The Hexosemonophosphate Shunt and Adaptive Hyperlipogenesis

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INTRODUCTION

A recent study of the effect of antecedent food intake pattern on hepatic lipogenesis (Tepperman and Tepperman)[1] revealed an extraordinary range of lipogenic activity of surviving liver slices derived from rats and mice which had been prepared in a number of ways. In confirmation of the work of others,[2] the incorporation of the C^{14} label of acetate into the lipid fraction was found to be negligible when the slices were obtained from starved rats. When rats were trained to eat their entire day's ration in one hour ("trained") their liver slices showed comparatively "supernormal" lipogenesis. Similarly, liver slices of aurothioglucose obese mice which had been overeating for several months incorporated much more of the precursor label into fat than did slices of lean fed controls (see also Mayer et al.).[3]

When rats were starved for forty-eight hours and refed a nutritionally complete high carbohydrate, low fat diet for twenty-four hours their liver slices exhibited strikingly high lipogenic activity when tested under the same conditions employed for the experiments described above.[1,4] We have called the "supernormal" lipogenesis of overnutrition, "training" and refeeding "adaptive hyperlipogenesis" because the lipogenic activity of the liver in each circumstance seems to us to be teleologically appropriate to the nutritional state of the animal.

The refeeding phenomenon appeared to be particularly useful for exploring the mechanisms involved in adaptive hyperlipogenesis, especially since a correlation plot of lipogenic activity versus initial glycogen level of the liver (figure 1) revealed that there is a good correlation (coefficient 0.84) between these parameters for many groups of control, fasted and "trained" rats, but that two refed groups were far outside the 3x S.E. of the Estimate boundary which presumably includes 99 per cent of the observations in a population. This suggested strongly that the slices from refed rats developed a lipogenic advantage over and above that afforded by their very high initial glycogen content.

The demonstration by Shaw, Dituri and Gurin[5] that the reaction crotonyl CoA→butyryl CoA is a locus of the defect in lipogenesis in diabetes and Langdon's[6] demonstration that there is a specific requirement for TPNH at this step suggested that this critically placed reaction might be a locus of "training" in those circumstances in which relatively "supernormal" lipogenesis occurs. The demonstration (Tepperman and Tepperman)[1] that most of the lipogenic advantage of the liver slices from "refed" rats disappears when one compares lipogenesis in nicotinamide-fortified whole homogenates of normal and "refed" livers emphasized the probability that variations in lipogenesis are more likely to be related to fluctuating supplies of critically needed cofactors than to variations in activity of the apoenzymes involved in the synthetic reaction. Accordingly, refed animals were prepared and their livers were assayed for TPN reducing activity of the hexosemonophosphate shunt dehydrogenases by the method of Glock and McLean.[7] (See Materials and Methods.) When the rats were sacrificed twenty-four hours after the beginning of refeeding (which oc-

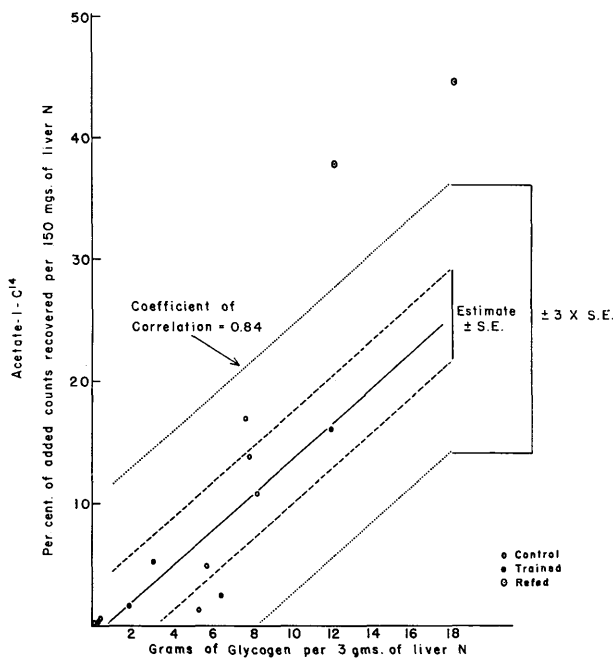

FIG. 1. Correlation of initial glycogen level and lipogenesis performance of liver slices. Data of Tepperman and Tepperman.[1]

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curred at the end of a forty-eight-hour fast) the enzyme activity was 300-400 per cent of the level found in fed controls, depending on the reference standard used for the calculation. Since these livers had a history of having had negligible lipogenic activity and nearly normal shunt activity only twenty-four hours before, the refeeding phenomenon seemed to us to offer an extraordinary opportunity to observe the change in lipogenic capacity over virtually its entire spectrum in a very short time, and to make observations on the concurrent reorganization of the cell's biochemical machinery which might be related to the process under study. The results of the following experiments, then, constitute a sort of moving picture of certain changes in chemical morphology and enzyme activity that occur during the first forty-eight hours of refeeding. They are presented in support of the view that a fluctuating supply of TPNH is one important determinant of lipogenic activity in liver.

