CYCLIC AMP METABOLISM AND ADENYLATE CYCLASE
CONCENTRATION IN PATIENTS WITH ADVANCED HEPATIC CIRRHOSIS

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Glucagon was tested for its effect on plasma adenosine 3',5'-cyclic monophosphate (cyclic AMP), insulin, and glucose in healthy subjects and in patients with advanced cirrhosis of the liver. In the normal subjects, intravenous infusion of glucagon caused a significant increase in plasma cyclic AMP, glucose, and insulin. In advanced cirrhotics, plasma cyclic AMP, glucose, and insulin did not increase. Adenylate cyclase concentration was measured in liver tissue from end stage cirrhotic patients and from brain-dead organ donors whose cardiovascular function was maintained in a stable state. Basal and total adenylate cyclase concentration were not different in the two groups. Adenylate cyclase from the livers of advanced cirrhotics was, however, significantly less responsive to glucagon stimulation than was that from donor livers. Hepatocytes in advanced cirrhosis have abnormal metabolic behavior characterized by abnormal adenylate cyclase-cyclic AMP response to hormonal stimulation.

Intracellular effects mediated by adenosine 3',5'-cyclic monophosphate (cyclic AMP) have been described after interaction of many hormones with their target tissues. Several studies have shown that cyclic AMP can pass from the intracellular compartment into plasma and that the rate of this passage can be altered by changes in plasma concentrations of certain hormones. Changes in rate of cyclic AMP passage might be attributable to changes in intracellular nucleotide concentration or in cell membrane permeability.

Intravenous infusion of glucagon into normal humans has been shown to increase plasma and urine concentrations of cyclic AMP. Liljenquist et al. have indicated that the liver is an important source of circulating cyclic AMP. Changes in rate of cyclic AMP passage might be attributable to changes in intracellular nucleotide concentration or in cell membrane permeability.

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Received June 29, 1976. Accepted July 1, 1978.

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This study was supported by Research Grants MRIS 8818-01 and 7227-01 from the Veterans Administration; by Grants AM-17260 and AM-07772 from the National Institutes of Health; and by Grants RR-0051 and RR-00269 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.
terol, serum total protein and protein electrophoresis, blood ammonia and serum bromsulphalein retention, and prothrombin time was carried out in all patients before glucagon infusion. Liver function tests in these cirrhotic patients are listed in table 1. All control patients had liver profiles within the normal range.

Liver biopsy was done in cirrhotics a few minutes before the infusion test whenever possible (prothrombin time up to 18 sec with control of 12 sec). In any case, all cirrhotic patients had liver biopsies confirming cirrhosis at some time before December 1978.

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In addition, adenylyl cyclase concentration was determined on liver tissue from 9 more patients with advanced cirrhosis at the time of liver transplantation. Liver profiles from these patients are shown in table 1. Cirrhotic liver adenylyl cyclase was compared with liver adenylyl cyclase concentration from 5 previously healthy brain-dead organ donors with stable cardiovascular function.

Experimental Procedures

Glucagon infusion tests were performed after an overnight fast. All patients had peripheral venous catheters placed and 0.9% saline infused. Control venous blood samples for plasma cyclic AMP, insulin, and glucose were drawn with heparinized syringes 20 and 10 min before glucagon infusion. A solution of glucagon (synthesized by Nova Industrials Laboratory, Denmark) in 0.9% saline was substituted for the saline and infused at a rate of 200 ng of glucagon per kg per min by means of a Dacson infusion pump (BV Dacson, Uden, Netherlands). Peripheral venous blood samples were obtained with heparinized syringes at 10-min intervals for 60 min. Plasma was immediately separated by centrifugation (3 to 5°C) and stored until analysis.

Liver taken from cirrhotic patients before liver transplantation and from brain-dead donors was immediately cooled in ice-cold saline and assayed.

