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Review

Biochemical mechanisms related to the homeostatic regulation of lipogenesis in animals

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Since most animals are intermittent eaters, they are confronted with the problem of temporarily storing much of the chemical energy ingested as food. Fat takes up less volume and weighs less per calorie of stored chemical energy than either carbohydrate or protein. Thus, in animals whose major foodstuff is carbohydrate, the use of lipogenesis as the primary means by which the energy of dietary carbohydrate is stored affords maximum efficiency. The major homeostatic function of lipogenesis is to store as fat the chemical energy of carbohydrate ingested in excess of the immediate energy requirements of the organism.

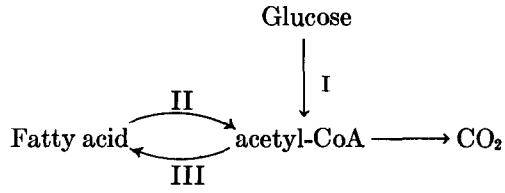
Clearly, a process as vital to caloric balance as fat synthesis must be precisely regulated to meet the ever-changing energy needs of the organism. While the experimental data on the regulation of lipogenesis are vast, they present a far from complete picture. The purpose of this review is to analyze these data and, when possible, formulate some general concepts concerning the biochemical mechanisms involved in this homeostatic regulation.

EFFECT OF FASTING ON LIPOGENESIS

That the rate of fatty acid synthesis can be markedly altered by changes in nutritional state was first demonstrated in 1944 by Boxer and Stetten (1) and Bernhard and Steinhauser (2). These investigators labeled the body water of rats with D_2O and found that either under-nutrition or a period of fasting greatly reduced the amount of deuterium incorporated into the body fat. The authors concluded that lipogenesis is greatly curtailed during conditions of restricted caloric intake.

In vitro studies by Bloch (3) and by Masoro *et al.* (4) using liver slices localized the impairment in lipogenesis to some point between acetyl-CoA[1] and fatty acids. Bloch found that liver slices from fasted rats had a markedly reduced ability to convert acetate-C^{14} to fatty acid. Masoro *et al.* (4) showed that, when liver slices prepared from rats fasted for 24 hours were incubated in the presence of 400 mg per 100 ml of glucose-C^{14}, almost no C^{14} was incorporated into the fatty acids. However, the conversion of the glucose-C^{14} to $C^{14}O_2$ was not altered by the 24-hour fast, a fact that indicates a normal ability to form labeled acetyl-CoA from glucose. It is evident that under these conditions the reduced rate of lipogenesis did not result from a lack of substrate (acetyl-CoA) derivable from glucose but rather from an inability to utilize acetyl-CoA in fatty acid synthesis.

The biological importance of the restricted utilization of acetyl-CoA for fatty acid synthesis becomes clear when one considers the metabolic problems facing the fasting animal. The following abbreviated metabolic scheme assists in this consideration:



During fasting, very little glucose is available to the tissues of the body. Therefore, little acetyl-CoA is formed from glucose by way of reaction sequence I, and the homeostatic requirement for storing glucose carbon as fatty acid is abolished. However, acetyl-CoA is generated by way of reaction sequence II at a much higher rate than in fed animals (5). Therefore, if reaction sequence III were not greatly inhibited during fasting, a great amount of the fatty acid undergoing β-oxidation would be needlessly recycled.

[1] The following abbreviations are used in the text: CoA, coenzyme A; TPN, oxidized triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P_i, inorganic orthophosphate; ATPase, adenosine triphosphatase; FFA, free fatty acid or acids.

The question of the mechanism by which lipogenesis is inhibited during fasting is a fundamental one of great interest. Several laboratories have used C^{14}-labeled substrates (6–9) to confirm the fact that lipogenesis is depressed in the intact fasting animal. Elucidation of the intimate mechanisms involved has, however, depended largely on *in vitro* studies of isolated liver preparations.

Lyon *et al.* (10) provided the initial evidence that depressed lipogenesis in the fasting state is related to depressed carbohydrate metabolism. They recognized that liver glycogen is greatly depleted during fasting and found that the administration of glucose by stomach tube to fasted rats 6 hours prior to sacrifice restored both the glycogen content of the liver and the ability of the liver slice to convert labeled acetate to fatty acid. Similar treatment with corn oil was without effect on lipogenesis, while casein hydrolysate was moderately stimulating. Masri *et al.* (11) found that the addition of unlabeled glucose to an incubation system containing liver slices from fasted rats partially restored the ability to incorporate acetate-C^{14} into fatty acids. From these studies, it was tentatively concluded that fatty acid synthesis fails in the livers of fasting rats simply because of a low rate of carbohydrate metabolism. It has been suggested that carbohydrate metabolism is required to generate the reducing environment required for lipogenesis; in particular, TPNH generation via the hexose monophosphate pathway has been considered the specific way in which carbohydrate metabolism promotes fatty acid synthesis.

This explanation, however, does not fit all the facts now at hand. Masoro *et al.* (12) reported that depressed lipogenesis in liver slices prepared from rats fasted for 24 hours at 0° to 2° was not even partially overcome by the addition of unlabeled glucose to the incubation system. Furthermore, Sauer (13) found that glucose-6-phosphate added to liver homogenates prepared from fasted rats did not overcome the depressed lipogenic activity. Moreover, the administration of glucose by stomach tube to fasting rats prior to sacrifice did not result in restored lipogenic activity in liver homogenates prepared subsequently.

In order to properly evaluate the contribution of depressed carbohydrate metabolism to the low lipogenic activity in the fasting state, it is essential to briefly review recent studies of the enzymatic sequence in the biosynthesis of fatty acids (14). An abbreviated version of what is now considered the major pathway of fatty acid biosynthesis is presented for reference:

$$(1)\quad \text{Acetyl-CoA} + \text{ATP} + CO_2 \xrightarrow{\text{acetyl carboxylase}} \text{malonyl-CoA} + \text{ADP} + P_i$$

$$(2)\quad 7\ \text{Malonyl-CoA} + \text{acetyl-CoA} + 14\ \text{TPNH} + 14\ H^+ \xrightarrow{\text{“synthetase”}} \text{palmitate} + 14\ \text{TPN}^+ + 7\ CO_2 + 8\ \text{CoA} + 6\ H_2O$$

This reaction sequence has been shown to take place mainly, if not exclusively, in the supernatant fraction of liver homogenate centrifuged at 105,000 × *g* for 1 hour.

In considering the regulation of fatty acid synthesis, it is important to recognize that the acetyl carboxylase reaction is far slower than the "synthetase" reaction (15, 16). It is theoretically possible that malonyl-CoA is also formed by reactions other than reaction 1, but none has been described in animal systems. Moreover, Numa *et al.* (16) found that, whereas the specific activity of acetyl carboxylase in rat liver supernatant was approximately equal to that of total fatty acid synthesis from acetate, the specific activity of the "synthetase" was considerably greater. It therefore seems reasonable to accept acetyl carboxylase as the rate-limiting step in lipogenesis and to expect that the regulation of fatty acid synthesis will involve this reaction.

