Effect of Administering 8-Azaguanine at Various Times on Liver Enzyme Activities of Starved-Refed Rats[1] (35126)

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In a previous communication it was noted that in rats starved for 2 days and then refed a diet containing 65% glucose and 25% casein, 8-azaguanine prevented the "overshoot" of normal, but not the recovery of normal or near normal activity of a number of enzymes (1). Since 8-azaguanine appears to inhibit protein synthesis by being incorporated into RNA, which may be nonfunctional (2, 3) and, since the effect of 8-azaguanine in the starved-refed response was not due to the slight reduction in food intake (1), it was suggested that the "overshoot" requires additional RNA synthesis, whereas this is not required for the recovery of normal enzyme activity (1). Implicit in these assumptions is that a 2-day period of starvation does not substantially decrease the RNA components necessary for the synthesis of these enzymes.

With respect to the mechanism of RNA synthesis apparently necessary for the overshoot response, a number of possibilities may be suggested: The first possibility in that all, or nearly all of the RNA is synthesized within a short period after refeeding. If such is the case then 8-azaguanine, injected after the bulk of the additional RNA is made, would not inhibit the overshoot. The second possibility is that the additional RNA is made during the entire refeeding period. In this case the inhibitory effect of 8-azaguanine should be progressivly smaller as its administration is delayed following refeeding. A third possibility is that the synthesis of the additional RNA does not begin until after a considerable delay and is then accomplished within either a relatively short time or continues throughout the rest of the overshoot phenomenon. This last proposed mechanism predicts that the effect of 8-azaguanine would be to inhibit the overshoot if the injection of the antibiotic was started before the onset of additional RNA synthesis, to inhibit the overshoot only partially if the antibiotic was first injected during the period of RNA synthesis and to have no effect if the administration of the antibiotic was delayed until the synthesis of additional RNA was completed. All three possible mechanisms and the expected effects of 8-azaguanine are based on the assumption that once the additional RNA is made it is relatively stable during the experiment. If, however, a fraction of the existing RNA is continuously being broken down then the inhibitory effect of 8-azaguanine will be somewhat larger than expected. The possibility of continuous RNA breakdown can be indirectly tested by treating *ad libitum*-fed animals with the antibiotic. The following experiments were undertaken in an attempt to distinguish between the three possible mechanisms of RNA synthesis required for the "overshoot" of enzyme activity in a starved-refed regimen.

Experimental Methods. Male Sprague-Dawley rats weighing 150–200 g were used throughout the experiments. The diet consisted of 65% glucose, 25% casein, 5% Mazola corn oil, 4% P.H. Salt (4) and 1% vitamins (5). The antibiotic 8-azaguanine was dissolved in dilute alkali and its pH was adjusted to 10. Injections were given intraperitoneally, 7.5 mg of 8-azaguanine in 0.5 ml/dose. All animals received the 65% glucose diet for at least 4 days before subjected to further treatment.

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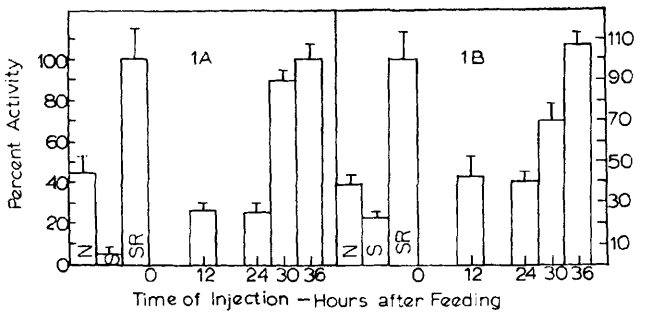

FIG. 1. Effect of 8-azaguanine on the starved-refed response of two rat liver enzymes: (A) glucose 6-phosphate dehydrogenase; (B) malic enzyme; N = activity in *ad libitum*-fed animals; S = activity in starved animals and SR = activity in starved refed, nontreated animals. The activity in starved-refed (nontreated) rats was defined as 100% and other activities were expressed on this basis. Vertical lines represent one standard error of the mean. The values for the normal control rats were 2.58 ± 4.5 units/100 g of body wt/min for glucose-6-phosphate dehydrogenase and 13.6 ± 2.4 units/100 g of body wt/min for malic enzyme.