MATERIALS AND METHODS

Adult male albino rats (Albino Farms) were used. Their mean weight at the beginning of the forty-eight-hour period of starvation was approximately 300 gm. A high carbohydrate, low fat, 21 per cent protein diet[8] was fed ad libitum during the refeeding period. Six rats were killed at the end of the forty-eight hour fast and 3, 6, 12, 24 and 48 hours after the beginning of refeeding, measurements were made of body weight, total liver weight, liver nitrogen, liver glycogen, liver total fat in some experiments, incorporation of acetate-1-C^{14} label into total fat and hexosemonophosphate shunt dehydrogenase activity by methods previously used in this laboratory.[1] At least one chow-fed control was studied each time an experiment was done. It should be emphasized that the liver measurements were made on aliquots of the same livers. The shunt enzyme measurement included the total reduction of TPN by both glucose-6-phosphate dehydrogenase and 6 phosphogluconate dehydrogenase at *p*H 7.6. A unit of enzyme activity is defined as the amount of enzyme which reduces 0.01 micromols of TPN per minute at 20° C. and *p*H 7.6 based on readings over the first five minutes. In all cases the mean values for six rats ± S.E. are given. Probabilities are estimated by Student's t test.

RESULTS

The numerical results are given in table 1. These data are presented as absolute values and include means and standard errors for each group. In every instance in which findings are related to body weight, the prestarvation weight was used. Figures 2 to 5 represent graphic summaries of some of the information presented in table 1 recalculated on the basis of percentage of control value.

The changes in body weight, liver weight and liver nitrogen are shown in figure 2. One can see that at the end of the antecedent forty-eight-hour fast all of these parameters were low as compared with the fed controls. The most striking fluctuation was shown by the liver weight, even when it is related to the weight of the rat. It is because of the variations shown in this figure that calculations in the next two figures are based on three different reference standards.

The recovery of lipogenesis and its "overshoot" above the level seen in that of chow-fed controls is well demonstrated in figure 3. By three hours there was significant recovery; by six hours, the slices had practically half the activity of the controls. At twelve hours, three times the control amount of acetate label appeared in the lipid at the end of three-hour incubation period. There was no increase in recovery of the acetate label between twenty-four and forty-eight hours in this experiment, in which 2 μM of acetate was added per flask and, in some experiments, 50 to 70 per cent of the added counts were recovered in the lipid fraction.

The changes in shunt dehydrogenase activity on refeeding are shown in figure 4. The significant elevation at twenty-four hours is confirmed, but this is seen to be only the beginning of a much more impressive rise.

The interrelations of lipogenic activity, shunt activity and initial glycogen content of the liver are well illustrated in figure 5. Here, one can see that in the early hours of refeeding, before any appreciable change in shunt enzyme activity has occurred, lipogenic activity is beautifully proportional to initial glycogen level. At twelve hours shunt activity is 135 per cent of the fed control level and lipogenesis is much higher than one would predict from a projection of the first three points. At twenty-four hours, glycogen content is extremely high, shunt activity is more than three times normal and lipogenesis is very rapid. At forty-eight hours glycogen is reduced, but lipogenesis persists at a high level. However, shunt enzyme activity is enormous. This, then, represents a clear dissociation of initial glycogen level and lipogenic activity, since a high rate of lipogenesis is seen in the face of a markedly diminished initial glycogen level.

Since more than half of the added acetate counts appeared in the lipid fraction in many experiments at twenty-four and forty-eight hours, it seemed probable that the 2 μM addition of acetate was limiting at high lipogenesis rates. Accordingly an experiment was done in which the same number of acetate counts was added in a total of 50 μM of acetate. The results are shown in

