An elevated plasma level of homocysteine is a risk factor for the development of cardiovascular disease. The purpose of this study was to investigate the effect of glucagon on homocysteine metabolism in the rat. Male Sprague-Dawley rats were treated with 4 mg/kg/day (3 injections per day) glucagon for 2 days while control rats received vehicle injections. Glucagon treatment resulted in a 30% decrease in total plasma homocysteine and increased hepatic activities of glycine N-methyltransferase, cystathionine β-synthase, and cystathionine γ-lyase. Enzyme activities of the remethylation pathway were unaffected. The 90% elevation in activity of cystathionine β-synthase was accompanied by a 2-fold increase in its mRNA level. Hepatocytes prepared from glucagon-injected rats exported less homocysteine, when incubated with methionine, than did hepatocytes of saline-treated rats. Flux through cystathionine β-synthase was increased 5-fold in hepatocytes isolated from glucagon-treated rats as determined by production of \(^{14}\text{CO}_2\) and \(\alpha-[1-^{14}\text{C}]\text{ketobutyrate}\) from \(L-[1-^{14}\text{C}]\text{methionine}\). Methionine transport was elevated 2-fold in hepatocytes isolated from glucagon-treated rats resulting in increased hepatic methionine levels. Hepatic concentrations of S-adenosylmethionine and S-adenosylhomocysteine, allosteric activators of cystathionine β-synthase, were also increased following glucagon treatment. These results indicate that glucagon can regulate plasma homocysteine through its effects on the hepatic transsulfuration pathway.

An elevated plasma concentration of homocysteine, a sulfur-containing amino acid derived from methionine, has been recognized as an independent risk factor for the development of vascular disease (1). Methionine is adenosylated by methionine adenosyltransferase to form S-adenosylmethionine, an important biological methyl donor. Numerous methyltransferases catalyze the transfer of a methyl group from S-adenosylmethionine to a methyl acceptor, producing a methylated product and S-adenosylhomocysteine, which is subsequently hydrolyzed to form adenosine and homocysteine. Homocysteine has several possible fates: 1) remethylation to form methionine via either the cobalamin-dependent methionine synthase (using \(N^5\)-methyltetrahydrofolate as a methyl donor) or betaine:homocysteine methyltransferase (using betaine as a methyl donor); 2) catabolism by the transsulfuration pathway, ultimately forming cysteine; 3) export to the extracellular space. Two vitamin B₆-dependent enzymes comprise the transsulfuration pathway: cystathionine β-synthase, which condenses homocysteine with serine to form cystathionine, and cystathionine γ-lyase, which cleaves cystathionine to cysteine, \(\text{NH}_2\), and \(\alpha\)-ketobutyrate.

Altered flux through the remethylation or transsulfuration pathways as a result of genetic mutations or impaired vitamin status has been shown to affect plasma homocysteine levels (2, 3). In recent years it has also become apparent that certain hormones can affect homocysteine metabolism. It has been shown that hypothyroid patients tend to have elevated plasma homocysteine and that these levels are normalized when thyroid levels are restored by thyroxine treatment (4, 5). Estrogen therapy for post-menopausal women has been shown to lower plasma homocysteine (6). Altered homocysteine metabolism has been observed in diabetes mellitus. Diabetic patients (Types 1 and 2) with signs of kidney dysfunction (i.e. elevated creatinine levels) tend to have increased plasma homocysteine (7). However, in the absence of renal dysfunction, patients with Type 1 diabetes exhibit decreased plasma homocysteine relative to normal subjects (8). Studies in our laboratory have shown that plasma homocysteine is decreased in the streptozotocin-diabetic rat (a model for Type 1 diabetes mellitus). Insulin treatment increased plasma homocysteine in these diabetic animals (9). We have also shown that enzyme activities of the hepatic transsulfuration pathway are increased during uncontrolled diabetes. These changes in activity were reversed by insulin treatment (9).

