoles; and all others, 15 μmoles. The total reaction volume in the flask was 3.0 ml with 0.2 ml of 10 per cent KOH and a 2 cm^2 filter paper added to the center well. The oxidations were carried out by standard Warburg techniques at 30°C with air as the gas phase.

To determine the extent of phosphorylation associated with oxidation, the incubations were continued until a significant amount of phosphate was esterified (usually 10 minutes but maximally 20 minutes with slowly oxidizing substrates). The incubations were then terminated by the addition of 1 ml of cold 20 per cent perchloric acid. The protein precipitate was removed by centrifugation and duplicate aliquots of the supernatant solutions were taken for phosphate analysis by the method of Gomori (13). The initial concentration of phosphate was determined routinely, and the phosphate esterified was calculated by difference.

The medium used for the assay of ATPase activity consisted of 6 μmoles ATP, 5 μmoles magnesium chloride, 20 μmoles tris(hydroxymethyl)aminomethane buffer (pH 7.4), 62.5 μmoles sucrose, and 0.2 ml of the mitochondrial suspension, giving a final volume of 1 ml. The incubation was conducted at 30°C without shaking. The reaction was terminated after 10 minutes by the addition of 1 ml of cold 5 per cent perchloric acid, and the resulting protein precipitate removed by centrifugation. The phosphate content of the supernatant solution was determined by the method of Gomori (13). The extent of nonenzymatic hydrolysis of ATP was determined routinely by the use of mitochondrial-free reaction mixture, and occasionally the phosphate content of the mitochondria was assayed. The latter, however, was insignificant relative to phosphate produced by the ATPase reaction. Mitochondrial nitrogen was determined by the method of Johnson (14).

Preparation of the mitochondria for electron microscopy was carried out essentially according to the method of DeLuca *et al.*, (15). In all cases, mitochondria from deficient rats and their respective

controls were prepared and treated in parallel, such that they were examined following identical treatments. Thin sections were cut with a Servall Porter-Blum microtome and examined with a Siemens Elmiskop I.

Liver slices were made by means of a razor microtome. Slices 0.3 mm thick were blotted on filter paper and weighed to give 200 mg of liver tissue per flask. The incubation mixture consisted of Krebs-Ringer phosphate solution, pH 7.4, which had been previously gassed for 10 minutes with oxygen. Substrate concentrations were as follows: succinate, 45 μmoles; glucose and caprylate, 30 μmoles; pyruvate and α-ketoglutarate, 15 μmoles. Where endogenous respiration was desired, the substrate was omitted. Total volume in the outer compartment of the flask was 3 ml; the center well contained 0.2 ml of 10 per cent KOH with a 1 cm^2 filter paper; the gas phase was oxygen. Oxidations were followed for 30 to 60 minutes at 37°C. Tissue nitrogen was determined according to a modification of the method of Johnson (14).

TABLE I

*Influence of Fat Deficiency on Substrate Oxidation by Rat Liver Mitochondria**

Substrate	Dietary fat: Fat free	Dietary fat: 5 per cent Corn oil
	μl O_2/hour/mg N	
Citrate	357 ± 12‡‖	262 ± 6
Succinate	665 ± 21‖	482 ± 18
α-Ketoglutarate	360 ± 30‖	251 ± 14
Glutamate	359 ± 24‖	241 ± 15
Malate	179 ± 7	162 ± 4
β-Hydroxybutyrate	111 ± 9	112 ± 12
Pyruvate§	374 ± 20‖	243 ± 19
Caprylate§	411 ± 66	437 ± 46

* Mitochondria were isolated in 0.44 M sucrose. The medium as described in the text included 330 μmoles sucrose and 6 μmoles of ATP. Oxidations were carried out for 30 minutes at 30°C. The rats were on their respective diets for 12 weeks.

‡ Standard error of the mean. Each value represents at least 5 determinations.

§ 6 μmoles fumarate added.

‖ $P < 0.001$.

RESULTS

Liver mitochondria isolated from rats maintained on a fat-free diet for 12 weeks showed an increased oxidation of citrate, succinate, α-ketoglutarate, glutamate, and pyruvate (Table I), as well as an increased ATPase activity (Table II). In contrast, the oxidation of malate, caprylate, and β-hydroxybutyrate was not increased by fat deficiency.

An increased citrate oxidation by liver mitochondria could be demonstrated as early as the fourth week on a fat-free diet and was concomitant with a rise in the ATPase activity (Fig. 1). By the 8th week, citrate oxidation had reached a maximum whereas the ATPase activity continued to rise through the eleventh week, although it should be noted that the difference between the mitochondria from EFA-deficient rats and those from normal rats in their ability to oxidize citrate is at least as great at 4 weeks as it is at 8 weeks.

