SELECTIVE INDUCTION AND PREVENTION OF ENZYME SYNTHESIS IN MAMMALIAN LIVER

George Weber, Gouri Banerjee, and Seymour B. Bronstein*

Departments of Pharmacology and Microbiology,
Indiana University School of Medicine, Indianapolis, Indiana

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Study of enzyme synthesis in microorganisms as well as of inducers and inhibitors affecting these processes has brought important information regarding the role enzymes play in homeostatic mechanisms. In recent reviews on the role of liver enzymes in mammalian adaptation mechanisms it was emphasized that hormonal factors appear to exert regulation in these systems (Knox et al., 1956) and pointed out that certain hepatic carbohydrate enzymes may be particularly suitable for the demonstration of hormonal regulation (Ashmore and Weber, 1959; Weber, 1959).

The present preliminary report describes the utilization of techniques of enzyme depletion and restoration and of hormonal induction in the study of enzyme regulation and synthetic mechanisms in mammalian systems.

Male Wistar rats were kept in separate cages with Purina Fox Chow and water available ad libitum; however, fasted animals had only water ad libitum. For fasting studies normal animals of 180 g, and for cortisone studies adrenalectomized rats of 120 g were employed. The weight of all animals at sacrifice was between 120-140 g. Adrenalectomies and postoperative treatment were carried out as described previously (Weber and Cantero, 1957 b).

Enzyme depletion was achieved by 6-day starvation; enzyme restoration was induced by refeeding. These procedures supplied a system for the study of synchronized enzyme breakdown and resynthesis processes (Weber and MacDonald,

*U. S. Public Health Service Postdoctoral Fellow.

1960). Enzyme synthesis was also induced in another series of experiments by administration of cortisone which had been shown to cause glucose-6-phosphatase (Weber et al., 1955; Ashmore et al., 1956) and fructose-1,6-diphosphatase (Mokrasch et al., 1956) increases interpretable in terms of enzyme synthesis (Weber, 1959; Kvam and Parks, 1960). Interference with enzyme synthesis was obtained by ethionine administration (Freedland and Harper, 1958; Kvam and Parks, 1960) in additional groups of experimental animals where enzyme increase was induced either by refeeding or cortisone administration.

Animals were killed by decapitation. Preparation of liver homogenate and supernatant fluid, cellularity, nitrogen determination and enzyme assay techniques were described previously (Weber and Cantero, 1957 a,b; 1959).

Results are summarized in Figures 1 and 2. Refeeding in 6-day fasted rats induced the following percentage increases in liver enzymes: glucose-6-phosphatase, 53; fructose-1,6-diphosphatase, 19; phosphoglucomutase, 79; phosphohexoseisomerase, 42; glucose-6-phosphate dehydrogenase, 566; and 6-phosphogluconate dehydrogenase, 121. The marked increase in shunt enzyme activities confirms previous data (Tepperman and Tepperman, 1958; Cohn and Joseph, 1959; Weber and MacDonald, 1960). Ethionine administration prevented enzyme increases except that of glucose-6-phosphate dehydrogenase.

Cortisone administration in adrenalectomized rats induced the following percentage increases in hepatic enzymes: glucose-6-phosphatase, 238; fructose-1,6-diphosphatase, 71; phosphoglucomutase, 5; phosphohexoseisomerase, 136; glucose-6-phosphate dehydrogenase, 33; and 6-phosphogluconate dehydrogenase, 59. The increase in dehydrogenases was not statistically significant. It is interesting that in adrenalectomized rats cortisone increased glucose-6-phosphatase to much higher levels than it did in normal animals (Weber et al., 1955). Administration of ethionine inhibited liver enzyme increases except for glucose-6-phosphatase where partial prevention occurred.

It appears that for these hepatic carbohydrate-metabolizing enzymes, increases (synthesis) can be achieved by both refeeding and cortisone administration.