The regulatory effects of glucagon on amino acid metabolism are well known. It can, for example, activate the glycine cleavage system (10), stimulate the \(\gamma^\text{m}^+\) transporter (11), and induce the five urea-cycle enzymes (12). Patients with a glucagonoma have diminished plasma amino acid levels, which are related to increased clearance by the liver (13). In light of the broad effects of glucagon on amino acid metabolism, it appears likely that homocysteine metabolism would be similarly regulated, given that it is a product of the metabolism of dietary essential methionine, and a precursor to cysteine. In addition, plasma glucagon is frequently elevated in Type 1 diabetes (14). We therefore examined the role of glucagon in regulating homocysteine metabolism in the rat. We show that glucagon treatment lowered plasma homocysteine levels and those of related amino acids. Glucagon administration elevated the activity of both
enzymes of the hepatic transsulfuration pathway. Increased hepatic cystathionine β-synthase mRNA was also observed. In addition, higher SAM and SAH concentrations provide positive allosteric modulation of cystathionine β-synthase, the committed step for the conversion of homocysteine to cysteine. The increased enzymes and positive effectors of the transsulfuration pathway could therefore provide a viable mechanism for the decrease in plasma homocysteine by stimulating flux through this pathway. Such an increased transsulfuration flux was directly demonstrated in isolated hepatocytes. Finally, we report novel data on the hepatic concentration of the relevant amino acids. In particular, we demonstrate that glucagon treatment, which decreases plasma levels of most amino acids, including methionine, actually results in an increase in hepatic levels of this amino acid. This, which would also contribute to the increased transsulfuration flux, was brought about by marked increase in methionine transport into hepatocytes.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Injectable glucagon was obtained from Eli Lilly Canada Inc. (Toronto, Canada). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Heparinized tubes (supplied from our University’s breeding colony) were centrifuged at 18,000 g at 50% output. Homogenates were prepared in 0.1 M potassium phosphate buffer and then homogenized with a Polytron (Brinkman Instruments, Ltd., Toronto, Canada) for 20 s at 50% output. All viability was at least 95% in all cases. Hepatocytes were preincubated for 20 min at 4–6 g of dry weight of cells/ml (1 ml of final volume) in Krebs-Henseleit medium equilibrated with 95% O2/5% CO2 and containing 1.25% (w/v) BSA. Following preincubation, 1 μmol methionine was added and cells were incubated for another 30 min. Cells were gassed with 95% O2/5% CO2 at the beginning of preincubation and at the addition of methionine. At the end of the incubation, the contents of the flasks were immediately centrifuged at 14,000 g for 2 min to sediment the cells. The supernatant was then frozen at –20 °C until analyzed. Homocysteine export was determined by subtracting a zero time point and is expressed as nanomoles of homocysteine per milligram of protein per minute.

**Homocysteine Export from Isolated Hepatocytes**—Hepatocytes were isolated by the method of Berry et al. (27), and viability was assessed by the trypan blue exclusion method. All viability was at least 95% in all cases. Hepatocytes were preincubated for 20 min at 4–6 g of dry weight of cells/ml (1 ml of final volume) in Krebs-Henseleit medium equilibrated with 95% O2/5% CO2 and containing 1.25% (w/v) BSA. Following preincubation, 1 μmol methionine was added and cells were incubated for another 30 min. Cells were gassed with 95% O2/5% CO2 at the beginning of preincubation and at the addition of methionine. At the end of the incubation, the contents of the flasks were immediately centrifuged at 14,000 g for 2 min to sediment the cells. The supernatant was then frozen at –20 °C until analyzed. Homocysteine export was determined by subtracting a zero time point and is expressed as nanomoles of homocysteine per milligram of protein per minute.

**In Vivo Effects of Glucagon on Homocysteine Metabolism**

**Animals**—All procedures were approved by Memorial University’s Institutional Animal Care Committee and were in accordance with guidelines of the Canadian Council on Animal Care. Male Sprague-Dawley rats (supplied from our University’s breeding colony) weighing between 225 and 275 g were used in all studies. Animals were placed in cages and had free access to water.

