Hormonal control of hepatic bilirubin transport and conjugation

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GARTNER, LAWRENCE M., AND IRWIN M. ARIAS. Hormonal control of hepatic bilirubin transport and conjugation. Am. J. Physiol. 222(5): 1091-1099. 1972.—The role of the pituitary and thyroid glands in the regulation of hepatic transport and metabolism of bilirubin in Sprague-Dawley rats was investigated with continuous bilirubin infusion and bile collection for estimation of cumulative hepatic uptake and maximal hepatic excretion of bilirubin in vivo and determination of glucuronyltransferase activity in vitro. Maximal hepatic excretion of bilirubin was reduced to 40% of normal by 7-11 days after hypophysectomy and 34-36 days after thyroidectomy. Both the volume of bile secreted and the maximal concentration of bilirubin in bile were reduced. Cumulative hepatic uptake of bilirubin was probably unaffected by hypophysectomy, but may have been reduced by thyroidectomy. 1-Thyroxine alone was capable of correcting the bilirubin transport defects in both groups of animals. Thyroid stimulating hormone (TSH) also corrected the defect in hypophysectomized rats. Defective glucuronyltransferase activity was observed only when ortho-aminophenol (OAP) served as the glucuronide receptor in vitro. Bilirubin glucuronide formation was either normal or increased following hypophysectomy or thyroidectomy. 1-Thyroxine administration returned glucuronyltransferase activity to normal. Other hormones were without effect.

hypophysectomy; thyroid; pituitary; hormones; excretion; liver; glucuronyltransferase; bile

REDUCED PLASMA CLEARANCE of administered bilirubin has been observed in hypophysectomized rats when compared with normals (4, 6, 10, 16). These observations suggest that the transfer of bilirubin from plasma to bile may be adversely affected by hypophysectomy. The following studies were performed to determine the role of the pituitary gland in regulating hepatic uptake, conjugation and excretion of bilirubin in rats, and the specific hormone(s) responsible for this control. Normal, hypophysectomized, and thyroidectomized rats were treated with various hormones, and hepatic uptake and excretion of bilirubin were estimated following intravenous administration of unconjugated bilirubin. The activity of hepatic UDP-glucuronyltransferase (UDPGTase), the microsomal enzyme responsible for transfer of glucuronic acid from uridine diphosphoglucuronic acid (UDPGA) to bilirubin, was estimated in vitro.

METHODS

Sprague-Dawley male rats between 6 and 12 weeks old were obtained from Charles River Breeding Laboratories (North Wilmington, Mass.). Hypophysectomy and thyroidectomy were performed (Charles River Breeding Laboratories) when animals weighed approximately 125 g. All animals were maintained on Purina Laboratory Rat Chow until studied. Thyroidectomized rats were offered 1% calcium lactate in water to prevent hypocalcemic tetany resulting from inadvertent removal of the parathyroid glands in some rats during surgery. Completeness of hypophysectomy was confirmed by virtually complete growth arrest in all animals used. Growth retardation was also observed in all thyroidectomized rats. Mean body weight of normal animals was 296 ± 42 (se) g; that of hypophysectomized rats 129 ± 7 g; and that of thyroidectomized rats 178 ± 10 g. The control animals were of comparable age range to that of the hypophysectomized and thyroidectomized rats.

All hormones were prepared in isotonic saline, pH 8.0, unless commercially prepared in diluent, and administered by subcutaneous injection over the back once a day.

