Regulation of Net Biosynthesis of Serum Albumin and Acute Phase Plasma Proteins

INDUCTION OF ENHANCED NET SYNTHESIS OF FIBRINOGEN, $\alpha_1$-ACID GLYCOPROTEIN, $\alpha_2$ (ACUTE PHASE)-GLOBULIN, AND HAPTOGLOBIN BY AMINO ACIDS AND HORMONES DURING PERFUSION OF THE ISOLATED NORMAL RAT LIVER

(Received for publication, June 16, 1969)

DAVID W. JOHN AND LEON L. MILLER

From the Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

SUMMARY

Livers isolated from normal, male, fed rats were perfused for 12 hours with bovine or rabbit red blood cells suspended in Krebs-Ringer-bicarbonate solution containing bovine serum albumin (3.0 g/100 ml) with L-lysine-l$^{14}$C and 500 mg of glucose continuously infused. Net synthesis of five specific plasma proteins was estimated by serological measurements of changes in their concentration in perfusate. The influence of insulin, cortisol, and growth hormone, and of a nutritionally complete mixture of amino acids on net synthesis of plasma proteins was evaluated. Control perfusions (no hormones or amino acids added) resulted in relatively linear net synthesis of the specific plasma proteins throughout the experiments. In experiments with full supplementation (insulin, cortisol, growth hormone, and the amino acid mixture) fibrinogen and haptoglobin synthesis increased 3-fold between 2 and 6 hours, $\alpha_1$-acid glycoprotein synthesis increased 3.5-fold between 4 and 8 hours, and $\alpha_2$ (acute phase)-globulin synthesis increased 11-fold between 8 and 10 hours. Increases in the latter four proteins were critically dependent upon the presence of cortisol and represented the first demonstration in vitro of hormone effects on net plasma protein synthesis. These effects may be important for understanding the increased blood levels of certain plasma $\alpha$ and $\beta$ globulins that occur in vivo in mammals after many forms of injury and may serve as a useful model for furthering our understanding of the influence of hormones and amino acids on protein synthesis.

Maximum net plasma protein synthesis occurred in perfusions with positive nitrogen balance (more net amino acid nitrogen loss from perfusate than urea nitrogen gain). Under the conditions employed the combination of amino acids and insulin was the minimal partial supplementation required for positive nitrogen balance. Additional data are presented on effects of amino acids and hormones on incorporation of L-lysine-l$^{14}$C into total plasma proteins and hepatic proteins, oxidation of L-lysine-l$^{14}$C to $^{14}$CO$_2$, production of bile, utilization of glucose, net urea synthesis, net changes in amino acid nitrogen, and on nitrogen balance as defined for the liver perfusion system.

Previous reports from this laboratory have described the use of the isolated rat liver perfused with heterologous blood in studies on net biosynthesis of the specific plasma proteins albumin, fibrinogen, $\alpha_1$-acid glycoprotein, and $\alpha_2$ (acute phase)-globulin (1, 2). More recently we have successfully extended the duration of routine liver perfusions to 12 hours (3). This change in technique has permitted us to simulate and define in vivo some of the hormonal, nutritional, and temporal factors which have been thought to play a role, whether direct or indirect, in the regulation of plasma protein biosynthesis in the intact animal.

In particular, we have examined the possibility that isolated livers from normal fed rats perfused under conditions favoring positive nitrogen balance (4) would show increased net synthesis of specific plasma proteins. For this purpose, glucose, a nutritionally complete amino acid mixture, growth hormone, insulin, and cortisol were added to 12-hour perfusions of livers from fed rats. Serological measurements of the plasma proteins named above and haptoglobin reveal 2-fold increases in rate of net synthesis of rat serum albumin. Of greater interest is the delayed onset (2 to 8 hours after start of perfusion) of striking increases in net synthesis of fibrinogen, $\alpha_1$-acid glycoprotein, $\alpha_2$ (acute phase)-globulin, and haptoglobin. The latter increases are critically dependent upon the presence of cortisol and appear to represent the first demonstration in vitro of induction (5) in net plasma protein synthesis. These results are pertinent for understanding the increased blood levels of certain
plasma α and β globulins ("acute phase" proteins) that occur in vivo in mammals after many forms of injury (6–8).

Also included are a series of experiments designed to identify the separate roles of the amino acid mixture and of the individual hormones in eliciting the above effects. Additional data is presented on effects of amino acids and hormones on incorporation of L-lysine-1-14C into total plasma proteins and hepatic proteins, oxidation of L-lysine-1-14C to 14CO2, production of bile, utilization of glucose, net urea synthesis, net changes in amino acid nitrogen, and on nitrogen balance as defined for the liver perfusion system (4).

