Adaptive changes in $\alpha$-glycerophosphate-generating enzymes in rat liver

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It is now well established that the enzymes of palmitate synthesis (1), as well as others which participate in the production of precursors for fatty acid synthesis or of reduced coenzyme necessary for the reductive steps in the synthesis (16), decrease in activity during food deprivation and increase during periods of hyperlipogenesis. The fact that 1-$\alpha$-glycerophosphate is an important intermediate in the synthesis of triglycerides and phospholipids suggests the possibility that enzymes which are involved in the production of this metabolite may fluctuate in activity in parallel with lipogenic activity.

Increases in the activity of the soluble 1-$\alpha$-glycerophosphate dehydrogenase had been described in certain forms of experimental obesity (7) and in animals fed high-carbohydrate, low-fat diets (6). We began by studying $\alpha$-glycerophosphate dehydrogenase and glycerokinase activities in livers of starved and re-fed rats. During the course of these studies, and partly as a result of our interest in the problem of glycerol-induced hypertriglyceridemia (11), we became interested in the effect of glycerol feeding on the two $\alpha$-glycerophosphate-generating enzymes. In this report we will describe an interesting reciprocal relationship between the adaptive responses of the two enzymes: after refeeding or glycerol feeding $\alpha$-glycerophosphate dehydrogenase activity increases markedly while glycerokinase activity decreases significantly. In the former case $\alpha$-glycerophosphate is generated from hexoses by way of dihydroxyacetone phosphate whereas in the latter instance glycerol utilization proceeds in large part by way of the reverse reaction.

METHODS

Young adult male rats (150-250 g starting wt) were used for all experiments. Studies on the effects of fasting and refeeding were carried out with rats obtained from Carworth Farms (CFE strain), New City (enzyme studies) or from the Holtzman Company, Madison, Wis. (glycerol uptake). Rats from both sources responded similarly to refeeding in previous experiments in our laboratory. All experiments on glycerol feeding effects were performed with rats from Charles River Breeding Laboratories, North Wilmington, Mass.

Diets. Table 1 gives the composition of the synthetic diets used. The laboratory chow fed was Rat and Mouse Diet RF, Agway, Inc., Syracuse, N. Y. Diets were fed ad lib. and drinking water was always available.

Enzyme assays. The supernatant fractions from cold sucrose fresh liver homogenates centrifuged at 21,500 X g for 30 min at 0-4 °C were used for all enzyme measurements. The two-stage method of Bublitz and Kennedy (4) was slightly modified for the glycerokinase (GK) assay. Stage II was carried out at room temperature with reagents in a final volume of 3 ml which included 0.01 M glycerol feeding; glycerophosphate dehydrogenase; glycerokinase; glucose-6-phosphate dehydrogenase; TPN malic enzyme; lipogenesis.
TABLE 1. Composition of diets given as % of calories

<table>
<thead>
<tr>
<th></th>
<th>Refeeding</th>
<th>Glucose</th>
<th>Glycerol</th>
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<tbody>
<tr>
<td>Casein</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Glucose</td>
<td>67</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>0</td>
<td>22</td>
<td>22</td>
</tr>
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Vitamin diet fortification mixture (Nutritional Biochemicals), 4% by weight; salt mixture (USP XIV), 5%; cellulose, 5%.

DPNH. The Fitch and Chaikoff (6) modification of the method of Beisenberg et al. (2) was used for measuring \( \alpha \)-glycerophosphate dehydrogenase (GDPH) activity at 25°C. TPN malic enzyme and hexose monophosphate-shunt dehydrogenase (HMPD) activities were measured at 25°C according to methods described in earlier papers from this laboratory (14, 15). For all enzymes 1 unit was considered to be the amount that oxidized or reduced 1 \( \mu \)mole of pyridine nucleotide per minute under the conditions employed.