The technique used for measurement of hepatic excretion is a modification of that of Weinbreu and Billing (11, 26). Cannulations of the common bide duct and external jugular vein were accomplished under pentobarbital anesthesia, following which isotonic saline was infused intravenously at 0.065-0.100 ml/100 g body wt per minute for 30 min. During the following 90 min, a 100 mg/100 ml aqueous solution of recrystallized bilirubin (Eastman Organic Chemicals) in 0.45% NaCl and 0.45% Na2CO3, pH 7.8, was continuously administered into the jugular vein at 0.065-0.100 mg of bilirubin per 100 g body wt per minute. The same rates of infusion were used for both normal controls and endocrinectomized rats. Body temperature was maintained with use of an incandescent light bulb, and rectal temperatures were measured in randomly sampled animals to insure normality. Bile was collected in precweighed tubes in ice in the dark for 30 min during saline infusion and for three consecutive 30-min periods during bilirubin infusion. At the conclusion of the study, blood was drawn from the heart, and the liver was promptly removed and perfused with 50 ml of cold isotonic saline through the hepatic veins. The volume of bile secreted in each period was determined by weight. A micromodification of the method of Malloy and Evelyn (18) was used to estimate total and direct-reacting bilirubin (TB, DRB) concentrations in serum and bile. The method of Hargreaves (14) was used for determination of total and direct-reacting bilirubin concentrations in liver. As previously reported, recovery of added unconjugated...
bilirubin ranged from 91 to 101 % and of added conjugated bilirubin from 84 to 112 % (11).

Bile flow and bilirubin excretion during the 30-min period of saline infusion are referred to as endogenous bile flow and endogenous bilirubin excretion, respectively. Bile flow during the 1-hr period following the start of bilirubin infusion is referred to as the 1-hr bile flow. Bilirubin excretory maximum (Em) was calculated as micrograms of bilirubin excreted in bile per 100 g body wt per minute during the 30-min period after maximum excretion was achieved. A plateau of maximum bilirubin excretion was attained in each study. Bile flow was stable throughout the 90-min period of bilirubin infusion.

Cumulative hepatic bilirubin uptake (CHUB) is a measure of the net rate of movement of unconjugated bilirubin from plasma into the liver and was calculated as follows:

\[
\text{uptake} = \left( \frac{\text{TB excr} - \text{endog TB excr} + \text{liver TB}}{100 \text{ g body wt} \times 90 \text{ min}} \right) \]

\[
\text{TB excr} = \text{milligrams total bilirubin excreted in bile during 90 min of bilirubin infusion}
\]

\[
\text{endog TB excr} = \text{milligrams total bilirubin endogenously excreted in bile during 90 min (i.e., 30-min endogenous bilirubin excretion during saline infusion \times 3)}
\]

\[
\text{liver TB} = \text{milligrams total bilirubin in liver at end of 90 min of bilirubin infusion}
\]

\[
\text{plasma DRB} = \text{milligrams direct-reacting bilirubin in total plasma volume at end of 90 min of bilirubin infusion}
\]

Plasma volume was estimated at 3.35 ml/100 g body wt for normal animals (8) and at 10 % less or 3.05 ml/100 g body wt for hypophysectomized and thyroidectomized rats (3, 7).

Cumulative hepatic uptake is expressed as milligrams bilirubin per 100 g body wt per minute. During the 90 min of bilirubin infusion, urine was not pigmented and, therefore, urinary excretion of bilirubin was considered negligible.

Theoretical extrahepatic conjugation of bilirubin and distribution of direct-reacting bilirubin in tissues other than liver and plasma were ignored in computation of CHUB.

Validity of this technique has been demonstrated in guinea pigs (11) and in rhesus monkeys (12).

Body weight of all animals was determined prior to hormone administration. Liver weight was determined immediately following removal of the liver.

Glucuronyltransferase activity was estimated using the following technique: a 20 % liver homogenate was prepared in ice-cold 0.154 M KCl using a motor-driven Teflon pestle homogenizer, and 1 ml of homogenate was incu-bated with 0.05 ml of 0.5 M Tris buffer (pH 8.0), 0.05 ml of 0.5 M MgCl₂, 0.1 ml of 1.25 \times 10⁻² M OAP in 0.01 M ascorbic acid, 0.20 ml H₂O, and 0.13 ml UDPGA (Sigma Chemical Co.) at concentrations of 5.5 \times 10⁻³, 1.1 \times 10⁻², 2.2 \times 10⁻², and 4.4 \times 10⁻² M at 37 °C for 30 min at 90 cycles shaking per minute in air. OAP glucuronide formation was determined according to Levy and Storary (17) and expressed as micromoles of OAP glucuronide formed per gram liver wet weight per 30 min.