In the light of this information on fatty acid synthesizing enzymes, it is profitable to re-evaluate whether or not the depressed lipogenesis of the fasting state is due to decreased generation of TPNH from the metabolism of carbohydrates. TPNH is needed for the "synthetase" reaction, which is not the rate-limiting enzyme. Thus, if a TPNH deficiency is to cause a marked reduction in lipogenesis, the concentration of TPNH would have to be low enough to make the "synthetase" reaction slower than that catalyzed by acetyl carboxylase. It would be extremely valuable to know the concentration of TPNH in the liver of the fasted rat, but, to the reviewer's knowledge, this measurement has not yet been made. For the following reasons, however, it seems unlikely that lipogenesis in the fasting state is limited by the rate of formation of TPNH via the hexose monophosphate pathway: (*a*) From a theoretical viewpoint, as discussed above, the "synthetase" step is not likely to be the control point. (*b*) The addition of a TPNH generating system to liver homogenates prepared from rats fasted at 0° to 2° did not overcome the depression in fatty acid synthesis (17), and the addition of substrates that should augment TPNH production failed to promote lipogenesis in liver homogenates from rats fasted at room temperature (13). (*c*) Lowenstein (18, 19) presented evidence that, in the extra-mitochondrial portion of the liver cell, TPNH can be produced by pathways other than the hexose monophosphate pathway and that this pathway is not a quantitatively important source of hydrogen for fatty acid synthesis. These findings

are not corroborated by the work of Foster and Bloom (20), which indicates that TPNH generation by the hexose monophosphate pathway is an important source of hydrogen for hepatic fatty acid synthesis. In very recent work by Lowenstein,[2] however, a comparison of glucose and isocitrate showed isocitrate to be a much more active source of hydrogen for hepatic fatty acid synthesis.

One may then consider other possible ways in which lipogenic activity could be limited. There are four general possibilities: lack of substrate, lack of co-factors, loss of fatty acid synthesizing enzymes, and presence of inhibitors of lipogenesis. Of these, the first has already been discussed and ruled out. A discussion of the other three will now be undertaken.

In 1958, Catravas and Anker (21, 22) obtained evidence for the presence of an hitherto unsuspected co-factor required for fatty acid synthesis. They found that this substance was present in livers of fed rats but not in those of fasted rats and that, when added to liver homogenates prepared from fasted rats, it promoted fatty acid synthesis from acetate. This lipogenic stimulator, which they call lipogenin, can also be prepared from yeast. When lipogenin was administered parenterally to fasted rats, it greatly increased lipogenesis from acetate *in vivo*, but similar treatment of fed rats caused no change in the lipogenic rate. Catravas and Anker believe that lack of lipogenin may be the cause of the failure in lipogenesis in fasted rats. Much work must still be done on this substance, however, before a physiological role can be assigned to lipogenin. Its chemical nature is unknown and there is still no knowledge of the biochemical site of its action.

Numa and co-workers (16) found that all enzymatic events associated with fatty acid synthesis were depressed during fasting and that acetyl carboxylase exhibited a specific activity equal to the over-all system. Moreover, when purified acetyl carboxylase was added to cell-free liver preparations from fasted rats, it stimulated fatty acid synthesis to values greater than those found for similar liver systems prepared from fed rats. It was suggested that the decreased activity of acetyl carboxylase is responsible for depressed lipogenesis during fasting. While this work has been confirmed in our laboratory by Korchak and Masoro,[3] certain findings force us to disagree with the interpretation of Lynen's group. At the end of a 24-hour fast, we found the rate of lipogenesis depressed far more than could be accounted for by the decrease in acetyl carboxylase activity noted at this time. Certainly something other than enzyme levels limited lipogenesis during the early interval of fasting; it would seem more likely that falling acetyl carboxylase levels are the result of a depressed lipogenesis than the cause of it.

Gibson and Hubbard (23) reported that the hepatic level of the enzyme or enzymes involved in the conversion of malonyl-CoA to fatty acids ("synthetase") was depressed during a 48-hour fast. They found no such deficiency of acetyl carboxylase. In a very recent report, Hubbard *et al.* (24) presented evidence showing that the restriction in lipogenesis during fasting is due to the loss in activity of the enzymatic step carrying out the condensation-decarboxylation reaction of the "synthetase" system. The reason for the marked discrepancy between the experimental findings of Gibson and Hubbard and those of Lynen's group and our laboratory is not clear. From theoretical considerations, as discussed earlier, it is difficult to accept the "synthetase" step as the site of regulation.

Masoro and Porter (25) reported that, during fasting, an activity that is inhibitory to lipogenesis appears in the liver cytoplasmic particulates and, in particular, the microsomes. Further work (26) showed that this lipogenic inhibitory system acts at the acetyl-carboxylase step and that at least part of the inhibitory action is attributable to an increased microsomal ATPase activity during fasting. Evidence has also been uncovered, however, for an interaction between the supernatant fraction of the cell and the microsomal inhibitory system which may not be related to ATPase. Further work on the chemical nature of the inhibitor or inhibitors and on the biochemical mechanism of action is in progress.

The physiological significance of inhibitory mechanisms in the regulation of hepatic lipogenesis can be evaluated by utilizing data on the rat fasted for 24 hours at 0° to 2° (27). The concentrations of fatty acid synthesizing enzymes are only moderately reduced in this condition. Loss of enzymes, reduced levels of co-factors and lipogenic stimulators or any combination of these can account for only part of the markedly depressed hepatic lipogenesis. One can only conclude that the lipogenic inhibitory mechanism or mechanisms play an important role in limiting the rate of hepatic lipogenesis in cold-fasted rats.

Since adipose tissue is quantitatively a far more important site of lipogenesis than the liver, the lipogenic response of adipose tissue to fasting is of great importance. Adipose tissue from fasted rats has a depressed ability to convert glucose-C^{14}, pyruvate-C^{14}, and acetate-C^{14} to fatty acids (28–30); the addition of unlabeled glucose to the incubation system partially

[2] Lowenstein, J. M., manuscript in preparation.

[3] Korchak, H. M., and E. J. Masoro, submitted for publication.

reactivates fatty acid synthesis from acetate but has no influence in the case of the other two substrates. Milstein (31) has shown that the activity of the hexose monophosphate pathway is depressed in the adipose tissue of fasting rats. There are no data, however, on the effect of fasting on enzyme levels or on the levels of lipogenic stimulators or inhibitors in adipose tissue. Until data in these areas are available, it is not possible to speculate on the mechanisms involved in the depressed lipogenesis in this tissue.

INFLUENCE OF EATING PATTERN ON LIPOGENESIS

Meal-eating as compared to continuous nibbling is known to alter metabolism markedly. On the basis of respiratory quotients, Tepperman *et al.* (32) found that far more of a glucose test load was converted to fat when rats were trained to eat the usual 24-hour ration in 3 hours or less. Also, Tepperman and Tepperman (33) found that liver slices from rats trained to eat their 24-hour ration in one hour had a higher rate of lipogenesis than liver slices from rats eating the same ration over a 24-hour period. Enzymatic evidence (33, 34) as well as isotopic evidence (33) shows the hexose monophosphate pathway of carbohydrate metabolism to be hyperactive in rats trained to eat spaced meals rather than allowed to nibble. At this time, it is not possible to say whether the high activity of the hexose monophosphate pathway is the cause of the "superlipogenesis" of meal eating or the result of it.

