BBA 4279

INHIBITORY MECHANISMS IN THE CONTROL OF LIPOGENESIS

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(Received July 22nd, 1963)

SUMMARY

Synthesis of fatty acids by supernatant prepared from livers of fed rats was measured when various cytoplasmic particles of livers from fed or fasted rats were present. Microsomal inhibition of lipogenesis occurs at the acetyl-CoA carboxylase (acetyl-CoA:CO_2 ligase (ADP), EC 6.4.1.2) step and involves at least two mechanisms: The action of a microsomal ATPase (ATP phosphohydrolase) which is elevated in fasting and an unidentified action upon the enzyme acetyl-CoA carboxylase. Inhibition by "light-" and by "intermediate" microsome fractions were tested separately. The inhibition due to the "light" microsomes was completely abolished by an ATP-generating system, indicating that the inhibitory mechanism here is ATP removal. Inhibition by intermediate fraction was only partially abolished by an ATP-generating system, indicating that both ATPase activity and interaction with the enzymes in the supernatant had occurred. Both microsomal fractions were more inhibitory when obtained from fasted rats than those from fed rats. The mitochondrial inhibition did not affect the reaction of the fatty acid synthetase system and probably acts directly on the enzyme acetyl-CoA carboxylase. Mitochondria from fasted rats were more inhibitory than those from fed rats.

INTRODUCTION

An explanation of the marked depression of fatty acid synthesis that accompanies even brief periods of starvation has been the aim of numerous investigations[1]. Some authors suggested deficiency of a cofactor, such as TPNH (see ref. 2); others have suggested loss of the enzymes synthesizing fatty acids[3,4]. Our experiments indicate that neither of the above deficiencies causes the low rate of hepatic lipogenesis in rats fasted for 24 h (see refs. 5–7). Rather, the production of one or more inhibitors of lipogenesis appears to be implicated[5–7]. Cytoplasmic liver particles from fasted rats inhibited hepatic lipogenesis, whereas those from fed rats stimulated lipogenesis. The inhibitory activity appeared to be predominantly in the microsomal fraction and to affect the acetyl-CoA carboxylase (acetyl-CoA:CO_2 ligase (ADP), (EC 6.4.1.2)) step. At least in part, restriction of the amount of ATP available for this reaction contributes to the inhibitory mechanism; microsomes from fasted rats have a higher

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ATPase (ATP phosphohydrolase) activity than do microsomes from fed rats. However, as brought out below, the mechanism is much more complex than indicated by these earlier studies.

MATERIALS AND METHODS

Adult male rats of the Wistar Strain were fed *ad libitum* for 10 days a diet composed of 25% casein, 10% lard, 51% dextrose, 3% salt mix[8], 5% cellulose, 6% brewer's yeast, 0.04% cod liver oil concentrate, and 0.01% α-tocopherol. After this period of stabilization, the animals were divided into two groups: the fasted group, which was deprived of food for 24 h, and the control group, which continued to feed *ad libitum* until sacrificed.

The rats were killed and liver homogenates were prepared in 0.25 M sucrose as previously described[7]. The homogenate was centrifuged for 10 min at 600 × *g* to remove nuclei, unbroken cells and debris. To obtain cytoplasmic particles the supernatant (S_{600}) was then centrifuged for 60 min at 105 000 × *g*; the resulting pellet was rinsed with 0.25 M sucrose. To prepare mitochondria from the S_{600}, a modification of SCHNEIDER AND HOGEBOOM's method[9], with a centrifugal force of 3550 × *g* for 15 min, was employed. After removal of the thrice-washed mitochondria, the supernatant (S_{3550}) was centrifuged for 60 min at 105 000 × *g* to secure a microsomal pellet, which was rinsed with sucrose. The supernatant fluid remaining after removal of mitochondria and microsomes is called the "particle-free" supernatant and is almost particle-free.

In some experiments, the S_{3550} was centrifuged for 15 min at 11 200 × *g*. The pellet thus obtained was resuspended in 0.25 M sucrose and spun for 15 min at 11 200 × *g*. This is termed the "intermediate" microsomal fraction. The supernatant remaining after removal of the mitochondria and the intermediate fraction was centrifuged at 105 000 × *g* for 60 min to secure the "light" microsomal pellet, which was rinsed with 0.25 M sucrose. All these homogenization and centrifugation steps were carried out at about 2°.

