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# Glucagon and Epinephrine Responsive Adenyl Cyclase Activity of Regenerating Rat Liver* (33704)

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(Introduced by C. A. Stetson)
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The effect of the liver on the regulation of blood glucose depends in large part upon the interaction between glucagon or epinephrine with hepatic adenyl cyclase (1). The activation of membrane-associated adenyl cyclase by either hormone is followed by a rapid rise in the intracellular concentration of 3′5′-cyclic adenosine monophosphate (cyclic A). This cyclic nucleotide is known to cause the enzymic activation of glycogen phosphorylase and the concomittant inactivation of glycogen synthetase. As a result of this change in the activities of the glycogen regulatory enzymes, hepatic glycogen undergoes stepwise phosphorolysis and glucose is made available to the hepatocyte and remote tissues.

Following 70% hepatectomy of the rat, a series of profound structural and biochemical alterations are observed to occur in a large number of the residual hepatocytes (2). Throughout the process of regeneration the hepatocyte glycogen stores are significantly depleted (3). This depression of glycogen appears to result from a greatly increased utilization of glucose which reflects the cells' heightened metabolic activity. During this time some areas of the hepatocyte membranes undergo degenerative change, subsequent to which a significant quantity of new membrane is elaborated (4, 5). Although it would appear advantageous to the cells' metabolic economy that they maintain their adenyl cyclase levels, the activity of this enzyme during the regenerative process has not been examined.

The present paper describes the behavior of the hepatic adenyl cyclase system at varying intervals following 70% hepatectomy.

*Materials and Methods.* Sprague-Dawley male rats weighing approximately 200 g were used throughout the studies except where specifically noted. They were fed and watered *ad libitum*. Seventy percent hepatectomy was performed as described previously. Sham operation was identical in all manipulations save for the handling and removal of the liver (6). Following sacrifice, the same liver lobes were utilized in each experimental

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group for the preparation of the enzyme systems.

The techniques employed in the preparation of murine adenyl cyclase were performed as previously described. Whole homogenates or washed membrane particles obtained at 1000*g* were tested. The activity of the enzyme was determined by the conversion of [$^{14}$C] ATP to [$^{14}$C] cyclic A which was purified by double descending thin-layer chromatography and counted in a scintillation counter (7). The specific activities of the enzyme systems were calculated on the basis of milligrams of membrane protein [determined by the method of Lowry (8)].

Phosphodiesterase activities were measured in an analogous manner with [$^{3}$H] labeled cyclic A as substrate and using the above chromatographic technique to measure the disappearance of labeled cyclic A as a function of time.

*Experimental Results.* The epinephrine and glucagon responsive components of the adenyl cyclase system were determined at varying intervals after 70% hepatectomy or sham operation. Three experiments were performed for each point; the data obtained from 200 g rats are shown in Table I. Neither the glucagon nor epinephrine responsive adenyl cyclase activities of the regenerating livers were significantly altered when compared to sham or normal rats. Determinations performed at these same points with the livers of weanling (60 g) and older rats (350 g) revealed similar results. Determination of these enzyme activities in 200 g rats at 12 and 50 hr after 70% hepatectomy also yielded comparable results.

The concentration of methyl xanthines employed routinely in the cyclase assays were sufficient to inhibit the major portion of phosphodiesterase activity. In order to ascertain that the observed results were in no way a reflection of compensatory depression of the activity of this enzyme, phosphodiesterase was directly assayed in normal sham operated and 70% hepatectomized, 200 g rats. No significant changes in the level of this enzyme were found at 3 and 24 hr following 70% hepatectomy.