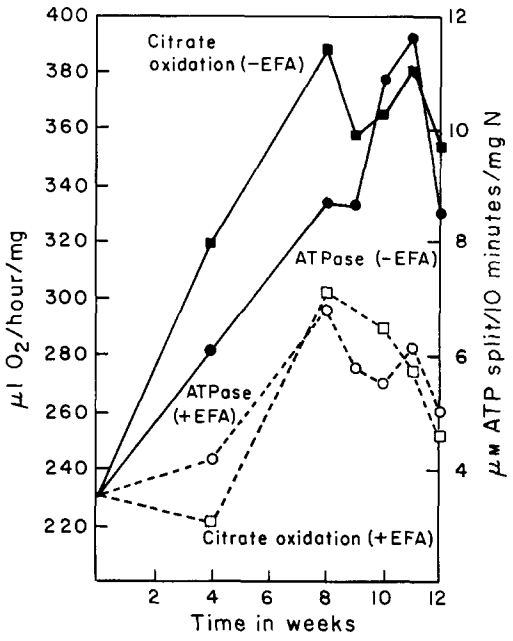
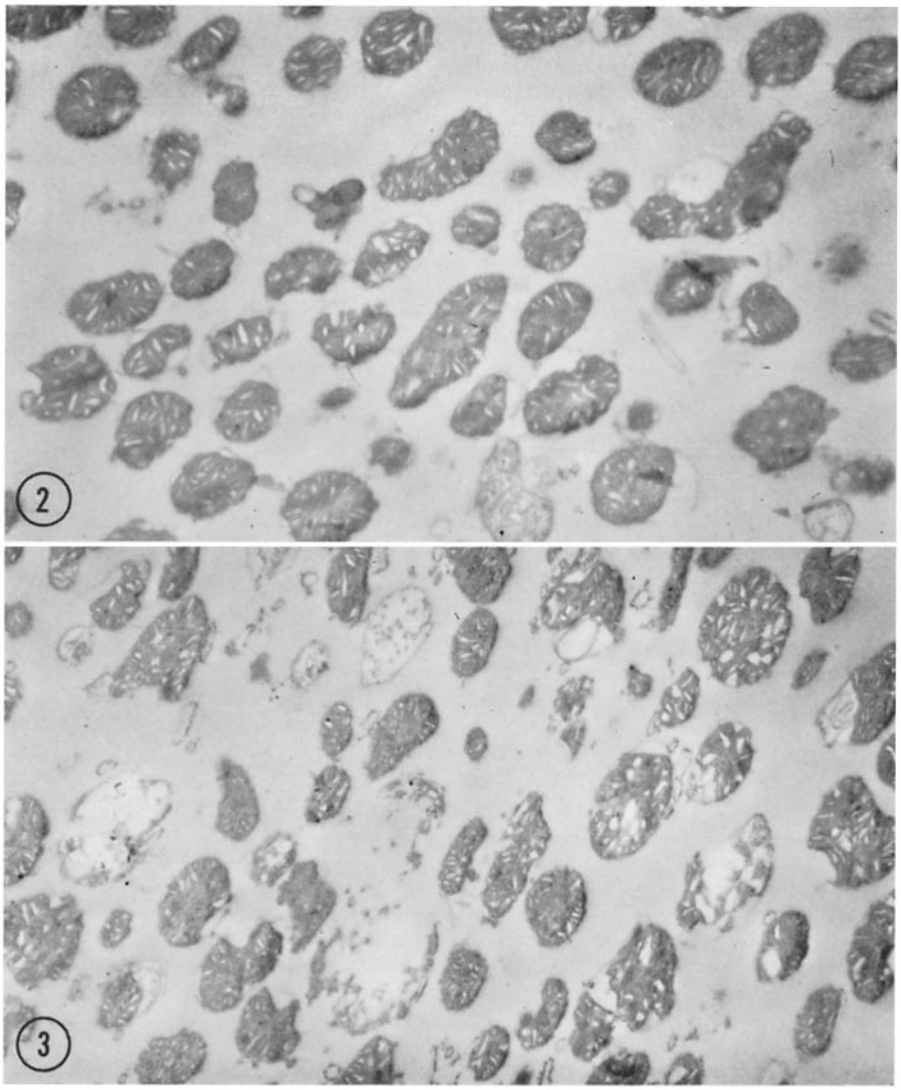


