Growth Hormone Stimulation of Amino Acid Transport and Utilization by the Perfused Rat Liver*

LEONARD S. JEFFERSON, CHARLES M. SCHWORER, AND EDWARD L. TOLMAN‡

From the Department of Physiology, College of Medicine, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

SUMMARY

The effects of growth hormone, administered in vivo or added in vitro, on amino acid transport and utilization have been studied in perfused livers of normal and hypophysectomized rats. A perfusion system employing a nonrecirculating medium was used in all of the studies.

Two nonmetabolizable amino acid analogues, α-aminoisobutyric acid (AIB) and 1-aminocyclopentane carboxylic acid (cycloleucine) were used to study transport. Accumulation of AIB increased linearly over a 60-min perfusion period, reaching distribution ratios of between 1 and 2 for both groups of animals. Treatment of both normal and hypophysectomized rats with growth hormone 60 min prior to the start of perfusion increased AIB distribution ratios by up to 84 and 108%, respectively. Accumulation of cycloleucine was linear for only about 20 min of perfusion and then plateaued. Steady state distribution ratios of this analogue ranged between 1 and 2 for both groups of animals. Growth hormone treatment had no apparent effect on the time necessary to reach these steady state levels, but significantly increased them in livers of both normal and hypophysectomized rats by 16 and 42%, respectively. Studies designed to analyze the kinetic properties of these hormone effects revealed that growth hormone treatment caused a 2-fold increase in the maximum velocities of both the AIB and cycloleucine transport systems. The substrate concentration for half-maximal transport velocity was increased slightly for both systems by growth hormone.

Direct effects of growth hormone were demonstrated in studies where livers of hypophysectomized rats were perfused under conditions simulating those of experiments in which the hormone was administered in vivo. Following an initial 45-min period of perfusion the medium was switched to one containing [14C]cycloleucine and the accumulation of this amino acid analogue was measured over the following 20 min. Growth hormone added to the medium during the entire 65-min perfusion at a concentration of 1 μg per ml caused a 30% increase in the cycloleucine distribution ratio.

Under similar experimental conditions growth hormone directly stimulated three hepatic pathways of amino acid utilization: (a) incorporation of [14C]valine into protein, (b) urea formation and (c) conversion of [14C]-amino-acids to labeled glucose. Intracellular concentrations of seven amino acids, including threonine, serine, proline, glycine, alanine, lysine, and arginine, were increased significantly in livers perfused with medium containing growth hormone. This finding and the observation that the hormone had no effect in livers perfused with levels of amino acids that were saturating for the pathways of protein synthesis, gluconeogenesis, and ureogenesis suggested that growth hormone increased amino acid utilization as a result of its stimulatory action on the transport of these compounds into liver cells.

When urea production was used to monitor the direct action of growth hormone on perfused liver, it was noted that a lag period of 20 to 25 min occurred before a significant effect of the hormone was manifested.

In vivo administration of growth hormone has been shown to increase the entry of amino acids into a variety of tissues including liver, muscle, and intestine (1, 2). The well documented (3-6) stimulation of protein synthesis in several tissues by this hormone suggests the possibility that at least a part of its action might involve an effect on amino acid transport. The effects of growth hormone on this process have been investigated most extensively in muscle. Kostyo et al. (7-9) and Hjalmarson and Ahrén (10, 11) have demonstrated that the uptake of both non-utilizable and naturally occurring amino acids is increased in diaphragms either taken from hypophysectomized rats treated with growth hormone or incubated in the presence of the hormone. Similar effects of the hormone on amino acid transport in perfused hearts of hypophysectomized rats have been observed (12). On the other hand, the control of amino acid transport in liver by growth hormone, as well as by other hormones, has been studied almost exclusively in vivo (1, 2, 13). In vito liver preparations have been employed for transport studies only recently. Chambers et al. (14-16) and Tews et al. (17) have observed stimulatory effects of hydrocortisone, insulin, catecholamines, glucagon, and adenosine 3':5'-monophosphate on amino acid transport in both the perfused rat liver and in

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‡ Recipient of National Institutes of Health Postdoctoral Fellowship HE-43762. Present address, Metabolic Disease Therapy Research Section, Lederle Laboratories, American Cyanamid Co., Pearl River, New York 10965.
amino acid utilization including protein synthesis, gluconeogenesis, and ureogenesis are dependent on the availability of the hormone on amino acid transport in a more direct manner.

