Persistence of Increased Inducibility of NADP-linked Dehydrogenases in Rat Liver (35226)

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(Introduced by D. L. Trout)

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It has been reported that in starved-refed rats the overshoot of *ad libitum*-fed levels of liver glucose-6-phosphate dehydrogenase activity persists for a number of days (1). In previous studies it was shown that treatment of rats with 8-azaguanine prevents the overshoot in glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME), but not the return to *ad libitum*-fed values (2, 3) indicating a requirement for *de novo* RNA synthesis for the overshoot. Preliminary results have indicated that the overshoot is strictly dependent on dietary carbohydrate and that if starved rats are refed a high-protein, low-fat, carbohydrate-free diet the overshoot in G6PD and ME activities is prevented. Another set of preliminary data showed that a second cycle of starvation-refeeding leads to an even greater overshoot than was observed after one cycle of starvation-refeeding (3).

It appears, therefore, that liver cells may acquire a type of cellular memory[1] of past dietary events. In this particular case (starvation-refeeding) we asked the following questions. (i) Does the liver retain the ability to develop the enzyme overshoot even if a dietary regimen which prevents the overshoot is interposed between starvation and refeeding the high-carbohydrate diet? (ii) Does the liver retain the ability to produce a larger overshoot following a second cycle of starvation-refeeding as the length of refeeding is increased, following the first starvation episode? The rationale to investigate these possibilities is as follows. If the ability to produce the overshoot following a starvation episode is retained, the overshoot should develop as a response to the high-carbohydrate diet even after interposing the carbohydrate-free diet which does not lead to an enzyme overshoot in G6PD and ME. Furthermore, if the ability to produce a larger overshoot following the second starve-refeed cycle is retained, the larger overshoot should develop even if refeeding after the first starvation episode is prolonged. To investigate these possibilities the following experiments were undertaken.

Procedure. Specific pathogen-free, male Wistar rats were purchased from Manor Research. The rats weighed 125–149 g before shipment and were provided during shipment with a food packet containing 30% ground lab chow and 70% water. These precautions were taken to prevent starvation during shipment. Following receipt the rats were equilibrated to laboratory conditions for 3 days. Three sets of experiments were performed. In series A, rats were starved for 2 days and were then refed a diet containing 65% glucose, 25% casein, 5% corn oil, 4% mineral mix, and 1% vitamins. Rats were then killed in the early morning at 0, 1, 2, 3, 4, and 7 days after refeeding. In series B, rats were starved for 2 days and were then refed in two stages: during stage 1, rats were fed a diet containing 90% casein and corn oil, salt, and vitamins for 1, 2, 4, or 7 days; this was then followed by feeding the rats the 65% glucose diet (described above) for 2 days. Rats in series C were starved 2 days, refed the 65% glucose diet for 2, 3, 4, or 7 days, starved again for 2 days and finally refed the 65% glucose diet for 3 days. Rats were anesthetized with 90 mg of sodium amytal/kg of

[1] The term cellular memory is used here to describe a condition during which the response to a dietary stimulus is influenced by the previous response to another dietary stimulus in the past.

TABLE I. Metabolic Memory in Rat Liver; Persistence of Increased Synthetic Capability.

Treatment	(units[b]/100 g of body wt) G6PD	ME	RLS[a]
	Expt. A		
Ad libitum fed, 65% glucose diet	22.4 ± 2.0[c]	14.4 ± 1.4	5.71 ± 0.2
Starved 2 days	6.0 ± 0.3	5.2 ± 0.5	3.22 ± 0.1
Refed 65% glucose diet for (days):			
2	57.6 ± 2.5	30.0 ± 1.4	5.84 ± 0.1
3	73.1 ± 1.3	39.2 ± 2.0	6.01 ± 0.1
4	63.7 ± 2.7	37.5 ± 3.0	5.83 ± 0.1
7	38.5 ± 5.1	17.4 ± 2.8	4.73 ± 0.1
	Expt. B[d]		
X			
1	70.2 ± 1.6	29.1 ± 0.5	5.36 ± 0.1
2	56.9 ± 2.9	31.5 ± 1.0	5.48 ± 0.1
4	61.9 ± 5.9	38.0 ± 2.9	5.25 ± 0.1
7	60.6 ± 3.0	30.1 ± 2.7	5.49 ± 0.1
	Expt. C[e]		
Y			
2	102 ± 3.9	53.0 ± 2.7	5.83 ± 0.1
3	118 ± 22	53.1 ± 11	5.65 ± 0.3
4	104 ± 13	51.6 ± 5.7	5.30 ± 0.1
7	117 ± 7.3	54.2 ± 2.1	5.28 ± 0.2