Ad libitum-fed rats (not subjected to starvation) were given two doses of 8-azaguanine daily, one dose was administered at 9 a.m., the second dose at 9 p.m. Control animals received no injections. Groups of rats were killed at 2 and 4 days after the first dose of 8-azaguanine was given.

Other rats were starved for 2 days and were then refed and killed at 2 or 4 days after refeeding was initiated. The rats refed for 2 days were injected with the first dose of antibiotic at 12, 24, 30, and 36 hr after refeeding was begun. Control rats for these groups received no injection. Rats which were first injected 30 hr after refeeding received a second dose at 36 hr after refeeding, those receiving their first dose at 36 hr after refeeding received no further treatment. Once treatment with 8-azaguanine was initiated it was repeated at 12-hr intervals until the animals were killed. Animals which were killed 4 days after refeeding were treated in a similar manner, except that treatment with the antibiotic was initiated at 24, 30, and 48 hr after refeeding.

Rats were killed between 9 and 10 a.m. by a sharp blow to the head and were decapitated. Carcasses were exanguinated for 30 sec, the liver was quickly removed, rinsed, blotted, weighed, and chilled over ice. The preparation of liver homogenate (1) and the assay procedures for glucose-6-phosphate dehydrogenase (6) and malic enzyme (7) have been previously described.

Enzyme activity is expressed as units per 100 g of body weight. One unit of enzyme is defined as that amount of enzyme which can produce 1 μmole of measured product/min under the conditions of the assay.

Results and Discussion. In the first series of experiments we sought to determine the effects of injecting 8-azaguanine at various times after refeeding was initiated. The results of these experiments are summarized in Fig. 1A and B. When 8-azaguanine was injected 12 or 24 hr after refeeding began, it completely prevented the overshoot. The results, therefore, argue against the probability that the *de novo* RNA synthesis required for the "overshoot" occurs shortly after refeeding. The fact that if the injection of the antibiotic was delayed 30 hr following refeeding the inhibition of the "overshoot" was only partial seems to rule out the probability that the increased *de novo* RNA synthesis occurs within a very short time and suggests rather that this process is prolonged and begins about 24 hr after refeeding. The pattern of response in glucose-6-phosphate dehydrogenase activity, however, suggests that the synthesis of RNA specific for the production of this enzyme may occur within a relatively shorter space of time than that for malic enzyme.

These hypotheses can also be tested by studying the effects of 8-azaguanine on the overshoot in cases in which it is still observable after 4 days of refeeding; glucose-

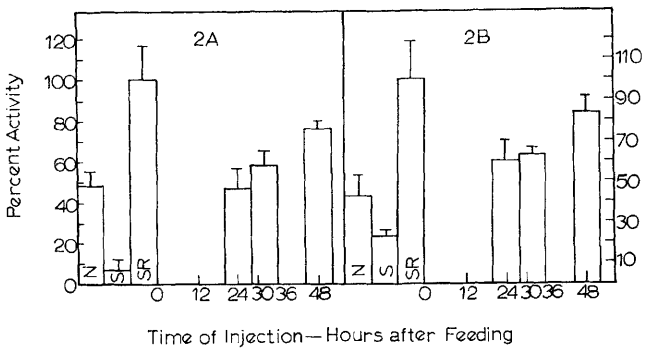

FIG. 2. Effect of 8-azaguanine on the 4-day starved-refed response of two rat liver enzymes: (A) glucose-6-phosphate dehydrogenase; (B) malic enzyme; N = activity in *ad libitum*-fed animals; S = activity in starved animals; SR = activity in starved-refed, nontreated animals. The activity in starved-refed, nontreated rats was defined as 100% and other activities were expressed on this basis. Vertical lines represent one standard error of the mean. See legend to Fig. 1 for control enzyme activities.