Studies in perfused liver have shown that various pathways of amino acid utilization including protein synthesis, gluconeogenesis, and ureogenesis are dependent on the availability of substrate in the perfusion medium (18–20). These findings indicate that entry of amino acids into liver cells may represent the rate-limiting step in these pathways and may provide an important site for regulation of hepatic amino acid metabolism. An effect of growth hormone on amino acid transport in perfused liver might be expected to increase the activities of these pathways by increasing the availability of intracellular substrates.

In the present series of experiments the effect of growth hormone on the accumulation of amino acids in the perfused rat liver has been studied. The properties of the transport process in this system and the relationship of the effects of the hormone on amino acid uptake to its influence on other amino acid metabolic pathways have been evaluated. To study transport, two nonmetabolizable amino acid analogues have been employed since naturally occurring amino acids are rapidly metabolized in liver. The properties of the uptake of a-aminoisobutyric acid and 1-aminocyclopentane carboxylic acid are such that at least two different transport systems are involved in their entry into a variety of cell types (13, 21, 22). This finding, confirmed in liver slices by Tews and Harper (23) and Crawhall and Segal (24), indicates that the use of both of these models allows for a more complete description of the actions of a hormone on amino acid transport. Hypophysectomized rats, as well as normal animals, have been used as liver donors since removal of the pituitary increases the sensitivity of the hepatic transport systems to growth hormone (2).

The points to be defined in the present study are (a) the effects of growth hormone, administered in vivo or added in vitro, on the accumulation of two model amino acids; (b) the mechanisms of these effects of the hormone as defined by their kinetic properties; (c) the influence of growth hormone on intracellular amino acid levels; (d) the effects of growth hormone on pathways of amino acid metabolism including protein synthesis, gluconeogenesis, and ureogenesis; and (e) the relationship of the effects of growth hormone on amino acid transport to its actions on amino acid metabolism in the perfused rat liver.

Portions of this work have appeared previously in preliminary form (25).

Materials and Methods

Animals—Male, normal and hypophysectomized rats of the Wistar strain (Carworth Farms) were used as liver donors. The animals were fed laboratory chow and water ad libitum. Hypophysectomized rats were received 2 days following surgery and were killed 14 to 16 days later. Livers from hypophysectomized rats were used only if the animals failed to exhibit gains in body weight. To maintain body weight, normal rats were used if they were 1 week younger than the hypophysectomized animals. All rats weighed 100 to 120 g at time of use.9

Hormone Treatment—Where noted, normal and hypophysectomized rats received intraperitoneal injections of bovine growth hormone (NIH-GH-B16) 1 hour prior to death at a dose of 1 mg/100 g body weight. The growth hormone was dissolved in a 0.9% NaCl solution made up to pH 8.0. Untreated control animals were injected with corresponding volumes of the saline solution.

To test the effects of growth hormone in vitro appropriate volumes of the hormone solution were added directly to the liver perfusion medium to give a concentration of 1 μg per ml.

Liver Perfusion—The technique of liver perfusion was based upon a method described previously (26). Methods for preparation of the basic perfusion medium were presented previously (27). Specific additions to the basic medium are presented along with the data collected from individual experiments. The medium was commonly gassed with a humidified mixture of 95% O2:5% CO2, continually mixed in rotating reservoirs, and kept at 37°. The perfusate was not recirculated and was pumped through the liver at flow rates which ranged between 6.8 and 7.0 ml per min. At appropriate intervals during perfusion, samples of effluent were collected from the outflow cannula. These samples were centrifuged at 2500 × g; the supernatants (“perfusion plasma”) were separated from the red cells and were stored frozen for later analyses. At the end of perfusion, livers were quickly excised and rapidly frozen between aluminum blocks cooled to the temperature of liquid nitrogen. The frozen livers were weighed and pulverized in a percussion mortar cooled to the temperature of liquid nitrogen. The tissues were stored at −70° until analyzed.

Amino Acid Transport—The accumulation of nonmetabolizable amino acid analogues was monitored by determining the intracellular to extracellular distribution ratios of these models in frozen liver samples as described previously (27).