TABLE I. Adenyl Cyclase Activity in Normal and Regenerating Liver.[a]

| | Hormone | | |
|---|---|---|---|
| | None | Glucagon | Epinephrine |
| A. Whole homogenate preparation | | | |
| Normal | 5.6[b] | 22.4 | 20.8 |
| 3 hr sham | 6.2 | 25.0 | 21.2 |
| HEP[c] | 5.2 | 26.0 | 19.4 |
| 24 hr sham | 5.8 | 24.6 | 18.4 |
| HEP | 5.0 | 23.1 | 22.8 |
| Av (range) | 5.6 (5.0–6.2) | 24.2 (22.4–26.0) | 20.5 (18.4–22.8) |
| B. Washed particle preparation | | | |
| Normal | 3.5[b] | 34.5 | 12.0 |
| 3 hr sham | 3.0 | 32.4 | 15.2 |
| HEP | 3.4 | 31.8 | 14.3 |
| 24 hr sham | 4.2 | 37.0 | 13.2 |
| HEP | 4.2 | 36.3 | 13.7 |
| Av (range) | 3.7 (3.0–4.2) | 34.4 (31.8–37.0) | 13.7 (12.0–15.2) |

[a] Reaction mixtures contained, in a volume of 20 $\mu$l: 0.045 *M* $PO_4$ buffer (pH 7.5), $3.5 \times 10^{-3}$ *M* $MgSO_4$, $4 \times 10^{-2}$ *M* glycylglycine, $10^{-4}$ *M* EDTA, $6.6 \times 10^{-3}$ *M* aminophylline, $2 \times 10^{-2}$ *M* phosphoenol pyruvate (PEP). Two $\mu$g of PEP-kinase (Sigma Type II), and $5.5 \times 10^{-4}$ *M* ATP-$^{14}$C. The concentration of the whole liver homogenate was adjusted to 800 $\mu$g of protein/20 $\mu$l. The washed particles' enzyme concentration was equivalent to a membrane concentration of 800 $\mu$g (protein) of whole homogenate. Hormone concentrations were $1.8 \times 10^{-6}$ *M* glucagon or $8 \times 10^{-6}$ *M* L-epinephrine bitartrate. Mixtures were incubated for 6 min at 30°.

[b] All figures represent the average result of 2 separate experiments; 3 rat livers were pooled at each determination. The results are expressed in terms of nanomoles of cyclic A synthesized per gram of protein/10 min.

[c] HEP = 70% hepatectomy.

*Discussion.* It would seem compatible with the metabolic needs of the dividing hepatocyte that it maintain an enzymatic capacity to prevent sequestration of glucose as glycogen. Such sequestration would divert the flow of glucose, uridine, and adenosine nucleotides away from those active metabolic pathways required for the support of the regenerative process and into a metabolic cul de

sac, *i.e.*, storage in the glycogen polymer (9). The absence or diminution of cyclic A during the regenerative process would constitute a serious metabolic disadvantage for the mitotic cell since its heightened metabolic and synthetic demands would be encumbered with all of the disadvantages of a simulated glycogen storage disease (10).

The above data indicate that adenyl cyclase activity is maintained throughout the process of liver regeneration. The requirement for active synthesis of membrane structures during this period must include replacement of the enzymic content as well as the structural components (11). The newly elaborated plasma membrane structure is endowed with physiological quanta of both the glucagon and epinephrine-responsive adenyl cyclase systems which are apparently synthesized and inserted into the newly formed membrane at rates sufficient to maintain functional levels.

The existence of two hormone regulators for the hepatic adenyl cyclase system suggests that there might exist separate cyclase systems in liver each responding specifically to a single class of hormone. Recently differences in the biophysical and biochemical properties of the epinephrine and glucagon responsive components of the adenyl cyclase systems have been found (7). During the regenerative process it appears that the components of both systems are synthesized with equal efficiency.