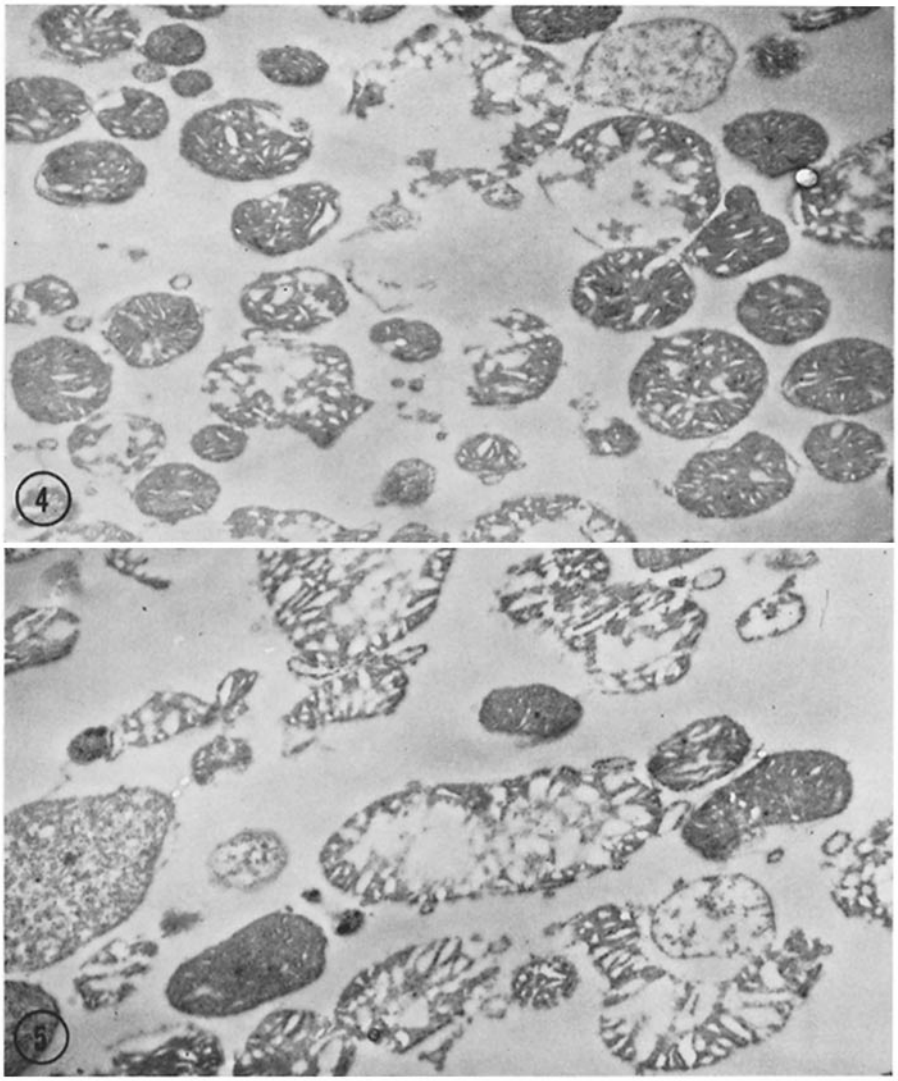
FIGURE 1 Correlation between ATPase and oxidative activities of rat liver mitochondria isolated in 0.44 M sucrose. EFA-deficient rats were fed a fat-free diet, while the controls were fed a 5 per cent corn oil diet. Experimental conditions were as given in the text.

Alterations in the biochemical characteristics of the mitochondria from EFA-deficient rats were accompanied by structural damage as revealed by electron microscopy. Fragmented forms were evident after the rats had been on a fat-free diet for 4 weeks (Figs. 2 and 3). Some of the mitochondria appeared slightly swollen and the spaces between the cristae were larger than those observed in control preparations. The majority of



FIGURES 2 AND 3 Liver mitochondria isolated, in 0.44 M sucrose, from rats which had received semi-synthetic diets for 4 weeks. Fig. 2, rat received diet containing 5 per cent corn oil as a source of EFA. Fig. 3, rat received fat-free diet. × 15,000.

the mitochondria, however, still appeared normal. By the 9th week, the swollen, broken forms became more numerous (Fig. 4). The mitochondria examined showed a wide range of variation in structure. The general distribution most often encountered, however, can be seen in Fig. 4. After 12 weeks on the fat-free diet, the majority of the mitochondria observed were damaged (Fig. 5), with only a few normal, intact forms still evident. At this time the mitochondria from EFA-deficient rats were two to three times larger than those from the corresponding control rats (Fig. 7). The internal structure had suffered serious derangement and was sometimes lacking entirely. It should be mentioned that, while only two rats from each group were used in any one week, several pellets from each were sectioned and examined.