Liver Analyses—The effect of growth hormone on intracellular amino acid concentrations was determined in perfused livers from hypophysectomized rats. Livers were perfused with medium containing the natural amino acids at levels 3 times those seen in normal rat plasma (see Ref. 27). Protein in samples of frozen, powdered liver was precipitated by homogenizing the tissue in 5% sulfosalicylic acid containing norleucine as an internal standard. Extracts were centrifuged at 10,000 X g for 10 min and the supernatants collected and neutralized to pH 6.8 to 7.0. A portion of the neutralized extract was mixed with 90 volume of 10 N HCl and was hydrolyzed for 2 hours at 100°. Hydrolysis converted asparagine and glutamine to aspartic acid and glutamic acid, respectively, and thus permitted an analysis of all of the natural amino acids except tryptophan and cysteine. The tissue concentrations of these two amino acids were below levels which could be measured accurately. Both the unhydrolyzed and the hydrolyzed extracts were analyzed by the methods described.

Portions of this work have appeared previously in preliminary form (25).

Radiochemicals and Reagents—a-Amino[3-14C]isobutyric acid (specific activity, 5.3 mCi per mmole), 1-aminocyclopentane [1-14C]carboxylic acid (specific activity, 10.8 mCi per mmole), uniformly 14C-labeled L-amino acid mixture and L-[U-14C]valine (specific activity, 228 mCi per mmole) were all obtained from New England Nuclear Corp.

The 20 naturally occurring amino acids, as their L isomers, and AIB2 were obtained from Mann Research Laboratories. Cycloleucine was purchased from Calbiochem. All other chemicals were of standard reagent grade.

1 NIH GH B16 was generously supplied by the Endocrinology Study Section of the National Institute of Arthritis, Metabolic and Digestive Diseases.

2 The abbreviations used are: AIB, α-aminoisobutyric acid; cycloleucine, 1-aminocyclopentane carboxylic acid.
Statistical Analyses—The data are presented as the mean of a given number of observations ±1 S.E. Differences between averages were tested for statistical significance by the Student t test. Differences whose p values were greater than 0.05 were not considered to be significant.

EXPERIMENTAL DESIGN AND RESULTS

Amino Acid Accumulation in Vivo—Steady state distribution between liver and plasma of one of the model amino acids employed in the present study was determined, in vivo, by injecting normal and hypophysectomized rats intraperitoneally with [14C]AIB at a dose of 1 μCi/100 g body weight, 24 hours prior to death. One hour before death, some of the animals were injected with bovine growth hormone at a dose of 1 mg/100 g body weight. This procedure was similar to the one described by Riggs and Walker (2). Liver and plasma samples were collected from anesthetized rats and the distribution ratios of AIB in the liver were determined. After 24 hours of exposure to [14C]AIB, distribution ratios were 4.6 ± 0.3 (n = 4) in normal rats and 5.6 ± 0.5 (n = 4) in the hypophysectomized animals. Growth hormone treatment increased AIB distribution ratios in both groups, by 19% in normal rat livers (to 5.4 ± 0.2, n = 9) and by 46% in livers of hypophysectomized rats (to 8.1 ± 0.7, n = 3). Both increases following growth hormone treatment were statistically significant (p < 0.05). These data confirmed those of Riggs and Walker (2) that AIB is strongly concentrated in the liver and that the process is enhanced by growth hormone.

Using a similar experimental approach (13), net transport of cycloleucine into liver, in vivo, was observed. Distribution ratios between liver and plasma ranged between 1 and 2 under steady state conditions and these values were attained within 30 min exposure of the tissue to [14C]cycloleucine. Growth hormone treatment, similar to that described above, increased the accumulation of this amino acid analogue by 48% in livers of hypophysectomized rats.

Amino Acid Accumulation in Vitro—In order to measure more directly the effect of growth hormone on hepatic amino acid transport in the absence of such complicating factors as amino acid excretion by the kidney and redistribution among various tissues, livers from normal and hypophysectomized rats were perfused with medium containing either [14C]AIB or [14C]cycloleucine. As described above, a portion of the animals received injections of growth hormone (1 mg/100 g body weight) 1 hour prior to use as liver donors. Whereas in vivo administration of growth hormone did not demonstrate a direct action of the hormone on the liver, this form of treatment facilitated the use of a greater number of animals and, thus, larger sampling for time course and kinetic analyses. A direct effect of growth hormone was investigated in other experiments and is reported below.

