Effects of Hypothyroidism, Hyperthyroidism, and Thyroxine on Net Synthesis of Plasma Proteins by the Isolated Perfused Rat Liver

MODULATION OF THE RESPONSE TO INSULIN PLUS CORTISOL IN THE NET SYNTHESIS OF ALBUMIN, FIBRINOGEN, $\alpha_1$-ACID GLYCOPROTEIN, $\alpha_2$-(ACUTE PHASE) GLOBULIN, AND HAPTOGLOBIN*

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SUMMARY

The isolated rat liver perfused for 12 hours has been used to study effects of added thyroxine and effects of hyperthyroidism and hypothyroidism in the liver donor on net biosynthesis of five specific plasma proteins, namely, rat serum albumin, fibrinogen, $\alpha_1$-acid glycoprotein, $\alpha_2$-(acute phase) globulin, and haptoglobin. Hypothyroidism was produced either by surgical thyroidectomy or by feeding methimazole (0.5 g per kg) in the powdered diet; hyperthyroidism was induced by injecting normal rats with thyroxine (10 $\mu$g/100 g body weight subcutaneously per day). The metabolic status of each animal was confirmed by $O_2$ consumption measurements. The basic perfusate consisted of washed bovine red cells suspended in Krebs-Ringer-bicarbonate solution containing bovine serum albumin (3.0 g/100 ml) to which was added nutritional (glucose, amino acids) and hormonal (cortisol, insulin) constituents. When used, thyroxine was added along with the other hormones at the start of perfusion as well as by constant infusion throughout the experiment.

Livers from hyperthyroid liver donors synthesized significantly more albumin than those from hypothyroid rats; however, addition of thyroxine to the perfusion of livers from euthyroid and hyperthyroid rats decreased albumin synthesis. Fibrinogen synthesis was not altered significantly among the experimental groups studied here, nor was there any change in the phenomenon of induction, shown previously to be dependent upon the presence of cortisol. Synthesis of $\alpha_1$-acid glycoprotein and $\alpha_2$-(acute phase) globulin was somewhat higher by livers from hypothyroid rats than by those from euthyroid controls, and maximum rates occurred in hyperthyroid livers given a total of 4.0 $\mu$g of thyroxine. Haptoglobin synthesis was greater by livers from hyperthyroid rats than by those from normal and hypothyroid donors; the addition of 4.0 $\mu$g of thyroxine to the perfusate of normal and hyperthyroid livers increased haptoglobin synthesis while 22.0 $\mu$g caused a decrease.

Nitrogen balance was estimated for the perfusion system in terms of net cumulative changes in amino acid nitrogen and urea nitrogen in the perfusate and bile. Hyperthyroidism in the liver donor and 4.0 $\mu$g of thyroxine in the perfusate resulted in negative nitrogen balance, simultaneously with maximum uptake of glucose and increased oxidation of L-[1-14C]lysine to $^{14}$CO$_2$. Positive nitrogen balance was not a requirement for normal or enhanced net synthesis of specific plasma proteins; however, under conditions of extremely negative nitrogen balance, synthesis of acute phase proteins was impaired.

Limited data on the dose-response relationship between thyroxine added to the perfusate and the metabolic response of the liver revealed that the addition of 2.0 $\mu$g of thyroxine at zero time plus the continuous infusion of an additional 2.0 $\mu$g over a 12-hour period elicited optimal anabolic responses.

Previous reports from this laboratory (1–6) have described effects of nutritional and hormonal factors on net synthesis of specific plasma proteins by the isolated rat liver perfused with heterospecies blood. The present study seeks further to elucidate some of the actions and interactions of hormones on net plasma protein synthesis by the isolated perfused rat liver. We have examined the effects of thyroid hormone on the liver without attempting to designate a specific mechanism or site of action. This report describes: (a) effects of hypothyroidism or hyperthyroidism in liver donors on net synthesis of rat serum albumin, fibrinogen, $\alpha_1$-acid glycoprotein, $\alpha_2$-(acute phase) globulin, and haptoglobin by the isolated perfused liver, (b) effects of adding thyroxine in vitro along with glucose, a complete amino acid mixture, insulin, and hydrocortisone to the medium-perfusing livers from normal and hyperthyroid rats. In addition,
data are included on the incorporation of L-[1-14C]lysine into hepatic proteins, oxidation of L-[1-14C]lysine to 14CO2, production of bile, glucose utilization, net urea synthesis, net changes in amino acid nitrogen, and over-all nitrogen balance as defined for the perfused liver system (4). Because of the known biphasic effects with increasing dose of thyroxine, a subarbitrate study was carried out to establish the dose of thyroxine necessary for maximum anabolic activity in the perfused liver.

**METHODS**

**Liver Donors**—Rats were adult males of Sprague-Dawley (Holstman) strain and weighed between 350 and 450 g. Hyperthyroidism was induced either by surgical thyroidectomy or by feeding methimazole (Eli Lilly-Tapazole), 0.5 g per kg of powdered Purina chow diet for 14 to 20 days. Preliminary experiments indicated that methimazole treatment of the liver donor or of the perfused normal liver had no adverse effect on the parameters of liver metabolism studied here, therefore Group 2 below is a composite of both surgically thyroidectomized and methimazole-treated liver donors. Hyperthyroidism was induced in normal animals by subcutaneous daily injection of 10 μg of thyroxine (Sigma) per 100 g body weight for 10 days. Food and tap water were available ad libitum until the day of killing. As a result of this treatment, a significant increase in albumin with no change in the other plasma proteins studied was noted.

