The Role of a High Fat Diet in the Rerepression[1] of Two Rat Liver Enzymes (37702)

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It has been reported that starvation followed by refeeding a low fat, adequate protein, high carbohydrate diet (the inducer diet) causes an increase (overshoot) in rat liver glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) activities (1). This increase in enzyme activity has been shown to be dependent on dietary protein and carbohydrate (2–6). A second cycle of starvation–refeeding leads to an even greater overshoot than is observed after one cycle of starvation–refeeding (7); however, neither dietary protein nor carbohydrate is necessary during the first refeeding to produce this greater overshoot after a second starvation–refeeding cycle (8). The increased inducibility of G6PD and ME can be obliterated by feeding a high fat diet during the first refeeding (9). If the antibiotic 8-azaguanine (8 AG) is administered during the feeding of the high fat diet in the first refeeding, the increased inducibility of the enzymes after the second refeeding can again be noted (B. Szepesi, unpublished data).

It has been suggested that starvation causes derepression[1] and that the high fat diet causes rerepression of the genes which code for G6PD and ME (10). If the process of derepression is due to the destruction of the repressor system for these genes, then during rerepression the complete repressor must be synthesized. Derepression has been shown not to be dependent on *de novo* RNA synthesis (B. Szepesi, unpublished data). However, since rerepression has been prevented by the administration of the antibiotic 8 AG, which leads to the formation of nonfunctional RNA (11, 12), it can be postulated that rerepression is a process which is dependent on *de novo* RNA synthesis.

The experiments reported in this communication were designed to test two hypotheses concerning the role of a high fat diet in the rerepression of the genes which code for G6PD and ME. The first hypothesis is that dietary fat induces the formation of the aporepressor(s), or an enzyme system necessary for the formation of the corepressor(s), or both. The second hypothesis is that dietary fat provides either the corepressor(s) or a precursor of the corepressor(s). This would imply that the molecules provided by the dietary fat would not be normally present in the liver, despite the large accumulation of fat which occurs when an inducer diet is fed after starvation.

As a corollary to either hypothesis, the experiments were designed to study whether or not dietary carbohydrate and/or protein is required for rerepression.

Methods and Materials. Specific pathogen-free, male Wistar rats were purchased from Hilltop Lab Animals, Inc., Scottdale, PA. The rats weighed 75–100 g before shipment and were delivered before noon of the day they were packed for shipment. In order to prevent starvation during shipment, the rats were provided with potatoes and pellets of laboratory chow. The rats were equilibrated to laboratory conditions for 1 week before

[1] The term derepression is used in this paper to denote the process(es) operating during starvation which render G6PD and ME inducible during refeeding an inducer diet. Hence, the term rerepression is used here to denote the effect of a treatment in counteracting those processes which render G6PD and ME inducible. These are operational definitions and do not imply a particular mechanism of action.

TABLE I. Experimental Protocol and Body Weights for Each Group of Rats.

Group	Treatment[a] 1st Starvation (2 days)	1st Refeeding 1st Diet (2 days)	1st Refeeding 2nd Diet (2 days)	2nd Starvation (2 days)	2nd Refeeding (3 days)	Initial body wt (g)	Body wt at killing (g)
Protein-fed groups							
A	—	5 F, 90 P	35 F, 60 P (No G)	—	5 F, 25 P, 65 G	110 ± 4[b]	138 ± 3
B	—	5 F, 90 P	35 F, 25 P, 35 G	—	5 F, 25 P, 65 G	112 ± 2	152 ± 4
C	—	5 F, 90 P	35 F, 25 P, 35 G + 8 AG	—	5 F, 25 P, 65 G	118 ± 2	143 ± 6
Carbohydrate-fed groups							
D	—	5 F, 90 G	35 F, 60 P (No P)	—	5 F, 25 P, 65 G	107 ± 4	101 ± 6
E	—	5 F, 90 G	35 F, 25 P, 35 G	—	5 F, 25 P, 65 G	107 ± 3	130 ± 4
F	—	5 F, 90 G	35 F, 25 P, 35 G + 8 AG	—	5 F, 25 P, 65 G	108 ± 2	111 ± 2

[a] The numbers under "Treatment" refer to the percentage (by weight) of each component of the diets; F = fat (corn oil), P = protein (casein), G = glucose. All diets contained 4% salt mix and 1% vitamins. A complete description of the diets is given in "Methods and Materials." The days during which 8-azaguanine was administered are indicated by 8 AG following the dietary regimen.