Fatty acid synthesis was studied with the following labeled substrates: [1-^{14}C]-acetate, [1-^{14}C]acetyl-CoA, and [2-^{14}C]malonyl-CoA. Except when specifically noted in the results, the preparation of the substrates, the reaction media, the incubation systems, the isolation of the fatty acid and the determination of their ^{14}C content were carried out as described previously[7]. Protein was determined by the method of LOWRY *et al.*[10].

In some experiments, an ATP generating system was used to study the incorporation of acetyl-CoA into fatty acids. Here the 1.5 μmoles ATP in the incubation medium were replaced by 10 μmoles creatine phosphate, 0.4 μmoles ADP and 0.5 mg creatine kinase (ATP:creatine phosphotransferase, EC 2.7.3.2) (≅ 15 units of enzyme).

RESULTS

In an earlier communication[7], we tentatively concluded that fasted microsomes restrict lipogenesis by means of a very active ATPase which limits the amount of ATP available. On the assumption that the effect of this ATPase would be overcome if enough ATP were available, a system synthesizing ATP was provided. To maintain an optimal level of ATP, the following reaction was utilized:

$$\text{creatine phosphate} + \text{ADP} \xrightarrow{\text{creatine kinase}} \text{creatine} + \text{ATP}.$$

The equilibrium of the reaction is such that at the concentrations of ADP and creatine phosphate used, the adenosine phosphate exists predominantly as ATP. When fasted microsomes were added to the particle-free supernatant from control tissue in the presence of the ATP-generating system, their inhibitory action on lipogenesis was much less than when a fixed amount of ATP was added to the medium (Table I).

Again, in a series of preincubation experiments conducted with [1-^{14}C]acetyl-CoA as the substrate, the inhibitory effect of fasted microsomes was reduced when the ATP-generating system was present (Table II). Preincubation of fasted microsomes with the medium containing a fixed amount of ATP almost abolished lipogenesis. Preincubation of fasted microsomes and medium also increased greatly the inhibition of fatty acid synthesis when the generating system was the source of ATP, but this effect could be abolished by doubling the concentration of the creatine phosphate. It should be noted that such an increase in creatine phosphate concentration did not affect lipogenesis in systems which were not preincubated.

The increase in lipogenesis following preincubation of the control supernatant has been observed previously[7] but it has yet to be explained.

TABLE I

MICROSOMAL INHIBITION OF LIPOGENESIS

"Fasted" microsomes were prepared from livers of rats fasted for 24 h and added in concentration necessary to reconstitute original supernatant to microsome ratio*.

Preparation	*Source of ATP*	*[1-^{14}C]acetyl-CoA converted to fatty acids (mμmoles)***
Control supernatant***	added	51.1 ± 7.3
Control supernatant + fasted microsomes§	added	10.1 ± 1.8
Control supernatant	generated	47.9 ± 5.2
Control supernatant + fasted microsomes	generated	34.9 ± 5.4

* Total volume of incubation system = 1.0 ml.
** Average value for 5 experiments ± standard error of the mean.
*** 0.3 ml of a 20% supernatant.
§ 0.15 ml of a 40% microsomal suspension.

TABLE II

EFFECT OF PREINCUBATION OF VARIOUS COMPONENTS ON LIPOGENESIS IN CONTROL SUPERNATANT*

System preincubated (30 min at 37°)	*Fasted microsomes added (ml of 40% suspension)*	*[1-^{14}C]acetyl-CoA converted to fatty acids***	
		*ATP added (mμmoles)****	*ATP generated (mμmoles)****
None	0	40.2	42.7
Control supernatant	0	47.3	52.0
None	0.15	8.3	23.5
Medium + fasted microsomes	0.15	2.9	4.6
Control supernatant + fasted microsomes	0.15	8.7	19.1

* 0.3 ml of 20% supernatant.
** Total volume of incubation system = 1.0 ml.
*** Average value for 3 experiments.

Lipogenesis was inhibited by the "light" microsomal fractions from livers of both the control and fasted rats, those from fasted rats being almost twice as inhibitory as those from control rats (Table III). This inhibition was demonstrable over a wide range of "light" microsome concentrations. In the presence of the ATP-generating system, however, the inhibitory effect of the fasted "light" microsomes was abolished, and those from control rats even stimulated lipogenesis.