**Perfusion Technique**—The perfusion apparatus, operative technique, and general conditions were the same as those described previously for 12-hour experiments (4). Typically the perfusate consisted of 38 ml of washed rabbit or bovine red cells suspended in 50 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3 g of bovine serum albumin (Armour Fraction V) per 100 ml. The basic starting perfusate also contained: glucose, 100 mg; heparin, 10,000 units; penicillin, 3,000 units; streptomycin, 3.0 mg; and enough Ringer solution to bring the total volume to 100 ml. Cortisol (5.0 mg), insulin (5.0 units), and thyroxine (0.2 to 2.0 μg) were added to the perfusate immediately after the initial sample at the outset of perfusion. The pH of the perfusate was maintained at 7.40 by a Radiometer titrator (type TTT1d; Copenhagen) equipped with a combined glass calomel electrode (type GK2026C) and an autoburette (type ABU 12) controlling an infusion of 0.75 milliunits NaHCO3 solution.

Table I indicates the treatment status of the liver donor, the quantity of thyroxine added, and time of addition. In all perfusions a total of 18.0 ml of Ringer solution was infused at a constant rate over the 12-hour period and contained: glucose, 500 mg; penicillin, 3,000 units; streptomycin, 3.0 mg; L-[1-14C]lysine HCl, 15 μCi (0.65 mg); hormones (6.8 units of insulin, 5.0 mg of cortisol); a complete amino acid mixture, 320 mg; and 1.0 N NaOH to adjust the pH to 7.40. The composition of the amino acid mixture has been detailed in earlier papers (4–6); it contained only pure L-amino acids obtained from General Biochemicals.

**Serological Measurement of Individual Plasma Proteins**—Specific plasma proteins synthesized by the rat liver and released into the perfusion medium over the 12-hour duration of all experiments were quantitatively measured in aliquots of the perfusate plasma by the single radial diffusion technique of Mancini et al. (8) as modified by Fahey and McKelvey (9). Details of the methods of preparation of the pure rat plasma protein antigens and rabbit antisera have been described by John and Miller (10).

**Other Methods**—Procedures for measuring 14CO2 production, incorporation of L-[1-14C]lysine into liver protein and bile, and for determining net changes in glucose, urea, and amino acid nitrogen have been described (11). Nitrogen balance for the perfusate is computed as previously reported (4) by subtracting net cumulative increase in urea nitrogen from net cumulative decrease of amino acid nitrogen in the perfusate. Thus positive perfusate nitrogen balance occurs when net loss of amino acid nitrogen from perfusate exceeds net gain of urea nitrogen. In addition to computing perfusate nitrogen balance on sequential samples, over-all nitrogen balance was determined at the end of each experiment by subtracting bile urea and amino acid nitrogen from the final perfusate nitrogen balance.

**RESULTS**

Table I presents an over-all summary of the data from 33 perfusions of 12-hour duration with perfusions in Group 1 serving as a general reference for comparison. The perfusate for this group, designated "full supplementation" in previous papers (4, 5), contained glucose, amino acids, insulin, and cortisol. Comparisons are made between groups of normal, hyperthyroid, and hyperthyroid liver donors, and with thyroxine present or absent in the perfusate. Table I includes the mean, standard deviation, and range of final values for each parameter measured in each group. Figs. 1 through 5 illustrate the course of net synthesis of serum albumin, fibrinogen, α1-acid glycoprotein, α2-(acute phase) globulin, and haptoglobin as well as the induction of four specific acute phase plasma proteins (fibrinogen, α1-acid glycoprotein, α2-(acute phase) globulin, haptoglobin).

**Net Biosynthesis of Rat Serum Albumin**—In addition to the final net cumulative synthesis of albumin for all groups presented in Table I, the time course of net changes in rat serum albumin of perfusate plasma for five groups are shown in Fig. 1. It can be seen that under conditions of adequate nutritional and hormonal supplementation livers from hyperthyroid donors (Groups 3) synthesized significantly more rat serum albumin than those from hypothyroid rats (Group 2), but not significantly more than normal livers (Group 1). It is apparent from these data that rat serum albumin synthesis was not substantially altered by the treatments which resulted in significant changes in the rate of synthesis of α1-acid glycoprotein, α2-(acute phase) globulin, and haptoglobin. However, it is noteworthy that for Groups 1, 3, 5, and 6 the rate of synthesis of albumin is roughly linear from the 2nd to the 6th or 8th hour; thereafter, the rate of synthesis decreases perceptibly for those groups in which marked induction of increased rates of synthesis of the acute phase proteins was observed.

**Net Biosynthesis of Fibrinogen**—Although Table I and Fig. 2 do not clearly indicate a significant difference between any treatment groups, Fig. 2 is included to show that the phenomenon of induction is unaltered and that between the 2nd and 6th hour of perfusion the rate of synthesis of fibrinogen increased approximately 3-fold.