When bilirubin served as glucuronyl receptor, the following technique was used: a 30 % homogenate of liver was prepared in 0.154 M KCl using a motor-driven Teflon pestle homogenizer, and 1 ml of homogenate was added to an incubation medium consisting of 0.3 ml of 0.5 M phosphate buffer (pH 7.4), 0.1 ml of 0.3 M MgCl₂, 1.0 ml of 6.0 mg/100 ml recrystallized bilirubin in 800 mg/100 ml crystalline bovine albumin solution (pH 7.6), and 0.6 ml uridine di-phosphoglucuronic acid (Sigma Chemical Co.) at each of four different concentrations (5.5 \times 10⁻³, 1.1 \times 10⁻², 2.2 \times 10⁻², and 4.4 \times 10⁻² M). Each assay was also performed in the absence of added UDPGA. Distilled water was added to a total volume of 3.4 ml. Incubation proceeded in the dark in a Dubnoff metabolic shaker at 37 °C at 90 cycles/min for 40 min. Following completion of incubation, the reaction tubes were immediately placed in ice, and 3.0 ml of McIlvaine’s buffer (0.96 M citric acid and 0.04 M Na₂HP³O₄, pH 2.2) were added to each tube. Unincubated controls were used for each homogenate. Total and direct-reacting bilirubin concentrations were determined according to the method of Hargreaves (14).

Glucuronyltransferase activity was expressed as micrograms of UDPGA-dependent direct-reacting bilirubin formed per gram of liver wet weight per 40 min after correction for unincubated blank. Optimal conditions and recoveries for this assay were established previously (11).

Glucuronyltransferase activity was also estimated in normal and hypophysectomized rats by modification of the technique of Heirwegh and Meuwissen (15). A 4 % homogenate of liver was prepared in 1 % digitonin solution, and 0.5 ml of homogenate was added to 0.15 ml of 9 ml triethanolamin in 1 N HCl (pH 7.4), 0.03 ml 0.3 M MgCl₂, 0.3 ml of 12 mg/100 ml recrystallized bilirubin in 800 mg/100 ml crystalline bovine albumin, 0.4 ml H₂O, and 0.1 ml 6.6 \times 10⁻² M UDPGA. Incubation proceeded at 37 °C in a Dubnoff metabolic shaker at 90 cycles/min in the dark for 40 min. Immediately following completion of incubation, the reaction tubes were placed in ice, 1.7 ml of McIlvaine’s buffer were added to each tube, and total and direct-reacting bilirubin concentrations were determined (14). Glucuronyltransferase activity was expressed as micrograms bilirubin glucuronide formed per gram liver wet weight per 40 min. Optimal conditions were established in regard to reagent concentrations, pH, duration of incubation, and homogenate concentrations using rat liver.

Results are expressed as the mean value \pm 1 se. The two-tailed Mann-Whitney U test was used for determination of statistically significant differences between groups at a significance level of \( P < 0.05 \) (13).