TABLE 1

Group	Body Weight, Gm. Initial	Body Weight, Gm. Final	Liver Weight Gm./100 Gm. Body Weight	Liver Nitrogen Per Cent Wet Weight of Liver	Liver Glycogen Per Cent Wet Weight of Liver	Liver "Lipogenesis" Per Cent Added Counts Recovered Per 15 Mg. Liver N	Liver "Lipogenesis" Mols Added Acetate Recovered Per 100 Gm. Rat	Liver HMP Shunt Dehydrogenase Activity Units Per Mg. Liver N	Liver HMP Shunt Dehydrogenase Activity Units Per 100 Gm. Rat
Control	296 ± 2.6	307 ± 10.3	3.7 ± .10	3.25 ± .05	5.8 ± .24	8.1 ± 1.09	1.4 ± .19	2.1 ± .16	250 ± 24.7
48 hr. fast	292 ± 9.4	263 ± 8.7	2.2 ± .06	3.68 ± .04	1.0 ± .33	0.3 ± .06	0.03 ± .0002	1.7 ± .16	144 ± 15.5
3 hr. refed	304 ± 9.3	283 ± 7.7	2.7 ± .04	3.48 ± .06	4.1 ± .13	1.4 ± .06	0.2 ± .02	1.8 ± .26	168 ± 31.2
6 hr. refed	305 ± 5.9	278 ± 3.1	2.7 ± .14	3.10 ± .05	6.7 ± .49	3.9 ± .68	0.5 ± .08	1.8 ± .16	153 ± 19.6
12 hr. refed	302 ± 4.6	286 ± 6.1	3.7 ± .12	2.45 ± .10	13.1 ± .46	25.6 ± 2.59	3.3 ± .37	2.8 ± .64	253 ± 59.5
24 hr. refed	305 ± 7.8	290 ± 6.0	4.6 ± .17	2.15 ± .11	14.6 ± .55	65.4 ± 4.71	9.3 ± .51	7.4 ± .99	720 ± 88.4
48 hr. refed	327 ± 3.4	315 ± 2.2	4.5 ± .22	2.45 ± .11	8.5 ± .75	65.5 ± 9.95	10.2 ± 1.37	28.9 ± 4.76	3,092 ± 635.6

Note: Each figure represents the mean of determinations on six rats ± S.E. of the mean.

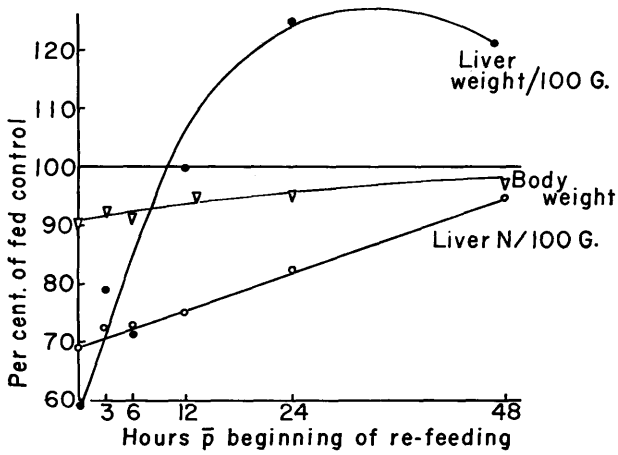

FIG. 2. Body weight, liver weight and liver nitrogen during refeeding. "/100 G" refers to initial body weight. Calculated from table 1.

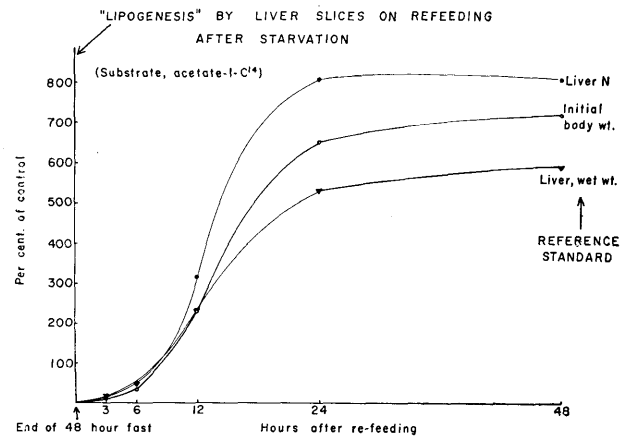

FIG. 3. Experiments done with two micromols of acetate per beaker. Lipogenesis referred to three different reference standards and calculated from data in table 1 as percentage of fed control.

figure 6. As before, there was a marked drop in initial glycogen level between twenty-four and forty-eight hours, but this time there was an *increase* in lipogenesis at the 5 per cent level of confidence. The apparently higher shunt activity at forty-eight hours in this experiment as compared with that shown in figure 4 is simply due to the fact that the control values for this experiment were slightly lower than were those for the previous one.