METHODS

Perfusion Technique—Conditions were similar to those previously described for 12-hour experiments (3) except that in most experiments defibrinated rabbit blood was replaced by bovine red cells which were washed three times in sterile Ringer's solution and suspended in Krebs-Ringer-bicarbonate, containing bovine serum albumin (Armour, Fraction V) 3.0 g/100 ml. Because results of experiments with bovine red cells were unaltered by substitution of washed rabbit red cells (Table I, Column 9 versus Column 2), the results of several experiments done with rabbit red cells are included in Table I.

Liver donors were adult male rats of Sprague-Dawley (Holtzmann Rat Company, Madison, Wisconsin) strain, and weighed between 329 and 456 g. They were allowed tap water and commercial rat food (Purina Checkers) ad libitum at all times.

Hormone and Amino Acids—Insulin, generously supplied by the Eli Lilly Company, Indianapolis, was a glucagon-free, porcine, crystalline zinc insulin dissolved with the aid of 0.1 N HCl and diluted to volume with Ringer's solution. Growth hormone was a purified, lyophilized preparation of bovine growth hormone generously supplied by Armour and Company, Chicago (Lot M10810); it was dissolved in Ringer's solution, 1 mg per ml of sodium succinate derivative (Solu-Cortef, Upjohn Company). L-Amino acids were the purest commercially available (General Biochemicals). For each perfusion the perfusate containing the amino acids consisted of 30 ml of Krebs-Ringer-bicarbonate-albumin solution (adjusted to pH 7.4 with 1.0 N NaOH), 50 ml; glucose, 100 mg; heparin, 10,000 units; penicillin, 3,000 units; streptomycin, 3.0 mg; insulin, 5.1 units; bovine growth hormone, 0.5 mg; cortisol, 5.0 mg; and enough Ringer's solution to bring the volume to 100 ml. For these full supplementation experiments, 18 ml of Ringer's solution were infused for 12 hours at a constant rate (1.5 ml per hour) and contained: glucose, 500 mg; penicillin, 3,000 units; streptomycin, 3.0 mg; insulin, 5.1 units; bovine growth hormone, 0.5 mg; cortisol, 5.0 mg; and enough Ringer's solution to bring the volume to 100 ml. For these full supplementation experiments, 18 ml of Ringer's solution were infused for 12 hours at a constant rate (1.5 ml per hour) and contained: glucose, 500 mg; penicillin, 3,000 units; streptomycin, 3.0 mg; L-lysine-1-14C-HCl, 15 μCi (0.62 mg); insulin, 6.8 units; bovine growth hormone, 1.0 mg; cortisol, 5.0 mg; and the complete amino acid mixture, 320 mg. Control experiments were set up exactly as detailed above except hormones and amino acids were omitted (from both perfusates and infusions). Other experiments involved omitting the amino acid mixture or certain of the hormones or all of them. Throughout this paper when these supplements are mentioned the above doses and methods of administration are implied.

Serological Measurement of Individual Plasma Proteins—Specific plasma proteins synthesized by the rat liver and released into the perfusion medium were quantitatively measured in aliquots of perfusion plasma by the single radial diffusion technique of Mancini, Carbonara, and Heremans (11) as modified by Fahey and McKelvey (12). Details of the method and of the preparation of the pure rat antigens and rabbit antisera to RSA, fibrinogen, α1-acid glycoprotein, and α2 (α2-macroglobulin) have been described (2). Rat haptoglobin was prepared by the method of Lombard, Moretti, and Jayle (13) and was further purified, as we have described for RSA (1), by elution from preparative acrylamide gels after electrophoresis. This haptoglobin was used to immunize rabbits by a technique similar to that we have described for preparation of anti-α1-acid glycoprotein (2). The antihaptoglobin sera were pooled and tested by immunoelectrophoresis (14) against whole rat serum. Because the antisera reacted weakly with α1-acid glycoprotein as well as strongly with haptoglobin, it was adsorbed with α1-acid glycoprotein (2). Subsequent tests of the adsorbed antisera showed reaction only against haptoglobin. The antisera was stored frozen in small glass ampules until used.

Evaluation of α1-Acid Glycoprotein with Anti-α1-Acid Glycoprotein Serum (Darcy)—To examine the possibility that the α1-acid glycoprotein measured in our experiments (2, 3) might be the same as that described by Darcy (15–17), samples of our α1-acid glycoprotein and its antisemur (2), were tested with a sample of antiserum against the α1-acid glycoprotein of Darcy. Although the antisemur supplied by Dr. Darcy reacted weakly with several α-globulin components of normal rat serum, its predominant reaction was with an α-globulin showing migration on immunoelectrophoresis (14) similar to our α1-acid glycoprotein. The two antisera set up against our purified α1-acid glycoprotein gave a reaction of identity on Ouchterlony diffusion (18). On this basis our α1-acid glycoprotein is apparently identical with that investigated by Neuhau$ and Liu (19) and Weimer, Benjamin, and Darcy (20).