Medes *et al.* (35) noted that very high rates of lipogenesis occurred in liver slices from rats that were refed for a short time after periods of fasting or food restriction. Tepperman and Tepperman (33, 36) carried out quite an extensive investigation of this phenomenon, which they term the "superlipogenesis" of refeeding. Following a 48-hour fast, rats were refed a high-carbohydrate, low-fat diet for 3, 6, 12, 24, and 48 hours. At the end of a given period of refeeding, groups of rats were sacrificed and liver slices were prepared. After 0 to 6 hours of refeeding, lipogenesis in the liver slices correlated well with the glycogen level. However, after 48 hours of refeeding, lipogenesis was just as high as it had been at 24 hours, even though the concentration of glycogen was only half as great. After 48 hours of refeeding, the dehydrogenase activity of the hexose monophosphate pathway was more than three times the level at 24 hours. The three-way correlation among these variables suggested to the authors that, by providing TPNH, the activity of the hexose monophosphate pathway might be an important determinant of lipogenic rate. In a very recent communication, however, Tepperman and Tepperman (37) modified their position somewhat. They pointed out the difficulty of determining whether the generation of TPNH "pushes" lipogenesis or whether lipogenesis, by oxidizing TPNH, "pulls" metabolism via the hexose monophosphate pathway. They further concluded that for the first 12 hours or so refeeding might be influencing regulators of lipogenesis other than the TPNH supply.

Hubbard *et al.* (24, 38) reported that refeeding fasted rats greatly increased the specific activity of "synthetase" and particularly the condensation-decarboxylation step. As a regulator of lipogenesis, however, the same reservation applies to the changes in activity of "synthetase" during refeeding as that discussed for the fasting state. Considerably more work must be done at the mechanistic level before the lipogenic response to refeeding can be properly interpreted.

Time studies carried out by Van Bruggen's group (39, 40) demonstrated that the rate of lipogenesis varied in relation to the time at which food was previously ingested. This work demonstrates the importance, in designing lipogenic experiments, of considering not only the quantity of food ingested but also the time at which ingestion occurred. It further emphasizes the homeostatic role of lipogenesis in the storage of calories ingested by intermittent eating.

EFFECT OF DIETARY FAT ON LIPOGENESIS

In 1950, Masoro *et al.* (4) found that liver slices prepared from rats fed a high-fat diet for as short a period as three days had almost no ability to convert glucose-C^{14} to fatty acids. Hausberger and Milstein (29) obtained similar results with adipose tissue. Whitney and Roberts (41) reported that liver slices from rats fed a high-fat diet for 2 to 3 months exhibited a depressed capacity to incorporate acetate-2-C^{14} into fatty acids, while Brice and Okey (42) found that rats fed a high-fat diet had a lower capacity to convert intraperitoneally administered acetate-2-C^{14} into fatty acids. Clearly, a high-fat diet markedly inhibits fatty acid synthesis, a fact completely consistent with the major homeostatic role of lipogenesis.

Further work (43, 44) has established the exquisite sensitivity of hepatic lipogenesis to fat ingestion. With as little as 2.5% fat in the diet, hepatic lipogenesis is measurably slower than it is when fat-free diets are fed. When the fat content of the diet is raised to only 15%, the liver loses about 90% of its ability to convert acetate to fatty acids; no change is noted in either hepatic fat or glycogen content. When corn oil or lard is administered by stomach tube to rats fed a fat-free

diet, a pronounced decrease in hepatic lipogenesis occurs as early as one hour after the fat administration, although only a small amount of the administered fat has reached the liver by this time.

While the biochemical mechanism by which fat ingestion inhibits fatty acid synthesis has not been established, work has appeared suggesting that increased FFA levels may be responsible. Cahill *et al.* (45) found that FFA added to adipose tissue *in vitro* inhibits lipogenesis. Langdon (46) reported that fatty acid synthesis from acetate-1-C^{14} in rat-liver homogenate was strikingly diminished by including potassium palmitate in the incubation system. FFA, as the serum albumin complex, was as inhibitory as the potassium soap. Addition of CoA to the liver homogenate partially overcame the inhibitory action of FFA, so it appears that a competition for CoA might, at least in part, be the basis of the inhibition.

Osborn *et al.* (47) reported that on the fourth day of insulin administration to diabetic rats, hepatic lipogenesis diminished abruptly. At this time, the liver-fat content had reached maximal levels and the authors suggested that the increased fat content might have suppressed lipogenesis. However, Shamoian *et al.* (48) found that, following insulin withdrawal from pancreatectomized dogs, the concentration of fat in the liver doubled without influencing the rate of hepatic lipogenesis.

It would appear that dietary fat does not influence lipogenesis by a simple feedback mechanism involving the concentration of fat; the FFA level may be the primary determinant.

LIPOGENESIS AND DIABETES MELLITUS

That the ability to biosynthesize fat from carbohydrate is lost in the diabetic state was first recognized by Drury (49). He found that pancreatectomized rats can derive adequate nourishment from a high-carbohydrate diet when they have access to food at all times, but they cannot maintain weight when subjected to alternate feeding and fasting periods. It is clear that the major homeostatic mechanism for storing chemical energy — lipogenesis — is grossly inadequate in diabetes mellitus. Stetten and his co-workers (50, 51) provided direct proof of this concept in a series of *in vivo* isotopic experiments that demonstrated the failure of fatty acid synthesis in diabetes.

In the search for a mechanistic explanation for this failure in lipogenesis, *in vitro* experiments utilizing the liver have provided most of our knowledge. Liver slices prepared from alloxan-diabetic rats are almost incapable of forming fatty acids from glucose-C^{14} (52), fructose-C^{14} (53), pyruvate-C^{14} (54), lactate-C^{14} (55), or acetate-C^{14} (56). Brady and Gurin (56) found liver slices prepared from pancreatectomized cats to be incapable of converting acetate-C^{14} to fatty acids, thus establishing that the hepatic lipogenic defect is not peculiar to alloxanized rats. Fatty acid synthesis was also found to be depressed in perfused livers (57) and in cell-free liver systems (58–62) prepared from diabetic rats. Nevertheless, Shamoian *et al.* (48) have recently shown that a loss of hepatic lipogenesis need not invariably accompany the diabetic state since liver slices prepared from pancreatectomized dogs deprived of insulin for $3^1/_2$ days converted acetate-C^{14} and glucose-C^{14} to fatty acids at a rate similar to that found for slices from normal dogs;[4] marked hyperglycemia, glucosuria, low liver glycogen, and high liver-fat content provided evidence of a diabetic state at the time of sacrifice.

Studies with liver slices indicate that, when insulin is administered to alloxan-diabetic rats prior to sacrifice, the defect in hepatic lipogenesis is completely repaired (54, 55, 63, 64). The direct addition of insulin to the *in vitro* system increases the lipogenic activity of liver slices from normal rats (65–68). This insulin effect cannot be demonstrated with slices from diabetic rats (67). Miller and his co-workers (57, 69–71) found, however, that the addition of insulin to the perfusion medium increased the lipogenic activity of perfused livers from both normal and diabetic rats and Scaife and Migicovsky (60) found that insulin added to liver homogenates prepared from either normal or diabetic rats stimulated the conversion of acetate-C^{14} to fatty acids.