The effects of various concentrations of intermediate fraction from fasted rats on the lipogenic activity of control supernatant are shown in Fig. 1. This inhibitory activity was heat labile.

The incorporation of [1-^{14}C]acetyl-CoA into fatty acids by the supernatant was linear with time up to 90 min. With intermediate fractions present (Fig. 2), the rate

TABLE III

EFFECT OF "LIGHT" MICROSOMES ON LIPOGENESIS FROM [1-^{14}C]ACETYL-CoA IN CONTROL SUPERNATANT

Each incubation flask contained 0.3 ml of 20 % control supernatant plus 0.15 ml of a 40 % suspension of control or fasted "light" microsomes*.

Tissue preparation	*Inhibition*		*Protein content of 40 % microsome suspension (mg/ml)*
	*ATP added (%)***	*ATP generated (%)***	
Control supernatant + control "light" microsomes	49.4 ± 10.8	−28 ± 30.0	11.48
Control supernatant + fasted "light" microsomes	71.6 ± 3.5	−8.4 ± 11.2	11.06

* Total volume of incubation system = 1.0 ml.
** Average values for 4 experiments ± standard error of mean.

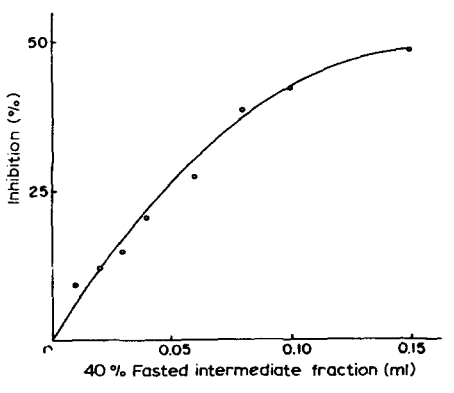

Fig. 1. Effect of varying concentrations of fasted intermediate fraction on fed supernatant lipogenesis from [1-^{14}C]acetyl-CoA. Average values from 3 experiments. (Total volume of incubation system was 1.0 ml and contained 0.3 ml of 20 % supernatant.)

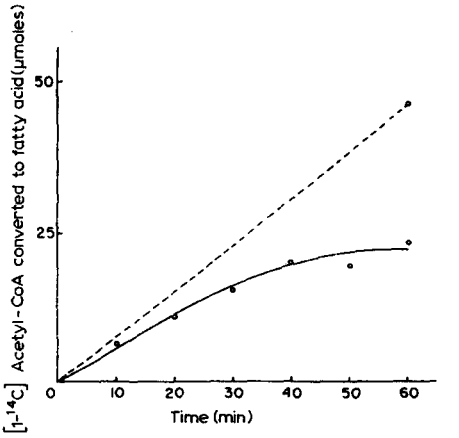

Fig. 2. Effect of fasted intermediate fraction on time course of lipogenesis from [1-^{14}C]acetyl-CoA. Broken line refers to fed supernatant and solid line to fed supernatant plus fasted intermediate fraction. Average values from 3 experiments. (Total volume of incubation system was 1.0 ml and contained 0.3 ml of 20 % supernatant; intermediate fraction was added as 0.15 ml of 40 % suspension and reconstituted the cellular supernatant: intermediate ratio.)

of incorporation clearly decreased and stopped after 40 min. Consequently, as shown by comparison of the first and third lines in Table IV, total lipogenesis was less in control supernatant when fasted intermediate fraction was present. If the intermediate fraction from fasted rats was preincubated with incubation medium (fifth line in Table IV) even less lipogenesis occurred. If, however, the control supernatant was preincubated with the intermediate fraction (sixth line in Table IV), lipogenesis approached that of the control supernatant. In view of the striking increase in lipogenesis produced by preincubation of the control supernatant alone (see also Table II) it seems clear that the fasted "intermediate" fraction was actually inhibitory in this case too. As with the "light" microsome fraction, the inhibitory effect of the control "intermediate" fraction was only half that of the fraction from fasted animals. Unlike the results with the "light" fractions, however, substitution of the ATP-generating system for added ATP only partially abolished the inhibitory effects of control and fasted intermediate fractions.