It was pertinent to inquire whether or not the rate of incorporation of the acetate label into the cholesterol fraction paralleled that into fatty acids. Accordingly, an experiment was done in which the incorporation into total lipid and into the digitonin-precipitable fraction was measured separately. The results, shown in figure 7, indicate that the counts recovered in the digitonide fraction of the control represented only a very small proportion of those recovered in the total lipid, and that, although the refeeding maneuver sharply increased incorporation in total lipid, it resulted in a significantly diminished incorporation in the digitonide. Thus at forty-eight hours after refeeding only 0.03 per cent of the counts recovered in the total lipid were in the digitonin-

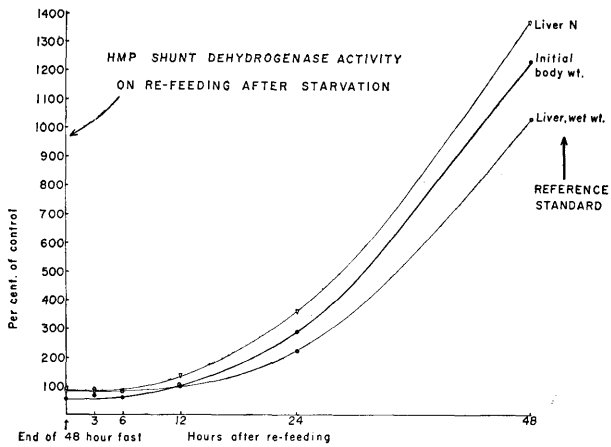

FIG. 4. Data are calculated from table 1 as percentage of fed control.

precipitable fraction.

The high lipogenic activity of the slices from refed rats suggested the possibility that a net increase in total liver fat might be observed during the refeeding period. Figure 8 shows a significant increase in liver fat from the twenty-fourth to the forty-eighth hour of refeeding.

The finding of a high HMP shunt dehydrogenase activity at forty-eight hours was the basis of an experi-

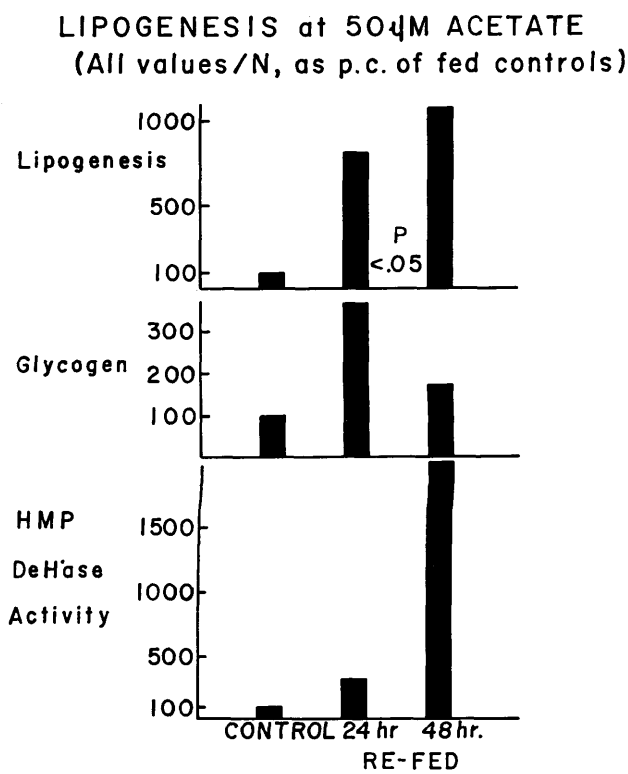

FIG. 6. Lipogenesis, glycogen and shunt enzymes. All values referred to liver nitrogen and expressed as percentage of control. Lipogenesis experiments done with 50 micromols of acetate per beaker.