Before discussing the possible biochemical mechanisms underlying the hepatic lipogenic alteration in diabetes, it might be well to consider the time course of the lipogenic response to both insulin administration and insulin deprivation. Renold *et al.* (72) found that, following parenteral administration of insulin to alloxan-diabetic rats, many hours elapsed before hepatic lipogenesis was stimulated. In contrast, glucose utilization by muscle was promoted in a matter of minutes. Glucose phosphorylation in the liver was also augmented sooner than lipogenesis. Williams *et al.* (73) supplied insulin to alloxan-diabetic rats by a continuous portal-vein infusion and found that 60 to 90 minutes elapsed before hepatic lipogenic activity started to increase. Carbohydrate metabolism responded in periods from 10 to 30 minutes, and choles-

[4] Shamoian, C., E. J. Masoro, and A. Canzanelli, unpublished observations.

terol synthesis was augmented in 30 to 60 minutes. On the other hand, Spiro *et al.* (74) found that, upon withdrawal of insulin therapy from alloxan-diabetic rats, an inability of the liver slice to synthesize fatty acids was the first fully developed metabolic lesion to appear. This finding was not confirmed by the work of Shamoian *et al.* (48) with pancreatectomized dogs deprived of insulin for $3^1/_2$ days; although the liver was low in glycogen and infiltrated with fat, it still had a normal lipogenic activity. Hepatic lipogenesis was depressed, however, when pancreatectomized dogs were maintained on an inadequate insulin dosage for several weeks. The studies discussed above, taken together, suggest that both the failure of hepatic lipogenesis in diabetes and the promotion of lipogenesis by insulin are not direct effects of the changing levels of this hormone but, rather, are responses secondary to other metabolic alterations induced by diabetes and insulin.

Considerable additional experimental evidence has accumulated that indicates that the changes in lipogenesis in diabetes are secondary to the altered carbohydrate metabolism characteristic of this condition. The most impressive evidence comes from the investigations of Chaikoff and co-workers (53, 75). They showed that fructose, unlike glucose, was catabolized in a normal manner by diabetic liver and that feeding diabetic rats a high fructose diet completely corrected the lipogenic defect without correcting the defective glucose metabolism. Gurin and co-workers (58, 59) found that the depressed lipogenesis in cell-free liver preparations of diabetic pigeons and rats could be partially overcome by adding glycogen and hexose phosphates to the system. Haft and Miller (71), however, do not agree with the concept that the promotion of fatty acid synthesis by insulin results from the influence of insulin on carbohydrate metabolism. They found that, while carbohydrate metabolism can be greatly increased in the perfused diabetic liver by adding glucose or fructose to the perfusate without increasing the rate of lipogenesis, the addition of insulin most effectively promotes fatty acid synthesis.

Bloom (76) and Felts *et al.* (77) found that the hexose monophosphate pathway of carbohydrate metabolism, which generates TPNH, was depressed more in the diabetic liver than was the Embden-Meyerhof pathway, which generates DPNH. Langdon (78) showed TPNH to be an essential co-factor for fatty acid synthesis; this led him to suggest that metabolic states characterized by decreased rates of TPNH generation may well be accompanied by decreased rates of lipogenesis. Siperstein and Fagan (79–82) developed this viewpoint further. They found that fatty acid synthesis occurred at a very slow rate in liver homogenates not supplemented with either DPN or TPN and that the normal homogenate exhibited somewhat more lipogenic activity than the diabetic. In the presence of added glucose-6-phosphate and DPN, liver homogenates still synthesized very little fatty acid, again the normal homogenate being somewhat more active than the diabetic. On the other hand, in the presence of added glucose-6-phosphate and TPN, the homogenates synthesized fatty acids at high rates, and the difference in lipogenic activity between homogenates from normal and diabetic rats was not great. Similar results were obtained when isocitrate and TPN were added in place of glucose-6-phosphate and TPN. Wenner and Weinhouse (83) found that TPN caused liver homogenates to utilize glucose-6-phosphate by way of the hexose monophosphate pathway. On the basis of this work and their own studies, Siperstein and Fagan concluded that the defect in fatty acid synthesis in the diabetic state is caused by a deficiency of TPNH generation because of a depressed rate of glucose oxidation via the hexose monophosphate pathway.

The following experimental findings, however, are in conflict with this interesting theory: (*a*) Although Glock and McLean (84) reported that diabetes resulted in a striking decrease in the activity of the hepatic hexose monophosphate pathway dehydrogenases, the TPNH level of the diabetic liver was within the normal range (85). (*b*) The extramitochondrial regions of the liver cell can produce TPNH by pathways other than the hexose monophosphate pathway (18); moreover, as discussed above, the hexose monophosphate pathway may not be the most or the only important source of hydrogen for hepatic lipogenesis. (*c*) Abraham *et al.* (86) found a depressed fatty acid synthesis in diabetic liver homogenates, in which TPNH generation is normal. (*d*) Shamoian *et al.* (48) found in diabetic dogs a normal rate of lipogenesis in liver slices that are so deficient in glycogen that the hexose monophosphate pathway cannot be very active.

The work of Shaw *et al.* (59) has been interpreted as providing supporting evidence for the theory that TPNH deficiency is a cause of the depressed hepatic lipogenesis in diabetes. They found that the defective lipogenesis in certain cell-free preparations of diabetic liver was repaired by the addition of butyryl-CoA. From this, it was inferred that the lipogenic failure resulted from a lack of TPNH at the reduction step that converts crotonyl-CoA to butyryl-CoA. This conclusion fails to take account of the fact that such a failure might logically be expected to occur also at the reduction steps involving hexenoyl-CoA, octenoyl-CoA, *etc.* Failure of lipogenesis due to defective reduction at these steps should not be overcome by butyryl-CoA

supplementation. It would seem, therefore, that other explanations for the action of butyryl-CoA supplementation should be considered.

Because of the many observations incompatible with it, it seems to the reviewer that the hypothesis that a lack of TPNH causes the lipogenic failure in diabetes must be discarded. A new avenue of approach to this problem has been provided by Chaikoff and his co-workers (61, 86, 87). They found that the particle-free supernatant fraction of the liver homogenates prepared from normal and diabetic rats synthesized fatty acids at about the same rate. The addition of normal microsomes to normal supernatant fractions caused a five- to ten-fold stimulation in lipogenesis, while the addition of diabetic microsomes to the diabetic supernatant fraction caused only one-and-one-half- to three-fold stimulation. Moreover, normal microsomes increased lipogenesis more when added to normal supernatant fractions than when added to diabetic supernatant fractions. These investigators concluded that there are two chemical lesions in the diabetic liver leading to impaired lipogenesis — one in the microsomes and the other in the supernatant fraction. Chaikoff and co-workers found a marked depression in the activity of the microsomal enzyme, which is involved in reducing the unsaturated fatty acyl-CoA derivatives in diabetic liver. They concluded that the loss in activity of this enzyme may be causally related to the defective lipogenesis. Another enzymatic defect has been reported by Gibson and Hubbard (23), who showed that the "synthetase" activity is markedly depressed in diabetic liver while the acetyl carboxylase activity is increased.

The detailed mechanisms involved in the depressed hepatic lipogenesis in diabetes still remain to be worked out. The new clues that have recently appeared from studies of microsomal and other enzymes should provide a fertile field for further research. Also, the roles of lipogenic inhibitors and stimulators in relation to this problem warrant investigation.

Although adipose tissue is quantitatively a far more important site of lipogenesis than liver, much less experimental effort has been devoted to the effect of diabetes on adipose tissue lipogenesis. Hausberger *et al.* (88) demonstrated that adipose tissue from diabetic rats cannot convert glucose-C^{14} to fatty acids but that diabetic rats treated with insulin have a high rate of adipose tissue lipogenesis. Winegrad and Renold (89) confirmed these results but found that insulin added *in vitro* promoted lipogenesis in diabetic adipose tissue to only a very limited degree. Adipose tissue from diabetic rats had a reduced capacity to oxidize glucose, and the hexose monophosphate pathway was inhibited more than the Embden-Meyerhof pathway (31).