FIGURES 4 AND 5 Liver mitochondria, isolated in 0.44 M sucrose, from rats on a fat-free diet. Fig. 4, after 9 weeks on the diet. Fig. 5, after 12 weeks on the diet. × 15,000.

The biochemical properties observed in liver mitochondria from fat-deficient rats can also be demonstrated when rats are fed a large quantity of saturated fat. The mitochondria from rats receiving 25 per cent of their diet as saturated fat showed an elevated ATPase activity (Table II) and an increased citrate oxidation (Fig. 6). The latter was apparent after 2 weeks on the diet and became maximal by the 4th week. This effect could be reversed readily by supplementing the deficient diet with a source of EFA. Citrate oxidation was normal after the deficient rats had received EFA for 3 weeks.

Feeding a diet containing saturated fat also failed to prevent the occurrence of damage in the isolated mitochondria and acted to accelerate its appearance. Although the changes in mitochondrial structure were analogous to those occurring in mitochondria from rats on a fat-free diet, the greatly swollen, fragmented forms appeared after only 8 weeks on the high-fat diet (Fig. 8). Mitochondria isolated from control rats

receiving the high-fat, EFA-supplemented diet were similar in appearance to those isolated from rats fed the 5 per cent corn oil ration (Figs. 2 and 7), and therefore examples of these are not included.

Marked improvement in the structure of the isolated mitochondria was noted after the deficient rats had received corn oil for 1 week (Fig. 9), in contrast to the effect of EFA on citrate oxidation (Fig. 6). The mitochondria appeared only slightly swollen and the cristae were evident although separated by large spaces. Again, the extensive variation in the appearance of the mitochondria examined should be stressed.

TABLE II

*ATPase Activity of Liver Mitochondria from EFA-Deficient Rats**

Diet	μmoles ATP split/10 minutes/mg N
Fat Free‡	9.4 ± 1.0‖¶
5 per cent Corn oil	5.3 ± 0.3
25 per cent Hydrol§	7.7 ± 0.4
20 per cent Hydrol–5 per cent corn oil	4.7 ± 0.1

* Experimental conditions were as described in the text.
‡ These rats were on their diets for 12 weeks.
§ These rats were on their diets for 8 weeks.
‖ Standard error of the mean.
¶ $P < 0.001$.

Although forms more severely damaged than those shown in Fig. 9 were observed, none of the greatly swollen mitochondria seen in Fig. 8 was noted, and a great many of the mitochondria appeared normal. The majority of the mitochondria examined possessed normal structure after the deficient rats had received EFA for 3 weeks (Fig. 10).

When a phosphate acceptor (hexokinase-glucose) was supplied to the medium, the effect of EFA deficiency on mitochondrial oxidation was largely eliminated except when citrate and succinate were used as substrates (Table III). There was a significant decrease due to EFA deficiency in the efficiency of phosphorylation coupled to the oxidation of citrate, glutamate in the presence of malonate, and β-hydroxybutyrate, whereas no significant impairment was noted with succinate, α-ketoglutarate, α-ketoglutarate plus malonate, and glutamate as substrates. Similar results were obtained when diets high in saturated fat were used to induce EFA deficiency. These results demonstrate that EFA deficiency does not induce a uniform uncoupling of phosphorylation, but rather under specific conditions the "uncoupling" can be demonstrated.

The fact that EFA deficiency markedly increases respiration of isolated mitochondria in the absence of phosphate acceptor is best illustrated by "respiratory control" measurements given in Table IV. The results leave no doubt that the deficiency markedly reduces the control of respiration by phosphorylation.

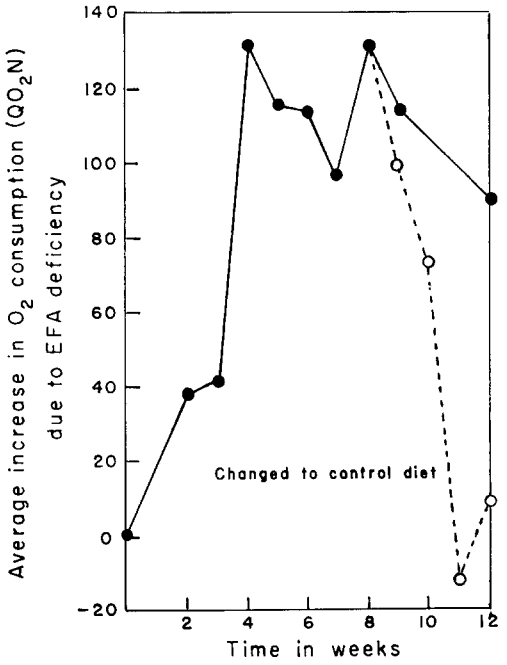


FIGURE 6 Rate of increase in citrate oxidation, resulting from EFA deficiency, and its reversal by feeding a source of EFA. Rat liver mitochondria were isolated in 0.44 M sucrose. Each point represents the difference between the average results from two deficient rats (25 per cent Hydrol) and two control rats (20 per cent Hydrol–5 per cent corn oil). Experimental conditions are described in the text.