Glucagon treatment followed the procedure of Snodgrass et al. (12). Glucagon (4 mg/kg/day, subcutaneously) was administered in three equal daily doses (at 8:00 a.m., 4:00 p.m., and 12:00 midnight) for 2 days while control rats received the vehicle (Eli Lilly Canada Inc.). Two hours following the last injection, animals were anesthetized with 65 mg/kg intraperitoneal sodium pentobarbital. Following a midline abdominal incision, a blood sample was collected from the abdominal aorta. The liver was then rapidly removed, and a portion was freeze-clamped at –70 °C while the remaining tissue was placed in ice-cold 50 mM potassium phosphate buffer (pH 6.9). Heparinated tubes containing the blood samples were placed on ice until homogenized by centrifugation in a clinical centrifuge (15 min, 3700 × g). The plasma was then frozen (–20 °C) for later use. Fresh tissues were diluted 1.5 with phosphate buffer and then homogenized with a Polytron (Brinkman Instruments, Toronto, Canada) for 20 s at 50% output. Homogenates were centrifuged at 18,000 g at 4 °C for 30 min, and the supernatant was retained. All enzyme assays were carried out on this, 18,000 × g, post mitochondrial supernatant.

**Analytical Procedures**—Total homocysteine and cysteine concentrations were determined in plasma and liver using reverse-phase HPLC and fluorescence detection of ammonium 7-fluoro-2-oxa-1,3-diazole-4-sulfonate thiol adducts, using the method of Vester and Rasmussen (15). For amino acid determination, plasma and freeze-clamped liver were first deproteinized with 10% sulfosalicylic acid. Following centrifugation, the tubes were frozen in liquid nitrogen and then the supernatant was retained and analyzed by HPLC using a Vydac C18 column (5 μm) (28). The samples were then centrifuged for 10 min at 13,000 × g. The supernatant was retained and analyzed by HPLC using a Yvad C18 column (model 21875PS) that was equilibrated with 96% 50 mM NaH2PO4, 10 mM heptane sulfonic acid (adjusted to pH 3.2 with concentrated sulfuric acid), and 4% acetonitrile. A 15-min gradient from 4% to 20% acetonitrile was used to separate SAM and SAH. Peaks were monitored by UV detection at 258 nm and quantitated using a 3390A Hewlett Packard integrator.

**Isolation of Total RNA and Northern Blot Analysis**—Total RNA was isolated from rat livers by the acid guanidinium thiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi (30). 15 μg

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2 The abbreviations used are: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; HPLC, high performance liquid chromatography; BSA, bovine serum albumin.

2 C. Wagner, personal communication.
In Vivo Effects of Glucagon on Homocysteine Metabolism

TABLE I

<table>
<thead>
<tr>
<th>Units</th>
<th>Control</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>g</td>
<td>240 ± 15</td>
</tr>
<tr>
<td>Final weight</td>
<td>g</td>
<td>272 ± 10</td>
</tr>
<tr>
<td>Food intake</td>
<td>g/day</td>
<td>26.3 ± 4.5</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>μg/ml</td>
<td>79.5 ± 12.1</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td>ng/ml</td>
<td>8.9 ± 1.7</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>mM</td>
<td>8.0 ± 0.8</td>
</tr>
</tbody>
</table>

*a Significant difference versus control rats, p < 0.05.

Rats were administered glucagon (4 mg/kg/day) for 2 days while control rats received the vehicle. Food intake and body weight were measured daily. Blood samples were taken from the abdominal aorta and centrifuged for plasma separation. Means ± S.D. are shown for three to six measurements.

RESULTS

Alterations in Plasma and Liver Amino Acids following Glucagon Treatment—Table I gives information on body weight, food intake, plasma glucose, insulin, and glucagon concentrations in control and glucagon-treated rats.

Hepatocytes Isolated from Glucagon-treated Rats Export Less Homocysteine—The liver is the central organ in sulfur amino acid metabolism. It contains a full complement of enzymes involved in the methionine cycle and the transsulfuration pathway and is the site of 85% of all methylation reactions in the body (32). In light of this, it is reasonable to assume that alterations in hepatic homocysteine metabolism would have a profound effect on circulating levels of this atherogenic amino acid. We therefore measured homocysteine output by isolated hepatocytes. Previously, our laboratory has shown that the half-maximal rate of homocysteine export occurs at a methionine concentration of 0.44 mM and is linear for at least 60 min (28). A methionine concentration of 1 mM was chosen for all experiments. Following incubation with methionine, hepatocytes isolated from the glucagon-treated rats exported less than half as much homocysteine as the control hepatocytes (Fig. 1). Our earlier studies have shown that addition of serine (a substrate for cystathionine β-synthase), together with methionine, reduced homocysteine export (28). We therefore undertook experiments with both serine and methionine in the incubation medium. Serine incubation decreased homocysteine export from the control hepatocytes by 50%. However, serine did not reduce, any further, the homocysteine export of hepatocytes from glucagon-treated animals. There was no change in cysteine export found in any of the experimental groups (data not shown).