When [2-^{14}C]malonyl-CoA rather than [1-^{14}C]acetyl-CoA was the substrate, lipogenesis was not measurably affected by intermediate fractions from fasted rats (Table V). Thus the inhibition evidently occurred at the acetyl-CoA carboxylase step.

TABLE IV

EFFECT OF PREINCUBATION OF VARIOUS COMPONENTS ON LIPOGENESIS IN CONTROL SUPERNATANT* IN PRESENCE OF FASTED "INTERMEDIATE" FRACTION

System preincubated (30 min at 37°)	*Fasted intermediate fraction added (ml of 40% suspension)*	*[1-^{14}C]acetyl-CoA converted to fatty acids***	
		*ATP added (mμmoles)****	*ATP generated (mμmoles)****
None	0	35.5	29.6
Control supernatant	0	67.3	62.3
None	0.15	24.1	21.0
Fasted intermediate fraction	0.15	27.6	23.1
Fasted intermediate fraction + medium	0.15	12.9	20.3
Fasted intermediate fraction + control supernatant	0.15	31.3	29.8

* 0.3 ml of 20% supernatant.
** Total volume of incubation system = 1.0 ml.
*** Average from 2 experiments.

TABLE V

EFFECT OF FASTED INTERMEDIATE FRACTION ON FATTY ACID SYNTHESIS FROM [2-^{14}C]MALONYL-CoA

Tissue preparation	*Incubation time (min)*	*[2-^{14}C]malonyl-CoA converted to fatty acids* (mμmoles)***
Control supernatant***	30	78
Control supernatant + fasted intermediate fraction§	30	71
Control supernatant	60	108
Control supernatant + fasted intermediate fraction	60	108

* Total volume of incubation system = 0.3 ml.
** Average values for 3 experiments.
*** 0.3 ml of 20% supernatant.
§ 0.15 ml of 40% intermediate fraction.

Moreover, the preincubation studies also suggested a relation of the inhibition exerted by the intermediate fraction to ATPase activity.

The effects of various concentrations of fasted mitochondria on the lipogenic activity of control supernatant are shown in Fig. 3. Inhibition occurred at all mitochondrial concentrations tested. Furthermore, although inhibition was drastically reduced by heating mitochondria for 15 min at 60° (from 49 % to 15 % at one concentration), it was not abolished. This result contrasts with the complete elimination of microsomal inhibition by similar heating.

The rate of incorporation of [1-^{14}C]acetyl-CoA into fatty acids in the presence of fasted mitochondria is shown in Fig. 4. Unlike the time curves with microsomal and intermediate fractions, the curve is linear to at least 60 min; however, the rate of incorporation was less than that for the control supernatant.

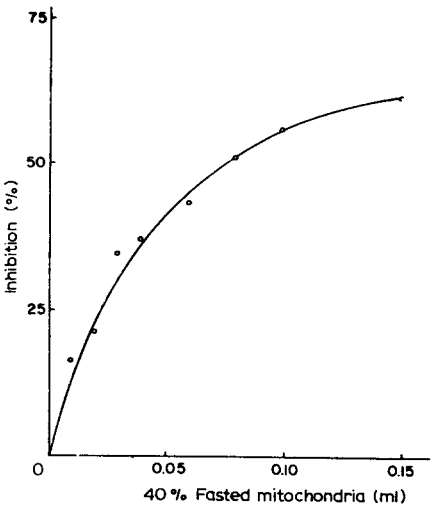

Fig. 3. Effect of varying concentrations of fasted mitochondria on fed supernatant lipogenesis from [1-^{14}C]acetyl-CoA. Average values from 3 rats. (Total volume of incubation system was 1.0 ml and contained 0.3 ml of 20 % supernatant.)