Fig. 1 illustrates the effect of in vivo growth hormone treatment upon the AIB distribution ratios in perfused livers of both normal and hypophysectomized rats. In normal rats the AIB distribution ratio, after growth hormone treatment, was significantly increased at all perfusion times when compared to the distribution ratio of controls. In hypophysectomized rats the AIB distribution ratio was significantly higher in the 15-, 30-, and 60-minute perfusions of growth hormone-treated animals as compared to that of hypophysectomized controls. The distribution ratios for the control hypophysectomized livers were lower than those of the control normals. However, growth hormone treatment of hypophysectomized rats increased the AIB distribution ratios to values slightly above those of control normal rats. In all four experimental conditions the AIB distribution ratios increased linearly over the entire time interval of perfusion. By extrapolating the line depicting the AIB distribution ratios in livers from normal rats to the value reported above for the steady state condition attained in vivo, it was estimated that at least 135 min of perfusion would have been required to reach this maximum value. This calculated time compared favorably with that observed in liver slices from normal rats by Tews and Harper (23).

The effects of growth hormone treatment on the accumulation of cycloleucine are shown in Fig. 2. The time course of cycloleucine accumulation differed markedly from that of AIB. Cycloleucine distribution ratios increased linearly for only about 20 min and then plateaued. Steady state distribution ratios ranged between 1 and 2 for all test conditions. Growth hormone treatment had no apparent effect on the time necessary to reach these steady state levels, but significantly increased them in livers of both normal (left panel) and hypophysectomized (right panel) rats by 16 and 42%, respectively. These relatively low distribution ratios for cycloleucine indicated a modest ability of the liver to transport actively this amino acid analogue.

Experiments of a similar design to the ones just mentioned were used in an attempt to evaluate the kinetic properties of the effect of growth hormone on amino acid accumulation. Livers were perfused with medium containing increasing concentrations of the nonmetabolizable amino acid analogues and their initial rates of entry were measured during short periods of perfusion. Fig. 3 shows the results that were obtained when normal rat livers were perfused 15 min with medium containing AIB at concentrations ranging from 0.25 to 10 mM and [14C]AIB. Hormone administration increased the maximum velocity of AIB accumulation more than 2-fold. The substrate concentration for half-maximal transport velocity was increased slightly in the presence of the hormone. Similar kinetic data for the in-
FIG. 2. Growth hormone (GH) stimulation of cycloleucine accumulation by the perfused rat liver. Hormone treatment of rats and methods of perfusion were similar to those described in the legend to Fig. 1 except that the perfusate contained L-amino-cyclopentane [1-14C]carboxylic acid (cycloleucine) at a level of 0.01 μCi per ml. Each point represents the mean of 4 to 6 observations and the vertical bars denote 1 S.E. After 30 min of perfusion, cycloleucine distribution ratios in livers from growth hormone-treated rats differed from those in livers from untreated animals by p < 0.02. These differences were maintained during longer periods of perfusion. HYPOX, hypophysectomized.

FIG. 3. The effect of growth hormone (GH) on the kinetics of AIB accumulation in the perfused rat liver. Livers from normal rats were perfused for 15 min with nonrecirculating medium containing all 20 natural amino acids at concentrations found in normal rat plasma and α-aminoisobutyric acid (AIB) at levels ranging from 0.25 mM to 10 mM. α-Amino[3-14C]isobutyric acid was added at concentrations increasing in proportion to the increasing levels of its unlabeled homolog. Where indicated (open circles), rats received intraperitoneal injections of bovine growth hormone at a dose of 1 mg/100 g body weight at 1 hour prior to death. The closed circles represent data obtained with livers of untreated normal rats. Each point represents the mean of 4 to 7 observations. The method of least squares (31) was used to fit the line to the data.

The effect of growth hormone on cycloleucine accumulation by livers from hypophysectomized rats are shown in Fig. 4. As was the case for AIB, treatment of hypophysectomized rats with growth hormone 1 hour prior to perfusion produced a 2-fold increase in the maximum velocity of cycloleucine transport while causing only a slight change in the substrate concentration required for half-maximal transport velocity.