Glucagon Treatment Increases Hepatic Enzyme Activities Involved in Methionine Catabolism—Such a decrease in homocysteine export by hepatocytes coupled with a decreased intracellular homocysteine concentration suggests an appreciably altered metabolism. Therefore, we assayed the major enzymes involved in producing (transmethylation) and removing (transsulfuration and remethylation) homocysteine in the liver. Glucagon-treated rats exhibited increased hepatic activities of enzymes involved in the catabolism of methionine to cysteine (Table III). The activities of glycine N-methyltransferase and cystathionine γ-lyase activity were elevated by 25% whereas cystathionine β-synthase activity was increased by 90%. These changes are still evident when activities are expressed per gram of liver or per 100 g of body weight (data not shown). Methionine adenosyltransferase activity was unaffected by glucagon treatment. These data suggest the importance of the hepatic transsulfuration pathway in glucagon’s regulation of homocysteine metabolism. No changes were observed in methionine synthase, betaine:homocysteine methyltransferase, or methylenetetrahydrofolate reductase activity.

mRNA Levels in the Glucagon-treated Rats—We measured the mRNA levels for cystathionine β-synthase and cystathionine γ-lyase following glucagon treatment by comparing their abundance to that of β-actin. Glucagon treatment increased cystathionine β-synthase mRNA levels by 90% (Fig. 2). This increase is similar to the increase in enzyme activity. No change was observed in cystathionine γ-lyase mRNA levels.
In Vivo Effects of Glucagon on Homocysteine Metabolism

Plasma and hepatic amino acids were determined using a Beckman amino acid analyzer, except for total homocysteine and total cysteine, which were determined by HPLC. Intracellular amino acid concentrations were calculated as described under “Experimental Procedures.” Liver:plasma (L:P) ratio is calculated by dividing the hepatic intracellular amino acid concentration (μM) by the plasma amino acid concentration (μM). Means ± S.D. for five rats are shown.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>Methionine</td>
<td>56.0 ± 2.9</td>
<td>56.0 ± 6.0</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>9.7 ± 1.1</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>315 ± 25</td>
<td>443 ± 110</td>
</tr>
<tr>
<td>Taurine</td>
<td>177 ± 19</td>
<td>2950 ± 435</td>
</tr>
<tr>
<td>Serine</td>
<td>180 ± 17</td>
<td>311 ± 51</td>
</tr>
<tr>
<td>Glycine</td>
<td>239 ± 38</td>
<td>920 ± 141</td>
</tr>
</tbody>
</table>

*Significant difference versus control, p < 0.05.

Allosteric Regulation of Cystathionine β-Synthase—Cystathionine β-synthase can also be regulated allosterically by S-adenosylmethionine and S-adenosylhomocysteine (33). Therefore, we measured the hepatic levels of these modulators. Hepatic concentrations of both S-adenosylmethionine and S-adenosylhomocysteine were elevated following glucagon administration (Fig. 3). However, the ratio of SAM:SAH, often described as the “methylation ratio,” was unchanged.