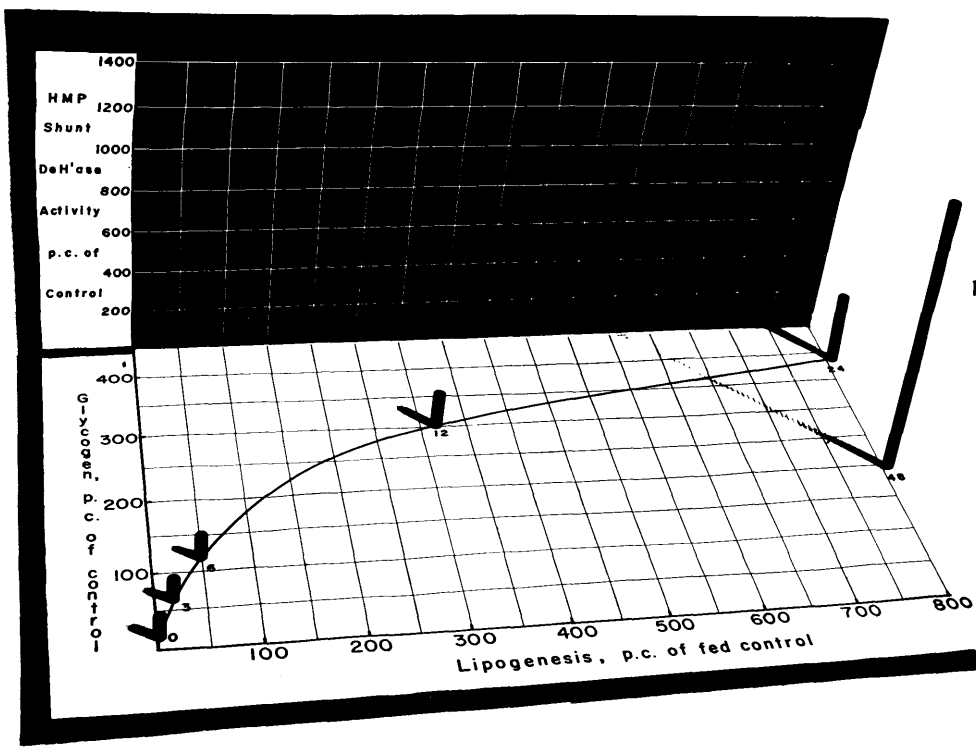

FIG. 5. Hexosemonophosphate shunt dehydrogenase activity, initial glycogen content and lipogenesis by liver slices. All values were referred to liver nitrogen and plotted as percentage of chow-fed control. Numbers at each point represent hours after the beginning of refeeding. See text for additional information.

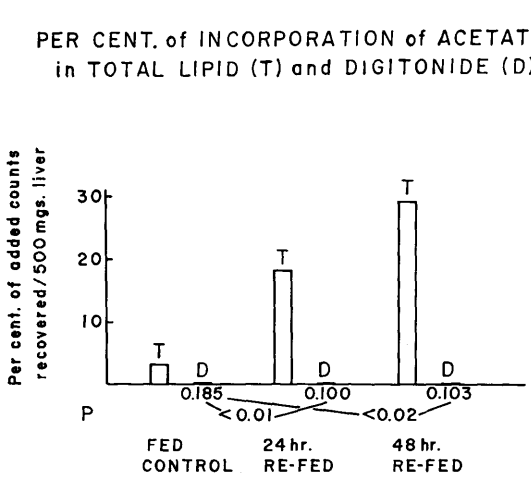

FIGURE 7

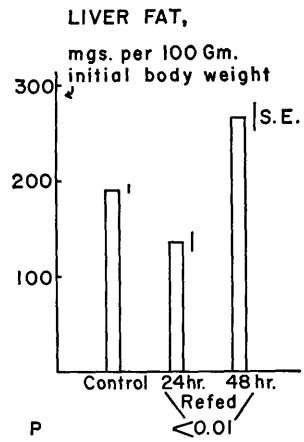

FIGURE 8

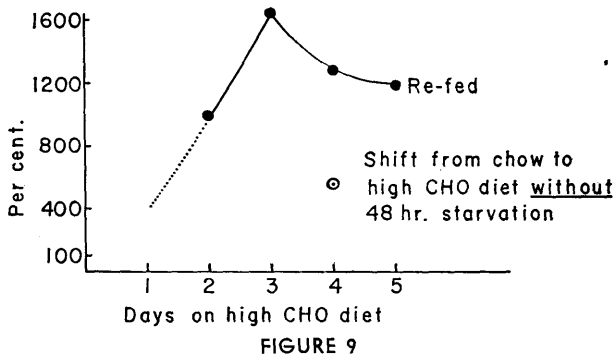

FIGURE 9

ment designed to show the duration of this change. Figure 9 shows that, in a separate series in which no lipogenesis studies were made, the shunt dehydrogenase activity did not reach its peak until seventy-two hours after the beginning of refeeding. Five days after the end of the starvation period the activity of the shunt enzymes was still very substantially above the control level. Since all animals in this study had been on a chow diet until the beginning of the starvation period, one group of six rats was shifted from a chow diet to a high carbohydrate diet without a prior period of starvation. The shunt dehydrogenase activity of the livers of these animals was significantly elevated, but it was well below the levels seen in rats with a history of prior starvation.