[b] Standard error of the mean. All groups contained 6 rats.

TABLE II. Effect of Dietary Treatments[a] and 8-Azaguanine Administration on Food Intake for Groups A–F.

Treatment	Food intakes (g/100 g body wt/day) A	B	C	D	E	F
Refed (90 P)	11.9 ± 0.47[b]	13.4 ± 1.01	12.9 ± 1.08			
Refed (90 G)				8.52 ± 0.42	9.04 ± 0.36	8.48 ± 0.36
(35 F, 60 P) following (90 P)	12.0 ± 0.46					
(35 F) following (90 P)		13.7 ± 0.70				
(35 F, 8 AG) following (90 P)			11.0 ± 0.69			
(35 F, 60 G) following (90 G)				6.66 ± 0.41		
(35 F) following (90 G)					15.4 ± 0.67	
(35 F, 8 AG) following (90 G)						8.06 ± 1.03
Refed for 2nd time, (65 G)	14.4 ± 0.38	14.8 ± 0.22	14.8 ± 0.30	17.7 ± 1.26	15.5 ± 0.28	15.6 ± 0.61

[a] See Table I for specific dietary treatments and 8-azaguanine administration schedule for each group.

[b] Standard error of the mean.

the experiment was begun.

Six experimental regimens were followed; these are summarized in Table I. All diets contained 4% Jones–Foster salt mix and 1% vitamin fortification mixture.[2] Groups A, B, and C were starved 2 days, and refed for 2 days a diet containing 90% casein and 5% corn oil but no carbohydrate (5 F, 90 P). This was followed by feeding for 2 days a high fat diet containing either 35% corn oil and 60% casein, but no glucose[3] (35 F, 60 P) (group A), or 35% corn oil, 25% casein, and 35% glucose (35 F, 25 P, 35 G) (groups B and C). Group C received 8 AG (7.5 mg ip at 8 AM and 8 PM in dilute NaOH, pH = 10) during the feeding of the 35% fat diet. All three groups were then starved 2 days and refed for 3 days a diet containing 65% glucose, 25% casein, and 5% corn oil (5 F, 25 P, 65 G, or the inducer diet).

Groups D, E, and F were starved 2 days and refed for 2 days a diet containing 90% glucose and 5% corn oil but no protein (5 F, 90 G). This was followed by feeding for 2 days either a diet containing 35% corn oil and 60% glucose but no protein (35 F, 60 G) (group D), or the high fat, adequate protein, and carbohydrate diet (groups E and F) (described above for groups B and C). Group F received 8 AG as described above for group C. All three groups were then starved 2 days and refed for 3 days the 65% glucose diet (described above).

Rats were killed by decapitation, carcasses were exsanguinated, and the liver was removed, blotted, chilled, and weighed. The preparation of a 10% liver homogenate and the assay of G6PD and ME activities have been previously described (13).

Results and Discussion. The body weights are presented in Table I. All rats weighed between 105 and 120 g when the treatments were begun.

The food intakes of the rats during the various experimental periods are presented in Table II. The 90% protein (casein) diet was well accepted by all the rats. Those rats refed the 90% glucose diet ate somewhat less food. The rats in group A had approximately the same intakes when fed the 35% fat, 60% protein diet as did group B when fed the 35% fat diet containing both protein and carbohydrate. When the rats in group C were administered 8 AG in conjunction with feeding the high fat diet, their intakes were slightly but not significantly lower than those of Group B when fed the high fat diet alone. Group D, when fed the high fat, protein-free diet, and group F, when given 8 AG injections in conjunction with feeding the high fat diet, had significantly lower intakes than those of the other four groups fed the high fat diet during the same period. The rats in group E showed a marked increase in food intake from previous levels when fed the high fat diet containing both protein and carbohydrate. The food intakes of all six groups were similar when they were refed the 65% glucose diet, although the intakes in group D were slightly but not significantly higher than those in the other five groups.