Increased Flux through Transsulfuration following Glucagon—The second step of the transsulfuration pathway involves the cleavage of cystathionine to produce cysteine, NH4, and α-ketobutyrate. Incubating cells with L-[1-14C]methionine will give rise to α-[1-14C]ketobutyrate, which may be metabolized via pyruvate dehydrogenase to produce 14CO2, which may in turn be readily collected and counted. Label in unmetabolized α-[1-14C]ketobutyrate can be released with H2O2. Such 14CO2 released from α-ketobutyrate, must also be included in measures of flux through the transsulfuration pathway (32). Following incubation with L-[1-14C]methionine, there was a 5-fold increase in 14CO2 production from hepatocytes isolated from the glucagon-treated rats as compared with control cells, indicating a profound activation of flux through the transsulfuration pathway (Table IV). To ensure that 14CO2 produced was actually a product of the transsulfuration pathway, propargylglycine, an irreversible inhibitor of cystathionine γ-lyase, was included in the incubations. Blocking cystathionine γ-lyase resulted in an 80% reduction in 14CO2 production from hepatocytes isolated from control and glucagon-treated rats. When hepatocytes were incubated with cyanocinnamate, an inhibitor of the mitochondrial α-ketoacid transporter, there was a reduction in 14CO2 released after the addition of perchloric acid with a corresponding elevation of 14CO2 release after addition of peroxide. This result further confirms 14CO2 production from [1-14C]methionine as a measure of flux through the transsulfuration pathway and emphasizes the need to determine 14C in α-ketobutyrate to fully quantify flux through this pathway.

Glucagon Activates Methionine Transport in Isolated Hepatocytes—Our calculations show that the hepatic intracellular concentration of methionine was elevated in the glucagon-treated rats (Table II). Of particular interest is the fact that the ratio of liver to plasma methionine was 5-fold greater in the glucagon-treated animal. This could be explained by an effect of glucagon on methionine transport. Methionine transport rates were, therefore, measured from hepatocytes isolated from control and glucagon-treated rats. Glucagon treatment resulted in a doubling of methionine uptake into hepatocytes (Fig. 4).

Discussion

The regulatory effects of glucagon on amino acid metabolism have been well documented. It increases the catabolism of a variety of amino acids (e.g. glycine, glutamine, arginine, and phenylalanine), it increases gluconeogenesis from amino acids, and it increases the rate of ureagenesis (10, 34–38). With this in mind, an investigation of the specific effects of glucagon on homocysteine metabolism is warranted. The dose of glucagon used in this study was indeed pharmacological. We observed a 35-fold increase in plasma glucagon using this dosage, well above circulating levels in healthy organisms. Although these levels are not observed in a healthy organism, patients with glucagon-producing tumors have been reported to have a 60-fold increase in plasma glucagon (13). Therefore, our model is a good reflection of human glucagonoma. This view is supported by the similar decreases in total and specific plasma amino acids in both the glucagon-treated rat and the human glucagonoma patient (13). The decrease in amino acids in glucagonoma patients has been linked to increased clearance from the plasma, likely by the liver, not due to decreased export from the muscles (13). Given the plethora of effects glucagon has on amino acid metabolism it is not surprising that glucagon had a profound effect on circulating homocysteine levels.

Regulation of the methionine cycle and of the transsulfuration pathway are thought to be exerted primarily through
alterations in substrate availability and in the levels of the effector molecules, SAM and SAH. This work demonstrates, for the first time, the effects of the catabolic hormone, glucagon, on these regulatory parameters and on the expression of the rate-limiting transsulfuration enzyme, cystathionine \( \beta \)-lyase. Our in vitro and in vivo experiments show a dramatic effect of glucagon on the metabolism of the atherogenic amino acid, homocysteine.

It is clear, from our data, that glucagon administration intervenes at a number of sites (reviewed in Fig. 5), in methionine and homocysteine metabolism, and, thus, the observed changes in homocysteine are a reflection of these different actions. Glucagon affects methionine transport. This is evident from the fact that the hepatic intracellular concentration of methionine was increased by 25\% and, more impressively, that the ratio of intracellular to plasma methionine was elevated 5-fold. This suggests a marked stimulation of methionine transport into liver cells, and, indeed, we found that this was increased 2-fold in the glucagon-treated rats. This is consistent with the observation that glucagon up-regulates the System A amino acid transporter (39), because this is the major hepatic transporter of methionine. It is likely that the increased hepatic methionine level has important metabolic consequences. In an elegant theoretical analysis of the control of hepatic methionine metabolism, Martinov et al. (40) showed that this system is extraordinarily sensitive to small changes in methionine concentration, largely due to the kinetic properties of the liver-specific \( S \)-adenosylmethionine synthetase. Therefore, even in the absence of a detectable increase in activity of this enzyme, it is expected that the increased intracellular methionine concentration will stimulate flux through \( S \)-adenosylmethionine synthase, which can account for the increased hepatic SAM levels.