Other Methods—Procedures for measuring 14CO2 production, incorporation of lysine-1-14C into plasma total protein, liver protein, and bile, and for determining net changes in glucose, urea, and amino acid nitrogen have been described (1). Net changes in glucose and amino acid nitrogen include the amounts infused during the 12-hour duration of these experiments. The ninhydrin method used for measurement of amino acids determines α-amino acid nitrogen with leucine as a standard. Total amino acid nitrogen was approximated by multiplying the latter by a factor (1.50) obtained by dividing the total calculated amino acid nitrogen of the mixture by the total calculated α-amino (ninhydrin) nitrogen of the mixture.

Nitrogen balance for the perfusion system is computed as previously reported (4) by subtracting net change in urea nitrogen from net change in amino acid nitrogen in perfusate. Thus, 1

1 The abbreviation used is: RSA, rat serum albumin.
positive nitrogen balance occurs when net loss of amino acid nitrogen exceeds net gain of urea nitrogen.

RESULTS

Table I presents an overall summary of the data from 41 perfusions all of 12-hours duration. In general, results of perfusions with full supplementation (see "Methods") will be compared to results of control perfusions, and the data from perfusions with partial supplementation will be compared to these first two types.

Net Synthesis of Rat Serum Albumin—Net changes in RSA of perfusate plasma are shown in Fig. 1. Full supplementation results in an average rate of synthesis of RSA between the 2nd and the 8th hour that is almost twice that found in control experiments during that time. Table I indicates that addition of the three hormones without the amino acid mixture does not elicit increased RSA synthesis. The combination of amino acids plus insulin and cortisol, gives albumin synthesis indistinguishable from synthesis seen with full supplementation (Column 5 versus Column 2). Lesser increases in RSA synthesis seen with the amino acid mixture and either insulin or cortisol are not clearly different from RSA synthesis with amino acids alone. The combination of amino acids plus growth hormone and insulin (Table I, Column 5) evokes a small but significant increase in RSA synthesis over that found with amino acids plus insulin alone (Table I, Column 8).

Net Biosynthesis of Fibrinogen—Net increases in fibrinogen of perfusate plasma are displayed in Fig. 2. Except for a small increase between 4 and 6 hours, synthesis in control perfusions is approximately linear for the entire 12 hours. Although the synthesis rate in experiments with full supplementation is the same as that of control studies for the first few hours, between the 2nd and the 6th hour of perfusion it increases to approximately 3 times that of controls. Fully comparable increases with partial supplementation are found only when amino acids, insulin, and cortisol are added (Table I, Column 6). Addition of amino acids without hormones or with insulin or cortisol does not differ significantly from RSA synthesis with amino acids alone (Table I, Column 5). The partial supplementation conditions give intermediate results.

Net Biosynthesis of α1-Acid Glycoprotein—In unsupplemented control perfusions, changes in α1-acid glycoprotein of perfusate plasma (Fig. 3) indicate a low initial rate of synthesis with a small increase in rate between the 4th and the 8th hours and a return to the initial rate after 8 hours. The average rate of synthesis of this protein in experiments with full supplementation is the same as that of control perfusions for the first 4 hours, however, between 4 and 8 hours the average rate of synthesis increases to approximately 3.5 times that of controls. Of the four acute phase plasma proteins studied in this paper, α1-acid glycoprotein showed the most variability in response to full supplementation. The wide standard deviation of the experiments with full supplementation (Fig. 3) is the result of large inductions in two of the eight experiments and little or no difference from controls in two other experiments. Table I indicates that a similar effect was obtained with partial supplementation only with addition of amino acids plus insulin and cortisol. Results similar to or lower than control data were obtained under all supplementation conditions characterized by omission of cortisol. Supplementation with amino acids plus cortisol gave intermediate results (Column 7 versus Columns 1 and 2).

Net Biosynthesis of α1 (Acute Phase)-globulin—In control perfusions net changes in α1 (acute phase)-globulin of perfusate plasma (Fig. 4) show great variability during the first 6 hours, but a low and roughly linear synthesis is apparent after that time. In experiments with full supplementation, synthesis is the same as that seen in controls for the first 8 hours; however, during the next 4 hours there is a striking increase in average synthesis rate to approximately 11 times that observed in control perfusions. The influence of partial supplementation (Table I) is similar to that discussed above for α1-acid glycoprotein. Here again in addition to amino acids the presence of cortisol is indispensable for any increase in synthesis above control.

Net Biosynthesis of Haptoglobin—Net changes in haptoglobin of perfusate plasma are presented in Fig. 5. As noted above for fibrinogen and α1-acid glycoprotein a small increase in rate of synthesis in control perfusions between 4 and 8 hours is not sustained. The average rate of haptoglobin synthesis in experiments with full supplementation is similar to that of controls for the first few hours; but between 2 and 6 hours it increases sharply, and after 6 hours it is approximately 3 times that of control perfusions. Similar increases in haptoglobin synthesis are obtained by supplementation with amino acids, insulin, and cortisol, or with amino acids and cortisol alone (Table I). Partial supplementation conditions lacking cortisol do not produce results different from controls.