The enzyme activities for each group of rats are presented in Table III. If the hypothesis were correct that dietary fat induces specific *de novo* protein synthesis, either synthesis of the aporepressor(s) or of an enzyme system necessary for the formation of the corepressor(s), or both, then the following results would have been expected from groups B, C, E, and F (the groups that were fed adequate protein and carbohydrate during the feeding of the high fat diet). If it is assumed that a high fat diet is necessary to induce specific *de novo* protein synthesis, then none of the groups would have synthesized the necessary proteins during the first 2 days of refeeding, when low levels of fat were fed. The groups to which 8 AG was administered during the feeding of the high fat diet (groups C and F) would also have been unable to synthesize the necessary proteins during the third and fourth days of refeeding. In groups B and E, however, which were not given 8 AG, the *de novo* synthesis of the necessary

[2] All dietary ingredients were purchased from Nutritional Biochemicals Corp., Cleveland, OH. Mention of a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture.

[3] It should be noted that the high fat, "carbohydrate-free" diet contained 3–4% carbohydrate as glycerol derived from the 35% triglycerides fed.

TABLE III. Effect of Dietary Treatments[a] and 8-Azaguanine Administration on Rat Liver Enzyme Activities[b] and Relative Liver Size.

Group	G6PD (units/100 g body wt)	ME (units/100 g body wt)	Relative liver size (g liver/100 g body wt)[c]
A	137 ± 12.2[d]	88.3 ± 11.1	5.76 ± 0.20
B	144 ± 12.2	82.2 ± 7.33	6.12 ± 0.20
C	158 ± 14.9	93.5 ± 9.07	6.25 ± 0.19
D	193 ± 9.54[e]	146.0 ± 11.0[e]	6.61 ± 0.22
E	156 ± 9.83	108.0 ± 6.09	6.03 ± 0.07
F	204 ± 10.0[e]	167.0 ± 8.56[e]	6.36 ± 2.17

[a] See Table I for a description of the specific dietary treatment for each group.

[b] One unit of enzyme activity is defined as that amount of enzyme which produces 1 μmole of measured product under the conditions of the assay. Enzyme activities can also be recalculated on a units per gram liver basis by dividing the enzyme activity as tabulated by the relative liver size.

[c] Relative liver size = (liver wt × 100)/(body wt).

[d] Standard error of the mean.

[e] These values differ significantly from the corresponding values in groups A, B, C, and E ($p < 0.05$).

proteins could have been induced by the feeding of the high fat diet. Hence, rerepression would have been established in groups B and E but not in groups C and F. Groups C and F would therefore have shown increased enzyme inducibility after the second starve–refeed episode, while groups B and E would not.

As shown in Table III, groups D and F had significantly higher activities for both G6PD and ME than those of the other four groups ($p < 0.05$). These results were interpreted to mean that groups D and F showed increased enzyme inducibility, while groups A, B, C, and E were able to establish rerepression. This evidence tends to disprove the first hypothesis, since group C apparently established rerepression despite the fact that 8 AG was administered during the feeding of the high fat diet.

If the alternative hypothesis were correct, that dietary fat provides either the corepressor(s) or a precursor of the corepressor(s), then the *de novo* protein synthesis could have occurred independently of the feeding of the high fat diet. The results from group C, which was given 8 AG in conjunction with the high fat diet and yet was able to establish rerepression, lend support to this hypothesis. In group C, the aporepressor(s), or an enzyme system necessary for the formation of the corepressor(s), or both, could have been synthesized during the first 2 days of feeding, when no 8 AG was administered. When the high fat diet was fed, rerepression could have been completed in one of two ways: if the preformed corepressor(s) were provided, then the complete repressor system would have been formed directly; alternatively, if a precursor for the corepressor(s) were provided, then the actual corepressor(s) could have been synthesized via the enzyme system which was made during the 2 days preceding the feeding of the high fat diet, and the complete repressor system could then have been formed.