The markedly decreased plasma methionine concentration, at a constant dietary intake, implies increased methionine catabolism, which is consistent with the general plasma amino acid picture in our rat model, as well as in human glucagonoma patients. This would involve increased flux through glycine \( N \)-methyltransferase, whose activity is modestly, though significantly, increased. \( S \)-Adenosylhomocysteine, produced in the glycine \( N \)-methyltransferase reaction, is also increased in the livers of these glucagon-treated rats. This may result partially from the increased flux though glycine \( N \)-methyltransferase but must also, in part, reflect its removal though methionine synthase, whose activity is modestly, though significantly, increased.

### Table III: Hepatic enzymes of methionine and homocysteine metabolism in glucagon-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S )-Adenosylmethionine synthase</td>
<td>1.20 ± 0.05</td>
<td>1.25 ± 0.10</td>
</tr>
<tr>
<td>Cystathionine ( \beta )-synthase</td>
<td>5.3 ± 0.9</td>
<td>9.3 ± 1.2*</td>
</tr>
<tr>
<td>Cystathionine ( \gamma )-lyase</td>
<td>15.2 ± 2.0</td>
<td>19.6 ± 3.2*</td>
</tr>
<tr>
<td>Glycine ( N )-methyltransferase</td>
<td>1.02 ± 0.04</td>
<td>1.25 ± 0.10*</td>
</tr>
<tr>
<td>Methionine synthase</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Betaine/homocysteine methyltransferase</td>
<td>2.6 ± 0.7</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Methyltetrahydrofolate reductase</td>
<td>0.16 ± 0.05</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

*Significant difference versus control rats, \( p < 0.05 \).
Hepatocytes were preincubated for 20 min in BSA-supplemented Krebs-Henseleit medium and in the presence and absence of propargylglycine (an inhibitor of CBS) and cyanocinnamate (an inhibitor of the mitochondrial ketoacid transporter). Following the addition of 1 mM [1-14C]methionine the flasks were incubated for 30 min. The flasks were then equipped with rubber septa in which plastic center wells containing NCS tissue solubilizer was suspended. Incubations were terminated by injection of PCA, and 14CO2 was collected for 1 h. Then a new center well was added to the flask and H2O2 was injected. 14CO2 was collected for another hour and radioactivity measured. The total 14CO2 produced was calculated by adding the 14CO2 collected after the addition of PCA with the 14CO2 collected after addition of H2O2. Data are expressed as nmoI of 14CO2 produced/mg of dry hepatocytes/30 min. Means ± S.D. for three measurements are shown.

<table>
<thead>
<tr>
<th></th>
<th>14CO2</th>
<th>14CO2</th>
<th>Total</th>
<th>14CO2</th>
<th>14CO2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methionine</td>
<td>Methionine +</td>
<td>Methionine +</td>
<td>Glucagon</td>
<td>Glucagon</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>3.31 ± 0.26</td>
<td>2.10 ± 0.11</td>
<td>1.89 ± 0.20</td>
<td>8.90 ± 0.65</td>
<td>13.7 ± 4.01</td>
<td>22.9 ± 7.61</td>
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<tr>
<td>glucagon</td>
<td>0.62 ± 0.12b</td>
<td>1.26 ± 0.11b</td>
<td>5.27 ± 0.74</td>
<td>0.49 ± 0.09a</td>
<td>3.42 ± 0.09c</td>
<td>3.90 ± 0.20</td>
</tr>
<tr>
<td>cyano</td>
<td>1.1 ± 0.18b</td>
<td>4.17 ± 0.58b</td>
<td>5.23 ± 1.93&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>24.2 ± 3.1&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>29.9 ± 4.99&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.05 versus same measurement in control group (Student’s t test).
<sup>b</sup> p < 0.05 versus control cells incubated with methionine alone (Student’s t test).
<sup>c</sup> p < 0.05 versus glucagon cells incubated with methionine alone (Student’s t test).