Table 1. Liver function tests in cirrhotic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein</th>
<th>Albumin</th>
<th>γ-Globulin</th>
<th>Serum cholesterol</th>
<th>Blood ammonia</th>
<th>BSP* retention</th>
<th>SGOT</th>
<th>SGPT</th>
<th>Alkaline phosphatase</th>
<th>Total bilirubin</th>
<th>PT*</th>
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<tbody>
<tr>
<td></td>
<td>U g/100 ml</td>
<td>%</td>
<td>%</td>
<td>mg/100 ml</td>
<td>µg/100 ml</td>
<td>%</td>
<td>mU/ml</td>
<td>IU</td>
<td>mg/100 ml</td>
<td>sec</td>
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<tr>
<td>Normal</td>
<td>6.6–8.7</td>
<td>55–68</td>
<td>14–19</td>
<td>150–250</td>
<td>21–86</td>
<td>&lt;4.7</td>
<td>&lt;12</td>
<td>&lt;12</td>
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<td>&lt;1.0</td>
<td>&lt;12.0</td>
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<td>43</td>
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<td>35</td>
<td>90</td>
<td>140</td>
<td>30</td>
<td>50</td>
<td>40</td>
<td>80</td>
<td>2.50</td>
</tr>
</tbody>
</table>

* BSP, bromsulphalein.

** PT, prothrombin time.
on both Dowexcation exchange resin according to Krishna et al.42 and aluminum oxide according to White and Zenser43 as previously described.44

Statistical procedures. The mean of values obtained at 10 and 20 min before glucagon infusion was considered as the control value for plasma cyclic AMP, insulin, and glucose. All results have been expressed as mean ± SEM. Differences between values were tested for significance by Student’s t-test.

Results

Glucagon infusion tests. Table 2 shows plasma cyclic AMP concentration in healthy subjects and cirrhotic patients during infusion of 0.9% saline at 20 and 10 min before glucagon infusion. In healthy subjects, plasma cyclic AMP rose promptly and remained significantly elevated throughout the glucagon infusion test. In contrast, cyclic AMP failed to rise in advanced cirrhotics (fig. 1).

In healthy subjects, insulin concentrations rose significantly and remained elevated throughout the glucagon infusion test. In cirrhotics, only minimal insignificant increases occurred (fig. 2).

Plasma glucose levels also rose rapidly and significantly in healthy subjects. Although base-line plasma glucose levels were significantly higher in cirrhotics than in healthy subjects, plasma glucose did not increase in response to glucagon infusion in these patients (fig. 3).

Hepatic adenylate cyclase concentration. Basal, glucagon-stimulated, and sodium fluoride-stimulated activities from donor livers are reported in table 3. Glucagon-stimulated activities increased steadily with increasing glucagon concentrations until near total (sodium fluoride-stimulated) activity was achieved at glucagon concentration of $10^{-8}$ M.

Adenylate cyclase concentrations from end stage cirrhotic livers were more heterogeneous (table 3). Although basal and total concentrations did not differ significantly from those of donor livers, they seemed to fall into high activity and low activity groups. In these cirrhotic livers, glucagon-stimulated concentrations rose less rapidly and to significantly lower levels with increasing glucagon concentration. Near total stimulation was never achieved with glucagon stimulation.

Discussion

Our results indicate that intravenous glucagon infusion causes different responses in healthy subjects and in patients with advanced cirrhosis. In normal subjects, glucagon infusion caused a rapid increase in both plasma cyclic AMP levels and plasma glucose. These increases were expected because it has been demonstrated that the effect of glucagon on the liver depends on an adenylate cyclase-mediated increase in cyclic AMP.14-17 Both plasma cyclic AMP and plasma glucose then plateaued with continued infusion of glucagon. Plasma insulin had substantially increased by that time. The effects on insulin have been attributed to glucagon administration,45 hyperglycemia,46-48 and in-

**Table 2. Basal plasma cyclic AMP in healthy subjects and in patients with advanced cirrhosis**

<table>
<thead>
<tr>
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<th>Saline infusion</th>
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<tr>
<td></td>
<td>20 mina</td>
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<tr>
<td><strong>pmole/ml</strong></td>
<td>6-23</td>
</tr>
<tr>
<td>Healthy subjects (n = 9)</td>
<td>13.55 ± 1.84</td>
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<tr>
<td><strong>pmole/ml</strong></td>
<td>6-18</td>
</tr>
<tr>
<td>Cirrhotic patients (n = 11)</td>
<td>12.54 ± 1.31</td>
</tr>
</tbody>
</table>

*a Time before glucagon infusion.

