PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 1159–1162 (1974)

# Regulation of Glucosamine Synthesis during the First Twenty-Four Hours Following Injury and Partial Hepatectomy[1] (38265)

HIDEO OKUBO[2] AND ALBERT M. CHANDLER

*Department of Biochemistry and Molecular Biology, University of Oklahoma School of Medicine and Biochemistry Section, Oklahoma Medical Research Foundation, 800 Northeast Thirteenth Street, Oklahoma City, Oklahoma 73190*

Because of the central role played by hexosamines and hexosamine derivatives in the structure of several macromolecules of biological importance including plasma glycoproteins, membrane glycoproteins, glycolipids and glycosaminoglycans, an increasing interest is being shown in the factors that regulate the metabolism of these compounds. Most of the effort has been focused on the initial and rate-limiting enzyme in the pathway, glucosamine synthetase (L-glutamine: D-fructose-6-phosphate aminotransferase, EC 2.6.1.16). Kornfeld *et al.* (1) were the first to show that this enzyme was subject to feedback inhibition by its major end product, uridine diphospho-*N*-acetylglucosamine (UDPAG). Later work has shown that this feedback inhibition is an extremely complex process involving various intracellular metabolites acting as secondary effectors (2–5).

A second mechanism for the regulation of glucosamine synthetase may be that of enzyme induction. Several investigators have observed that the activity of this enzyme is increased after various kinds of stress or partial hepatectomy (6–9) and that this increase can be blocked by the appropriate administration of actinomycin D or cycloheximide (8, 9). Concomitant measurements of changes in UDPAG pool sizes and glucosamine synthetase activity as a function of time following stress or partial hepatectomy showed that the concentration of the feedback inhibitor remained relatively constant despite marked fluctuations in enzyme activity (9). These studies were restricted to time intervals of between 1 and 8 days.

The present communication is an extension of the previous study (9) and reports on the early changes that occur in glucosamine synthetase activity and the UDPAG pool within the first 24 hr following surgery.

*Experimental procedure.* Male, albino rats, 150–250 g, were maintained under controlled conditions for at least 2 weeks prior to use on standard laboratory chow. Partial hepatectomies were performed according to the method of Higgins and Anderson (10) and injuries (laparotomies) were carried out in a similar manner but stopped short of ligation and removal of liver lobes. Following surgery, the animals were given free access to water, but food was withheld. At various intervals between 6 and 24 hr, groups of rats were sacrificed and hepatic glucosamine synthetase activities and uridine diphospho-*N*-acetylhexosamine (UDPAH) concentrations were assayed as previously described (9).

*Results.* The results of two representative experiments are shown in Figs. 1 and 2. Although the degree of the response varied from experiment to experiment, the general qualitative pattern of the response was quite reproducible over several trials.

*Response to injury.* A detectable increase

[1] Supported by Public Health Grant No. AM-11540.

[2] Postdoctoral Fellow of the Oklahoma Medical Research Foundation. Present address: First Department of Medicine, Kyushu University, Fukuoka, Japan.