Glycerol uptake. The method of Longmore and Hastings (10) was adapted for measuring glycerol uptake by liver slices. We used 2 ml Krebs-Henseleit bicarbonate buffer with 10 mM glycerol to incubate 200 mg liver slices for 90 min at 95% O\(_2\), 5% CO\(_2\) at 37°C in a Dubnoff shaker. Preliminary experiments indicated that the uptake of glycerol was the same in this medium as in that used by Longmore and Hastings and that the rate of uptake was linear over the 90-min period. The method of Korn (9) was used for glycerol analyses.

Lipogenesis. Incorporation of acetate-\( ^{14} \)C into fatty acids by liver slices in 3 hr was measured by methods previously described (15). Bicarbonate buffer with 100 mg/100 ml glucose and 4 \( \times \) \( 10^{-4} \) m sodium acetate was used.

Statistics. Results are reported as means ± standard error. The Student t test was used to test significance. Differences between means were considered to be non-significant (NS) when \( P \) was > .05.

EXPERIMENTAL DESIGN AND RESULTS

The effects of starvation and refeeding on hepatic \( \alpha \)-glycerophosphate dehydrogenase activity and glycerokinase activity are summarized in Fig. 1. Forty-eight hours of starvation resulted in a significant decrease in the activities of both enzymes in comparison with those of rats fed laboratory chow ad lib. Liver weight showed the usual fall in the starved animals. On refeeding a 67% glucose, fat-free diet for 48 hr after a 48-hr fast a highly significant increase in GDPH activity was observed. This was accompanied by a statistically significant decrease in GK activity. As one can see from a comparison of the scales for the two enzymes GK has only a fraction of the activity of GDPH.

The observed increase in GDPH activity was consistent with the observations of others (6, 7) who had described increases in the activity of this enzyme in the presence of hyperlipogenesis. It was also in harmony with the concept that hyperlipogenesis requires a sustained input of comparatively large amounts of \( \alpha \)-glycerophosphate for the esterification of fatty acids. The fall in GK activity in starved rats and the subsequently greater fall in re-fed ones was difficult to reconcile with the only working hypothesis we had at the time; i.e., that the rate of delivery of free glycerol to the liver participated in regulating the level of hepatic GK. This theory has some appeal in the case of the re-fed animals, in which adipose tissue
Fig. 2. Effects of glycerol fed in drinking water on liver enzyme activity. All rats were fed laboratory chow. Control rats were given tap water to drink and glycerol or glucose was added to the drinking water of experimental groups as indicated. Rats were studied after 7 days of feeding. GDH and GK as in Fig. 1.

In the first attempt to answer this question animals were given 15% glycerol in their drinking water for 1 week. Other animals were given an equal amount of glucose by the same route. The control group and the glycerol and glucose groups were fed a laboratory chow diet. The results are shown in Fig. 2. The livers of the glycerol animals showed a significant rise in GDH activity and (only in comparison with the glucose group) a marginally significant fall in GK activity. Since glycerol might be expected to stimulate lipogenesis these findings appeared to be consistent with those of the refeeding experiment though their magnitude was smaller.

Since no attempt had been made to measure fluid intake by all animals in this experiment and since the intra-group variability of response was so large the experiment was repeated with synthetic diets which were designed to provide approximately the amounts of glucose and glycerol consumed spontaneously by rats in the preceding experiment. The new design had the effect of magnifying differences among experimental groups as one can see in Fig. 3. This time, glycerol feeding for 1 week produced a striking increase in GDH whereas the glucose-starch combination resulted in only a modest increase. Both synthetic diets caused a fall in GK activity, but the fall in the glycerol-fed rats was significantly greater than that seen in the glucose-diet animals. It is noteworthy that, since glycerol feeding caused a significant degree of liver hypertrophy, the aggregate hepatic GDH activity increased even more than did the activity per milligram N. By the same token, the aggregate hepatic GK activity per rat decreased to a smaller, but still statistically significant, extent.