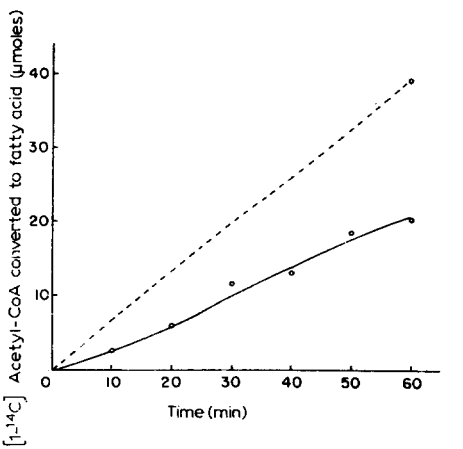

Fig. 4. Effect of fasted mitochondria on the time course of lipogenesis from [1-^{14}C]acetyl-CoA. Broken line refers to fed supernatant and solid line to fed supernatant plus fasted mitochondria. Average values from 3 experiments. (Total volume of incubation system was 1.0 ml and contained 0.3 ml of 20 % supernatant; mitochondrial fraction was added as 0.15 ml of 40 % suspension and reconstituted the cellular supernatant: mitochondria ratio.)

When fasted mitochondria were preincubated for 30 min with incubation medium (Table VI), lipogenesis was inhibited far more than it was by fasted mitochondria alone. Moreover, preincubation of the fasted mitochondria with control supernatant offset the augmentation of lipogenesis produced by preincubation of control supernatant alone.

Again, the incorporation of [2-^{14}C]malonyl-CoA into fatty acids was not significantly affected (Table VII). The substrates for the acetyl-CoA carboxylase step are acetyl-CoA, HCO_3^- and ATP. Of these three components, acetyl-CoA and HCO_3^- were present in excess in the reaction mixture. However, it seemed possible that the mitochondria were removing acetyl-CoA rapidly enough to cause the rate of lipo-

TABLE VI

EFFECT OF PREINCUBATION ON LIPOGENESIS IN PRESENCE OF FASTED MITOCHONDRIA

Fasted mitochondria were prepared from livers of rats fasted for 24 h and added to the system in a concentration that reconstituted the original ratio of the mitochondrial fraction to the supernatant*.

System preincubated (30 min at 37.5°)	*Fasted mitochondria added (ml of 40% suspension)*	*[1-^{14}C]acetyl-CoA converted to fatty acids** (mμmoles)****
None	0	32.9
Control supernatant	0	56.1
None	0.15	22.6
Fasted mitochondria	0.15	12.1
Fasted mitochondria + medium	0.15	5.9
Fasted mitochondria + control supernatant	0.15	23.0

* 0.3 ml of 20% supernatant.
** Total volume of incubation system = 1.0 ml.
*** Average values for 3 experiments.

TABLE VII

EFFECT OF FASTED MITOCHONDRIA ON FATTY ACID SYNTHESIS FROM [2-^{14}C]MALONYL-CoA

Mitochondria addition is the same as described in Table VI.

Tissue preparation	*Incubation time (min)*	*[2-^{14}C]malonyl-CoA converted to fatty acids* (mμmoles)****
Control supernatant***	30	62
Control supernatant + fasted mitochondria	30	56
Control supernatant	60	82
Control supernatant + fasted mitochondria	60	78

* Total volume of incubation system = 1.0 ml.
** Average values from 3 experiments.
*** 0.3 ml of 20% supernatant.

genesis to fall. An obvious route for the removal of acetyl-CoA is via the citric acid cycle, the acetyl-CoA condensing with oxaloacetate to form citrate. However, addition of malonate in concentrations sufficient to block the citric acid cycle did not affect the mitochondrial inhibition of lipogenesis. Furthermore, the addition of more acetyl-CoA to the incubation medium did not reverse mitochondrial inhibition.

In the presence of an ATP-generating system, the inhibition by the mitochondria was enhanced (Table VIII). The reason for this increase is not clear, but obviously mitochondrial inhibition of lipogenesis is not related to an ATPase action.

Since acetyl-CoA carboxylase was the rate-limiting enzyme, it may have generated only a small pool of malonyl-CoA, which was rapidly destroyed by a mitochondrial malonyl-CoA decarboxylase (malonyl-CoA carboxylyase, EC 4.1.1.9). The presence of this enzyme would probably not be demonstrated by an assay of fatty acid synthetase system like that reported in Table VII, since the amount of malonyl-CoA is present in excess. However, activity of malonyl-CoA decarboxylase should affect incorporation of [2-^{14}C]malonyl-CoA into fatty acids when its concentration is suboptimal. As seen in Table IX, addition of fasted mitochondria did not inhibit the reaction at any concentration tested. Thus, the possibility that the mitochondrial inhibitor is malonyl-CoA decarboxylase can be ruled out.