Lysine-1-14C Incorporation into Perfusate Plasma Total Protein—and Total Hepatic Protein after 12 Hours of Perfusion (Table D)—Since 320 mg of the amino acid mixture contained 35.9 mg of nonradioactive L-lysine, predictable specific activity differences prevent direct comparison between perfusions in which the amino acid mixture was added and those in which it was not present. A striking positive effect of hormones on incorporation of lysine-14C into perfusate plasma total proteins and into total hepatic protein is, however, apparent when controls are compared with experiments in which the three hormones are added without added amino acids (Column 1 versus Column 3). Further evidence of positive effects of hormones in the presence of added amino acids is also summarized in Table I (Column 2 versus Column 4).

In general, in perfusions with added amino acids gross changes in incorporation of lysine-14C into total plasma protein are in harmony with the observed changes in net synthesis of specific plasma proteins. Addition of amino acids, insulin, and cortisol results in 14C incorporation similar to that found with full supplementation; addition of amino acids and other hormone combinations results in incorporation variably greater than that obtained with added amino acids and no hormones (Columns 5, 7, and 8 versus Column 4).

Incorporation of L-lysine-1-14C into total hepatic protein in perfusions with added amino acids is also substantially increased by the mixture of three hormones. In each case where insulin is present, incorporation into liver protein is greater than that noted with controls (Columns 5, 6, and 8 versus Column 4). Only with the addition of cortisol alone is incorporation depressed below controls (Column 7 versus Column 4).

The absence of precise information concerning liver lysine-14C specific activities and pool sizes at best allows only presumptive gross inferences to be drawn with respect to correlations between lysine-14C incorporation and synthesis of liver and plasma proteins. Such presumptive correlation is supported by our finding that in experiments with added amino acids, positive over-all
### Table I

**Composite summary of hepatic protein synthetic and metabolic activity; net changes in 12 hours of perfusion.**

Columns 1 and 2 are averages and standard deviations. Columns 3 through 9 are averages; values in parentheses are ranges.