The question of whether EFA deficiency results in uncoupled oxidative phosphorylation in intact tissue is of greatest importance with regard to both an explanation for the increased basal metabolic rates in deficiency and the question of whether the mitochondrial changes observed to

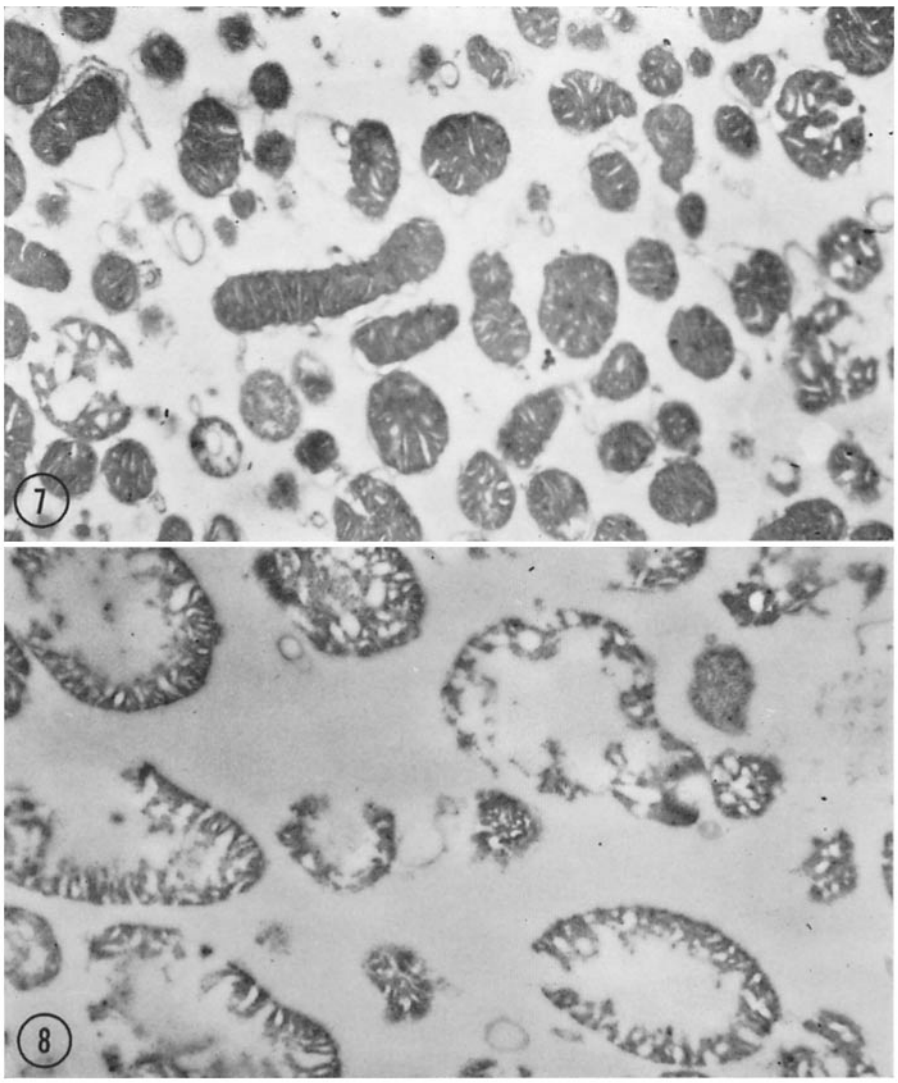


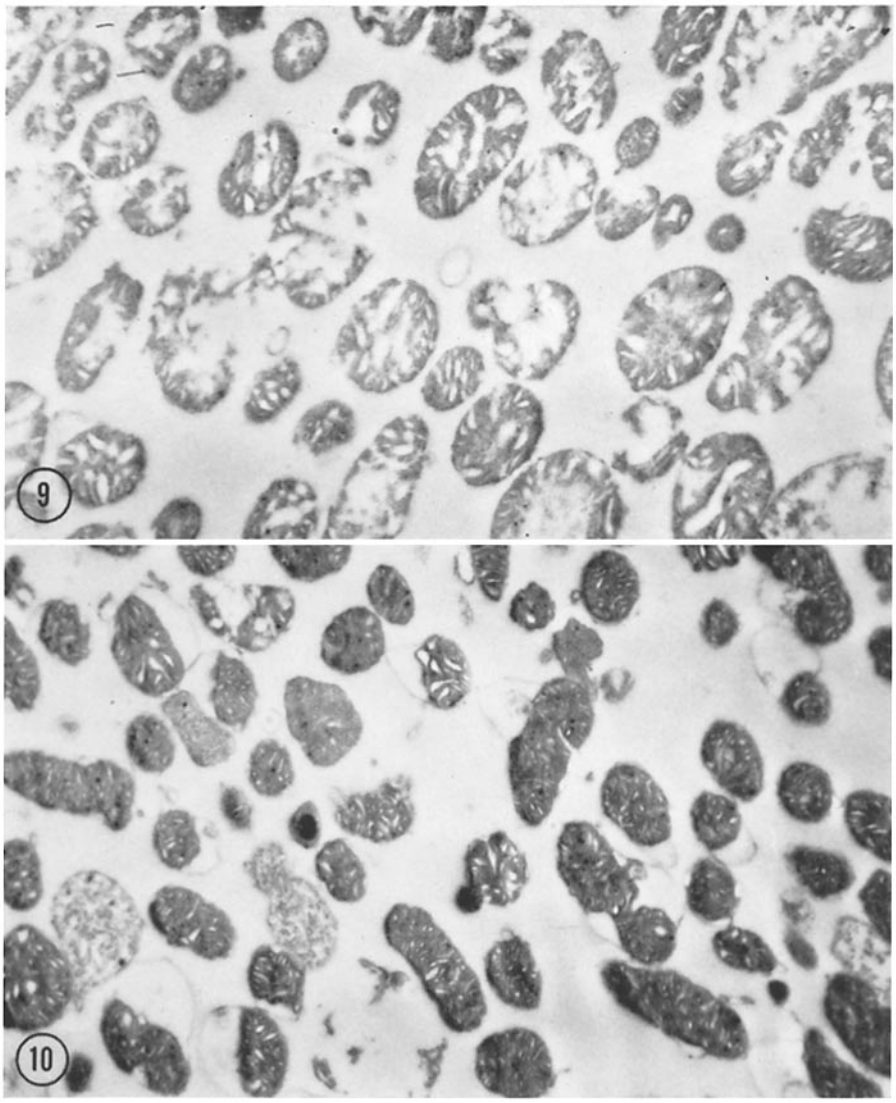
FIGURE 7 Liver mitochondria, isolated in 0.44 M sucrose, from a control rat receiving the 5 per cent corn oil diet for 12 weeks. × 15,000.

FIGURE 8 Liver mitochondria, isolated in 0.44 M sucrose, from a rat which had been fed the 25 per cent saturated fat diet for 8 weeks. × 15,000.

date are of physiologic significance. Certainly the morphological changes in isolated mitochondria are not readily apparent in intact tissue. studies on the effect of EFA deficiency on respiration in liver slices were therefore carried out in the presence and absence of substrates. The results shown in Table V clearly demonstrate that EFA deficiency does not increase the respiratory rate of liver slices whether or not the animals had been fasted for 24 hours prior to killing. Similar results were obtained with kidney slices and with rats made deficient with diets high in saturated fat.

DISCUSSION