DISCUSSION

The large changes in liver weight as a result of starvation and subsequent refeeding are attributable in part to deglycogenation followed by very extensive glycogen deposition. The amounts of glycogen in the livers of twenty-four-hour refed rats were often in excess of 15 per cent of the wet weight of the organ. Table 1 shows that, in the presence of these amounts of glycogen, there was a significant decrease in percentage of liver nitrogen. The mechanisms involved in this extraordinary glycogen deposition are not known. It is possible that the phenomenon may be caused by the combination of realimentation with a high carbohydrate diet (which can cause a significant elevation of liver glycogen levels without prior starvation) and the persistence into the refeeding period of an inappropriately *high level* of gluconeogenesis initiated during the preceding fast.

Inspection of the time curves for lipogenesis and shunt enzyme activity (figures 3 and 4) shows that the rearrangement of the biochemical machinery of the cell which enables it to synthesize fat very rapidly takes place over a period of hours in much the same way that the recovery of the lipogenic activity of the diabetic liver following insulin[9] or fructose[10] administration requires a period of hours.

The three-dimensional plot of lipogenesis, shunt activity and glycogen (figure 5) reemphasizes the importance of initial liver glycogen as a determinant of the lipogenic performance of liver slices in vitro, particularly during the period of relative constancy of shunt enzyme activity. When the shunt activity is markedly increased, and when the glycogen levels are high, the slices appear to have additional lipogenic activity. The fact that persistently high lipogenic activity was demonstrated when the glycogen fell and the shunt activity increased markedly strongly suggests that lipogenic performance is not determined simply by how much glycogen was in the slice at the beginning of the incubation period, but also by the available enzyme sys-

tems for its metabolism via the hexosemonophosphate shunt. In order for a continuing supply of TPNH to be maintained, a pool of glucose-6-phosphate must be provided, but if substrate traffic over the shunt is maintained at a high level by the presence of a large amount of shunt dehydrogenase, just as large a TPNH supply can be maintained with a smaller initial potential glucose-6-phosphate pool. It should be emphasized that, at the moment, it is a matter of conjecture that the level of shunt dehydrogenase activity as measured in these experiments determines the rate of substrate metabolism via the hexosemonophosphate shunt. Experiments with C_1- and C_6-labeled glucose are now being done to study this problem.

There are many other experiments in which the differentially labeled glucose technic has been used that suggest that (a) in diabetic liver shunt traffic is low[11] and (b) in adipose tissue and in lactating mammary gland high rates of fatty acid synthesis are accompanied by high rates of metabolism via the shunt pathway.[12,13] In the diabetic liver[14] and in the lactating mammary[15] gland good correlations have been shown between hexosemonophosphate shunt enzyme activity and glucose metabolism via the shunt as estimated by the differentially labeled glucose technic. These observations, and those of Siperstein,[16] who was able to stimulate lipogenesis in cell-free preparations by providing TPN reducing systems, generally support the view that such systems play an important physiologic role in adaptive fluctuations in lipogenesis such as the ones described here. It should be pointed out that TPNH is required specifically not only for the reduction of crotonyl CoA but also for ethylene reductions that occur on subsequent two carbon additions all the way up to long chain fatty acids.[6,17]

Siperstein[16] has suggested that TPNH is required for cholesterol synthesis as well as fatty acid synthesis. Therefore it was of great interest to discover whether or not cholesterol synthesis from acetate was enhanced in liver slices in which hexosemonophosphate shunt activity was known to be extremely high. It has already been pointed out that there is a dissociation between cholesterol synthesis and fatty acid synthesis in the diabetic liver,[18] in which the former is high and the latter vanishingly low. In the twenty-four- and forty-eight-hour refed liver a reverse dissociation is apparent, for here (figure 7) the rate of lipogenesis is very high while there is a significant *decrease* in rate of incorporation of the acetate label into the "cholesterol" fraction. Obviously, the factors that influence the rate of fatty acid synthesis in the liver do not necessarily affect the cholesterol synthetic mechanisms in the same direction.

A diagrammatic hypothesis concerning the failure of lipogenesis in diabetes and starvation and the adaptive enhancement of the process in the refeeding phenomenon is presented in figure 10. The variation in width of the arrows in this diagram is not intended to have precise quantitative significance. This is merely an attempt to show how the potential metabolic activity of the individual liver cell is changed by the conditions under discussion and how these changes relate to the chemical economy of the whole organism. While emphasis is placed on the specific ethylene reduction that occurs between crotonyl S CoA and butyryl S CoA, it should be recalled that similar reactions occur on further two carbon fragment addition as the fatty acid is progressively lengthened.[17] In the diabetic cell, gluconeogenesis from amino acids proceeds at a high rate. There is diminished glucose utilization via both Emden-Meyerhof and HMP shunt pathways.[11] This (as Langdon,[6] Siperstein[16] and others have suggested) has the effect of failing to provide a sufficiently large pool of reduced cofactors to support reductive synthesis. That the lack of TPNH is most critical is shown by the failure of butyryl CoA formation[5] while ketone body formation, which is independent of TPNH, proceeds apace. The increase in glucose-6-phosphatase activity that accompanies this condition[19] has the effect of marking glucose produced from amino acids "for export only." This in turn ensures a continuing supply of glucose to tissues like the central nervous system, whose metabolism, not requiring insulin, is mainly dependent on glucose. There is an accentuation of fatty acid catabolism in liver and, probably, muscle by analogy with the adaptation that occurs in fat feeding.[20]