The full availability of liver cyclase throughout the regenerative process and absence of changes in phosphodiesterase activity support the concept that the normal turnover mechanisms of the hepatocyte are not diminished during the preparation of the cell for mitotic activity and division [although decreased catabolism of these components is possible (12)]. It has been postulated that adenyl cyclase is located primarily in the plasma membrane (1). During the process of regeneration it was observed that the subsinusoidal surfaces of the hepatocyte plasma membrane are subject to considerable disruptive changes especially involving the microprojections (4). During the first 3–6 hr following hepatectomy these processes undergo extensive degeneration and actual sloughing. If adenyl cyclase were associated with this area then considerable loss of cyclase activity might reasonably be expected during this phase of regeneration. Since the cyclase apparatus remains intact throughout all phases of regeneration other possibilities must be considered.

Cyclase would be spared if associated primarily with the intermicroprojection membrane or with the interhepatocytic membranes. It is also possible that the synthesis of cyclase systems is sufficiently accelerated to compensate for the expected loss during the membrane alterations of the 3–6 hr after 70% hepatectomy.

The liver is known to be the main locus of metabolism of circulating glucagon and catecholamines (13, 14). Following 70% hepatectomy, one would expect an alteration of the circulating levels of these hormones. It is not known at present whether such alterations occur and whether these hormones are in any way associated with the process of hepatocyte regeneration.

*Summary.* The membrane-associated enzyme adenyl cyclase gives rise to 3′5′ cyclic adenosine monophosphate (cyclic A) within the hepatocyte; and the availability of intrahepatocytic glucose is determined to a considerable extent by the mobilization of glycogen via cyclic A. Following 70% hepatectomy, a significant turnover of membrane structures occurs concurrently with loss of cellular glycogen. The present study revealed that glucagon and epinephrine activated adenyl cyclase remains at a normal level throughout the regenerative process. These findings suggest that the regenerating hepatocyte is capable of producing adenyl cyclase and inserting it into newly formed membranes.

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## The Effect of Hydroxyurea on Differentiated Marrow Erythroid Precursors* (33705)

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Hydroxyurea (OHU) an inhibitor of DNA synthesis, is used in the treatment of melanoma and resistant chronic myelocytic leukemia (1, 2). Its action at the cellular level has been studied with several strains of proliferating cells (3–5). It has been amply documented that OHU interferes with DNA synthesis, presumably at the level of conversion of ribonucleotides to deoxyribonucleotides, without influencing the synthesis of RNA and protein. Philips *et al.* (6) reported acute selective cytotoxic effects in rats treated with OHU. Injury was sustained by those tissues with high rates of cellular proliferation. They further concluded that the cytotoxic effects were time related to the effective concentrations of circulating drug. The half clearance in the plasma of the rat was of the order of 1 hr and reflected both excretion and metabolism. The observations of Philips led us to consider that OHU would serve as a useful adjunct in the study of erythroid differentiation. Critical to this consideration was documentation of the selective action of OHU on marrow erythroid precursors in DNA synthesis. Measurement of erythroid cell cycle kinetics after OHU are the subject of this report.

*Materials and Methods.* Ten-week-old female $CF_1$[2] mice weighing 20–25 g were used. All injections were given intravenously via the tail vein in the following amounts: OHU, 0.9 g/kg; colchicine, 1 mg/kg; and 2.5 $\mu$Ci/g of tritiated thymidine (TDR-$^3$H) with a specific activity of 6.7 Ci/mmole. Blood samples were collected in versenate by cardiac puncture from anesthetized animals. Reticulocytes were estimated by the method of Brecher (7). Cellularity of tibial marrow contents was measured by electronic particle counting after saponization as described by Fruhman (8). Bone marrow smears prepared by a brush technic were stained with Wright's and Giemsa. The percentage of erythroid precursors was estimated from differential counts on 500–1000 nucleated cells. The mitotic index was measured in these same preparations. For the studies with TDR-$^3$H, autoradiographs were prepared with Kodak NTB[3] nuclear track emulsion and then processed as described by Ebbe and Stohlman (9). The labeling index was determined on each slide from the observation of a minimum of 250 basophilic normoblasts. For TDR-$^3$H incorporation into DNA, marrow

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