Direct Effects of Growth Hormone—The data presented thus far indicated that growth hormone stimulated the hepatic accumulation of two model amino acids. These observations did not preclude the possibility that the hormone was acting indirectly through an intermediate derived from extrahepatic tissues. A direct effect of the hormone was monitored by measuring the accumulation of cycloleucine in the presence and absence of 1 μg per ml of growth hormone in the perfusion medium. In these studies the effects of growth hormone on protein synthesis, gluconeogenesis, and ureogenesis were also evaluated. These three pathways of amino acid metabolism provide an indirect assessment of the hormone effect on transport since they are limited in perfused liver by the availability of substrate.

In these studies, an attempt was made to simulate the conditions of the experiments described above in which an effect of growth hormone on amino acid transport was observed in perfused livers 60 min after in vivo administration of the hormone. Livers from hypophysectomized rats were perfused for an initial 45-min period with medium containing all 20 natural amino acids at concentrations 3 times those seen in the plasma of normal rats. In preliminary experiments it was found that these relatively high perfusate amino acid levels were necessary to maintain intracellular amino acid concentrations at in vivo levels, especially when longer perfusion times were used. During a second 20-min period of perfusion employing medium containing the amino acids at normal rat plasma concentrations, effects of the hormone were measured. Where indicated, growth

* L. S. Jefferson, unpublished observations.
hormone (1 μg per ml) was added to the perfusate during the entire 65-min perfusion.

As is demonstrated in Table I, growth hormone significantly increased the cycloleucine distribution ratio by 30% in livers of hypophysectomized rats indicating a direct effect of the hormone on liver to stimulate the accumulation of this amino acid analogue.

Using similar experimental conditions, the possibility that growth hormone affected some of the pathways of amino acid utilization was investigated. In order to monitor the effect of the hormone on protein synthesis, the perfusing medium was switched during the final 20 min to one containing [14C]valine. It had been reported earlier (20) that the rate of incorporation of this amino acid accurately reflected the rate of protein synthesis in the perfused liver. Addition of growth hormone caused a 23% stimulation in the incorporation of valine into total protein of livers of hypophysectomized rats (Table I). These data confirmed the earlier work of Jefferson and Korner (4) which first demonstrated an effect of growth hormone on protein synthesis in the perfused rat liver. Similar results were also reported for studies using liver slice preparations (32, 33).

The conversion of a mixture of [14C]-amino acids to [14C]glucose was used to estimate flux through the pathway of gluconeogenesis. When the rate of gluconeogenesis was determined between 44 and 45 min of perfusion of livers from hypophysectomized rats, it was noted that the presence of growth hormone markedly stimulated [14C]glucose production (Table I). These data suggested that growth hormone stimulated not only protein synthesis, but also amino acid gluconeogenesis as well.

The effect of growth hormone on ureogenesis was also studied. Table II shows the results obtained when livers of hypophysectomized rats were perfused with medium containing varying concentrations of amino acids, in the presence or absence of growth hormone. The rate of urea production doubled when the perfusate amino acid levels were raised from normal rat plasma concentrations to 3 times those levels and again when the amino acid levels were increased to 6 times (6 X AA) those seen in the plasma of normal rats. It had been shown previously that urea production by normal rat livers was increased by raising perfusate amino acid levels until they reached about 5 times those seen in normal rat plasma (20). The addition of growth hormone to the perfusate stimulated rates of urea production at the two lower amino acid levels, by about 40 to 50%. At 6 X AA, the rates of urea production by livers perfused with and without growth hormone were the same, indicating that the hormone effect was dependent upon the presence of levels of amino acids that were not saturating for the pathway.

Earlier studies in perfused liver had shown that the pathways of protein synthesis, gluconeogenesis, and ureogenesis were usually limited by the availability of intracellular amino acids (18–20). Therefore, the results obtained in the preceding studies (Tables I and II) could have resulted from the effect of growth hormone to increase transport of amino acids into liver cells. If this were the mechanism responsible for the increased flux of amino acids through these metabolic pathways, then under conditions where transport was no longer rate-limiting one would expect to find elevated concentrations of intracellular amino acids. When livers of hypophysectomized rats were perfused for 45 min with medium containing amino acid concentrations at 3 times normal plasma levels (Table III), growth hormone significantly increased the intracellular concentrations of seven amino acids, including threonine, serine, proline, glycine, alanine, lysine, and arginine. Transport of other amino acids may have been stimulated by growth hormone, but if utilization kept pace with their rate of entry then their intracellular concentrations would not have changed. Earlier studies had shown that protein synthesis in perfused liver was influenced by the availability of 11 amino acids and these included threonine, proline, lysine, and arginine (18). Included within the group of seven amino acids that increased in the presence of growth hormone