The cell of the refed animal makes a remarkable adaptation to the presence of a plethora of glucose. Refeeding probably diminishes glucose-6-phosphatase activity (by analogy with insulin treatment of the diabetic)[21] but possibly does not immediately "shut off" accelerated gluconeogenesis. At the same time a large amount of glucose is absorbed from the gastrointestinal tract. All of these events collaborate to provide a very large pool of glucose-6-phosphate, much more than the cell can possibly dispose of conveniently at first. Some of the excess goes into an expanded glycogen compartment. With the passage of time, more hexosemonophosphate shunt enzyme activity appears and substrate traffic over the shunt pathway increases. This has the effect of generating increasingly large amounts of TPNH which progressively increases the cell's potential for fatty acid synthesis and thus helps the cell dispose

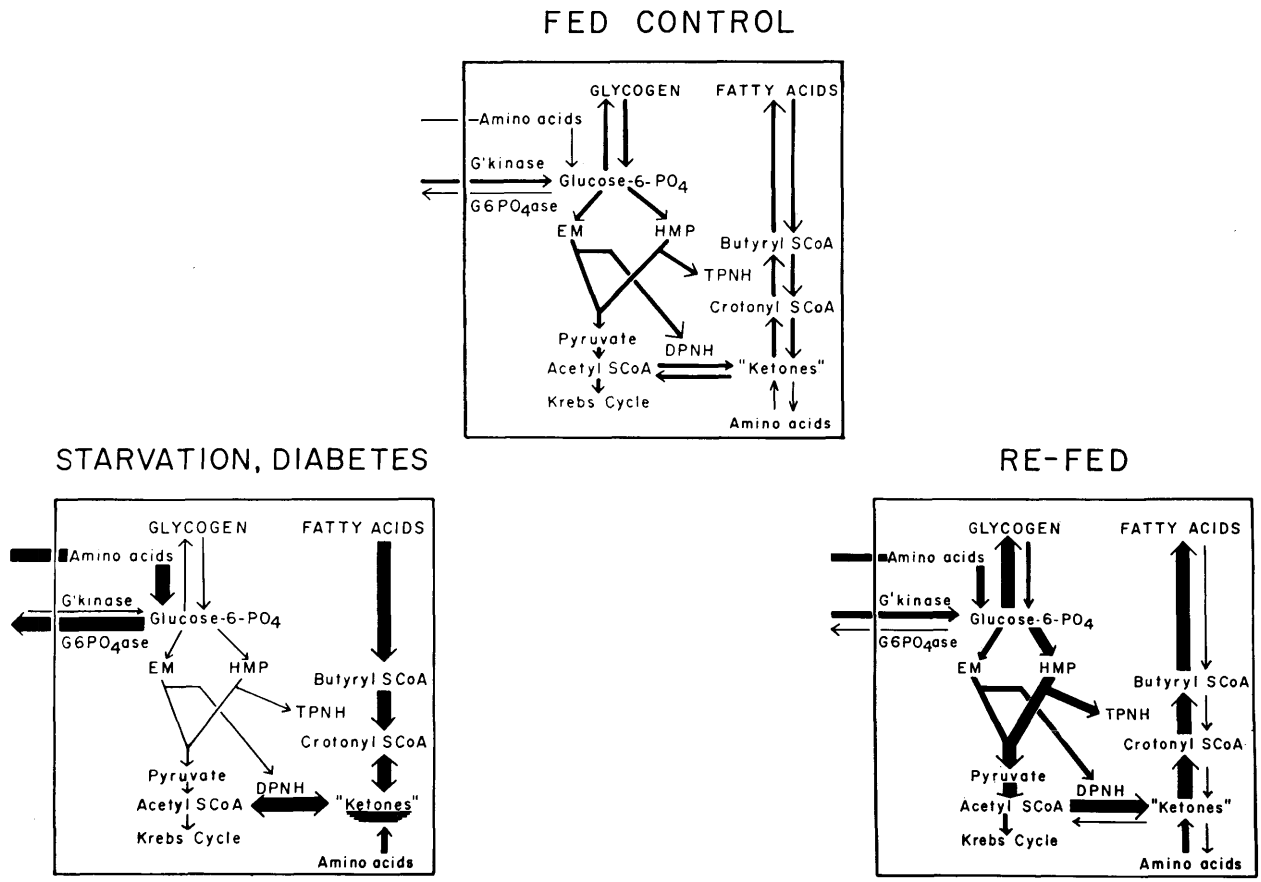

FIG. 10. Hypothetical schemes of cellular adaptation. See text.