**Net Biosynthesis of α1-Acid Glycoprotein**—Table I and Fig. 3 illustrate that synthesis of α1-acid glycoprotein was maximum by livers from hyperthyroid donors given 4.0 μg of thyroxine in the perfusate (Group 6). These livers synthesized significantly
TABLE 1

Composite Summary of Protein Synthesis and Metabolic Activity; Net Changes in 12 Hours of Perfusion

<table>
<thead>
<tr>
<th>Treatment Status of Liver Donor</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine Added (μg)</td>
<td></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>Stat</td>
<td>Infused</td>
<td>Stat</td>
<td>Infused</td>
<td>Stat</td>
</tr>
<tr>
<td>WGA (mg/300 cm²)</td>
<td></td>
<td>45.6 ± 9.0</td>
<td>33.4 ± 3.8</td>
<td>49.9 ± 7.8</td>
<td>37.9 ± 7.5</td>
<td>40.0 ± 2.8</td>
<td>14.4 ± 6.9</td>
<td>20.7 ± 2.1</td>
<td>40.4 ± 5.3</td>
</tr>
<tr>
<td>(33.2 to 56.0)</td>
<td></td>
<td>(28.6 to 38.0)</td>
<td>(41.0 to 34.1)</td>
<td>(28.8 to 46.2)</td>
<td>(37.2 to 40.8)</td>
<td>(31.3 to 45.6)</td>
<td>(28.3 to 32.2)</td>
<td>(33.9 to 44.4)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/300 cm²)</td>
<td></td>
<td>20.4 ± 3.8</td>
<td>18.0 ± 2.0</td>
<td>20.0 ± 3.9</td>
<td>14.8 ± 5.3</td>
<td>17.4 ± 0.9</td>
<td>21.3 ± 3.6</td>
<td>14.5 ± 1.6</td>
<td>14.2 ± 5.0</td>
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<td></td>
<td>(16.5 to 20.9)</td>
<td>(16.4 to 24.1)</td>
<td>(9.0 to 21.4)</td>
<td>(16.5 to 18.1)</td>
<td>(15.7 to 24.3)</td>
<td>(12.6 to 15.6)</td>
<td>(8.2 to 19.6)</td>
<td></td>
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<tr>
<td>α₂-Acid Glycoprotein (mg/300 cm²)</td>
<td></td>
<td>13.7 ± 5.2</td>
<td>17.4 ± 2.8</td>
<td>24.6 ± 10.4</td>
<td>10.1 ± 3.8</td>
<td>21.4 ± 3.8</td>
<td>31.1 ± 9.3</td>
<td>17.2 ± 0.2</td>
<td>5.8 ± 2.2</td>
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<td>(7.2 to 22.1)</td>
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<td>(14.6 to 20.2)</td>
<td>(12.6 to 31.1)</td>
<td>(5.0 to 14.5)</td>
<td>(17.5 to 25.2)</td>
<td>(17.9 to 36.9)</td>
<td>(16.9 to 17.4)</td>
<td>(4.0 to 8.9)</td>
<td></td>
</tr>
<tr>
<td>Globulin (units/300 cm²)</td>
<td></td>
<td>2441 ± 177</td>
<td>670 ± 183</td>
<td>380 ± 356</td>
<td>278 ± 308</td>
<td>379 ± 179</td>
<td>1161 ± 118</td>
<td>476 ± 52</td>
<td>143 ± 307</td>
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<td>(194 to 715)</td>
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<td>(407 to 824)</td>
<td>(0 to 704)</td>
<td>(0 to 809)</td>
<td>(419 to 778)</td>
<td>(734 to 1427)</td>
<td>(429 to 531)</td>
<td>(0 to 638)</td>
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<tr>
<td>Haptoglobin (mg/300 cm²)</td>
<td></td>
<td>7.0 ± 0.7</td>
<td>5.3 ± 0.8</td>
<td>12.1 ± 3.0</td>
<td>5.5 ± 1.7</td>
<td>10.1 ± 0.7</td>
<td>11.8 ± 1.0</td>
<td>9.7 ± 1.6</td>
<td>3.7 ± 1.1</td>
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<td>(3.9 to 9.7)</td>
<td></td>
<td>(4.8 to 6.5)</td>
<td>(9.2 to 15.1)</td>
<td>(3.8 to 7.3)</td>
<td>(9.5 to 10.8)</td>
<td>(9.3 to 13.8)</td>
<td>(8.6 to 11.5)</td>
<td>(2.3 to 4.8)</td>
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</tr>
<tr>
<td>L-lysine-1-¹⁴C - Infused</td>
<td></td>
<td>-46.2 ± 1.0</td>
<td>-43.9 ± 3.3</td>
<td>-43.1 ± 3.3</td>
<td>-45.1 ± 3.1</td>
<td>-45.3 ± 0.7</td>
<td>-41.7 ± 1.2</td>
<td>-46.2 ± 0.3</td>
<td>-43.3 ± 2.3</td>
</tr>
<tr>
<td>Nitrogen (mg)</td>
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<td>(-47.7 to -44.9)</td>
<td>(-47.5 to -42.2)</td>
<td>(-46.1 to -43.3)</td>
<td>(-46.1 to -43.3)</td>
<td>(-46.0 to -44.6)</td>
<td>(-43.2 to -40.5)</td>
<td>(-44.5 to -44.0)</td>
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<tr>
<td>Bile Amino Acid</td>
<td></td>
<td>1.9 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>3.3 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>3.0 ± 1.0</td>