[a] RLS = relative liver size = (liver wt × 100)/(body wt).

[b] One unit of enzyme activity = amount of enzyme which can produce 1 μmole of NADPH/min under the conditions of the assay.

[c] SE of mean; numbers represent the average of 4 to 8 animals.

[d] Rats were starved for 2 days, refed the 90% casein diet for *X* days then were fed the 65% glucose diet for 2 days.

[e] Rats were starved 2 days, refed the 65% glucose diet for *Y* days then were starved for 2 days and refed the 65% glucose diet for 3 days.

body weight given intraperitoneally, and then were killed either by heart puncture or by decapitation; 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 (4).

Results and Discussion. The results are summarized in Table I. The series A experiments show that the enzyme overshoot is maximum 3 days after refeeding and begins to decline thereafter. No overshoot was observed with either enzyme when the 90% casein diet was refed. In this case G6PD did not rise above 25 units/100 g of body weight even after feeding the 90% protein diet for 4 days (data not shown). When the 90% casein diet was interposed between starvation and refeeding the high-carbohydrate regimen (Expt. B), the overshoot was still observed even after interposing the high-protein diet for as long as 7 days. Although the high-carbohydrate diet does cause an increase in these enzymes when the antecedent diet is the high-protein diet and the rats were not previously starved, the levels of G6PD and ME in the high-carbohydrate-fed rats under these circumstances do not exceed one half of the overshoot level (data not shown). This indicates that starvation causes a number of events which lead to the overshoot during refeeding and that the cellular memory of these events is not obliterated during dietary conditions which prevent the overshoot. It appears, therefore, that the rat retains a cellular memory of having been starved. The

liver, apparently, also retains a cellular memory that it has been refed after starvation and this is illustrated by the results of Expt. C; the increased overshoot in each group of animals persisted as the duration of refeeding between the two starvation episodes was increased to 7 days. The significance of the last value is that by 7 days of refeeding after the first starvation episode, both G6PD and ME are nearly back to *ad libitum*-fed levels (Expt. A). The increased overshoot after the second starve-refeed episode, therefore, cannot be attributed to a simple leftover of increased enzyme amount from the first refeeding to the second, but it is due to an increased ability to produce the overshoot. Based on currently available data (2) the increased overshoot is probably due to the persistence of newly formed RNA during the first refeeding episode and the addition of newly formed RNA during the second refeeding episode.

The existence of a cellular memory of increased capacity to reach higher levels of G6PD and ME could explain the occasional rat in which one known starvation-refeeding episode causes a seemingly greater response in liver G6PD and ME. In the case of man, where the liver is believed to be the major site of fatty acid production, increased inducibility of G6PD and ME due to periods of meal-skipping or severe caloric deficiency could lead to increased fat synthesizing capability (so far as increased reducing power is concerned) and possibly obesity.

Summary. Liver cells of rats starved and then refed a high-protein diet retain the cellular memory of starvation and respond to a high-carbohydrate diet by developing the enzyme overshoot. A second cycle of starvation causes an even greater overshoot and the increased capability after one starve-refeed cycle is retained during and after a second cycle. The best explanation of the data is that during refeeding the high-carbohydrate diet after starvation new RNA is formed which persists during subsequent dietary manipulations.

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