**Table I**

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Hypophysectomized</th>
<th>Hypophysectomized + GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloleucine, distribution ratio</td>
<td>0.807 ± 0.027</td>
<td>1.117 ± 0.032</td>
</tr>
<tr>
<td>Valine incorporation, dpm/mg protein</td>
<td>324 ± 11</td>
<td>398 ± 23</td>
</tr>
<tr>
<td>[14C]Glucose production, dpm/min/g</td>
<td>1502 ± 194</td>
<td>2886 ± 260</td>
</tr>
</tbody>
</table>

* Differs from control by p < 0.01.

<table>
<thead>
<tr>
<th>Additions to perfusate</th>
<th>Urea production at perfusate amino acid concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 X AA</td>
</tr>
<tr>
<td></td>
<td>μmol/g/min</td>
</tr>
<tr>
<td>None</td>
<td>0.43 ± 0.04 (6)</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>0.60 ± 0.03 (4)*</td>
</tr>
</tbody>
</table>

* Differs from control by p < 0.01.

b Differs from control by p < 0.001.
concentrations 3 times those seen in normal rat plasma. Each nonrecirculating medium containing all 20 natural amino acids at

Direct effects of growth hormone (GH) on intracellular concentrations of amino acids in perfused livers of hypophysectomized rats

Livers of hypophysectomized rats were perfused for 45 min with nonrecirculating medium containing all 20 natural amino acids at concentrations 3 times those seen in normal rat plasma. Each value represents the average of 8 to 9 observations ± 1 S.E.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Intracellular concentration, 10^4 M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypophysectomized</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>290 ± 47</td>
</tr>
<tr>
<td>Threonine</td>
<td>159 ± 8</td>
</tr>
<tr>
<td>Serine</td>
<td>404 ± 10</td>
</tr>
<tr>
<td>Asparagine</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>830 ± 60</td>
</tr>
<tr>
<td>Proline</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1150 ± 75</td>
</tr>
<tr>
<td>Glycine</td>
<td>250 ± 12</td>
</tr>
<tr>
<td>Alanine</td>
<td>382 ± 29</td>
</tr>
<tr>
<td>Valine</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>Methionine</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Leucine</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Lysine</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>Histidine</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>Arginine</td>
<td>20 ± 2</td>
</tr>
</tbody>
</table>

* Differs from control by p < 0.005.

† Differs from control by p < 0.01.

‡ Differs from control by p < 0.02.

were ones that serve as precursors for the synthesis of glucose and urea (30, 34). Alanine was quantitatively the most important glucose precursor (19, 30, 35) and its concentration increased 70% in the presence of growth hormone. These data suggested that in perfused liver the direct effect of growth hormone on gluconeogenesis, ureogenesis, and protein synthesis resulted from its stimulatory action on amino acid transport. In diaphragm muscle from hypophysectomized rats, a period of 20 min or more was required for the demonstration of a direct effect of growth hormone on amino acid transport (36). Using urea production to monitor the action of the hormone, a similar lag period was noted in perfused livers of hypophysectomized rats (Fig. 5). A significant difference between rates of urea production by livers perfused with growth hormone and those perfused in its absence required between 20 and 25 min of perfusion. The initial increase in the rate of urea production was related to the fact that the perfusate contained amino acid levels that were 3 times those seen in normal rat plasma.

**Discussion**

The data obtained in this study confirmed and extended earlier work which had shown a stimulation of hepatic amino acid transport in vivo in growth hormone-treated rats (2, 13). Since the liver plays a prominent role in amino acid metabolism, an effect of growth hormone on transport in this tissue may have contributed significantly to the lowering of plasma amino acid levels observed following treatment with the hormone (37, 38). The time course for the accumulation of either AIB or cycloleucine in perfused livers of normal rats was in agreement with that reported for rat liver slices (23, 24). AIB accumulated more slowly than cycloleucine, but reached higher distribution ratios both in vivo and during a 1-hour perfusion. On the basis of other studies (13, 21, 22) it was suggested that at least two distinct transport systems were involved in the entry of these amino acid analogues into cells. By analogy to the well studied Ehrlich cells (21), AIB would have entered liver cells by the sodium-dependent "A" system (alanine preferring) and cycloleucine, at least in part, by the "L" system or that which prefers leucine. The latter was shown to exhibit a high rate of efflux, due apparently to an active component of exchange diffusion. This property may explain the low levels of cycloleucine accumulated in perfused liver and liver slices (23). Whereas kinetic analysis of amino acid entry is difficult to interpret, growth hormone appeared to accelerate the maximal transport rate rather than to increase the affinity of the carrier system. It is possible that a greater number of carrier sites became active following exposure to growth hormone or that the rate of carrier movement was accelerated. More details of hepatic amino acid transport are needed before the mechanism of the effect can be defined.