**RESULTS**

**Hepatic bilirubin excretion.** Maximal hepatic excretion of bilirubin was determined in 9 normal rats, 27 hypophysectomized rats 2–63 days following surgery, and 22 thyroidectomized rats 4–36 days following surgery (Fig. 1).
HEPATIC BILIRUBIN TRANSPORT AND CONJUGATION

EM<sub>B</sub> in normal rats was 37.3 ± 3.9 (SE) µg/100 g per min.
Seven to eleven days following hypophysectomy, EM<sub>B</sub> was reduced to 16.1 ± 2.1 (SE) (<i>P</i> < .05) and remained at that level throughout the period of study of up to 63 days following hypophysectomy. Following thyroidectomy, EM<sub>B</sub> declined more slowly, with a significant reduction occurring at 14–23 days following thyroid removal, when the EM<sub>B</sub> was 25.0 ± 2.3 (SE) (<i>P</i> < .05). A degree of reduction comparable to that observed in the 7- to 11-day posthypophysectomy rats was not achieved in thyroidectomized rats until 34–36 days when the EM<sub>B</sub> was 14.4 ± 2.9. Thus, removal of either thyroid or pituitary resulted in 60% reduction in the hepatic capacity to excrete bilirubin.

Bile flow. Bile flow declined by approximately 50% after hypophysectomy or thyroidectomy as compared with the normal rats which had a mean bile flow of 0.0047 ± 0.0006 (SE) ml/100 g per min (Fig. 2). As with EM<sub>B</sub>, the rate of bile flow declined more rapidly in hypophysectomized rats than in thyroidectomized animals.

Bile bilirubin concentrations. The mean bilirubin concentration achieved in bile during maximal hepatic excretion of bilirubin decreased following hypophysectomy or thyroidectomy; however, the decrease was not statistically significant after thyroidectomy (<i>P</i> > .05) (Fig. 3). Normal rats achieved a mean bile bilirubin concentration of 843 ± 150 (SE) mg/100 ml, while rats 42–63 days posthypophysectomy achieved a mean bile bilirubin concentration of 562 ± 53. The capacity to concentrate bilirubin in bile was relatively less affected than the capacity to secrete a volume of bile. From 2 to 4 days after hypophysectomy and 9–10 days after thyroidectomy, bile flow decreased, and there was a transient rise in mean bilirubin concentration in bile to 1,058 ± 188 in hypophysectomized rats and 1,156 ± 166 in thyroidectomized rats.

Cumulative hepatic uptake. The cumulative hepatic uptake of bilirubin was 41 ± 7 (SE) µg/100 g per min in normal rats and was unaffected by hypophysectomy (Fig. 4). By 34–36 days following thyroidectomy, CHU<sub>B</sub> decreased to 21 ± 2 (<i>P</i> < .05).

Replacement therapy. In order to elucidate the role of the pituitary and thyroid glands in control of hepatic transport of bilirubin, a variety of pituitary and end-organ hormones were examined for their ability to restore bilirubin transport to normal in hypophysectomized rats.

Two different preparations of porcine growth hormone (PGH) (Sigma Chemical Co.) were administered during the study. One PGH preparation given to 12 hypophysectomized rats at a dose of 2 mg/day for 6 days restored EM<sub>B</sub> to normal (40.1 ± 3.1 (SE) µg/100 g per min). A second preparation of PGH also administered at a dose of 2 mg/
day was entirely without effect (11.0 ± 1.5 μg/100 g per min). Both preparations restored weight gain rates to normal (2.59 and 1.97%/day, respectively). The first preparation had been shown previously to be contaminated with significant quantities of thyroid stimulating hormone (TSH) (10). The second preparation was not studied for TSH content. Administration of the first preparation of PGH at a dose of only 0.2 mg/day for either 6 or 15 days was entirely without effect on EMB and only partially restored growth. Unfortunately, at the time of these studies, growth hormone preparations were not assayed for growth hormone effect in international units.

Bovine growth hormone (BGH) administered to five hypophysectomized rats in a dose of 1.5 mg/day for 6 days partially restored EMB to normal (27.5 ± 5.7 μg/100 g per min) and completely restored growth to normal. This preparation had also been previously demonstrated to contain significant quantities of TSH (10).