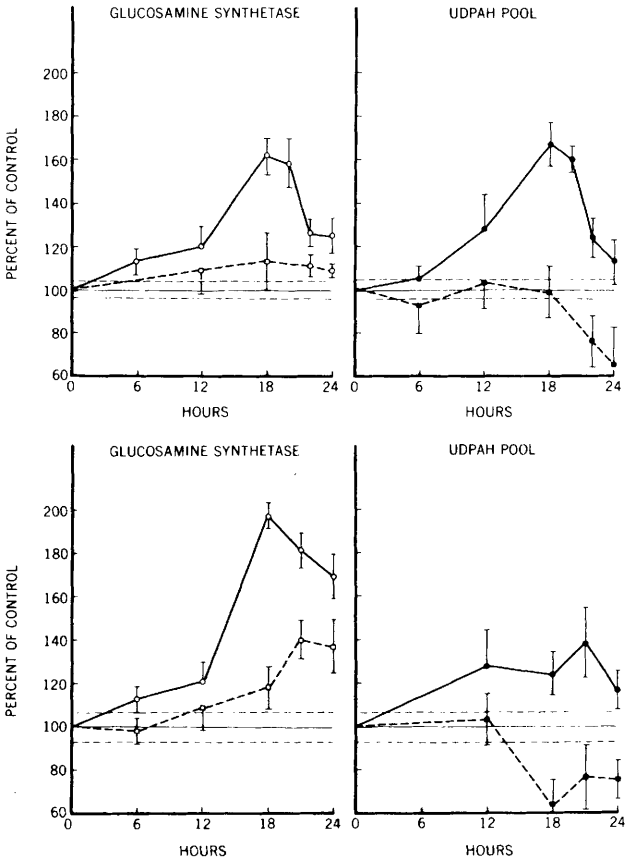


FIG. 1. Relative glucosamine synthetase activity and relative UDPAH concentration as a function of time following laparotomy and partial hepatectomy. Laparotomy ———, partial hepatectomy - - - - - -. Values are presented as means ± SEM. Each point represents the average of 8 rats. Assays for UDPAH were carried out in duplicate on the same 105,000*g* supernatants used to assay glucosamine synthetase activities. The specific activity of glucosamine synthetase in liver extracts from control rats averaged 58.0 ± 2.2 nmoles/mg protein/hr and the average UDPAH concentration was 345 ± 17 nmoles/g liver.

FIG. 2. Relative glucosamine synthetase activity and relative UDPAH concentration as a function of time following laparotomy and partial hepatectomy. Laparotomy ———, partial hepatectomy - - - - - -. Each point represents the mean of 4 rats. In this experiment the average specific activity of glucosamine synthetase in liver extracts of control rats was 52.8 ± 1.4 nmoles/mg protein/hr and the average UDPAH concentration was 630 ± 24 nmoles/g liver.

in enzyme activity was observed as early as 6 hr following laparotomy. The activity rose slowly to about 20% above control values at 12 hr and then increased much more rapidly to a peak value of 60–100% above control levels at 18 hr ($P < 0.001$). The enzyme activity then declined rapidly at variable rates to reach 24 hr values of 25–70% above control levels depending on the rate of decline of activity and the peak value attained.

Changes in the hepatic UDPAH concentrations generally paralleled the changes observed in enzyme activity. The degree of response varied from experiment to experiment but was always in the direction of the change in enzyme activity.

*Response to partial hepatectomy.* As com-

pared to injury, the response of glucosamine synthetase activity to partial hepatectomy was considerably delayed and quite variable during this time period. Although increases in activity occurred, these were usually not significantly different from control values until about one day.

The hepatic UDPAH concentrations remained at control levels until about 14–18 hr after which time a sharp decline occurred, reaching 24 hr levels of 25–35% below control values.

*Discussion.* Previous work has shown that the increase in glucosamine synthetase activity observed following injury (9) or partial hepatectomy (8) can be blocked by the administration of actinomycin D or cycloheximide and thus it is assumed that they are the result of *de novo* synthesis. Some caution should be observed in accepting this assumption, however, since the regulation of this enzyme by several intracellular metabolites is extremely complex.

The glucosamine synthetase activity rose to a peak at 18 hr following laparotomy after which a relatively sharp decline occurred. It is interesting to note that this pattern of response resembles that reported by Liu and Neuhaus (11) in their studies on the effects of injury on hepatic protein synthesis. They observed that 18 hr was the point of maximal polysomal aggregation and maximal $^{14}$C-leucine incorporation into isolated microsomal fractions. This time period also coincided with minimal activity of alkaline ribonuclease.

The sharp decline in the activity of glucosamine synthetase following the 18 hr maximum indicates that the activity induced above the basal level is quite unstable. The factors that control the *in vivo* stability of this enzyme are unknown, although several investigators have commented on its lability *in vitro,* especially after one or two stages of purification (5, 12, 13, 14). Previous studies have shown that control animals fasted for 24 hr or treated with actinomycin D for the same time period lost 15–20% of the enzyme activity (9). Bates and Handschumacher (15), on the other hand, observed that the activity of glucosamine synthetase in control animals given puromycin did not decline significantly over a 12 hr period and thus concluded that the enzyme did not turn over rapidly *in vivo.*

The response of glucosamine synthetase to partial hepatectomy was quite different from its response to injury. Although the tendency was for the enzyme activity to increase above the control level, this response was quite variable during the first 24 hr. Any increases observed appeared to be correlated with the first wave of mitosis which usually occurs around 24 hr (16, 17). Earlier work has shown that the increase in glucosamine synthetase activity following partial hepatectomy continues to rise to a maximum at around 48 hr after which it plateaus or declines slightly (9). This pattern of response correlates well with the rate of plasma glycoprotein synthesis measured under similar conditions (18).