The possibility that full rerepression was established during the feeding of the high protein diet was excluded by previous work, where it was shown that interposing the high protein diet between starvation and feeding the inducer diet did not prevent the enzyme overshoot (14, 15). Other studies in this laboratory have shown that if 8 AG is given throughout the first refeeding, regardless of the dietary manipulation, transcriptional rerepression is blocked (B. Szepesi, unpublished data). These observations, along with the results from group C, suggest that at least two processes are operating to establish rerepression: first, the *de novo* synthesis of one or more proteins; and second, the synthesis of

the complete repressor system when either the corepressor(s) or its precursor(s) is provided by dietary fat. The data from this study do not indicate whether the protein(s) which must be synthesized *de novo* is the aporepressor (which may have been destroyed during starvation), or an enzyme system necessary for the production of the corepressor(s) from dietary precursors, or both.

If it is postulated that synthesis of the aporepressor(s) or the enzyme system must occur for rerepression to be established, then two corollary hypotheses can be tested: first, that dietary carbohydrate is required for synthesis of these proteins; second, that dietary protein is required. If dietary carbohydrate were required, then those groups which were fed essentially no carbohydrate during the first refeeding (group A), or were fed carbohydrate only in conjunction with 8 AG injections, thus preventing *de novo* protein synthesis at that time (group C) would have been unable to synthesize the necessary proteins and hence would not have been able to establish rerepression. However, as discussed previously, groups A and C were both apparently able to establish rerepression. It, therefore, appears that dietary carbohydrate is not necessary for the *de novo* synthesis of the aporepressor(s), or the enzyme system necessary for the formation of the corepressor(s), or both.

If dietary protein were required for synthesis of the aporepressor(s) or the enzyme system, then the following results would have been expected. Those groups which were fed no protein during the first refeeding (group D), or were fed protein only in conjunction with 8 AG injections (group F), would have been unable to synthesize the necessary proteins and hence would not have been able to establish rerepression. Groups D and F would therefore have shown increased enzyme inducibility after the second refeeding.

Since groups D and F did show increased inducibility of G6PD and ME, it may be concluded that dietary protein is required for synthesis of the aporepressor(s), or an enzyme system necessary for the formation of the corepressor(s), or both. The interpretation of these results is made more difficult by the fact that in group D consumption of the high fat, protein-free diet, was relatively low. It would appear, however, from the comparison of groups E and F that the aporepressor(s) or the enzyme system capable of making the corepressor(s) are not made during the feeding of the high carbohydrate, protein-free diet, but that the system retains its ability to make these proteins, as evidenced by the results in group E. The dependence of synthesis of the aporepressor(s) or the enzymes responsible for corepressor synthesis on dietary protein is further illustrated by a comparison of groups C and F. The only difference in treatment between these two groups was that group C was fed a high protein, carbohydrate-free diet during the first 2 days of refeeding, while group F was fed a high carbohydrate, protein-free diet. Group C was able to synthesize the necessary proteins during the first 2 days of refeeding, but group F was not.

The evidence from the study, therefore, suggests that the role of a high fat diet in the rerepression of the genes which code for G6PD and ME is to provide either the preformed corepressor(s) or precursors of the corepressor(s). The data also suggest that neither the corepressor(s) nor their precursors are normally present in rat liver, despite the large accumulation of fat which occurs when an inducer diet is fed after starvation; or, that if the corespressor(s) or their precursor(s) are present, they are unavailable for the purposes of rerepression. Such a situation could arise if these fat molecules were sequestered in metabolically inert form, or if they were stored in compartments which were open to extracellular, but not intracellular, transport. It further appears that dietary protein but not carbohydrate is necessary for the synthesis of the aporepressor(s) or an enzyme system necessary for the formation of the corepressor(s), or both.

Summary. The results of the experiments described herein suggest that the role of a high fat diet in the rerepression of the genes which code for G6PD and ME is to provide either the corepressor(s) or a precursor of the corepressor(s). It appears that this substance is not normally present in rat liver, despite the large accumulation of fat which occurs after starvation and refeeding an in-

ducer diet. Synthesis of the aporepressor(s) or an enzyme system necessary for the formation of the corepressor(s), or both, requires dietary protein but not carbohydrate.

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