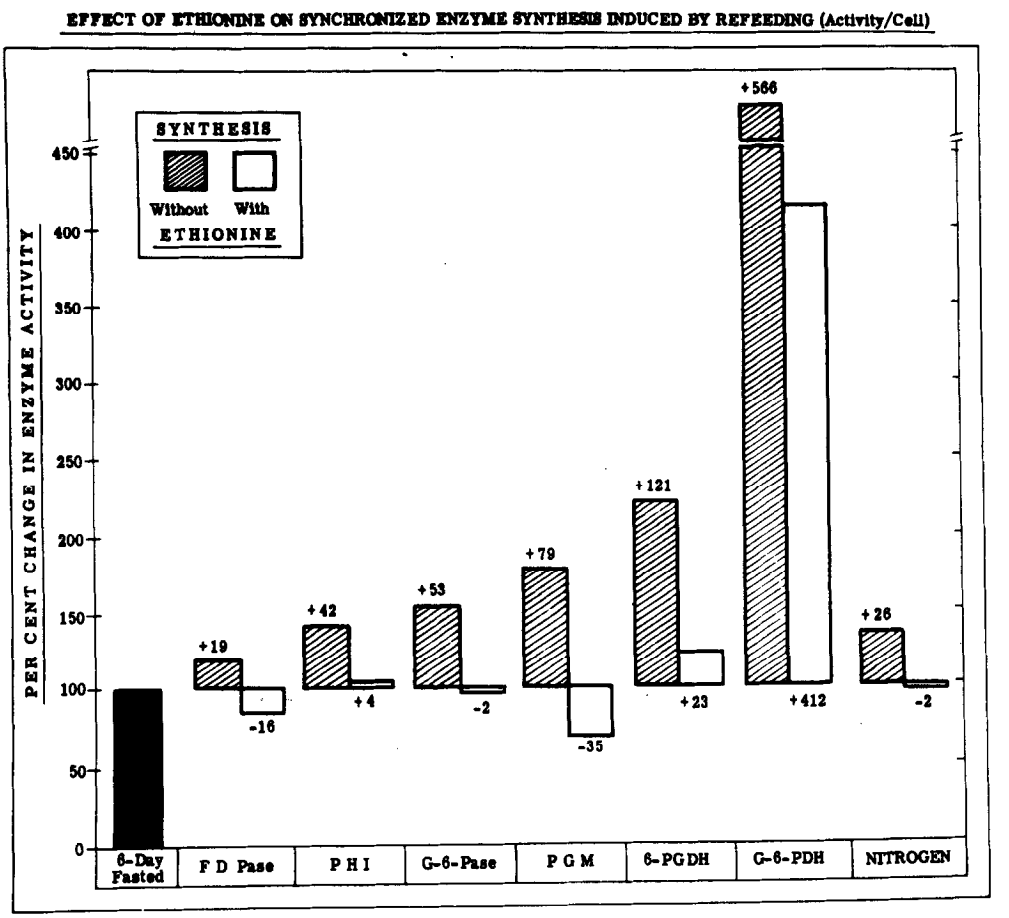

Fig. 1. Effect of ethionine on synchronized enzyme synthesis induced by refeeding. Rats were fasted for 6 days and killed after 1 day of refeeding. In the inhibitory studies 2 ethionine injections were given intraperitoneally in 100 mg. doses, one on the last day of starvation and the other on the day of refeeding. Enzymatic activities and nitrogen content were calculated per average cell and expressed in % taking the values of 6-day fasted animals as 100. The bars indicate the alterations from the 6-day fasted levels.