<td>1.6 ± 0.3</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Nitrogen (mg)</td>
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<td>(1.3 to 2.6)</td>
<td>(0.3 to 0.9)</td>
<td>(3.0 to 3.6)</td>
<td>(1.7 to 2.4)</td>
<td>(1.3 to 2.7)</td>
<td>(2.0 to 4.4)</td>
<td>(1.2 to 1.9)</td>
<td>(1.3 to 2.1)</td>
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<tr>
<td>Perfusate Urea Nitrogen (mg)</td>
<td></td>
<td>37.3 ± 4.7</td>
<td>34.0 ± 4.5</td>
<td>43.2 ± 5.4</td>
<td>43.4 ± 3.3</td>
<td>39.9 ± 3.1</td>
<td>46.9 ± 4.4</td>
<td>38.9 ± 0.6</td>
<td>62.4 ± 6.1</td>
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<td>(34.3 to 46.6)</td>
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<td>(30.5 to 40.6)</td>
<td>(39.3 to 49.4)</td>
<td>(40.1 to 48.5)</td>
<td>(38.8 to 43.0)</td>
<td>(43.5 to 53.4)</td>
<td>(38.2 to 39.6)</td>
<td>(34.4 to 69.0)</td>
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<tr>
<td>Bile Urea Nitrogen (mg)</td>
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<td>3.9 ± 0.7</td>
<td>0.9 ± 0.5</td>
<td>5.8 ± 0.9</td>
<td>4.7 ± 1.0</td>
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<td>6.6 ± 2.0</td>
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<td>(3.0 to 5.2)</td>
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<td>(0.4 to 1.4)</td>
<td>(4.8 to 6.3)</td>
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<td>(4.0 to 5.8)</td>
<td>(4.6 to 8.8)</td>
<td>(2.7 to 4.2)</td>
<td>(2.5 to 5.1)</td>
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</tr>
<tr>
<td>Overall Nitrogen Balance (Perfusate and Bile) (mg)</td>
<td></td>
<td>2.9 ± 3.3</td>
<td>8.5 ± 2.2</td>
<td>-9.2 ± 9.7</td>
<td>-5.0 ± 4.7</td>
<td>-1.4 ± 2.8</td>
<td>-14.9 ± 7.2</td>
<td>0.3 ± 1.1</td>
<td>-24.7 ± 4.5</td>
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<tr>
<td>(4.1 ± 1.5)</td>
<td></td>
<td>(5.5 ± 1.5)</td>
<td>(-10.4 to 7.1)</td>
<td>(-11.9 to -0.1)</td>
<td>(4.2 ± 1.4)</td>
<td>(-25.1 ± 9.0)</td>
<td>(1.0 ± 1.1)</td>
<td>(26.0 ± 18.2)</td>
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<tr>
<td>Glucose (mg)</td>
<td></td>
<td>-390 ± 49</td>
<td>-257 ± 52</td>
<td>-514 ± 92</td>
<td>-289 ± 89</td>
<td>-360 ± 64</td>
<td>-520 ± 61</td>
<td>-324 ± 22</td>
<td>-649 ± 65</td>
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<tr>
<td>(446 to -305)</td>
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<td>(-319 to -212)</td>
<td>(-585 to -410)</td>
<td>(-427 to -204)</td>
<td>(-432 to -305)</td>
<td>(-619 to -483)</td>
<td>(-338 to -298)</td>
<td>(-513 to -371)</td>
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<tr>
<td>Bile Volume (ml)</td>
<td></td>
<td>13.2 ± 2.5</td>
<td>3.6 ± 1.9</td>
<td>17.6 ± 0.7</td>
<td>12.5 ± 1.9</td>
<td>15.0 ± 2.3</td>
<td>17.6 ± 3.5</td>
<td>11.6 ± 2.0</td>
<td>9.4 ± 1.6</td>
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<tr>
<td>(9.2 to 16.7)</td>
<td></td>
<td>(1.7 to 6.2)</td>
<td>(16.9 ± 18.4)</td>
<td>(11.2 to 15.6)</td>
<td>(12.7 to 27.4)</td>
<td>(13.4 to 20.7)</td>
<td>(9.5 to 13.6)</td>
<td>(7.4 to 11.1)</td>
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</tr>
<tr>
<td>¹⁴CO₂ Production (mg/300 cm²)</td>
<td></td>
<td>7560 ± 1720</td>
<td>6650 ± 670</td>
<td>7690 ± 1780</td>
<td>8920 ± 730</td>
<td>8980 ± 500</td>
<td>10,040 ± 1040</td>
<td>9920 ± 180</td>
<td>11,790 ± 510</td>
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<tr>
<td>(5090 to 9430)</td>
<td></td>
<td>(5830 to 7400)</td>
<td>(6150 to 9520)</td>
<td>(7960 to 9800)</td>
<td>(8690 to 9710)</td>
<td>(8870 to 11,370)</td>
<td>(9720 to 10,060)</td>
<td>(9,080 to 12,290)</td>
<td></td>
</tr>
</tbody>
</table>

a) Number of replicates.

b) Mean ± standard deviation. Ranges in parentheses.