Ovine growth hormone (OGH) was available in two different preparations. One preparation of OGH previously shown to contain only trace amounts of TSH was without effect on EMB (13.0 ± 0.7 μg/100 g per min) when administered to hypophysectomized rats in a dose of 1.5 mg/day for 6 days, although it effectively restored growth to normal. A second preparation of OGH partially restored EMB toward normal (25.7 ± 5.4 μg/100 g per min) and completely restored growth of hypophysectomized rats.

This variability in effect of growth hormone preparations on EMB with a regular restoration of weight gain as well as the demonstration that most of the effective preparations were contaminated with TSH suggested that thyroid stimulating hormone, rather than growth hormone, was the effective agent in restoring hepatic excretory function in the hypophysectomized rat.

Therefore, ovine TSH was administered in three dosage schedules to groups of five hypophysectomized rats. One unit per day for 6 days did not affect EMB (16.9 ± 3.9 μg/100 g per min); the same dose for 15 days increased EMB slightly, but not significantly (24.6 ± 7.7 μg/100 g per min). Four units per day for 6 days restored EMB to normal with an excretion rate of 32.3 ± 6.4 (SE) μg/100 g per min, and CHUB also increased to 52 ± 3 (SE) μg/100 g per min as compared with 41 ± 7 in normal rats (P < 0.05). TSH administration at 1 U/day restored weight gain to 0.85%/day and 4 U/day to 1.65%/day. These weight gains are less than those achieved following growth hormone administration, however.

The importance of thyroid hormone in hepatic bilirubin transport is suggested by the effects of thyroidectomy and TSH replacement therapy (Figs. 1–4). Confirmation of the role of thyroid hormone was established by administration of l-thyroxine (Sigma Chemical Co.) to hypophysectomized and thyroidectomized rats. EMB returned to normal following administration of 1.25, 2.50, 5.0, and 10.0 μg of thyroxine per day for 6 days (Fig. 5). A single dose of 1 mg of thyroxine did not increase EMB 24 hr later. Bile flow increased significantly following administration of 10.0 μg of thyroxine for 6 days (Fig. 5). Smaller doses were without significant effect on bile flow. The mean concentrations of bilirubin in bile increased significantly in animals following administration of thyroxine for 6 days (Fig. 5). Six days of low and moderate dosage thyroxine treatment restored EMB to normal by increasing bile bilirubin concentration above normal without increasing bile flow significantly.

Thyroidectomized rats were studied only with thyroxine and porcine growth hormone (the preparation of PGH effective in hypophysectomized rats), the two hormones which restored EMB to normal in hypophysectomized rats. Thyroxine in doses of 1.25 μg/day for 15 days, 2.50 μg/day for 6 days, and 5.0 μg/day for 6 days returned EMB in thyroidectomized rats to normal (60.2 ± 7.5, 37.7 ± 6.2, and 44.4 ± 3.7 μg/100 g per min, respectively). A dose of 1.25 μg/day for 6 days was without significant effect. Porcine growth hormone in a dose of 2 mg/day for 6 or 12 days also did not significantly increase the mean EMB of thyroidectomized rats.

Significantly different from untreated hypophysectomized (P < 0.05)
The following preparations were also administered to hypophysectomized rats and were entirely without effect in restoring EMb, bile flow, and bile bilirubin concentration: adrenocorticotropic hormone (ACTH), 4 IU/day × 6 days; leucocrotic hormone, 75 IU/day × 7 days and 14 days; oxytocin, 0.2 IU/day × 6 days; vasopressin (Pitressin), 0.05 IU/day × 15 days; diethylstilbestrol, 0.5 mg/day × 6 days; hydrocortisone, 2.5 mg/day × 15 days; and protamine zinc insulin, 1 U/day × 6 and 15 days and 2 U/day × 6 days.