<table>
<thead>
<tr>
<th></th>
<th>1. Controls with no amino acids, growth hormone, insulin, or cortisol (6 experiments)</th>
<th>2. Full supplementation with amino acids, growth hormone, insulin, and cortisol (6 experiments)</th>
<th>3. Supplementation with growth hormone, insulin, or cortisol, but no amino acids (4 experiments)</th>
<th>4. Supplementation with amino acids, growth hormone, insulin, or cortisol (4 experiments)</th>
<th>5. Supplementation with amino acids, growth hormone, insulin, or cortisol, but no growth hormone (4 experiments)</th>
<th>6. Supplementation with amino acids, growth hormone, insulin, cortisol, but no growth hormone or insulins (4 experiments)</th>
<th>7. Supplementation with amino acids and insulin, but no growth hormone or cortisol (8 experiments)</th>
<th>8. Supplementation with amino acids and insulin, but no growth hormone or cortisol (8 experiments)</th>
<th>9. Rabbit red blood cells plus amino acids, growth hormone, insulin, or cortisol (4 experiments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSA (mg/300 cm³)</td>
<td>27.4 ± 4.9</td>
<td>45.9 ± 8.8</td>
<td>28.4</td>
<td>35.1</td>
<td>40.6</td>
<td>46.7</td>
<td>39.6</td>
<td>36.5</td>
<td>50.8</td>
</tr>
<tr>
<td>Fibrinogen (mg/300 cm³)</td>
<td>9.4 ± 2.4</td>
<td>19.9 ± 3.7</td>
<td>(14.9-32.3)</td>
<td>(15.7-23.3)</td>
<td>(10.9-16.3)</td>
<td>(12.1-14.0)</td>
<td>(13.9-21.9)</td>
<td>(12.1-18.0)</td>
<td>(8.4-13.3)</td>
</tr>
<tr>
<td>α-Acid glycoprotein (mg/300 cm³)</td>
<td>7.11.8</td>
<td>21.2 ± 10.1</td>
<td>(7.5-11.7)</td>
<td>(11.1-31.3)</td>
<td>(2.0-10.1)</td>
<td>(7.2)</td>
<td>(19.6)</td>
<td>(13.1)</td>
<td>(18.1)</td>
</tr>
<tr>
<td>Haptoglobin (mg/300 cm³)</td>
<td>9.6 ± 0.4</td>
<td>6.5 ± 1.2</td>
<td>(2.9-3.7)</td>
<td>(3.1-5.8)</td>
<td>(2.6-4.9)</td>
<td>(5.6-8.7)</td>
<td>(3.4-6.2)</td>
<td>(2.5-3.9)</td>
<td>(4.8-7.2)</td>
</tr>
<tr>
<td>¹⁴C activity in perfusate plasma total protein at 12 hrs (dpm/ml)</td>
<td>1258 ± 202</td>
<td>2666 ± 438</td>
<td>(1050-1400)</td>
<td>(2219-2902)</td>
<td>(1031-2104)</td>
<td>(1829)</td>
<td>(2600)</td>
<td>(1952)</td>
<td>(2317)</td>
</tr>
<tr>
<td>¹⁴C in total hepatic protein (% total ¹⁴C dose)</td>
<td>8.8 ± 2.0</td>
<td>10.6 ± 1.7</td>
<td>(6.8-10.8)</td>
<td>(6.9-12.8)</td>
<td>(14.7-17.0)</td>
<td>(9.1-12.5)</td>
<td>(11.8-14.2)</td>
<td>(10.7-13.1)</td>
<td>(11.9)</td>
</tr>
<tr>
<td>Perfusate amino acid nitrogen (mg)</td>
<td>+14.8 ± 2.1</td>
<td>+42.0 ± 2.9</td>
<td>(-44.9 to +39.1)</td>
<td>(+5.2 to +7.8)</td>
<td>(-45.7 to -25.2)</td>
<td>(-45.9 to -37.1)</td>
<td>(-46.8 to -38.1)</td>
<td>(-39.7 to -37.1)</td>
<td>(-44.7 to -38.6)</td>
</tr>
<tr>
<td>Bile amino acid nitrogen (mg)</td>
<td>1.5 ± 0.4</td>
<td>2.1 ± 0.6</td>
<td>(1.1-1.9)</td>
<td>(1.6-2.6)</td>
<td>(0.5-1.5)</td>
<td>(0.8-1.6)</td>
<td>(0.9-2.7)</td>
<td>(2.4-3.2)</td>
<td>(0.7-1.1)</td>
</tr>
<tr>
<td>Perfusate urea nitrogen (mg)</td>
<td>31.1 ± 3.0</td>
<td>27.6 ± 4.5</td>
<td>(28.3-34.1)</td>
<td>(28.3-32.1)</td>
<td>(18.7-20.9)</td>
<td>(24.8-43.7)</td>
<td>(31.9-35.2)</td>
<td>(24.3-39.4)</td>
<td>(46.6-75.1)</td>
</tr>
<tr>
<td>Bile urea nitrogen (mg)</td>
<td>2.0 ± 0.7</td>
<td>3.3 ± 1.1</td>
<td>(1.3-2.7)</td>
<td>(2.2-4.4)</td>
<td>(2.2-4.6)</td>
<td>(0.4-1.4)</td>
<td>(0.9-1.2)</td>
<td>(2.4-4.4)</td>
<td>(3.6-4.0)</td>
</tr>
<tr>
<td>Over-all nitrogen balance (perfusate and bile) (mg)</td>
<td>-49.4 ± 2.8</td>
<td>+9.0 ± 4.4</td>
<td>(-53.2 to -46.6)</td>
<td>(+4.0 to +14.3)</td>
<td>(-30.9 to -27.4)</td>
<td>(-17.6 to +1.8)</td>
<td>(0.0 to +10.8)</td>
<td>(+1.8 to +16.1)</td>
<td>(-11.6 to +26.1)</td>
</tr>
<tr>
<td>Glucose (mg)</td>
<td>-136 ± 133</td>
<td>-329 ± 133</td>
<td>(-259 to -77)</td>
<td>(-462 to -106)</td>
<td>(-292 to -186)</td>
<td>(-256 to -13)</td>
<td>(-380 to -105)</td>
<td>(-483 to -272)</td>
<td>(-80 to +106)</td>
</tr>
<tr>
<td>Bile volume (ml)</td>
<td>8.1 ± 1.5</td>
<td>14.6 ± 3.2</td>
<td>(6.6-9.6)</td>
<td>(11.4-17.8)</td>
<td>(12.1-18.3)</td>
<td>(2.8-7.4)</td>
<td>(4.8-6.0)</td>
<td>(12.9-17.7)</td>
<td>(5.7-7.5)</td>
</tr>
<tr>
<td>¹⁴C in bile (% total ¹⁴C dose)</td>
<td>1.3 ± 0.4</td>
<td>1.7 ± 0.5</td>
<td>(0.9-1.7)</td>
<td>(1.2-2.2)</td>
<td>(1.3-1.5)</td>
<td>(0.2-0.9)</td>
<td>(0.5-0.7)</td>
<td>(1.1-1.9)</td>
<td>(0.5-1.4)</td>
</tr>
</tbody>
</table>

By guest on August 28, 2017
nitrogen balance (see below) is seen in all cases where substantially increased lysine-\(^{14}\)C incorporation was observed. Conversely, the greatest negative nitrogen balance was observed in the experiments in which lysine-\(^{14}\)C incorporation is similar to or less than controls.