It was possible that the stimulation of amino acid transport in perfused livers taken from rats treated in vivo with growth hormone was the result of the hormone acting indirectly through its influence on other tissues or endocrine glands. It had been suggested that growth hormone acted on protein metabolism by promoting the secretion of insulin, which in turn, served as the effector agent at the level of the target tissue (39, 40). More recently, Salmon and DuVall (41) demonstrated that a partially purified preparation of sulfation factor, a substance now termed somatomedin (42), directly stimulated leucine incorporation into protein in the isolated rat diaphragm preparation. This observation led Merimee and Rabin (43) to suggest that the effects of
growth hormone on protein metabolism were not direct, but were mediated through the intermediate humoral substance, somatomedin. However, effects of growth hormone on protein synthesis in several tissues of hypophysectomized rats has been demonstrated within 30 min of exposure to the hormone in vivo, a time when neither somatomedin activity nor insulin levels were affected (44). This observation suggested that the acute stimulatory effects of growth hormone on amino acid transport and protein synthesis in several tissues including liver were not mediated by somatomedin or insulin. That growth hormone was directly affecting amino acid transport in liver, as well as other pathways involved in the subsequent metabolism of these compounds, was demonstrated in the present study by the elevated cycloleucine distribution ratios and the enhanced rates of valine incorporation into protein, urea formation, and conversion of amino acids into glucose in livers perfused with medium containing growth hormone. The report that somatomedin was produced by liver during perfusion with growth hormone (45) raised the possibility that this factor mediated these in vivo effects. However, McConaghey and Sledge (45) found that it was necessary to recirculate the medium containing growth hormone through the liver three times to produce a detectable level of somatomedin activity. Production of this factor would not appear to account for the results obtained in the present study because the perfusate was not recirculated but passed through the liver a single time.

Most of the work dealing with the question of the relationship of an acceleration of amino acid transport to protein synthesis has involved the use of muscle. Hjalmarson (46) observed that in isolated diaphragms taken from rats injected 3 hours prior to killing with growth hormone, the incorporation of leucine and glycine into protein was increased, but their distribution ratios did not change correspondingly. Knobil (47) reported similar findings for other natural amino acids and Kostyo (48) demonstrated that while the effect of growth hormone on transport was abolished by incubating diaphragm in sodium-free medium, the stimulation of protein synthesis was still apparent. In the diaphragm the stimulatory effect of growth hormone on amino acid transport was shown to be a transitory phenomenon, lasting only 2 to 3 hours (49), whereas the protein synthetic effect of the hormone persisted for a number of hours (44). These findings supported the argument that the growth hormone effect on protein synthesis in diaphragm muscle was independent of its effect on amino acid transport.

The situation in liver may be different from diaphragm. The literature suggests a direct relationship between levels of intracellular amino acids and protein synthetic activity in liver. For example, the rate of incorporation of amino acids into protein in rat liver slices was dependent on the availability of even single amino acids (50, 51). The omission of certain individual amino acids from the diet of rats impaired the protein synthetic machinery of the liver (52). Studies in the perfused rat liver demonstrated that increasing amino acid levels in the medium raised the rate of protein synthesis by stimulating peptide chain initiation (4, 20). This effect was observed with a complete mixture or with a mixture of 11 amino acids in the perfusate (18). Omission of a single one of these 11 amino acids impaired the rate of protein synthesis and caused disaggregation of polysomes.