**Fig. 1.** Plasma cyclic AMP concentration in 9 control subjects and in 11 cirrhotic patients at various intervals during glucagon infusion. Values are mean ± SEM and show the probability that concentrations during fasting do not differ from those at zero time (paired Student’s t-test); n.s., not significant.
The increase in insulin levels could account for the transient nature of the glucagon mediated effect because insulin has been demonstrated to decrease hepatic cyclic AMP and the glycogenolytic and gluconeogenic effect of cyclic AMP. This antagonism between insulin and glucagon represents one of the most important factors controlling liver metabolism.\textsuperscript{54-57} Wahren et al. have demonstrated, however, that the effect of glucagon on splanchnic glucose production is evanescent even in the face of absolute insulin deficiency.\textsuperscript{58}

On the other hand, plasma cyclic AMP, glucose, and insulin all failed to respond to glucagon infusion in the patients with advanced cirrhosis. This unresponsiveness might be attributed to several factors. Glucagon might bypass the liver by way of portal systemic shunts because all of these patients had demonstrated esophageal varices. This is probably not of major importance in our studies because infusion times were long and hormone dose high. Impaired responsiveness of hepatic adenylate cyclase has been demonstrated in hyperglucagonemic states from either endogenous or exogenous glucagon.\textsuperscript{31-34} We have demonstrated decreased responsiveness of hepatic adenylate cyclase to glucagon stimulation in our group of end stage cirrhotic patients submitted to liver transplantation. Although basal and
that hepatocytes from patients with advanced cirrhosis have abnormal metabolic behavior or, more specifically, surgery reported by Davies et al. 27 more like the cirrhotics who had undergone shunt spontaneous portal systemic shunting, responded much more heterogeneously than did their generally well compensated patients. The mechanism for this impairment is speculative. Srikant et al. have recently demonstrated a reduction in glucagon-binding sites in livers of rats made chronically hyperglucagonemic. 39 Rather surprisingly, these investigators were unable to show a correlation between terminal plasma glucagon concentration and the reduction in receptor concentrations. In their hyperglucagonemic models, the ability of glucagon to stimulate adenylate cyclase was not reduced proportionately by the reduction of glucagon-binding sites.

We can only speculate on why our patients with cirrhosis responded significantly differently to glucagon infusion from those reported by Strange et al. 38 who found no significant difference in cyclic AMP or glucose response between cirrhotics and controls. We employed higher doses of glucagon and achieved much higher plasma cyclic AMP levels in our normal controls. A more likely explanation is that our patients had more heterogeneous cirrhosis than did their generally well compensated patients. Our patients, with demonstrated spontaneous portal systemic shunting, responded much more like the cirrhotics who had undergone shunt surgery reported by Davies et al. 27 Our results suggest that hepatocytes from patients with advanced cirrhosis have abnormal metabolic behavior or, more specifically, an abnormal adenylate cyclase system. This unresponsiveness to known hepatotrophic factors 37 could further damage the already injured liver.

These findings suggest new prospects for the glucagon infusion test in determining the degree of parenchymal dysfunction in patients with liver disease and cirrhosis.

**REFERENCES**


<table>
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<tr>
<th>No.</th>
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<th>NaF stimulated</th>
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<td></td>
<td></td>
<td></td>
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<td>10^-9</td>
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<tr>
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<td>49.5</td>
<td>51.5</td>
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* Basal activity reflects receptor and catalytic components of adenylate cyclase concentration; glucagon-stimulated values reflect receptor activity at different molar concentrations of glucagon; sodium fluoride (NaF)-stimulated values reflect the full (catalytic) concentration of adenylate cyclase.

* Compared to donor liver by Student's t-test.

NS, not significant.
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