6-phosphate dehydrogenase and malic enzyme fall into this category. The rationale for these experiments is as follows. If the newly made RNA necessary for the overshoot is made between 24 and 48 hr after refeeding, then if 8-azaguanine were administered 24 hours or less after refeeding the overshoot should not occur; whereas if the antibiotic were given 48 hr after refeeding, the overshoot should not be inhibited. These experiments were then performed and the results are depicted in Fig. 2A and B. It is evident that injection of 8-azaguanine 24 or 30 hr after refeeding completely abolished the overshoot, whereas when the antibiotic was injected 48 hr after refeeding the overshoot was only partially inhibited. This partial inhibition could have been caused either by inhibition of some RNA synthesis necessary for the overshoot and still not completed at 48 hr after refeeding, although the RNA synthesis necessary for the glucose-6-phosphate dehydrogenase overshoot may be completed before 48 hr after refeeding as shown in Fig. 1, or by the inhibition of synthesis due to normal turnover of RNA. To obtain a preliminary indication as to which of these possibilities may be more likely, we treated *ad libitum*-fed rats with 8-azaguanine. After 2 days of treatment with 8-azaguanine, glucose-6-phosphate dehydrogenase activity was decreased by 16% and activity fell to 76% of normal after 4 days of treatment. It may be significant that in the starved-refed rat the injection of 8-azaguanine 2 days after refeeding and 2 days before termination of the experiment also causes about 15–25% reduction in glucose-6-phosphate dehydrogenase activity (Fig. 2). This may be an indication that a fraction of the RNA specific for glucose-6-phosphate dehydrogenase is constantly turning over. The overshoot of malic enzyme was also decreased by 8-azaguanine (Fig. 2), but no decrease was noted in *ad libitum*-fed rats treated with 8-azaguanine. Whichever may be the case with respect to the possible turnover of RNA specific for glucose-6-phosphate dehydrogenase, it appears that the bulk of the RNA necessary for the overshoot is synthesized between 24 to 48 hr after refeeding. Although such a delayed response in RNA production is unlike the responses noted in hormone-induced changes (8–12), there is evidence of delayed increase in cellular RNA following refeeding of previously starved rats (13).

It is very interesting that the overshoot in enzyme activity on the second day appears almost simultaneously with the production of RNA necessary for the overshoot. Since, 2 days after refeeding, the level of glucose-6-phosphate dehydrogenase is approximately twice that of normal, and since enzyme activity seems to double between the first and second day of refeeding (1), one should account for the relatively rapid increase in enzyme activity in terms of the kinetics of enzyme synthesis and degradation. It may

either be assumed that the turnover of glucose-6-phosphate dehydrogenase is very fast in the starved-refed experiment or that once the enzyme is synthesized it remains relatively stable for a number of days.

Summary. In rats starved for 2 days and then refed a 65% glucose diet for 2 days the activities of liver glucose-6-phosphate dehydrogenase and malic enzyme reached approximately twice normal levels. This overshoot of normal activity was totally prevented if 8-azaguanine was administered 24 hr or sooner after refeeding. If the antibiotic was administered 30 hr after refeeding, the overshoot was still inhibited, although to a lesser extent, while if 8-azaguanine was administered 36 hr after refeeding, no noticeable reduction in the overshoot was noted. The results indicate that the overshoot in the starved-refed experiment requires *de novo* RNA synthesis and that this *de novo* RNA synthesis seems to occur between 24 and 36 hr after refeeding. To further check this hypothesis, the effect of 8-azaguanine was studied on the 4-day overshoot in glucose-6-phosphate dehydrogenase and malic enzyme activities. It was found that the overshoot was totally prevented by injecting the antibiotic 30 hr or sooner after refeeding. If the antibiotic was injected 48 hr after refeeding, the activities of glucose-6-phosphate dehydrogenase and malic enzyme reached about 75% of the levels in untreated rats refed for 4 days. Since, the administration of 8-azaguanine for 2 days to *ad libitum*-fed rats caused about an equal reduction in glucose-6-phosphate dehydrogenase activity, but not in malic enzyme activity, the results could be interpreted in one of two ways: (i) that the *de novo* RNA synthesis necessary for the overshoot is not entirely completed by 48 hr after refeeding, or (ii) that the above mentioned RNA synthesis is completed within 48 hr after refeeding, but that 8-azaguanine reduces the level of functional RNA by interfering with the normal turnover of RNA.

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