of its large glucose load. (There is, as yet, no definite information about the quantities of glucose that traverse the Emden-Meyerhof pathway and are oxidized in the refed liver.) The net effect of the accentuation of the shunt pathway is to facilitate lipogenesis when it is physiologically advantageous for this process to proceed briskly. The repletion of fat stores is especially important for an animal that has depleted them as a result of a forty-eight-hour period of starvation.

The nature of the increase in shunt enzyme activity described here is as yet unknown. We have been careful to refer to this phenomenon as an increase in activity, rather than a de novo synthesis of enzyme, although the latter view represents our current working hypothesis. Certainly, the persistence of high levels of shunt activity over a five-day period shown in figure 9 suggests the possibility that new enzyme has been synthesized. At present, we are inclined to view the shunt pathway as an inducible enzyme system similar to those seen in microorganisms.[22] Possibly, glucose-6-phosphate can function as an inducer. Our current studies are concerned with this hypothesis.

SUMMARY

Liver slices of rats starved for forty-eight hours and refed a high carbohydrate diet for forty-eight hours show extremely high rates of lipogenesis from acetate-1-C^{14} when compared with those of chow-fed controls. In order to test the hypothesis that glycogenolysis may influence the rate of lipogenesis by providing TPNH for critically placed ethylene reductions in the synthesis of fatty acids, rats were starved for forty-eight hours, refed and killed in groups of six at 3, 6, 12, 24, and 48 hours after the beginning of refeeding. Lipogenesis by liver slices, glycogen content, and hexosemonophosphate shunt dehydrogenase activity and other pertinent measurements were made on the same livers. From 0 to six hours lipogenesis correlated well with initial glycogen level, but at forty-eight hours lipogenesis was as high as it had been at twenty-four, though the glycogen had dropped by half. Hexosemonophosphate shunt dehydrogenase activity at this time was more than three times the twenty-four-hour level. The three-way correlation among these variables suggests that substrate traffic over

the shunt may be an important determinant of lipogenesis rate. A hypothetical scheme is presented in which an attempt is made to relate the phenomenon of adaptive hyperlipogenesis to the problem of failure of lipogenesis observed in starvation and diabetes.

SUMMARIO IN INTERLINGUA

Le Shunt De Hexosa-Monophosphato E Hyperlipogenese Adaptatori

Trenchos de hepate ab rattos non alimentate durante quaranta-octo horas e re-alimentate durante quaranta-octo horas con dietas ric in hydrato de carbon revela un extrememente intense lipogenese ab acetato-1-C^{14} in comparation con specimens de controlo. Esseva formulate le hypothese que glycogenolyse exerce un influentia super le intensitate del lipogenese per provider TPNH pro reductiones ethylenic de position critic in le synthese de acidos grasse. Pro testar iste hypothese, rattos non alimentate durante quaranta-octo horas esseva re-alimentate e occidite in gruppos de sex a periodos de 3, 6, 12, 24, e 48 horas post le recomenciamento del alimentation. Le lipogenese per trenchos de hepate, le contento de glycogeno, le activitate de dishydrogenase del shunt de hexosa-monophosphato, e altere pertinente factores esseva determinate in le mesme hepates. Usque a sex horas post le recomenciamento del alimentation, le lipogenese se monstrava ben correlationate con le nivello initial de glycogeno, sed post quaranta-octo horas le lipogenese esseva tanto intense como post vinti-quatro horas, ben que le nivello de glycogeno habeva descendite per un medietate. Le activitate de dishydrogenase del shunt de hexosa-monophosphato a iste tempore esseva plus que tres vices illo trovate post vinti-quatro horas. Le correlation tridirectional inter iste variabiles suggere que migration de substrato via le shunt es possibilemente un factor importante in determinar le intensitate del lipogenese.

Es presentate un schema hypothetic que tenta relationar le phenomeno del hyperlipogenese adaptatori con le problema del collapso lipogenetic que ha essite observate in affamation e in diabete.

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