c) L-lysine-1-¹⁴C - HCl, 15 μCi (0.62 mg), added by constant infusion over 12 hours.
more α₁-acid glycoprotein than livers from normal (Group 1) and hypothyroid (Group 2) rats. Induction of an increased rate of synthesis of α₁-acid glycoprotein occurred by the 4th hour, and although the newly established rate was different for some treatment groups, the rate for any one group remained almost constant for 8 hours after induction. The rate of synthesis after induction ranged from approximately 1.5 (Group 1) to 3.5 (Group 6) times the original rate. Effects of hyperthyroidism in the liver donor and 4.0 μg of thyroxine in the perfusate (Group 6, Table I) were additive in causing increased α₁-acid glycoprotein synthesis. However, 22.0 μg of thyroxine in the perfusate of normal livers (Group 4), or in hyperthyroid livers with a simultaneous reduction in the dose of cortisol and insulin in the perfusate (Group 8, Table I) acted to attenuate induction.

Net Biosynthesis of α₁-Acute Phase Globulin—The over-all pattern of induction and synthesis of α₁-(acute phase) globulin shown in Fig. 4 was qualitatively similar to that of α₁-acid glycoprotein with some important exceptions. Synthesis of α₁-(acute phase) globulin by hyperthyroid livers given 4.0 μg of thyroxine in the perfusate (Group 6) was maximum and significantly greater than that of all other groups except hypothyroid livers (Group 2). However, hyperthyroidism (Group 3) alone caused no change in α₁-(acute phase) globulin synthesis as did the addition of 22.0 μg of thyroxine to the perfusate of normal livers (Group 5) and hyperthyroid livers with lowered levels of insulin and cortisol in the perfusate (Group 8). Except for hyperthyroid livers given 4.0 μg of thyroxine (Group 6), the rate of α₁-(acute phase) globulin synthesis subsequent to induction was much the same in all groups.

Net Biosynthesis of Haptoglobin—Table I and Fig. 5 show that livers from hyperthyroid donors (Group 3) synthesized maximum quantities of haptoglobin. The addition of 4.0 μg of thyroxine
to the perfusate of hyperthyroid livers (Group 6) caused no further increase, while normal livers given 4.0 μg of thyroxine (Group 5) synthesized significantly more hepatogliser than normal livers without thyroxine (Group 1). A comparison of hepatoglogin synthesis by normal livers given 0 to 22.0 μg of thyroxine in the perfusate (Groups 1, 4, 5, 7; Table I) reveals a biphasic effect with 22.0 μg being detrimental while 4.0 μg enhanced synthesis. Fig. 5 also shows that the newly induced rate of synthesis varied from no change for hyperthyroid livers given 22.0 μg of thyroxine and decreased levels of cortisol and insulin (Group 8) to twice the initial rate in normal livers given 4.0 μg of thyroxine (Group 5).

[1-3H]Lysine Incorporation into Hepatic Protein—Table I shows that hypothyroidism in the liver donor (Group 2) resulted in the minimum incorporation of [1-3H]lysine into hepatic protein which was significantly below the incorporation by normal and hyperthyroid livers given 4.0 μg of thyroxine in the perfusate (Groups 5 and 6).

Perfusate Nitrogen Balance—Perfusate nitrogen balance shown in Fig. 6 was calculated for sequential perfusate samples from analyses for α-amino acid nitrogen and urea nitrogen. The net removal of amino acid nitrogen from the perfusate of hyperthyroid livers containing 4.0 μg of thyroxine (Group 6) (Table I) was significantly less than for normal livers given the various doses of thyroxine (Groups 4, 5, 7) in the perfusate. There was no significant difference between any other groups in total amino acid nitrogen removal or in the rate at which removal occurred. Although not detailed here, the rate of removal remained remarkably constant after the 1st hour of perfusion and approximated 3.5 mg of amino acid nitrogen per hour.

Net Production of Perfusate Urea Nitrogen—This was significantly reduced by hypothyroidism below that seen with hyperthyroidism in the liver donor, although neither was significantly different from perfusate urea nitrogen of normal livers (Table I). However, perfusate urea produced by livers from hyperthyroid rats (Group 2) was significantly below that produced by normal livers given 22.0 μg of thyroxine (Group 4) and hyperthyroid livers given 4.0 μg of thyroxine (Group 6). Maximum urea production, significantly above all other groups, occurred when hyperthyroid livers were given 22.0 μg of thyroxine along with reduced insulin and cortisol doses (Group 8). Although not
documented in detail here, the rate at which urea nitrogen accumulated in the perfusate also remained virtually constant for every group after the 2nd hour of perfusion; hypothyroid livers (Group 2) produced urea nitrogen at a rate of approximately 3.0 mg per hour, while the rate for hyperthyroid livers given 22.0 μg of thyroxine and lowered insulin and cortisol (Group 8) was approximately 5.0 mg per hour of perfusion.