Since the levels of Mn^{2+} were suboptimal in these assays, an additional series of experiments was conducted with excess Mn^{2+}. The inhibitory effect of fasted mitochondria was not diminished. Since the concentration of Mn^{2+} was also suboptimal in our previous work[7] with [1-^{14}C]acetate several of its aspects were re-explored with 3 mM $MnCl_2$ rather than the 0.6 mM previously used. This higher concentration caused a shift in the optimal ATP concentration from 3 mM to 4 mM; thus the high concentration of ATP was used in the systems containing supernatant, and ATP was deleted from the homogenate system, where it is not required for optimal activity. Since the concentration of [1-^{14}C]acetate used in the previous work, 1 mM, was also slightly suboptimal, the concentration was raised to 6 mM. Finally, lipogenic activity in the supernatant was increased slightly by increasing the isocitrate concentration from 17 mM to 34 mM; as lipogenesis with the homogenate did not change at the higher isocitrate concentration, only 17 mM was used in this system.

TABLE VIII

EFFECT OF ATP-GENERATION ON MITOCHONDRIAL INHIBITION OF LIPOGENESIS FROM [1-^{14}C]ACETYL-CoA IN CONTROL SUPERNATANT*

Mitochondria addition is the same as described in Table VI.

Mitochondrial preparation	*Protein in 40% mitochondrial suspension (mg/ml)*	*Inhibition***	
		*ATP added (%)****	*ATP generated (%)****
Control	9.40	14.2 ± 3.1	41.6 ± 5.8
Fasted	8.88	49.1 ± 7.4	56.9 ± 7.9

* 0.3 ml of 20% supernatant.
** Total volume of incubation system = 1.0 ml.
*** Average values ± standard error of mean.

TABLE IX

EFFECT OF FASTED MITOCHONDRIA ON FATTY ACID SYNTHETASE SYSTEM WITH SUBOPTIMAL CONCENTRATIONS OF [2-^{14}C]MALONYL-CoA

Concentration of [2-^{14}C]malonyl-CoA 10 mM	*[2-^{14}C]malonyl-CoA converted to fatty acids**	
	Without mitochondria (mμmoles)	*With mitochondria** (mμmoles)*
0.10	105	94
0.06	78	71
0.04	50	46
0.02	23	23

* 0.15 ml of 20% supernatant; total volume of incubation system = 1.0 ml.
** 0.15 ml of a 40% suspension.

When control or fasted microsomes or mitochondria were added individually, the effect on lipogenesis in the control supernatant was the same with this revised incubation medium as with that used previously. However, when both mitochondria and microsomes were added simultaneously to the revised medium a rather striking difference was found, namely, fasted cytoplasmic particles stimulated rather than

inhibited lipogenesis of the control supernatant. Indeed, in the revised incubation medium, the lipogenic activity of the fasted liver homogenate was much higher than that of the fasted supernatant (Table X), which is just the opposite of our previously reported findings[7]. In studies to determine which change in the incubation medium was responsible for this difference, it became apparent that no single change but rather a combination of two or more changes was responsible.

TABLE X

EFFECT OF FASTING ON LIPOGENESIS BY RAT-LIVER HOMOGENATE AND SUPERNATANT

For each rat, 3 ml of 20% homogenate and 3 ml of supernatant, prepared from the 20% homogenate, were studied simultaneously*.

Physiological state	*No of rats*	*[1-^{14}C]acetate converted to fatty acid*	
		*Homogenate (mμmoles)***	*Supernatant (mμmoles)***
Control	5	1750 ± 438	610 ± 62
Fasted	5	1450 ± 356	710 ± 159

* Total volume of incubation system = 5.0 ml.
** Average values ± standard error of mean.

DISCUSSION

The combined cytoplasmic particles (mitochondria plus microsomes) of the liver of fasted rats were shown to inhibit fatty acid synthesis by supernatant prepared from the livers of fed rats while cytoplasmic particles from livers of fed rats were shown to stimulate supernatant lipogenesis[7]. On the other hand, present work shows that by appropriate modifications of the incubation medium, the combined cytoplasmic particles from either fed or fasted rats can promote incorporation of [1-^{14}C]acetate into fatty acids. However, when [1-^{14}C]acetyl-CoA was the labeled substrate, conditions whereby the combined particles from fasted rats increased rather than decreased incorporation of ^{14}C into fatty acids were not attained.