**Serum and liver bilirubin concentrations.** At the completion of each infusion study, bilirubin concentrations were determined in liver and serum (Fig. 6). Normal control rats had a mean indirect-reacting serum bilirubin concentration at the completion of the 90 min of bilirubin infusion of 22.7 ± 2.9 (SE) mg/100 ml and a mean direct-reacting serum bilirubin concentration of 4.2 ± 1.1 (SE) mg/100 ml. Hypophysectomy and thyroidectomy did not significantly alter the serum indirect-reacting bilirubin concentration. Serum direct-reacting bilirubin was significantly increased in hypophysectomized rats, but not thyroidectomized, rats. Thyroxine treatment of hypophysectomized rats restored direct-reacting bilirubin concentrations toward normal.

The mean concentration of indirect-reacting bilirubin in liver at completion of the study in normal animals was 139 ± 31.4 (SE) µg/g, and the mean direct-reacting bilirubin concentration in liver was 127.1 ± 30.0 (SE) µg/g (Fig. 6). Hepatic indirect-reacting bilirubin concentration on completion of bilirubin infusion were not significantly altered by either hypophysectomy or thyroidectomy. Hepatic direct-reacting bilirubin concentration in hypophysectomized rats was increased to 397.8 ± 30.0; however, thyroidectomy did not alter hepatic direct-reacting bilirubin concentrations. Thyroxine administration to hypophysectomized rats returned hepatic direct-reacting bilirubin concentrations to those observed in normal rats following bilirubin infusion.

**Liver weights.** Liver weight relative to body weight was examined in normal, hypophysectomized, and thyroidectomized animals and following hormone therapy (Fig. 7). Mean relative liver weight in normal adult male rats was 4.14 ± 0.63 (SE) %. No change in relative liver weight was observed during the first 11 days following hypophysectomy or thyroidectomy (P > .05). By 14–17 days following hypophysectomy, relative liver weight declined to 3.37 ± 0.10% (P < 0.05). During the following 46 days, there was a small progressive increase in liver weight in this group. Relative liver weight in thyroidectomized animals decreased throughout the first 36 days following extirpation. The patterns of development of relative liver weight and CIU (Figs. 4 and 7) were similar following both hypophysectomy and thyroidectomy.

Maximal hepatic excretion has been calculated on the basis of body weight. If EMb were calculated relative to liver weight rather than body weight, the reduction in EMb following hypophysectomy and thyroidectomy would be slightly less pronounced (a difference of 15%), but would still show a significant reduction of greater than 50%.

Replacement therapy with those hormone preparations shown to restore hepatic excretory function either partially or completely (growth hormone, thyroxine, and TSH) resulted in no significant increase in relative liver weight as compared with untreated hypophysectomized rats. The only exception was the preparation of porcine growth hormone which completely returned EMb to normal; it increased liver weight to 3.84 ± 0.09% (P < .05).

**Hepatic glucuronyltransferase activity.** Ortho-aminophenol glucuronide formation by rat liver homogenate decreased following hypophysectomy or thyroidectomy, and UDPGA concentrations were not rate limiting (Fig. 8). OAP glucuronide formation in normal adult male rats was 0.543 ± 0.027 (SE) µmoles OAP glucuronide formed per gram liver per 30 min; in male rats hypophysectomized 83–121 days previously, 0.170 ± 0.001 µmoles/g liver per 30 min; and in male rats thyroidectomized 95 days previously, 0.150 ± 0.012 µmoles/g liver per 30 min. Following hypophyse-
enzyme activity increased during the first 4 days, returned to normal on days 6–8, and then decreased to approximately 30% of normal activity during the ensuing 110 days (Fig. 9). Thyroidectomy resulted in a similar decline beginning at about 8–10 days following surgery (Fig. 10). The lowest levels of enzyme activity, reached after approximately 3 months, were similar in both groups of animals (Figs. 9 and 10). Combined removal of both glands in the same animal resulted in the observed initial rise noted in the hypophysectomized rats and a relatively rapid decline in OAP glucuronide formation thereafter (Fig. 11).