When insulin is added *in vitro* to adipose tissue of normal rats, a marked increase in lipogenesis occurs from glucose-C^{14} but not from acetate-C^{14} or pyruvate-C^{14} (89). In the presence of unlabeled glucose, however, insulin promotes lipogenesis from acetate-C^{14} and pyruvate-C^{14} in normal adipose tissue. Winegrad and Renold (90) have also found that insulin causes normal adipose tissue to oxidize more glucose by both the Embden-Meyerhof and the hexose monophosphate pathways.

It seems clear that insulin promotes lipogenesis in normal adipose tissue through some action on carbohydrate metabolism. The stimulatory effect of insulin on the activity of the hexose monophosphate pathway, however, could as easily be the result of increased lipogenesis as the cause of it. The data are insufficient to permit speculation concerning the cause of the defective lipogenesis of adipose tissue in diabetes. Moreover, it is likely that the mechanisms involved are at least as complex as those being found for hepatic tissue.

EFFECT OF COLD EXPOSURE ON LIPOGENESIS

At the outset of this discussion, it seems advisable to clarify the meaning of the terms *cold-stressed animal* and *cold-acclimated animal*. *Cold-stressed animal* refers to an animal undergoing its initial exposure to low environmental temperatures. These animals are losing weight, and their liver glycogen is disappearing. Part of the animal population dies during the first week or so. *Cold-acclimated animal* refers to an animal that has been living in the cold environment for several months. These animals are gaining weight, and liver glycogen is at normal levels. The death rate of the population does not markedly differ from that of rats living at more usual ambient temperatures.

In 1954, Masoro *et al.* (91) reported that liver slices from cold-stressed rats have almost no ability to convert acetate-C^{14} to fatty acids. This finding has been confirmed by Kline *et al.* (92), and similar results have been obtained with hamsters by Denyes and Carter (93).

A clue to the mechanism of the depressed lipogenesis came from the finding that the hepatic glycogen content of cold-stressed rats was only about 40% that of rats living at normal temperatures (91). Moreover, addition of high concentrations of unlabeled glucose to the incubation system partially repaired the defective lipogenesis. It would seem, therefore, that the failure in hepatic lipogenesis in cold-stressed rats is probably related to a low rate of carbohydrate metabolism.

Investigation of this problem by using liver homogenates (17) revealed that, in the presence of all the necessary co-factors, homogenates from cold-stressed rats formed fatty acids at rates similar to those from rats living at the more usual ambient temperatures. This finding suggests that the enzymes involved in fatty acid synthesis are present at normal or near normal concentrations.

Therefore, the defective lipogenesis in the intact liver cell of cold-stressed rats is probably caused by a co-factor environment that is unfavorable to fatty acid synthesis. The nature of this co-factor defect is not clear. It would seem, from evidence of an indirect nature, that a lack of TPNH is probably the cause of the restricted rate of lipogenesis. On theoretical grounds, however, it is difficult to believe that a TPNH lack could be the major determinant of hepatic lipogenesis, as discussed earlier. Measurement of hepatic TPNH levels in cold-stressed rats would yield the necessary data for a more definitive answer to this question.

Masoro *et al.* (94) studied the effect of cold-stress on adipose tissue lipogenesis. In contrast to its inhibitory effect on hepatic fatty acid synthesis, cold-stress has no inhibitory effect on adipose tissue lipogenesis. This finding supports our earlier *in vivo* results (95) showing that cold stress does not reduce the overall rate of lipogenesis in the intact rat.

In studies on the cold-acclimated rat, liver slices showed a greatly reduced ability to convert acetate-C^{14} to fatty acids (96). Since the glycogen content of the liver of cold-acclimated rats is the same as that of rats acclimated to 25°, the inhibition of hepatic lipogenesis cannot be related in any simple way to a lack of carbohydrate substrate. Studies of glycogen metabolism and of the oxidation of exogenous glucose-1-C^{14} and glucose-6-C^{14} indicate that both the rate of carbohydrate metabolism and the pathways of carbohydrate metabolism are normal. It would seem that there are mechanisms in the cold-acclimated rat that limit the rate of hepatic lipogenesis even in the presence of a high rate of carbohydrate metabolism.

This problem was investigated further by using liver homogenates (97) to which all necessary co-factors for lipogenesis were added. Liver homogenates from cold-acclimated rats did not exhibit a depressed ability to synthesize fatty acids. One must conclude that the enzymes involved in lipogenesis are present at near normal levels in the liver of cold-acclimated rats. It would appear that the decreased lipogenesis in the intact liver cell of cold-acclimated rats results from an improper co-factor environment, although the specific nature of co-factor alteration is not known. Brady *et al.* (98) have found that a high CoA level inhibits lipogenesis while stimulating fatty acid oxidation. Campbell *et al.* (99) reported that the CoA level of the liver of cold-acclimated rats is greatly increased. It is quite possible that this change in CoA level serves to promote heat production from catabolism of fatty acids and, at the same time, restricts hepatic lipogenesis in the cold-acclimated state.

Patkin and Masoro (100) found that adipose tissue from cold-acclimated rats converted acetate-C^{14} to fatty acids at three times the rate of adipose tissue from rats living at 25°. This finding may seem surprising at first, but it takes on new meaning when considered in terms of the major physiologic problem facing the cold-acclimated animal — *i.e.*, the high rate of energy metabolism required for thermogenesis.

It is known that cold-acclimated rats eat much more food than do rats living at 25°. The isotopic studies of Stetten and Boxer (101) demonstrate that at least 30% of the dietary glucose of animals living in a steady state at the usual ambient temperature of around 25° follows the metabolic pathway of fatty acid synthesis. If these findings can be applied to the cold-acclimated animal, one would predict that a greater absolute amount of dietary glucose would be utilized in fatty acid synthesis by the cold-acclimated rat simply because such rats eat much more than do animals living at 25°. Since fat synthesis occurs primarily in the adipose tissue, a substantial increase in fat synthesis by adipose tissue of the cold-acclimated animal is to be expected. It follows further that the increased amounts of fatty acids synthesized by the adipose tissue depots are rapidly mobilized, presumably as FFA, to meet the high caloric needs of the cold-acclimated animals. The biochemical mechanisms involved in this increased lipogenic activity remain to be explored.

FATTY ACID SYNTHESIS BY LACTATING MAMMARY GLAND

Because the mammary gland shows rapid and large fluctuations in lipogenic activity, it provides excellent experimental material for the study of the regulation of lipogenesis. At the onset of lactation, for example, the lipogenic activity of the mammary gland suddenly increases (102). Mammary-gland slices from pregnant rats, one day before term, form fatty acids at about one-fifth the rate of slices from lactating rats; within one day of weaning the young, the mammary-gland slices prepared from the mother rat show a markedly reduced capacity to synthesize fat.