It is now clear, from these studies on isolated liver mitochondria, that mitochondria undergo extensive structural damage in EFA deficiency. Further, the damage is evident early in the



FIGURES 9 AND 10 Liver mitochondria, isolated in 0.44 M sucrose, from EFA-deficient rats (25 per cent saturated fat diet) which had been fed EFA after 8 weeks on the deficient diet. Fig. 9, after 1 week of EFA feeding. Fig. 10, after 3 weeks of EFA feeding. × 15,000.

development of the deficiency and is accompanied by an increase in both citrate oxidation and ATPase activity. It is essential to note that in the presence of excess phosphate acceptor the effect of EFA deficiency on mitochondrial oxidations is largely eliminated (Table III), resulting in decreased respiratory control values (Table IV). This may be caused by an uncoupling of phosphorylation in deficiency or by an increased ATPase activity which regenerates more phosphate acceptor (ADP), and, under these conditions, Lardy and Wellman (16) have shown that the rate of respiration is dependent on the availability of a phosphate acceptor. Of these possibilities, the latter appears the greater contributor, since with α-ketoglutarate and glutamate there was only slight uncoupling of phosphorylation in EFA deficiency while respiratory control was

TABLE III

*Oxidation and Associated Phosphorylation by Liver Mitochondria from Normal and EFA-Deficient Rats**