**Perfusate Amino Acid Nitrogen**—Amino acids arising from liver and blood proteins accumulate slowly in perfusate during the entire 12 hours of the control perfusions (Fig. 6). In experiments with full supplementation there is a significant net loss of amino acids from the perfusate which contrasts with the net gain observed in control experiments. Table I reveals that a large net loss of amino acids from perfusate is observed only when amino acids are added without hormones. Controls is apparently related to an effect of one or more of the three hormones added without amino acids (Table I, Column 7). Table I indicates that when amino acids are added, urea production as low as found in full supplementation experiments is elicited by addition of insulin alone or insulin plus cortisol; the combination of amino acids, growth hormone, and insulin results in production of slightly more urea than found with addition of amino acids and insulin alone (Table I, Column 5 versus Column 8). The combination of amino acids and cortisol results in far more urea synthesis than occurs when amino acids are added with no hormones.

**Total Urea Nitrogen of Bile** (Table I)—These values are all lower than 4 mg, and, as in the case of bile amino acid nitrogen described above, they tend to parallel the bile volumes.

**Nitrogen Balance**—Fig. 8 presents data on nitrogen balance of perfusate. Since bile was not sampled with each perfusate sample, losses of urea nitrogen and amino acid nitrogen in bile are not included in the figure. The rather small total losses in bile are, however, included in over-all nitrogen balance (perfusate and bile) of Table I. Control perfusions (Fig. 8) show an approximately negative nitrogen balance, while full supplementation results in a progressive positive nitrogen balance after 2 hours. Table I (Columns 3 and 4) indicates nitrogen balance less negative than that of controls if the three hormones are added without amino acids, and considerably less negative than that of controls if amino acids are added without hormones. Both amino acids and hormones are necessary, but neither alone is sufficient for positive nitrogen balance.

Table I reveals that positive nitrogen balance seen in full supplementation experiments is matched when amino acids and insulin are added, with or without growth hormone or cortisol. Because cortisol alone results in a strongly negative nitrogen balance (Table I, Column 7), it is striking that insulin plus cortisol produces a positive nitrogen balance equal to that seen with insulin alone.

Although highest values for net plasma protein synthesis are found in experimental groups with positive nitrogen balance (Table I, Columns 2, 6, and 9), magnitude of net synthesis of specific plasma proteins is not consistently correlated with overall positive nitrogen balance. For example, average synthesis of acute phase proteins is higher in experiments with added amino acids and cortisol (Table I, Column 7) with a strongly negative nitrogen balance, than in some experimental groups.
FIG. 4 (left). Cumulative average net change in rat α1 (acute phase)-globulin in perfusate per 300 cm² of body surface area of liver donor rat.

FIG. 5 (right). Cumulative average net change in rat haptoglobin in perfusate per 300 cm² of body surface area of liver donor rat.

with less negative nitrogen balance (Table I, Columns 3 and 4), or with positive nitrogen balance (Table I, Columns 5 and 8).

Glucose—Fig. 9 shows a sharp increase in average net perfusate glucose during the 1st hour in control experiments which is not seen in full supplementation experiments. Controls then show a decrease from 2 to 8 hours; thereafter there is a more rapid net loss similar in rate to that of the full supplementation experiments. The data of Table I indicate that in all experiments in which insulin was used, net change in glucose approximates the losses seen in experiments with full supplementation. A possible explanation for this is presented in the discussion. Supplementation with amino acids alone results in net loss of glucose not different from that of controls (Table I, Column 4 versus Column 1). Addition of amino acids and cortisol produces almost no over-all net change in glucose.

Bile Volume (Table I)—Bile volume is considerably larger in experiments with full supplementation than in controls. The average volume of bile is not influenced by growth hormone and insulin (Column 5 versus Column 4), doubled by cortisol (Column 7 versus Column 4), and tripled by cortisol plus insulin (Column 6 versus Column 4).

Percentage of Lysine-14C Dose in Bile (Table I)—14C activity in bile varies directly with bile volume and seems not to be influenced specifically by any of the variables studied.

Oxidation of Lysine-14C to 14CO2—Percentage of the total dose of lysine-14C converted to 14CO2 varied between 13.3 and 24.8% for the 41 perfusions with no significant differences between any of the experimental groups and the controls.

DISCUSSION

Increase in rate of net synthesis of plasma proteins occurring during perfusions has been referred to in this report as induction. This term is used in the sense discussed in detail by Greengard (5) to mean an increase in the amount of specific proteins due to increases in the ratios of their synthesis and degradation rates without implication of any specific mechanism by which this might occur. It is likely that the increases reported herein are primarily the result of more rapid synthesis, because suppression of the relatively low rate of plasma protein catabolism known to occur in isolated liver perfusions (1, 21) would be inadequate to account for the large changes found. Neuhaus, Bologo, and Chandler (22) have used the term “induction” to describe increases in synthesis of the seromucoid plasma protein fraction (6, 23) after injury of rats by laparotomy. The seromucoid fraction is known to contain two of the acute phase proteins studied in the present report, α1-acid glycoprotein and haptoglobin (19).