Folley and French (103) found that glucose stimulated the utilization of acetate for fatty acid synthesis by the mammary gland; they suggested that glucose

might act by providing glycerol for glyceride synthesis. In recent years, a different interpretation of their finding has come to the fore — *i.e.*, by generation of TPNH, the hexose monophosphate pathway stimulated the lipogenic system. Indeed, there is much experimental evidence that the hexose monophosphate pathway has a greatly increased activity during lactation. On the basis of studies on glucose-1-C^{14} and glucose-6-C^{14} metabolism by mammary-gland slices, Abraham *et al.* (104) concluded that the hexose monophosphate pathway of carbohydrate metabolism is very active during lactation. Glock and McLean (105, 106) showed that the level of glucose-6-phosphate dehydrogenase increases sixty-fold from the end of pregnancy until the end of lactation and then falls off abruptly after weaning. Similar but less marked changes were found in the case of 6-phosphogluconate dehydrogenase. Glock *et al.* (107) also found changes in glucose-1-C^{14} and glucose-6-C^{14} metabolism during the time sequence involving pregnancy-lactation-weaning. There was a great rise in the activity of the hexose monophosphate pathway during lactation, followed by a sharp drop in activity immediately after weaning. That the generation of TPNH by the hexose monophosphate pathway during lactation could be stimulating fatty acid synthesis is to some extent supported by the work of Lowenstein (18). He found that the hydrogen attached to carbon number 1 of glucose contributes greatly to the fatty acids synthesized by the lactating mammary gland.

On the other hand, no experimental evidence has yet appeared to prove *unequivocally* that formation of TPNH via the hexose monophosphate pathway causes the high rate of lipogenesis in the lactating mammary gland. All data presently available could just as easily be interpreted as evidence for the hypothesis that the increased rate of lipogenesis occurring during lactation promotes the hexose monophosphate pathway by making oxidized TPN available. The increase in lipogenesis may be stimulated by mechanisms quite independent of the hexose monophosphate shunt. Consistent with this latter view is the finding of Abraham and Chaikoff (102) that non-lactating mammary-gland slices, which show no evidence of hexose monophosphate pathway activity, converted significant amounts of glucose carbon to fatty acids. The addition of unlabeled glucose to these slices appreciably stimulated fatty acid synthesis from acetate. In this case, glucose metabolism must have stimulated lipogenesis by a mechanism other than that of TPNH generation via the hexose monophosphate pathway.

The rate of lipogenesis in the mammary gland is influenced by certain hormones, and the increased lipogenic activity in the mammary gland during lactation depends upon the presence of specific hormones. Balmain *et al.* (108, 109) found that the addition of insulin to an incubation system containing mammary-gland slices increased the rate of incorporation of acetate-C^{14} into fatty acid when unlabeled glucose was present but did not increase the rate when it was absent. This finding provides further evidence for the theory that insulin does not act directly on lipogenesis but influences fatty acid synthesis indirectly through its action on carbohydrate metabolism. McLean (110) showed that the addition of insulin to mammary-gland slices promoted the oxidation of glucose by way of the hexose monosphosphate pathway and she presented some evidence supporting the concept that this increased activity of the hexose monophosphate pathway resulted from the fact that the increased rate of lipogenesis generated TPN from TPNH at high rates.

McLean (111) found that the addition of prolactin to the *in vitro* system increased both $C^{14}O_2$ production from glucose-1-C^{14} and the incorporation of glucose-C^{14} into fatty acids by mammary-gland slices from pregnant rats. This action of prolactin disappeared with the onset of lactation. In contrast, insulin became even more effective during lactation.

Abraham *et al.* (112) hypophysectomized rats in mid-pregnancy and, during the postpartum period, investigated the hormonal requirements for the development of a lactating mammary gland metabolically similar to that of normal postpartum animals. The authors achieved this result by the simultaneous administration of prolactin and Δ^1-hydrocortisone acetate.

As yet, the full potentiality of the mammary gland as a tool for the study of the biochemical mechanisms involved in the regulation of lipogenesis has not been realized. From what is known, it would seem that this tissue offers a unique opportunity to study the mechanisms involved in the stimulating action of carbohydrate metabolism on fatty acid synthesis. Of course, caution should be used in making generalizations because a tissue with such a specific functional need for lipogenesis may well have control mechanisms that are peculiar to it alone.

BIOCHEMICAL DATA NOT YET RELATED TO PHYSIOLOGIC REGULATION

There are a great many data on the effects of a variety of substances on lipogenesis that cannot at present be related to its physiologic regulation. Some of these data are examined here because certain clues to the control of lipogenesis might emerge from such a review.

It has long been felt that the glycogen content of a tissue is in some way related to lipogenesis (113).

Haugaard and Stadie (114) altered the glycogen content of the liver of rats by varying the diet and found a significant positive correlation between the glycogen content and the ability of the liver slice to synthesize fatty acids from acetate. Masoro *et al.* (91) observed a similar positive correlation between the liver-glycogen content and the lipogenic activity in the case of liver slices prepared from cold-stressed rats. With pigeon-liver systems, Von Brand and Helmreich (115) found a close relationship between the rate of glycolysis and rate of fatty acid synthesis. On the other hand, Spirtes *et al.* (116) could find no direct relationship between hepatic-glycogen levels and hepatic lipogenesis in hyperthyroid rats, and Whitney and Roberts (41) reported that no such correlation exists in rats fed high-fat diets. Also, as mentioned earlier (36), there is no simple correlation between liver glycogen and hepatic lipogenesis in rats that have been refed after a period of fasting. Clearly, the relationship, if any, between liver glycogen and hepatic lipogenesis is very complex.

In the absence of added glucose, adipose tissue *in vitro* converts very little acetate-C^{14} to fatty acids; with the addition of glucose to the system (at concentrations similar to those found in the extracellular body fluids), adipose tissue converts acetate to fatty acids at a high rate (89, 94, 117). Similarly, *in vitro* studies by Popják and Tietz (118) showed that unlabeled glucose promotes fatty acid synthesis in lactating mammary gland. It might be considered that the addition of glucose stimulates lipogenesis in such tissues as mammary gland and adipose tissue because they do not have large reserve stores of carbohydrate. Masoro *et al.* (91) found, however, that the addition of glucose also markedly stimulated fatty acid synthesis in liver slices containing high levels of glycogen.

Further information on the possible site of interaction between carbohydrate metabolism and lipogenesis comes from the work of Bloch and Kramer (65), who reported that the addition of unlabeled pyruvate increased by several-fold the incorporation of acetate into fatty acids in liver slices. This result could not be confirmed by Masoro *et al.* (119). Popják and Tietz (118) found that pyruvate stimulated lipogenesis in mammary gland slices, while Miller and Cooper (117) found that pyruvate had no effect on fatty acid synthesis by adipose tissue. Obviously more work is needed to clarify these conflicting pieces of evidence.

Balmain *et al.* (109, 120, 121) found that the addition of glycerol to mammary gland slices increased their lipogenic activity; this is a rather suprising finding since Hirsch *et al.* (122) showed that glycerol is poorly utilized by mammary gland. However, the possibility that carbohydrate metabolism promotes lipogenesis by providing L-α-glycerophosphate for the esterification of FFA is intriguing and merits more experimental investigation.

It is also possible, in view of the experiments discussed below, that carbohydrate metabolism could be influencing fatty acid synthesis by increasing the pool size of the tricarboxylic acid cycle intermediates. The carboxylation reactions utilizing pyruvate should lead to a *de novo* production of malate and oxalacetate. Bloch and Kramer (65) found that the addition of oxalacetate to liver slices promoted fatty acid synthesis while the addition of malate had no effect. Miller and Cooper (117) obtained similar results with adipose tissue, and Popják and Tietz (123) found that oxalacetate stimulated lipogenesis in cell-free preparations of mammary gland.