Substrate	Diet			
	Fat free		5 per cent Corn oil	
	μA O_2‡	P/O	μA O_2	P/O
Citrate	38.0 ± 1.6§‖	2.10 ± 0.04¶	30.8 ± 1.6	3.03 ± 0.17
Succinate	83.1 ± 1.1‖	1.46 ± 0.11	75.4 ± 2.7	1.63 ± 0.15
α-Ketoglutarate	47.6 ± 1.6	3.02 ± 0.08	42.1 ± 1.9	3.25 ± 0.19
α-Ketoglutarate-malonate	22.6 ± 0.8	3.19 ± 0.19	20.6 ± 1.4	3.50 ± 0.15
Glutamate	60.1 ± 3.2	2.75 ± 0.16	54.0 ± 2.2	2.85 ± 0.05
Glutamate-malonate	9.1 ± 0.7¶	1.23 ± 0.36¶	12.6 ± 0.2	2.71 ± 0.15
Malate	18.8 ± 2.7	2.36 ± 0.15‖	17.7 ± 0.4	2.88 ± 0.10
β-Hydroxybutyrate	13.8 ± 0.4¶	1.75 ± 0.08¶	16.0 ± 0.5	2.28 ± 0.07

* Mitochondria were isolated in 0.25 M sucrose. The medium, as described in the text, included 2 μmoles ATP and 296 μmoles sucrose. 50 μmoles glucose and 0.5 mg hexokinase (Sigma, type III) were added from the side arm at 0 time.

‡ μA O_2/hour/mg mitochondrial N.

§ Standard error of the mean. Each value represents at least 5 determinations.

‖ $P < 0.05$.

¶ $P < 0.01$.

TABLE IV

*Respiratory Control in Mitochondria from EFA-Deficient Rats**

Diet	Substrate	
	α-Ketoglutarate	Glutamate
Fat Free	2.18 ± 0.16‡§ (6)	3.06 ± 0.28§ (6)
5 per cent Corn Oil	3.68 ± 0.30 (6)	4.51 ± 0.26 (5)
25 per cent Hydrol	3.08 ± 0.23§ (6)	4.50 ± 0.38 (7)
20 per cent Hydrol–5 per cent Corn Oil	4.47 ± 0.24 (6)	5.62 ± 0.61 (7)

* Control of respiration refers to the ratio of respiration in the presence of a phosphate acceptor to that in its absence. Hexokinase-glucose was used as the phosphate acceptor. Mitochondria were isolated in 0.25 M sucrose, and the medium described in the text included 2 μmoles ATP, 296 μmoles sucrose and, when indicated, 0.5 mg hexokinase (Sigma, type III).

‡ Standard error of the mean. Numbers in parenthesis indicate the number of determinations.

§ $P < 0.01$.

greatly affected (see Tables I and IV). Moreover, under some conditions the activation of ATPase may be the result and a measure of mitochondrial damage (17, 18). Thus, in agreement with the suggestion of Levin *et al.* (5), the observed biochemical changes in EFA deficiency may well be the result of injury sustained by the mitochondria during their isolation. However, it should also be pointed out that, whereas the loss of structural integrity becomes more extensive as the deficiency progresses, the biochemical changes do not increase in magnitude after the eighth week on a fat-free diet. This would indicate that severe damage is not necessary in order to obtain maximal oxidation rates, so long as the ATPase is adequate to provide sufficient phosphate acceptor.

The presence of saturated fat in the diet not only failed to prevent structural damage of the isolated, EFA-deficient mitochondria but appeared to accelerate it. This would be expected from previous reports on the effect of saturated fat on other parameters of EFA deficiency (19) and

would further indicate that the mitochondrial fragility resulted from a lack of EFA *per se* rather than of fatty acids. The fact that feeding a source of EFA to deficient animals resulted in a reversal of the biochemical lesions and in improved structural arrangement of mitochondrial membranes is added evidence that EFA are necessary for structural integrity and that the biochemical defects are directly related to mitochondrial structure.

On the other hand, if loss of EFA from the phospholipids of mitochondrial membranes was the only factor involved in the observed structural disorganization, then these structural changes could be expected to be most pronounced by the 6th week on a fat-free diet. Klein and Johnson (8) have observed that the greatest loss of EFA from subcellular particles occurs within this period, with little or no loss occurring thereafter. However, Collins (20) noted that the incorporation of P^{32} into liver phospholipids was greater in EFA-deficient rats than it was in normal controls, suggesting an increased turnover of the phospholipids. This may also contribute to the membrane fragility of the deficient mitochondria and, together with other yet poorly understood phenomena, may account for the progressive damage which has been found to occur over the entire 12-week period of the present study.