The fact that the glycerol-fed rats showed hepatic GDH levels which were markedly greater than were those seen in the glucose-fed animals suggested that...
glycerol was being activated and metabolized by the cell in spite of the apparent decrease in activity of the only known liver glycerol activating enzyme. It was pertinent to inquire whether or not glycerol uptake by the livers of glycerol-fed rats was unimpaired in spite of the clearly demonstrated decrease in the activity of GK. Therefore, liver slices of chow-fed, refed, and glycerol-fed rats were tested for their ability to remove added glycerol from the incubation medium with results as shown in Fig. 4. In both the refeeding experiment and the glycerol-feeding experiment decreased GK activity was associated with a significant decrease in glycerol uptake from the medium. This suggests that GK catalyzes the rate limiting reaction for glycerol uptake.

Finally, since glycerol feeding was observed to increase GPD activity to such a striking extent we elected to test the effect of this procedure on the ability of liver slices to incorporate acetate-1-14C into fatty acids ("lipogenesis") and on the associated activity of the major TPN-generating enzymes, the hexose monophosphate-shunt dehydrogenases (HMPD) and the TPN malic enzyme. Figure 5 represents a graphic summary of the results of this experiment. Both glucose and glycerol produced marked increases in the activity of the HMP dehydrogenases over levels seen in rats on a chow diet. Also, in both cases, there was a small increase in lipogenesis which was no higher in the glycerol fed rats than in glucose fed ones though the increase in GDH was much greater in the former than in the latter.

Glycerol feeding resulted in a significantly greater increase in malic enzyme activity than did isocaloric glucose feeding.

**DISCUSSION**

The rate of production of α-glycerophosphate is certainly an important component of the coordinated response we have called adaptive hyperlipogenesis (16). The availability of this metabolite can influence the overall process in at least two ways: first, by providing precursor for esterifying fatty acid thioesters which are inhibitory to acetyl CoA carboxylase (3), the rate-limiting step in palmitate synthesis and second, possibly by acting allosterically to stimulate the same enzyme as suggested by Wakil and his colleagues (20). Therefore, it was pertinent to examine the two enzymes which are known to generate α-glycerophosphate from the point of view of their adaptability to drastic changes in diet.

The response of cytosol GDH to refeeding was not unexpected since Fitch and Chaikoff (6) had seen a similar increase in the activity of this enzyme in rats shifted from a chow diet to glucose and fructose diets. It is interesting that in this instance, as well as in the case of glucose-6-
phosphate dehydrogenase and TPN malic enzyme, a 48-hr period of starvation has the effect of exaggerating the effect of the response to hexose diets. The reason for this enhancement is not yet known. The association of a high GDH activity with the hyperlipogenesis of refeeding is consistent also with previous findings of increased activity of this enzyme in the tissues of certain experimentally obese animals which characteristically show very active lipogenesis (7).

The fall in glycerokinase on refeeding is understandable teleonomically if one concedes that the phosphorylation of glycerol is not a particularly advantageous reaction when α-glycerophosphate is being generated in large amounts from carbohydrate precursors. In this connection it is interesting to note that Fain, Reed, and Sapirstein (5) demonstrated in an acute experiment with isolated white fat cells that glucose and insulin depressed the incorporation of glycerol-1,3-14C into glyceride glycerol. This finding makes it possible to conceive of a "quick" control mechanism associated with the sudden entrance of large amounts of glucose into the cell followed by the operation of a long range control mechanism which resets the level of glycerokinase. It is somewhat surprising that glycerol feeding also has the effect of increasing markedly the activity of GDH while it decreases GK activity. Clearly a sufficient amount of glycerol gained access to the interior of the cell to produce a stimulation of lipogenesis and adaptive increases in the activity of both malic enzyme and the hexose monophosphate-shunt dehydrogenases. Therefore, the reaction catalyzed by GDH was operating in the direction α-GP → DHAP. In the livers of rats fed a hexose diet the reverse reaction would be expected to be predominant. This, then, is a rare example of an adaptive increase in the activity of a bidirectional enzyme.