L-Thyroxine administration to hypophysectomized rats (1.25–10 μg for 15–22 days) restored OAP glucuronide formation toward normal; 22 days of treatment were required for complete restoration (Fig. 9). The same doses for 5–6 days were without effect. Thyroidectomized rats responded more promptly (Fig. 10). Fifteen and 22 days of thyroxine administration resulted in exaggerated enzyme activity. Administration of the following hormones failed to increase OAP glucuronide formation in hypophysectomized rats: corticosterone, 2.5 mg/day for 15 days; adrenocorticotropin gel, 4 IU/day for 21 days; diethylstilbestrol, 0.5 mg/day for 15 days; porcine growth hormone, 2 mg/day for 15 days; oxytocin, 0.2 IU/day for 15 days; and Pitressin, 0.05 mg/day for 15 days.

Whereas OAP glucuronide formation was reduced to 30% of normal following hypophysectomy and/or thyroidectomy, bilirubin glucuronide formation was either unaffected or increased following removal of pituitary or thyroid glands (Figs. 12 and 13). Using the standard method for estimation of glucurononyltransferase activity with bilirubin as substrate, enzyme activity was not altered (Fig. 12). Concentrations of UDPGA needed for optimal activity were also unaffected (Fig. 12). Enhancement of enzyme activity at suboptimal concentrations of UDPGA was higher in the hypophysectomized and thyroidectomized groups than in normals (Fig. 12). Enhancement of enzyme activity at suboptimal concentrations of UDPGA was not ob-
HEPATIC BILIRUBIN TRANSPORT AND CONJUGATION

Bile have been studied, and only excretion of conjugated bilirubin from the liver cell into bile is markedly reduced by hypophysectomy and/or thyroidectomy. The slow decline in hepatic excretory capacity following thyroidectomy (5 weeks) as compared with hypophysectomy (1 week) suggests that the pituitary may affect hepatic excretion through more than just TSH secretion.

Neither hypophysectomy nor thyroidectomy reduced bilirubin conjugation as estimated in vivo or in vitro. This is in contrast with the suggestions of Lazard and Sobotka (16) that exaggerated retention of administered bilirubin in the hypophysectomized rat resulted from reduced glucuronic formation. Brown and Dix (1) also found normal glucuronyltransferase activity in hypophysectomized rats, but did not specify the substrate used.

Studies of sulfobromophthalein (BSP) excretion by euthyroid humans with various defects in pituitary secretion suggest that growth hormone deficiency was responsible for reduced hepatic BSP excretion (24). BSP clearance is reduced in hypophysectomized rats (4). Increased BSP excretion occurs in acromegalic humans and in dogs treated with growth hormone (19, 22). In the present studies, administration of growth hormone from cow, sheep, and pig failed to restore hepatic excretory function to normal, despite doses greater than required for restoration of growth. Since physiologic doses of thyroxine completely restored hepatic excretory function, it was not possible to determine whether thyroxine and growth hormone act synergistically.

One preparation of porcine growth hormone returned excretory function to normal, while another preparation did not. All growth hormone preparations which restored hepatic excretory function were contaminated with TSH (10). All other hormones studied were without significant effect.

Reduced hepatic excretion in hypophysectomized and thyroidectomized rats resulted primarily from decreased volume of bile secreted. The capacity to concentrate bilirubin in bile was less severely reduced. Reduced bile volume which occurs in hypophysectomized rats could result from reduced bile acid synthesis (1, 2). Studies of bile acid synthesis in hypophysectomized rats suggest that diminished bile acid synthesis may be secondary to reduced hepatic excretion of bile acids and feedback inhibition of synthesis. Thus, reduced bile acid excretion and bilirubin excretion following hypophysectomy may result from a common excretory defect.

The effect of thyroid hormone replacement in increasing bile bilirubin concentration to a greater degree than bile flow in hypophysectomized animals is opposite to the effect of hypophysectomy itself. Data from the current study offer no explanation for this observation, but suggest either that the hypophysectomized rat liver suffers permanent and irreversible damage to the bile secretory mechanism or that bile flow can only be restored by combined hormone treatment. Thus, thyroid hormone may restore excretion to normal by a compensatory mechanism.