To explain increases in hepatic synthesis of acute phase plasma proteins after injury a number of authors have conjectured that “humoral factors” are released from damaged cells, circulate in the blood, and then act on the liver (16, 22, 24). Although two groups have reported apparent increase in rate of synthesis of acute phase plasma proteins when livers isolated from normal rats were perfused with blood from acutely injured rats (25, 26) subsequent investigations (27, 28) including unpublished studies in our laboratory have not confirmed these observations. Thus,
until now, clear and reproducible increases in rate of plasma protein synthesis occurring during perfusions of isolated rat livers have not been reported.

Demonstration of induced increases in net synthesis of specific plasma proteins reported herein has depended upon the following specific conditions. (a) Liver donors were screened before perfusions and were used only if the plasma level of $\alpha_1$-acid glycoprotein was not abnormally elevated. This minimized the possibility that pre-existing illness or trauma may have stimulated an acute phase response. This possibility was again reduced at time of surgery by careful examination of liver donors for gross evidence of disease, and by serological measurement of five specific plasma proteins in a sample from the liver donor to detect changes consistent with injury or illness (6, 7). These procedures have helped assure relatively stable, low base-line synthesis of acute phase proteins in the early hours of all experiments (Figs. 2 through 5) against which increases can be easily observed. (b) The 12-hour duration of the perfusions has also favored demonstration of inductive changes. Figs. 2 through 5 indicate that differences between full supplementation experiments and controls are far less apparent at 6 hours than by 10 to 12 hours. (c) The use of fed liver donors appears to be important in the demonstration of significant induction of some of the acute phase proteins. Unpublished results of a series of four full supplementation perfusions of livers from rats fasted 18 hours indicate less definite induction of fibrinogen and $\alpha_1$ (acute phase)-globulin; for haptoglobin and $\alpha_1$-acid glycoprotein induction is similar to that seen with livers from fed donors. (d) Addition of the amino acid mixture is essential. (e) Hormones, especially the combination of insulin and cortisol, are important.

The following are some indications that the inductive changes described here for the isolated liver are comparable to increases seen in vivo in rate of hepatic synthesis of acute phase proteins after injury. (a) The timing of increases in rate of synthesis of acute phase proteins during perfusions (Figs. 2 through 5) is consistent with some observations of Neuhaus et al. (22) on induction in vivo after laparotomy in rats. Our observation that none of the four acute phase proteins is induced before 2 hours of perfusion parallels their finding that actinomycin C administered up to 2 hours after injury blocked an expected increase in synthesis of acute phase proteins. (b) There is little data from experiments in vivo on how soon after injury the increase in hepatic protein synthesis of specific plasma proteins increases, but early increases in synthesis of $\alpha_1$-globulins and fibrinogen in rats 4 to 9 hours after injury are consistent with this time elapsing in our perfusions before induction. (c) Other points of similarity with the acute phase response in vivo are that all four acute phase proteins are induced, and that increased rates of synthesis of these proteins roughly match rates we have reported for the same proteins during isolated perfusions of livers from rats injured in vivo (2, 29).

Although a number of factors discussed above are required for maximal induction of acute phase plasma protein synthesis in vivo, cortisol is essential. Cortisol seems to be required for induction of at least one of the acute phase proteins in vivo in that there is little response of $\alpha_1$ (acute phase)-globulin in injured, adrenalectomized rats unless the animals are injected with cortisol (30, 31). On the other hand, high blood concentration of $\alpha_1$-acid glycoprotein with enhanced response to injury (17) and increased rate of incorporation of leucine-$\text{H}^4$C into $\alpha_1$-acid glycoprotein (15-17, 28) have been observed in adrenalectomized rats.

At present the mechanism by which cortisol increases the rates of hepatic synthesis of acute phase proteins is obscure; the timing and relative magnitude of the changes may be compared with those produced by cortisol in the induction of the hepatic enzymes tryptoaphan pyrrolase and tyrosine transaminase, both in vivo (32, 33), and in the isolated perfused rat liver (34, 35). Because actinomycin C blocks the induction of these enzymes by cortisol, and has been reported to block the acute phase response to injury in rats (22), it has been suggested (35, 36) that cortisol acts somehow by enhancing transcription of DNA to produce more functional messenger RNA for the synthesis of the specific proteins in question.