Fig. 6 illustrates perfusate nitrogen balance (perfusate amino acid nitrogen minus perfusate urea nitrogen). This should not be confused with over-all nitrogen balance (Table I) since bile nitrogen was not determined until the end of each perfusion. Fig. 6 shows that by the 12th hour the nitrogen balance in the perfusate of livers from normal and hypothyroid donors (Groups 1 and 2) was the same while the nitrogen balance for livers from hyperthyroid animals (Group 3) was significantly lower. Table I reveals that addition of varying amounts of thyroxine in the perfusate elicited a graded response in nitrogen balance. In general the higher the dose of thyroxine in the perfusate of normal livers (Group 4, 22.0 μg of thyroxine; Group 5, 4.0 μg of thyroxine; Group 7, 0.4 μg of thyroxine) the more negative the perfusate nitrogen balance. Hyperthyroidism in the liver donor plus thyroxine in the perfusate (Group 6, 4.0 μg of thyroxine) were additive in decreasing perfusate nitrogen balance while in the case of lowered cortisol and insulin levels (Group 8) the nitrogen balance became extremely negative (Fig. 6).

Over-all Nitrogen Balance—Since bile contains significant amounts of α-amino acid nitrogen and urea nitrogen these values were used in estimating over-all nitrogen balance. As summarized in Table I, bile volume, total bile urea nitrogen, and amino acid nitrogen levels were significantly lower in perfusates of livers from hypothyroid donors than those from normal donors (Group 2 versus 1). Also, hyperthyroidism in the liver donor (Group 3) caused a significant increase in bile volume (and amino acid nitrogen and urea nitrogen) with a maximum in perfusates using livers from hyperthyroid donors and thyroxine (Group 6). Addition of 22.0 or 4.0 μg of thyroxine to the perfusate of normal livers (Groups 4 and 5) had no effect on bile amino acid nitrogen and urea nitrogen.

Table I shows that hypothyroidism in the liver donor (Group 2) resulted in positive over-all nitrogen balance while hyperthyroidism (Group 3) caused a negative nitrogen balance. As with respect to perfusate nitrogen balance, there appeared to be a dose-response relationship between over-all nitrogen balance and thyroxine in the perfusate. Higher doses of thyroxine (Group 4, 22.0 μg of thyroxine) resulted in a more negative over-all nitrogen balance while lower doses (Group 5, 4.0 μg of thyroxine)
roxine; Group 7, 0.4 μg of thyroxine) allowed a slightly positive balance in normal livers. Hyperthyroidism in the liver donor and thyroxine added to the perfusate (Group 6) were additive in causing negative nitrogen balance and with lowered insulin and cortisol doses the nitrogen balance became extremely negative (Group 8).

Oxidation of L-[1-14C]-Lysine to 14CO2—Table I summarized the cumulative conversion of L-[1-14C]lysine to 14CO2. Production of 14CO2 by livers from hypothroid or hyperthyroid donors was not different from that by livers from normal liver donors (Group 2 versus 3 versus 1). However, when thyroxine was added to the perfusate, 14CO2 production increased slightly in normal livers (Groups 4 and 5) and was further increased by hyperthyroidism in the liver donor (Group 6) and by a decrease in the dose of insulin and cortisol (Group 8). Although not documented in detail, a constant rate of conversion was established in each group by the 2nd hour of perfusion; the maximum rate was seen in hyperthyroid livers given 22.0 μg of thyroxine and decreased doses of insulin and cortisol (Group 8) and was approximately twice that seen in hypothyroid livers (Group 2).

Net Change in Perfusate Glucose—Table I summarizes the net cumulative utilization of glucose at the close of 12 hours of perfusion and shows that net removal of glucose from the perfusate was significantly lower with livers from hypothroid donors (Group 2) and significantly higher with livers from hyperthyroid rats (Group 3) than for normal livers (Group 1). In addition, the summarized data show that with hyperthyroid liver donors, the addition of thyroxine to the perfusate had no additional effect on glucose balance by the liver (Group 3 versus 6) even in conditions of lowered insulin and cortisol (Group 8). However, 22.0 μg of thyroxine in the perfusate of normal livers (Group 4) decreased net glucose disappearance from the perfusate.

Bile Volume—As revealed in Table I (uncorrected for small differences in liver size), bile volume was least in livers from hypothyroid donors (Group 2), increased severalfold in hyperthyroid donors (Group 3), and was unaffected by thyroxine in the perfusate (Groups 4 and 5) of normal livers.

### DISCUSSION

In vivo studies of effects of hormones on plasma protein synthesis estimated in terms of radioisotope incorporation or turnover are difficult to interpret because gland removal and hormone treatment could be associated with altered nutrition, blood volume, radioactive precursor specific activity, or other secondary factors, and not be due to direct effects of specific hormones on the liver. In contrast, the use of the isolated perfused liver has resulted in the direct demonstration of optimal conditions for maximum net synthesis of rat serum albumin, fibrinogen, α1-acid glycoprotein, α1-(acute phase) globulin, and haptoglobin (4); among the factors necessary for maximum induction of synthesis of acute phase plasma proteins, cortisol was essential. This report extends the view that the synthesis of specific plasma proteins by the isolated liver reflects interactions among a complex of factors not only including the supply of amino acids, and glucose, but also the quality and quantity of hormones. In particular, data presented here support the view that net synthesis of each of several specific plasma proteins by the isolated perfused liver is altered differently by the thyroid status of the liver donor, and by the addition of thyroxine to the perfusate. In addition, this study reveals that the thyroid status of the liver donor or the dose of thyroxine in the perfusate can also significantly affect nitrogen balance, glucose uptake, bile production, L-[1-14C]lysine oxidation to 14CO2, and the gross incorporation of L-[1-14C]lysine into hepatic protein.