Other studies (19, 20) in the perfused rat liver have demonstrated that the hepatic pathways of gluconeogenesis and ureogenesis are also influenced by the availability of amino acids in the perfusate. Maximal effects on these pathways were not observed until the concentrations of amino acids in the perfusate were raised to 3 to 5 times those found in the plasma of normal rats. We have observed a similar dependence of these pathways on amino acid availability in perfused livers of hypophysectomized rats. The finding (Table II) that no effect of growth hormone was observed when perfuse amino acid concentrations were raised to levels that gave maximal rates of flux through the pathway of ureogenesis strongly suggested that the hormone effect on this metabolic pathway resulted from its stimulatory action on amino acid transport.

Additional support for this view is provided by the data presented in Table III. The fact that increased flux through the various pathways was associated with increased intracellular levels of amino acids indicated that the primary action of the hormone was on amino acid accumulation rather than utilization. It is particularly noteworthy that the list of amino acids that were increased in livers perfused with growth hormone is very similar to the list of amino acids whose uptake by diaphragm was stimulated by the hormone (53). The list for diaphragm included glycine, alanine, serine, threonine, proline, histidine, tryptophan, glutamine, and asparagine. Thus, growth hormone stimulated the accumulation of glycine, alanine, serine, threonine, proline, and histidine in both tissues. Since the rapid metabolism of amino acids by liver makes it difficult to assess the net transport of these substances, it is not possible to say with certainty that the hormone has an effect on the transport of only certain amino acids. Nonetheless, the similarity between the effects of the hormone on diaphragm and liver is striking.

The mechanism of the stimulatory effect of growth hormone on amino acid transport is poorly understood but may involve the synthesis of a specific peptide as an obligate intermediate step, at least in muscle. When diaphragm was preincubated in the presence of puromycin or cycloheximide prior to exposure to growth hormone, the stimulation of amino acid uptake usually associated with the addition of the hormone was either markedly diminished or abolished (54, 55). Kostyo (54) suggested that the effects of growth hormone on amino acid transport in rat diaphragm required the synthesis of specific peptides or whole proteins which may have been involved in the activation of the transport mechanism. This possibility provides an explanation for the fact that effects of growth hormone in perfused liver (4) and liver slices (32) are seen best when experiments are carried out in the presence of higher than the normal plasma levels of amino acids. We have found that intracellular levels of amino acids in livers perfused in a nonrecirculating system are maintained at or above in vivo levels only if 3 times the normal plasma concentrations of amino acids are added to the perfusate. At lower amino acid levels, intracellular concentrations decrease and rates of protein synthesis decrease. It is possible that these conditions limit synthesis of a peptide which activates the transport process and therefore masks the effect of growth hormone.

The time required for a significant effect of growth hormone on amino acid metabolism in the perfused rat liver was 20 to 25 min. This figure agreed well with the observations of others on parameters other than urea production. Leucine incorporation into protein of thigh muscles, diaphragm, and liver was significantly increased 30 min following the intravenous administration of the hormone to hypophysectomized rats (44). A direct stimulation of phenylalanine incorporation into liver protein was observed 30 min after the addition of the hormone to the perfusing medium (4). For diaphragm, Rillema and Kostyo (36) observed that the enhancement of both amino acid transport and incorporation
into protein required more than 20 min to become manifest once the tissue had come in contact with the hormone. It would appear that a lag period of a few minutes is necessary for growth hormone to exert its effect on the transport process.

It must be pointed out that the results obtained in the present study represent early effects of growth hormone on liver. These types of effects in other tissues have classically been termed "insulin-like" and their relationship to the physiological role of growth hormone is unknown. Growth hormone has other effects in liver which are manifested only after several hours of growth hormone treatment. For example, Korner (56) found that several hours of treatment of hypophysectomized rats were required for growth hormone to stimulate the ability of liver ribosomes to synthesize protein in a cell-free system. Perfused livers of hypophysectomized rats have a restricted rate of peptide chain elongation which is corrected only after 48 hours of growth hormone treatment (20). Increased ability of liver ribosomes to bind aminoacyl-tRNA (67), increased RNA polymerase activity of rat liver nuclei (58, 59), increased ornithine decarboxylase activity (60, 61), and decreased activities of alanine-α-ketoglutarate and tyrosine-α-ketoglutarate transaminases (62, 63) all are manifested only after several hours of growth hormone treatment. Further studies are required to determine whether these effects of the hormone are mediated through its early action on amino acid transport, or whether other mechanisms are involved.

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