Microsomes from fasted rats markedly inhibited fatty acid synthesis from acetate by liver supernatant[7]. From preincubation studies, it appears that the microsomal inhibition involves an essential component of the incubation medium; of the components necessary for lipogenesis, TPNH and ATP are most critical[7]. This inhibitory action affected the incorporation of acetyl-CoA into fatty acids, but not the incorporation of malonyl-CoA (see ref. 7). Since TPNH is needed for both systems, but ATP is required only with acetyl-CoA, removal of ATP seems the probable mechanism of microsomal inhibition. Further evidence is supplied by the following findings: in the presence of fasted microsomes, the optimal concentration of ATP for lipogenesis is markedly elevated[7], and fasting greatly increases the microsomal ATPase[7]. Moreover when an ATP-generating system is included in the incubation medium, the inhibitory action of the fasted microsomes is greatly reduced.

After separation of the microsomes into "light" and intermediate fractions, further evidence on the inhibitory mechanisms was obtained. Since the inhibition of lipogenesis due to the "light" microsomes could be completely reversed by an

ATP-generating system, it appears that this inhibition of lipogenesis is due to ATPase activity.

It is very difficult to assess the significance *in vivo* of this inhibitory action. KORCHAK AND MASORO[11] reported that the decrease in levels of hepatic enzymes for fatty acid synthesis observed in 24-h starved rats is not sufficient to entirely account for the depression of lipogenesis in liver slices from similarly fasted rats. Moreover, direct inhibitory mechanisms may well be major causes of the marked depression of lipogenesis in fasted cold-stressed rats[6]. Several authors[12,13] have shown that during fasting a decline in hepatic ATP and increases in hepatic ADP and AMP occur concurrently. A reasonable conclusion is that the microsomal ATPase regulates lipogenesis *in vitro* but owing to the experimental difficulties precluding direct observations of its role within the living cell, its physiological function remains unknown.

The experiments with the "intermediate" fraction using acetyl-CoA and malonyl-CoA as substrates indicated that this fraction also has an inhibitory action on lipogenesis related to an ATPase action. However, preincubation studies carried out in the presence of generated ATP also indicated an interaction of the intermediate fraction with the control supernatant but not with the incubation medium. It therefore can be tentatively concluded that the "intermediate" fraction also inhibits lipogenesis through an unidentified action upon hepatic enzymes contained in the supernatant.

Mitochondria from both the control and fasted rats inhibited lipogenesis from [1-^{14}C]acetyl-CoA, the fasted mitochondria being the more inhibitory. This inhibition occurs at the acetyl-CoA-carboxylase step but was not reversed in the presence of an ATP-generating system. Consequently, the active factor is probably not an ATPase.

With fasted mitochondria, the concentration of ATP optimal for supernatant lipogenesis was not increased, as it was with microsomes. Also, the mitochondria apparently do not act by removing the substrate acetyl-CoA, since the requirement for this compound was not altered; nor do they inhibit by decarboxylating malonyl-CoA. These data point to the possibility that the mitochondria inhibit the supernatant enzyme, acetyl-CoA carboxylase. The preincubation studies did indicate an interaction of the mitochondria with enzymes in the supernatant. However, these studies also showed enhanced inhibition when the fasted mitochondria were preincubated with the incubation medium. This finding is hard to reconcile with others which indicated mitochondria did not interact with the incubation medium. Perhaps incubation with some components of the medium caused an increase in the permeability of the mitochondrial membrane. Activation of the latent mitochondrial ATPase or synthesis of an inhibitor could also very well occur under these conditions. The latter would be consistent with the heat lability studies. It could be postulated that a heat-stable inhibitor was generated by a heat-labile system. Obviously more work is needed before any conclusions can be drawn regarding the mode of action of the mitochondrial lipogenic inhibitors.

ACKNOWLEDGEMENTS

This investigation was supported by Grant AM06917-02 of the National Institutes of Health.

The authors are indebted to Drs. F. FUCHS and F. W. BRIGGS for the gift of the creatine kinase A. The competent technical assistance of SUSAN BURR is gratefully acknowledged.

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