From a direct effect on cystathionine β-synthase expression. Goss et al. (44) showed that cAMP, glucagon’s intracellular messenger, can increase cystathionine β-synthase activity in cultured hepatoma cells. Work in our laboratory has shown that cAMP increases cystathionine β-synthase mRNA, protein, and activity in H4IIE cells. Insulin levels were unchanged in our glucagon-treated rats so that changes in this hormone cannot account for the increased activity of cystathionine β-synthase, although we have shown that insulin treatment of diabetic rats can reduce cystathionine β-synthase activity to normal levels (9). It is currently unknown whether glucagon’s effects on cystathionine β-synthase are mediated by an increase in gene transcription, enhancement of mRNA stability, or both. It has been reported that liver cystathionine β-synthase mRNA is increased in rats fed a high protein diet (45). Because such a diet is known to elevate plasma glucagon (46) our results provide an explanation for this dietary regulation of cystathionine β-synthase.

That flux through the transsulfuration pathway is stimulated by glucagon treatment is directly shown in isolated hepatocytes (Table IV). These data also provide evidence that the α-ketobutyrate produced by cystathionine γ-lyase must enter mitochondria on the mitochondrial ketoacid transporter for decarboxylation. A key finding is that glucagon treatment caused a 4-fold increase in flux through the transsulfuration pathway even though the increased activity of cystathionine β-synthase and cystathionine γ-lyase was less than 2-fold. Clearly, additional factors must play a role. In this context, the doubling of the rate of methionine transport into the hepatocyte is key. It is possible that glucagon exerts effects at other sites, because it activates cellular protein kinases. Cystathionine β-synthase does contain a protein kinase A consensus sequence, but phosphorylation of this enzyme has yet to be shown. We can eliminate the idea that glucagon increases flux through the transsulfuration pathway by mediating changes in serine, the co-substrate for cystathionine β-synthase. Indeed, hepatocytes isolated from the glucagon-treated rats exported less homocysteine, when incubated with methionine, than did control hepatocytes. When serine was included in the incubation, homocysteine export was reduced in control hepatocytes but not in the hepatocytes isolated from the glucagon-treated rat. However, the possibility that such a mechanism is important in vivo is eliminated by the finding that the hepatic concentration of serine was unaffected by glucagon treatment.

The decreased plasma homocysteine, upon glucagon treatment, follows from the decreased hepatic homocysteine concent-

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3 S. Ratnam, unpublished.
tation. Very little is known about the transport of homocysteine across hepatocyte membranes. In rat cortical tubules homocysteine has been shown to share the γ+ system transporter with the dibasic amino acids (47). The uptake of homocysteine is plausible, because most of the free homocysteine in plasma exists in an oxidized disulfide form. However, uptake of homocysteine by liver cells has not been reported. Furthermore, because hepatocytes have such low activity of the γ+ transporter, another mechanism may be required for homocyst(e)ine uptake (48). Given the reduced nature of the intracellular milieu (the cytoplasmic NADPH/NADP+ ratio is about 100 (49) and that of reduced glutathione/oxidized glutathione is about 150 (50), most of the intracellular homocysteine is believed to be in the reduced form. This necessitates a transporter that recognizes reduced homocysteine to effect homocysteine export. At present this carrier has not been identified. However, glucagon treatment caused no change in plasma homocysteine levels. It is not necessary to ascribe any role to changes in homocysteine transport.

Activated flux through the transsulfuration pathway also requires increased disposal of cysteine because this substance does not accumulate (Table II). Pyruvate is the carbon product of cysteine catabolism and is a good gluconeogenic precursor. In view of glucagon's well-established role in stimulating gluconeogenesis (36) and given that gluconeogenesis is the likely fate of much of our dietary amino acids (51), we suggest that glucose production is the carbon end-product of cysteine oxidation in these glucagon-treated rats.

This report illustrates the effects of glucagon on sulfur amino acid metabolism and provides a plausible explanation of how this catabolic hormone regulates circulating homocysteine concentrations. Our previous work (9) emphasized the role of insulin in regulating plasma homocysteine. This anabolic hormone increases plasma homocysteine in the Type 1 diabetic rat. Taken together, it is clear that the opposing effects of these metabolic or "fuel" hormones can be very important in controlling plasma homocysteine metabolism and that the liver is the site of this hormonal regulation.

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In Vivo Effects of Glucagon on Homocysteine Metabolism

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