The major fate of hepatic UDPAH is to supply hexosamines and hexosamine derivatives as components for plasma and membrane glycoproteins and membrane glycolipids. Under normal conditions the UDPAH pool remains quite constant, indicating that the removal of UDPAH from the pool is balanced by the entrance of newly synthesized UDPAH. After injury and partial hepatectomy, however, the rate of synthesis of several of the plasma glycoproteins is increased from five- to sevenfold (18). The increased demand for hexosamines should, then, cause a decline in hepatic UDPAH concentrations unless compensated for by an increase in synthesis. The intact liver apparently is able to respond to the increased demand by inducing the activity of the rate limiting enzyme, glucosamine synthetase. The pattern of this induction closely parallels that of the increase in plasma glycoprotein synthesis and, in fact, precedes it by a few hours (Okubo, unpublished results). This causes a temporary increase in the UDPAH pool which is brought back to normal levels by 24 hr.

The regenerating liver, on the other hand, is unable to induce glucosamine synthetase activity significantly during the first 18–24 hr even though plasma protein production has been stimulated to a high degree (18). This leads to a transient, but significant,

depletion of the UDPAH pool until glucosamine synthetase activity has been induced sufficiently to meet the demand for hexosamines. This may take up to 3–4 days after partial hepatectomy (9).

*Summary.* The activity of glucosamine synthetase and the concentration of UDPAH were measured concomitantly during the first 24 hr following laparotomy or partial hepatectomy. Glucosamine synthetase activity increased significantly above normal by 12 hr following laparotomy, rose rapidly to peak values of 60–100% above normal by 18 hr and then declined at variable rates thereafter. UDPAH concentrations rose and fell in parallel with the fluctuations in enzyme activity. Glucosamine synthetase activity showed little or no response after partial hepatectomy until about 20–24 hr when variable rates of increase occurred. The UDPAH concentrations remained constant until 14–18 hr after which a significant decrease occurred.

1. Kornfeld, S., Kornfeld, R., Neufeld, E. F., and O'Brien, P. J., Proc. Nat. Acad. Sci. USA **52**, 371 (1964).

2. Winterburn, P. J., and Phelps, C. F., Nature (London) **228**, 1311 (1970).

3. Winterburn, P. J., and Phelps, C. F., Biochem. J. **121**, 711 (1971).

4. Winterburn, P. J., and Phelps, C. F., Biochem. J. **121**, 721 (1971).

5. Miyagi, T., and Tsuiki, S., Biochim. Biophys. Acta **250**, 51 (1971).

6. Shönhöfer, P., and Anspach, K. F., Arch. Int. Pharmacodyn. Ther. **166**, 382 (1967).

7. Nishii, Y., Shikoku Igaku Zasshi **24**, 266 (1968).

8. Akamatsu, N., and Maeda, N. R., Biochim. Biophys. Acta **244**, 311 (1971).

9. Bley, R. L., Okubo, H., and Chandler, A. M., Proc. Soc. Exp. Biol. Med. **144**, 134 (1973).

10. Higgins, G. M., and Anderson, R. M., Arch. Pathol. **12**, 186 (1931).

11. Liu, A. Y., and Neuhaus, O. W., Biochim. Biophys. Acta **166**, 195 (1968).

12. Gryder, R. M., and Pogell, B. M., J. Biol. Chem. **235**, 558 (1960).

13. Kornfeld, R., J. Biol. Chem. **242**, 3135 (1967).

14. Winterburn, P. J., and Phelps, C. F., Biochem. J. **121**, 701 (1971).

15. Bates, C. J., and Handschumacher, R. E., Adv. Enzyme Reg. **7**, 183 (1969).

16. Glinos, A. D., *in* "The Chemical Basis of Development" (McElroy and Glass, eds.), p. 813. Johns Hopkins Press, Baltimore (1958).

17. Buchler, N. L. R., N. Eng. J. Med. **277**, 686 (1967).

18. Chandler, A. M., and Snider, G. A., Proc. Soc. Exp. Biol. Med. **135**, 415 (1970).

Received Mar. 11, 1974. P.S.E.B.M., 1974, Vol. 146.