### Biosynthesis of Albumin

Altered albumin levels in vivo have not been clearly associated with hyperthyroidism or hypothyroidism; however, hyperthyroidism has been shown to accelerate albumin turnover in man and rats (12, 13). Direct study of the isolated liver reveals that albumin synthesis was altered to a greater degree by the thyroid status of the liver donor (Group 2 versus Group 3) than by the addition of thyroxine to the perfusate; this contrasted to the greater influence of specific thyroxine treatments on the synthesis of α1-acid glycoprotein, α1-(acute phase) globulin, and haptoglobin. The rate of synthesis of albumin was maximal at the start and generally decreased over the last 4 to 6 hours of 12-hour perfusions. During the same later interval the rate of synthesis of acute phase plasma proteins was greatly increased. One may reasonably speculate whether the late decreased synthesis of albumin is, at least in part, secondary to a substantial increase in the competition for available substrate to support the increased synthesis of acute phase proteins after induction.

### Biosynthesis of Fibrinogen, α1-Acid Glycoprotein, α1-(Acute Phase) Globulin, and Haptoglobin

The net biosynthesis of fibrinogen by the isolated liver was almost unaffected by either the prior thyroid status of the liver donor or by supplementation with thyroxine. In contrast, synthesis of α1-acid glycoprotein, α1-(acute phase) globulin, and of haptoglobin was manifestly more responsive to the thyroid hormone. The response was most pronounced in the livers from hyperthyroid donors perfused with a total of 4 μg of thyroxine (Group 6); here, the induction of increased synthesis referable to the cortisol supplement (4, 5) was exaggerated, and net synthesis of protein was significantly greater than that observed with livers from normal donors (Group 1). Without knowing what functions are served by α1-acid glycoprotein and α1-(acute phase) globulin, speculation about the possible biological significance of thyroid hormone increasing their rates of synthesis is hardly warranted. It is the synthesis of haptoglobin which most remarkably reveals a graded biphasic response of livers from normal donors to the dose of thyroxine. Furthermore, in comparison with the latter (Group 4) an increased requirement for substantial levels of insulin and cortisol became evident in experiments of Group 8; the combination of hyperthyroidism in the liver donor, high thyroxine dose in the perfusate, one-fifth and one-tenth the control doses of cortisol and insulin, respectively, caused a decrease in all anabolic protein synthetic responses and an increase in all catabolic responses studied. Under the latter conditions haptoglobin synthesis decreased to approximately one-half that of normal livers, and one-third that in hyperthyroid livers given the highest dose of thyroxine and the control doses of cortisol and insulin. It is of interest that in the absence of added thyroxine, the reduced quantities of insulin and cortisol used in the experiments of Group 8 have, in unpublished studies, been found adequate to induce maximally increased rates of synthesis of haptoglobin and of all the other acute phase proteins studied.

Although thyroid hormones have been implicated in the general regulation of protein synthesis (14, 15), in changes in the activity of enzymes of oxidative energy metabolism (16–18), and in the assembly and function of polyribosomes (10), the limited state of our knowledge affords at present no unequivocal molecular basis for the observed qualitative and quantitative effects in the synthesis of the five specific plasma proteins. Despite this, it is clear that the synthesis of the acute phase proteins: α1-acid glycoprotein, α1-(acute phase) globulin, and es-
especially haptoglobin may be quantitatively modulated by thyroxine. These observations are of added significance because they identify thyroid hormone as a humoral substance which, like cortisol, may have a direct effect on biosynthesis of acute phase proteins by the liver. As such, these observations bring us closer to understanding some of the more prominent and as yet unexplained changes in the plasma proteins resulting from injury (20).

Hepatic Protein Synthesis—In an earlier study John and Miller (4) observed that insulin alone enhanced L-[1-14C]lysine incorporation into hepatic protein without increasing synthesis of specific plasma proteins examined; in contrast, cortisol alone decreased L-[1-14C]lysine incorporation into hepatic protein and increased net synthesis of acute phase proteins. Together the hormones insulin and cortisol elicited effects which were dominated by the proanabolic influence of insulin; in fact, insulin allowed the induction by cortisol of enhanced synthesis of fibrinogen and the other acute phase proteins without the liver suffering the overt catabolic effects of cortisol. Now it is clear that the combined effects of insulin and cortisol on the liver can be modified by thyroid hormone.

A biphasic effect of thyroid hormone is manifest here insofar as the lowest incorporation of L-[1-14C]lysine into liver protein was seen in perfusion of livers from hypothyroid rats (Group 2) and in livers of hyperthyroid rats given increased doses of insulin plus cortisol along with the highest dose of thyroxine, 22 µg (Group 8); in contrast the total dose of 4 µg of thyroxine given to perfused livers from normal or hyperthyroid liver donors resulted in significantly higher incorporation of labeled lysine into liver protein (Groups 5 and 6).

It is known that chronic administration of either cortisol or thyroxine to intact rats elicits a loss of carcass protein and a simultaneous gain in liver protein and ribonucleic acid, but it is impossible to discern whether deposition of liver protein is related more to increased flow of amino acids from catabolism of carcass protein or to direct effects of these hormones on hepatic protein synthesis.