Although glycerol feeding cannot be considered a physiologically "normal" circumstance this maneuver can be used to secure basic information about enzyme regulation as Takeda and his colleagues (12) have recently demonstrated. These investigators have shown convincingly that glycerol feeding not only increases glucose-6-phosphate dehydrogenase (as we also demonstrate in this report) but also glucokinase, pyruvate kinase, citrate cleavage enzyme, and acetyl CoA carboxylase. In other words, glycerol feeding, like fructose feeding, is a very powerful stimulus to lipogenesis. It is not surprising that this should be so, for fructose is metabolized to "instant trioses" by 1-phosphofructoaldolase, so that the ultimate effect of either fructose feeding or glycerol feeding is to present the cell with a large-scale triose disposal problem.

In this connection, it is interesting to observe that fructose was seen to have a greater effect on TPN malic enzyme of liver than glucose while it had approximately the same effect on glucose-6-phosphate dehydrogenase (6). In this study exactly the same pattern was seen as a result of glycerol feeding. This suggests the possibility that some event associated with the necessity to dispose of large quantities of trioses may have special significance in setting the level of activity of the TPN malic enzyme, for, although the activity of this enzyme often fluctuates in parallel with that of glucose-6-phosphate dehydrogenase, one can often dissociate the responses of the two (13, 19).

The identity of the signal or signals which elicit increased GDH activity is not known. It seems possible that a high rate of product removal for esterification of newly synthesized fatty acids might first stimulate α-GP formation and subsequently result in an increase in GDH. In support of this hypothesis Zakim et al. (21) have found that animals maintained on high hexose diets for 3 weeks show a significantly lower concentration of α-glycerophosphate in their livers than do animals on chow diets. There is every reason to suppose that both lipogenesis and GDH activity were high under the conditions of their experiment. Moreover, our time studies of GDH adaptation and recovery of lipogenesis on refeeding (unpublished data) show that lipogenesis recovery nearly reaches its maximum at 24 hr whereas GDH activity continues to increase for 72 hr after the beginning of refeeding.

Since, to our knowledge, this is only the second report of an adaptive fluctuation in hepatic glycerokinase (Treble and Ball, 17) found an increase in the enzyme in cold-exposed rats there has been little speculation on the mechanisms involved in regulating the level of activity of this enzyme. However, there are two bacterial model systems which are relevant to the discussion. Zwaag and Lin (22) have described inhibition of glycerokinase in Escherichia coli by fructose 1,6-diphosphate. The studies reported here, together with those of Fain et al. (5), are consistent with the possibility that an intermediary metabolite generated either by hexose feeding or glycerol feeding may inhibit mammalian glycerokinase in a manner analogous with that shown for the E. coli enzyme by Zwaag and Lin. This hypothesis would require that an "acute" regulation be translated into a "chronic" one in the form of a decrease in glycerokinase.

In another study on E. coli Hayashi and Lin (8) suggest that glycerol kinase is inducible by its product, α-glycerophosphate. If this were true of the mammalian enzyme the finding of Zakim et al. (21) that hepatic α-glycerophosphate concentration is low in high lipogenesis situations could be helpful in formulating a theory of glycerokinase regulation, for the activity of the enzyme is clearly low when the concentration of its product is low. The finding of Tzur et al. (18) that α-GP concentration is increased within 2 hr after the beginning of refeeding does not invalidate Zakim's theory that α-GP concentration and lipogenesis vary inversely for, at the time the analyses of Tzur et al. were performed, there had been little time for lipogenesis recovery to occur. Application of these ideas to the glycerol experiment will require more complete information about changing concentrations of α-GP during glycerol feeding in combination with variably lipogenic diets.

The reciprocal responses of GDH and GK to refeeding
and to glycerol feeding represent another example of a coordinated adjustment of the cell's metabolic machinery to an abrupt change in its substrate environment. An elucidation of the intimate mechanisms involved in the regulation of the levels of activity of these enzymes will depend on further work.

REFERENCES