Some question exists whether the improvement of mitochondrial structure observed after feeding EFA to deficient rats is due to the repair of existing mitochondria or the formation of new mitochondria. Fletcher and Sanadi (21) reported the half-life of liver mitochondria to be approximately 10 days. This would allow a significant synthesis of new mitochondria to occur within 1 week, accounting for the improvement in structure observed in Fig. 9. In contrast, if Collins's data (20) indicate an increased turnover of phospholipids in existing mitochondria rather than an increased turnover of mitochondria as a whole, the possibility remains that EFA would be incorporated into existing mitochondria, constituting a repair. In either case the reversal would be incomplete, especially if the amount of supplemented EFA was insufficient to meet the need. Thus the present data do not resolve this question. It has already been shown that homogenate oxidations return to normal after feeding deficient rats EFA for 1 week (11). The present requirement of 3 weeks for reversal of citrate oxidation would seem to be in disagreement. However, it may be that the improved mitochondrial structure is sufficient to withstand homogenization but that further manipulations necessary for isolation of the mitochondria constitute a damaging stress. This would also indicate that the biochemical properties of the mitochondria respond quite sensitively to structural defect.

Since the biochemical properties of EFA-deficient mitochondria appear related to mitochondrial structure, it would be of interest to

TABLE V

*Lack of Effect of EFA Deficiency on Respiration of Liver Slices from Non-fasted and Fasted Rats**

Substrate	Dietary conditions			
	Non-fasted		Fasted	
	Fat free‡	5 per cent Corn oil	Fat free (4)	5 per cent Corn oil
	μlO_2/hour/mg N			
Endogenous	40 ± 4‡	42 ± 3	32 ± 4	41 ± 4
Pyruvate	57 ± 4	54 ± 3	40 ± 5§	59 ± 3
Succinate	240 ± 13	230 ± 7	295 ± 17	274 ± 20
α-Ketoglutarate	47 ± 4	54 ± 4	39 ± 2§	57 ± 3
Glucose	50 ± 6	45 ± 4	36 ± 5	42 ± 3
Caprylate	74 ± 8	64 ± 2	58 ± 8	72 ± 3

* Experimental conditions were as given in the text.
‡ Standard error of the mean.
§ $P < 0.05$.

determine whether the swollen, broken forms observed are an artifact due to isolation procedures or occur *in situ*. Preliminary observations in this laboratory did not reveal any abnormalities in mitochondria in intact liver from EFA-deficient rats, whereas Wilson and Leduc (10), working with whole cells of liver from EFA-deficient mice, noted greatly swollen mitochondria with an increased number of centrally located cristae. However, these forms could be demonstrated only in severely deficient mice rather than in the earlier stages of the deficiency. Thus, even if analogous forms were to be found in severely deficient rats, they would not account for the increased metabolic rate associated with EFA deficiency since Wesson and Burr (1) and Panos and Finerty (22) report this to be a fairly early symptom of the deficiency disease.

It should be noted that the effects of EFA deficiency on the oxidative activity of liver mitochondria reported here are similar to those observed by Levin *et al.* (5), with two exceptions. Whereas in the present experiments the oxidation of malate was unchanged, the oxidation of pyruvate was significantly increased by EFA deficiency. Levin and his coworkers obtained directly opposite results. Further, an increased oxidation of both malate and caprylate by EFA-deficient homogenates has been observed in this laboratory. These discrepancies may have resulted from the use of a hypertonic isolation medium which has been shown to reduce the effect of EFA deficiency on mitochondrial oxidations (11). The data on increased ATPase activity and oxidative phosphorylation are in accord with the report of Klein and Johnson (3).

The basic question enveloping the present and previous investigations of this type is: Can the changes in biochemical behavior observed in isolated mitochondria account for the increased basal metabolic rate found in EFA deficiency? Undoubtedly, many if not all of the biochemical alterations in mitochondria from EFA deficient rats thus far reported are an artifact resulting from damage during isolation. Certainly the drastic changes in the morphology found in isolated mitochondria do not appear in mitochondria of intact tissue, although subtle changes may be found by more careful and refined electron microscopy. The failure to observe an increase in the respiration of liver slices from EFA-deficient rats also provides strong evidence that the observed biochemical properties of isolated mitochondria are not reflected in intact tissue. Certainly, uncoupled phosphorylation or increased ATPase should have resulted in an elevated respiration in liver slices. These results suggest therefore that a reexamination of the biochemical basis for the elevated metabolic rate in EFA deficiency is needed and that strong consideration be given to alternative explanations.

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