Net Amino Acid Uptake and Urea Synthesis—The rate of net removal of amino acid nitrogen from the perfusate remained remarkably constant for all treatment groups after the 1st or 2nd hour of perfusion. It is of some interest that after 12 hours of perfusion, amino acid nitrogen in the perfusate was significantly lower (less net removal) for hyperthyroid livers given 4.0 µg of thyroxine (Group 6) than for normal livers with or without thyroxine (Groups 1, 4, 5, 7). While final perfusate amino acid nitrogen levels reflect both uptake and release by the liver, in general it appears that thyroxine treatment of the liver donor or the isolated liver did not in a major way alter net amino acid transport in these experiments.

Grossly, appearance of urea nitrogen in the perfusate correlated more directly with the relative dose of thyroid hormone than with disappearance of amino acid nitrogen. Decreasing the doses of insulin and cortisol while increasing the dose of thyroid hormone (Group 8) superimposed conditions which dramatically increased urea production without altering amino acid uptake.

In the isolated liver as in the intact animal it appears that there is an optimal level of thyroid hormone compatible with most efficient utilization of amino acid nitrogen for protein synthesis while gross excess of thyroid hormone enhances amino acid catabolism and urea synthesis.

Oxidation of L-[1-14C]Lysine to 14CO2—The pattern of oxidation of [14C]lysine to 14CO2 in general paralleled the net production of urea nitrogen. Oxidation increased with the addition of thyroxine was additive with hyperthyroidism in the liver donor, and reached maximum levels under the conditions of lowered insulin and cortisol.

Nitrogen Balance—Previous reports from this laboratory (4–6) have indicated that positive nitrogen balance can be elicited in the isolated perfused liver system and that this is a useful index of over-all protein metabolism. Over-all nitrogen balance as defined for the perfused liver (4) is calculated on the basis of net cumulative changes in amino acid nitrogen and urea nitrogen content of both perfusate and bile. Positive nitrogen balance may be favored by: (a) increasing amino acid uptake; (b) decreasing protein breakdown and urea synthesis; (c) increasing protein synthesis. Miller and Griffin (21) have presented evidence for the view that insulin is the hormone essential for positive nitrogen balance, while cortisol alone or in combination with glucagon seriously impairs net uptake of amino acids, enhances urea production, and accentuates negative nitrogen balance. It appears that insulin may permit the liver to respond more selectively to thyroxine as well as to glucagon or cortisol without incurring the metabolic liability of severely negative nitrogen balance.

It is notable that positive nitrogen balance is not a require-ment for normal or enhanced net synthesis of the acute phase plasma proteins examined. A moderately negative nitrogen balance (Group 6) was not detrimental to the increased synthesis of plasma proteins, but in the case of Group 8 (decreased insulin and cortisol dose), extremely negative nitrogen balance was attributable to greatly increased urea production and synthesis of acute phase proteins was impaired. The observation made here that livers from hypothyroid donors (Group 2) maintained the highest positive nitrogen balance concurrently with minimum albumin synthesis and minimum incorporation of L-[1-14C]-lysine into hepatic protein emphasized that positive nitrogen balance per se cannot be used for assessing detailed segments of over-all protein metabolism.

Net Glucose Uptake—Net glucose uptake from the perfusate increased significantly when livers were taken from hyperthyroid donors, while hypothyroidism in the donor resulted in net glucose uptake significantly below control values. Although we can refer only to net glucose uptake since the rate of uptake and output were not measured, these observations are in harmony with increased oxidative activity and calorigenic effect of excess thyroid hormone. Oxidative metabolism as a primary target of thyroid hormones has attracted much attention, and the present findings invite speculation about the action of thyroxine. Under conditions with control quantities of insulin and cortisol and excessive thyroxine (Group 6), maximum oxidation of L-[1-14C]-lysine to 14CO2 occurred simultaneously with maximum uptake of glucose and amino acids, with the highest urea production, and with the most negative nitrogen balance. One may conjecture that these results relate not only to an increased energy demand because of increased anabolic protein synthetic activity, but also to decreased ATP-generating efficiency referable to uncoupling of oxidative phosphorylation. Although it is generally agreed that thyroxine can uncouple oxidative phosphorylation, there is neither general agreement that it occurs with physiological levels of thyroxine, nor on the mechanism by which it is brought about.

Thyroxine Levels—A subordinate goal of this study was to estimate the level of thyroxine in the perfusate necessary to elicit optimal anabolic responses by the liver. Since thyroxine is metabolized and excreted rapidly by the liver, mainly through a
conjugation pathway, constant infusion of thyroxine was considered necessary. The total quantities of thyroxine added during a 12-hour perfusion ranged from 0.4 to 22.0 ng. If all the thyroxine added as a primary dose plus that infused existed in the perfusate as free thyroxine at the end of perfusion, the maximum concentration would have been between $10^{-9}$ and $10^{-7}$ M. It is unlikely that the concentration of free thyroxine ever reached such levels, because binding to rat plasma proteins could occur, and because some of the hormone was undoubtedly metabolized during the perfusion.

In any case, it is of considerable interest that this concentration range corresponds to levels of thyroid hormone which prevail under physiological conditions in the intact animal.

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