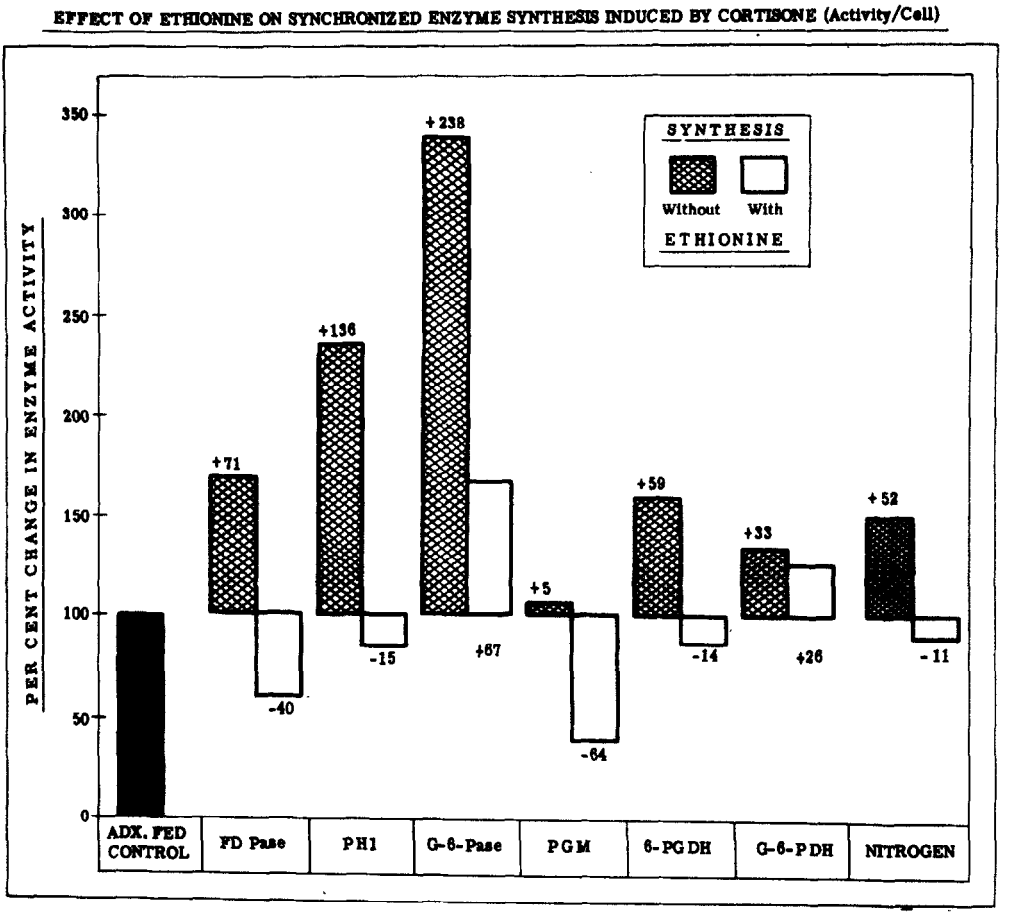

Fig. 2. Effect of ethionine on synchronized enzyme synthesis induced by cortisone. Cortisone was given intramuscularly in 25 mg. doses daily for 5 days (Weber et al., 1955). In the inhibitory studies ethionine was injected intraperitoneally in 100 mg. daily doses for 5 days, concurrently with cortisone administration. Liver enzyme activities and nitrogen content were calculated per average cell and expressed in % taking the values of control adrenalectomized rats as 100. The bars show the actual increase found in %.

However, the two procedures differ in acting as inducers for the various enzymes. Refeeding stimulated particularly the shunt enzymes which may have a role in lipid synthesis. On the other hand, cortisone administration was the most potent inducer for glucose-6-phosphatase, fructose-1,6-diphosphatase and phosphohexoseisomerase which are key enzymes in gluconeogenesis.

The nitrogen data demonstrate that enzyme activity changes do not merely reflect alterations in total cellular protein content. The Figures show that as a result of different induction processes certain of the examined hepatic enzymes may move parallel with the behavior of protein content, but some enzymes are preferentially synthesized whereas others may be preferentially broken down. This is consistent with reports on behavior of these enzymes (Weber and Cantero, 1957 c; Freedland and Harper, 1958; Weber, 1959).

The results indicate that in this mammalian system there is selectivity for both enzyme induction and inhibition of induction. In refed animals ethionine can interfere with synthesis of the examined hepatic enzymes, but is ineffective in antagonizing the increase in glucose-6-phosphate dehydrogenase. In contrast, with cortisone induction the synthetic mechanisms of glucose-6-phosphatase appear to be in part refractory to ethionine interference.

On the basis of the available data it would be premature to speculate whether the described liver enzyme increases and interference with these increases may be explained in terms of enzyme synthesis and breakdown and induction and repression as established in microorganisms. Investigation is in progress to utilize the presented experimental systems in the analysis of factors involved in influencing enzyme activity in mammalians.

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