Succinate has been shown to stimulate lipogenesis by adipose tissue (117) and by cell-free preparations of mammary gland (123), but it has no such effect on liver slices (65, 119). Fumarate promotes the synthesis of fatty acids in cell-free liver preparations (98) but not in liver slices (65, 119) or in isolated adipose tissue (117). Bucher (124) reported that α-ketoglutarate greatly promotes fatty acid synthesis in liver homogenates; others have also noted stimulation of lipogenesis by α-ketoglutarate in cell-free preparations of liver (98, 125) and mammary gland (123), but no such effect has been found in liver slices (119) or in isolated adipose tissue (117).

The effects of citrate on lipogenesis are especially provocative. The addition of citrate to liver slices (119) or surviving adipose tissue (117) has no effect on lipogenesis, but a similar addition to cell-free liver systems very markedly promotes lipogenesis (98, 125). In fact, in the case of certain cell-free liver preparations, Shaw *et al.* (59) found citrate to be required for fatty acid synthesis. Porter *et al.* (126) found that isocitrate was required for the synthesis of fatty acids from acetate by reconstituted fractions of pigeon-liver particle-free supernatant; if aconitate were added, citrate could be used in place of isocitrate. They also found that isocitrate could not be replaced in these systems by TPNH or a TPNH generating system such as the glucose-6-phosphate dehydrogenase system; however, it could be replaced by a combination of α-ketoglutarate, CO_2, and a TPNH generating system. Abraham *et al.* (127) found that citrate and TPN were essential for lipogenesis in a particle-free supernatant fraction of lactating mammary gland. Then they studied the effect of inhibiting citrate oxidation by glucose-6-phosphate and found a decreased lipogenesis, even though the total amount of TPNH produced in the presence of glucose-6-phosphate was the same or higher

than that produced in its absence. They further found (128) that the requirement for citrate could be satisfied by *cis*-aconitate and could be partially satisfied by isocitrate or a combination of α-ketoglutarate, bicarbonate, and a TPNH generating system.

The mechanism by which citrate promotes lipogenesis in the cell-free system is not known. Hülsmann (129) suggested that the oxalsuccinate generated from citrate may be involved in transcarboxylation reactions, yielding malonyl-CoA by a pathway that bypasses the rate-limiting acetyl carboxylase step. In accord with this possibility is the finding of Lowenstein[5] that citrate promotes the conversion of acetate and acetyl-CoA but not malonyl-CoA to fatty acids. Such transcarboxylation reactions have been shown in microorganisms, but no evidence for this kind of reaction has been found as yet in the case of animal tissues (130, 131). If such a reaction were discovered in animal tissue, it would become less likely that acetyl carboxylase is the most important site of regulatory interaction.

In general, tricarboxylic acid cycle intermediates appear to influence lipogenesis in cell-free systems but are much less effective in preparations containing intact cells. There seem to be two possible explanations: (*a*) the exogenous tricarboxylic acid cycle intermediates cannot permeate the intact cell to the region where they promote lipogenesis, (*b*) these intermediates, although necessary for lipogenesis, are usually present in the intact cell at adequate levels. The latter possibility would suggest that the tricarboxylic acid cycle intermediates do not play an important role in the physiologic regulation of lipogenesis.

The level of CoA has also been considered a possible factor in the control of lipogenesis. Klein and Lipmann (132) found a positive correlation between the CoA level and lipid synthesis in the livers of pantothenic acid-deficient rats. On the other hand, Brady *et al.* (98) reported that high concentrations of CoA inhibit fatty acid synthesis, and Hubbard *et al.* (24) found that CoA inhibits the condensation-decarboxylation step of the "synthetase" reaction. Thus, an optimal concentration of CoA appears to be required for a maximal rate of lipogenesis, and changing levels of CoA may well play a physiologically important regulatory role in the control of lipogenesis. Hepatic lipogenesis in cold-acclimated rats may be regulated in this manner (96–99). A similar argument can be made for the importance of changing ATP levels (26, 133).

In 1958, Lachance *et al.* (134) found that the addition of liver microsomes greatly increased the ability of an enzyme system from mammary gland to synthesize short-chain fatty acids, a finding that they related to the presence in liver microsomes of a TPNH-dependent enzyme that catalyzes the reduction of crotonyl-CoA to butyryl-CoA. Abraham *et al.* (61, 86) reported that liver microsomes stimulated long-chain fatty acid synthesis by the particle-free supernatant fraction of liver homogenates; they related this finding to a microsomal enzyme that catalyzes the reduction of the unsaturated fatty acyl-CoA derivatives (87). This lipogenic stimulatory action of liver microsomes has been confirmed by the work of Fletcher and Myant (135) but not by Gibson and Hubbard (23) or Masoro and Porter (25).

ENDOCRINOLOGIC EXPERIMENTS NOT YET RELATED TO PHYSIOLOGIC REGULATION

Throughout this discussion, endocrinologic information has been examined whenever such considerations aided in the understanding of the physiologic problem at hand. A large body of endocrinologic experimentation on lipogenesis, however, has not yet been related to the problems of physiologic regulation of fatty acid synthesis. A review of these data is included in the belief that it may provide important leads for future research.

Brady *et al.* (136) reported in 1951 that liver slices from hypophysectomized rats had a greater lipogenic activity than slices from normal rats. Other workers have not confirmed this finding (137–141). Hill *et al.* (138) found that livers of hypophysectomized rats fed an adequate diet, with whole ground wheat as its main carbohydrate source, showed an impaired ability to convert acetate carbon to fatty acids. This defect in lipogenesis was readily overcome by feeding a synthetic diet containing 60% glucose but not by a diet containing only 25% glucose. They concluded that the hypophysectomized rat exhibits an increased sensitivity to glucose requirements for the maintenance of hepatic lipogenesis. They further showed (139) that the conversion of hexose carbons to fatty acids by the liver requires the concurrence of one or more of the anterior pituitary hormones.

In 1950, Welt and Wilhelmi (142) found that the administration of growth hormone to rats led to a depressed fatty acid synthesis. Since then, several workers (143–146) have shown that liver slices prepared from rats treated with growth hormone have a reduced lipogenic activity, while growth hormone added to adipose tissue *in vitro* decreases fatty acid synthesis (147). Indeed, all reports but one (148) show that the administration of growth hormone leads to a re-

[5] Lowenstein, J. M., submitted for publication.

duced lipogenic capacity, but nothing is known about the physiologic meaning of this alteration in fatty acid synthesis.

The administration of corticotropin to the rat inhibits fatty acid synthesis (142). That this action may not be mediated through the adrenal cortex is indicated by the fact that the *in vitro* addition of corticotropin to adipose tissue (147, 149, 150) also inhibits fatty acid synthesis.

Prolactin added *in vitro* to adipose tissue has been reported to stimulate the conversion of glucose to CO_2 and fatty acids (151). No effect of prolactin on the synthesis of fatty acids from acetate-C^{14} or pyruvate-C^{14} by adipose tissue occurred unless unlabeled glucose was also present. It seems unlikely, however, that adipose tissue is a major site of prolactin action.

Brady *et al.* (136) reported that the depressed hepatic lipogenesis of the pancreatectomized cat could be reversed by removing the hypophysis. They further found that hepatic fatty acid synthesis was inhibited by administering growth hormone to these Houssay cats. Spiro (152) noted only a very slight increase in hepatic fatty acid synthesis from fructose following the hypophysectomy of diabetic rats, while Bauman *et al.* (153) found that hypophysectomy increased hepatic lipogenesis only in the case of diabetic rats fed a high carbohydrate diet.