Chronic administration of cortisol to intact rats causes loss of protein from skeletal muscle and gain of protein by the liver (37). It is not clear whether the latter is related more to increased flow of amino acids from skeletal muscle breakdown or to a direct effect on cortisol on hepatic protein synthesis. Experiments reported herein show that in perfusions supplemented with amino acids addition of cortisol (Table I, Column 7 versus Column 4) is associated with decreased incorporation of lysine-$\text{H}^4$C into total hepatic protein, with decreased loss of amino acids from perfusate, and with increased production of urea resulting in more negative nitrogen balance. This suggests that increased hepatic protein synthesis in cortisol-treated rats cannot be solely the result of a direct action of cortisol on the liver and favors the suggestion of Goodlad and Munro (38) that increases in hepatic protein in cortisol-treated animals can be attributed in part to the additional supply of amino acids coming from the carcass. Probably administration of cortisol to intact rats also elicits increased secretion of insulin, secondary to elevated blood levels of amino acids and glucose, and the combination of insulin and cortisol favors hepatic protein synthesis as in the liver perfusions presented here.

In general several actions of the combination of insulin and cortisol are different from those that might be predicted from actions of the individual hormones. Although insulin alone elicits no increase in net synthesis of four specifically measured plasma proteins, and cortisol alone elicits only moderate increases in acute phase proteins, the combination of insulin and cortisol enhances increases of four of five proteins specifically measured (Table I, Columns 8 and 7 versus Column 6); haptoglobin synthesis is as high with cortisol alone as with cortisol plus insulin.

Noteworthy is the observation that insulin alone enhances lysine-$\text{H}^4$C incorporation in hepatic total protein without major increase in net synthesis of specific plasma proteins (Table I, Column 8 versus Column 4). In contrast, cortisol alone decreases lysine-$\text{H}^4$C incorporation into hepatic protein yet increases net synthesis of acute phase proteins (Column 7 versus Column 4). These contrasting results suggested the possibility that hepatic plasma protein synthesis was stimulated by insulin, but that release of plasma proteins from the liver was favored by cortisol. If this were the case, more of the specific plasma proteins would remain in the liver at the end of the insulin experiments than at the end of those with cortisol; to evaluate this, liver homogenates prepared at the close of those perfusions were examined with our quantitative serological method for $\alpha_1$-acid glycoprotein, $\alpha_1$ (acute phase)-globulin, and haptoglobin. Since no significant differences were found, it seems more likely that the data reflect
Different effects of the hormones on hepatic and on plasma protein synthesis, rather than on the mechanism of release of plasma proteins.

Liver from partially pancreatectomized rats have shown increased incorporation of leucine-1-14C into hepatic protein when insulin was added to incubated liver slices (39) or to isolated liver perfusions (40). The present report, however, appears to present the first published data showing insulin-induced stimulation of radioactive amino acid incorporation into hepatic protein during isolated perfusion of livers from normal rats. This may be due to the long duration of the experiments which allows residual effects of insulin from the liver donor to diminish in control perfusions, but not in those supplemented with insulin.

Cortisol causes changes in perfusate amino acid loss and urea nitrogen formation that result in severely negative nitrogen balance; insulin causes positive nitrogen balance and, surprisingly, their combination results in equally positive nitrogen balance. Insulin causes considerable net disappearance of glucose, and cortisol causes little net change in glucose, yet their combination is associated with a net loss of glucose that is greater than that produced by insulin alone. The larger average net loss of glucose in all perfusions supplemented with insulin (Table I, Column 2) shows essentially no difference from full supplementation (Table I, Column 6) and indicates that the effects of cortisol may be a consequence of the residual effects of insulin from the liver donor to diminish with an adequate period of time. Data presented here indicate the powerful protein anabolic action of the combination of insulin and cortisol, and suggest that administration of insulin or oral hypoglycemic agents to patients receiving cortisol might alleviate some of these effects.

Acknowledgments—We wish to express our appreciation to Dr. Kimball B. Temple, Strong Memorial Hospital, Rochester, New York, for assistance in the preparation of rat haptoglobin, and to Dr. Douglas A. Darcy, Chey, Beatty Research Institute, London, England, for the sample of his anti-al-acid glycoprotein antiserum. We thank Donna M. Eddy, Louise Finch, Catherine S. Plane, Drusilla Wyemott, and Janice White for their invaluable technical assistance, and Leon Schwartz and Gerald Cooper for their preparation of the illustrations.

REFERENCES

Regulation of Net Biosynthesis of Serum Albumin and Acute Phase Plasma Proteins: INDUCTION OF ENHANCED NET SYNTHESIS OF FIBRINOGEN, α1-ACID GLYCOPROTEIN, α2 (ACUTE PHASE)-GLOBULIN, AND HAPTOGLOBIN BY AMINO ACIDS AND HORMONES DURING PERFUSION OF THE ISOLATED NORMAL RAT LIVER

David W. John and Leon L. Miller

J. Biol. Chem. 1969, 244:6134-6142.

Access the most updated version of this article at http://www.jbc.org/content/244/22/6134

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/244/22/6134.full.html#ref-list-1