With the exception of the inhibitory action of growth hormone, the effects of the pituitary hormones on lipogenesis have not yet been clearly outlined. Most certainly the physiologic role of the pituitary gland in the regulation of fatty acid synthesis has still to be defined.

In 1950, Welt and Wilhelmi (142) reported that lipogenesis, *in vivo*, is greater in adrenalectomized rats than in normal rats. Subsequently, Bates *et al.* (141), in a study on intact rats, reported that adrenalectomy does not significantly change the rate of lipogenesis. Cohn and Joseph (154) may have resolved this difference by showing that the nutritional state of the adrenalectomized animal is a prime determinant of lipogenic activity. Adrenalectomized rats allowed to eat *ad libitum* did not gain weight as well as normal animals but incorporated more acetate-C^{14} into fat. When adrenalectomized rats were force-fed so that they gained weight normally, however, their lipogenic activity was not different from that of normal rats.

Perry and Bowen (155) reported that liver slices from adrenalectomized rats incorporated less acetate-C^{14} into fatty acids than did slices from normal rats. Jeanrenaud and Renold (156), however, could find no influence of adrenalectomy on fatty acid synthesis by adipose tissue, provided the nutritional condition of the rats was carefully controlled.

It has been reported that liver slices prepared from normal rats treated with cortisone have a reduced ability to synthesize fatty acids (136, 157). Certain glucocorticoids, when added *in vitro* to lactating mammary gland slices, inhibit fatty acid synthesis (158); similar treatment does not inhibit adipose tissue lipogenesis (156). The addition of cortisone to the perfused liver does not influence fatty acid synthesis from acetate, but the combination of cortisone and insulin causes a greater increase in lipogenesis in perfused liver than does insulin alone (69). The depressed hepatic lipogenesis in the diabetic state can be partially overcome by removal of the adrenal glands (92, 136), but such treatment does not influence the lipogenic activity of adipose tissue (156). The role of the adrenal cortex in regulating lipogenesis is certainly far from clear.

Mayer and co-workers (141, 159) found that lipogenesis was depressed in thyroidectomized rats and increased in thyroxine-treated rats. In a study of normal, thyroid-fed, and thiouracil-treated rats, Dayton *et al.* (160) found fatty acid synthesis to be directly proportional to the basal O_2 consumption. Spirtes *et al.* (116) reported an undiminished rate of fatty acid synthesis in liver slices prepared from hyperthyroid rats. Fletcher and Myant (161), however, found a depressed lipogenesis in liver slices from rats given large doses of thyroxine. Moreover, liver homogenates prepared from thyroxine-treated rats from which the particles sedimenting at $10{,}000 \times g$ had been removed, showed a depressed fatty acid synthesis (162). The authors (133) believe that this defect in lipogenesis results from a lack of glycogen, which in turn reduces both the ATP and TPNH supply.

Epinephrine added *in vitro* inhibits fatty acid synthesis by liver (68) and adipose tissue (45, 150). This effect on adipose tissue is particularly interesting since epinephrine increases glucose uptake and does not depress glyceride-glycerol formation from glucose (45, 147, 150). Cahill *et al.* (45) suggested that the depressed adipose tissue lipogenesis may be secondary to elevated FFA levels induced by epinephrine.

Glucagon has been shown to inhibit hepatic lipogenesis (68, 163), but the reports on its effects (147, 150) on adipose tissue lipogenesis are conflicting. Glucagon promotes lipogenesis in normal intact mice (148). Castration of the male rat stimulates lipogenesis in liver and adipose tissue; testosterone administration inhibits it (164). Estradiol administered to female rats promotes lipogenesis in liver and adipose tissue (164).

GENERAL SUMMARY

The homeostatic regulation of lipogenesis is determined to a large extent by the availability of carbo-

hydrates to the tissues involved in fatty acid synthesis and by the ability of these tissues to metabolize the carbohydrates. The amount of fat ingested and the state of the energy balance also serve in a regulatory capacity. The specific chemical mechanisms by which these factors regulate the rate of fatty acid synthesis have yet to be defined.

New information on the enzymatic sequence of events involved in the biosynthesis of fatty acids greatly aids in considering the nature of such specific chemical mechanisms. The enzymatic conversion of acetyl-CoA to malonyl-CoA, a reaction requiring the participation of ATP and CO_2, appears to be the rate-limiting step in fatty acid synthesis. This step, catalyzed by acetyl carboxylase, would seem to be a more likely site of regulatory interaction than the subsequent reductive steps.

On the basis of both theoretical considerations and available experimental evidence, several mechanisms can now be excluded as lipogenic regulators. There is no evidence that the intracellular level of acetyl-CoA plays a role in the regulation of lipogenesis. In the opinion of the reviewer, the rate of generation of TPNH by the hexose monophosphate pathway of carbohydrate metabolism rarely, if ever, appears to be of physiologic importance in the regulation of lipogenesis. Hormonal factors probably have no direct regulatory effect on lipogenesis, although they can markedly influence fatty acid synthesis indirectly by their action on carbohydrate metabolism and other aspects of fat metabolism.

From a detailed analysis of possible biochemical mechanisms involved in regulating lipogenesis, the following possibilities seem to be worthy of further investigation:

(*a*) Changes in concentration of acetyl carboxylase may be involved in regulating fatty acid synthesis. Changes in the concentration of this enzyme do not appear to be primary in alterations in lipogenic activity, but it may serve in a regulatory capacity after the level has been altered by other factors.

(*b*) Lipogenic inhibitors that act at the acetyl carboxylase step have been described. Such substances are potentially very important regulators and already have proved to be quantitatively important in certain physiologic conditions.

(*c*) At least one substance serving as a lipogenic stimulator has been described. This or similar substances may play an important role in the control of fatty acid synthesis, but our knowledge in this area is too fragmentary for a thorough evaluation at this time.

(*d*) Experimental evidence suggests that the levels of tricarboxylic acid cycle intermediates may serve as lipogenic regulators. Such a mechanism is easily linked to the lipogenic action of carbohydrate metabolism. Tricarboxylic acid intermediates may act by generating malonyl-CoA from acetyl-CoA via transcarboxylation reactions, thus circumventing the slow acetyl carboxylase step. No evidence has yet been found, however, for the existence of this kind of reaction in animal tissues.

(*e*) The rate of lipogenesis is very sensitive to the concentration of CoA, and there appears to be an optimal concentration above and below which the rate of lipogenesis decreases. Such a property makes the CoA level a very likely regulator; there is evidence that it may function in certain physiologic states.

(*f*) FFA are powerful inhibitors of fatty acid synthesis. This suggests that the intracellular level of FFA may serve as a lipogenic regulator. Carbohydrate metabolism may, then, promote lipogenesis by removing FFA through the generation of L-α-glycerophosphate for their esterification. By the same token, the ingestion of fat may inhibit lipogenesis by generating FFA. Evidence has been found to indicate that FFA may limit fatty acid synthesis by competing for CoA.

To a great extent, investigators in this field have been looking for *the* mechanism by which carbohydrate metabolism promotes fatty acid synthesis. The concept of a single mechanism may be retarding development in this field. It is probable that carbohydrate metabolism influences lipogenesis by the interaction of multiple mechanisms. While in any given physiologic state one or more such mechanisms may be particularly important, it seems likely that the problem cannot be understood without taking into consideration the multiple nature of the specific chemical events controlling fatty acid synthesis.

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