Temperature control is critical in studies of hepatic excretion of bilirubin and bile volume (23). Hypophysectomy and thyroidectomy produce hypothermia; however, in the present studies reductions in hepatic excretion persisted, despite artificial maintenance of body temperature at nor-

DISCUSSION

These studies define a role for the pituitary and thyroid glands in control of hepatic excretion of bilirubin in the rat. Thyroid hormone is apparently directly responsible. The major hepatic steps in transfer of bilirubin from plasma to
nal levels. Bauman et al. (1) found bile flow in nonanesthe-
sitized hypophysectomized animals 1–2 days after recovery
from surgery also to be markedly reduced. Thus, anesthetis-
and immediate surgical trauma may be excluded as signifi-
cant factors in reducing bile flow and bilirubin excretion in
hypophysectomized rats.

Despite decreased hepatic excretion of bilirubin follow-
ing hypophysectomy, the rate of cumulative hepatic uptake
of bilirubin appeared to be unaffected. The computation of
cumulative hepatic uptake understimates total transfer of
unconjugated bilirubin from plasma to liver by ignoring
distribution of conjugated bilirubin into extracellular tis-

sues as well as possible flux of unconjugated bilirubin from
liver back to plasma without conjugation.

In these studies a single determination of CHUB was per-
formed through the 90-min period of bilirubin infusion. The
same technique has been used in monkeys, but 3 hourly
determinations were performed demonstrating that the 1st
hr of CHUB is lower than the 2nd hr, suggesting that the
uptake is relative to the serum bilirubin concentration (12).
The 3rd hr CHUB in monkeys is again lower than the 2nd
hr, probably as a result of saturation of the hepatic storage
sites (12). In the primate studies, the rate of infusion of
bilirubin had no effect on the rate of uptake, providing that
the rate of infusion of bilirubin was twice that of the maxi-
mal CHUB (12). Thus, in the rat studies, the use of a single
90-min period for determination of CHUB may be accepted
as a suitable estimate of true maximal hepatic up-
take. The calculated normal cumulative hepatic uptake and
reduced excretion of bilirubin are supported by the ob-
servations that both liver and plasma direct-reacting bili-
rubin concentrations were 3 times higher at the end of the
study in hypophysectomized rats than in normals (Fig. 6).
Only slightly increased plasma and liver direct-reacting
bilirubin concentrations were noted in thyroidectomized
rats, probably because cumulative hepatic uptake of bili-
rubin declined following thyroidectomy. Hepatic concen-
trations of indirect-reacting bilirubin were slightly lower
and serum indirect-reacting bilirubin slightly higher in
thyroidectomized rats as compared with hypophysectomized
rats, also suggesting that hepatic uptake of unconjugated
bilirubin was lower in thyroidectomized rats. More detailed
studies of hepatic uptake of bilirubin would be needed to
confirm these observations.

Although not conclusive, these observations again sup-
port the suggestion that hypophysectomy and thyroidectomy
produce somewhat different hormonal effects on hepatic
transport mechanisms.

The parallel changes in cumulative hepatic uptake and
relative liver weight following surgery in both hypophyse-
tomized and thyroidectomized rats suggest that hepatic up-
take may be related to hepatic cell mass and/or protein syn-
thesis. Defective hepatic excretion of bilirubin is independent
of liver mass, however.

With standard techniques used to assay glucuronyltrans-
ferase activity with bilirubin as glucuronide receptor in
vitro, hypophysectomy and thyroidectomy did not change
enzyme activity or UDPGA concentrations required for
optimal activity. Hypophysectomized and thyroidectomized
rats achieved greater enzyme activity at suboptimal levels
of UDPGA than did normal rats or hypophysectomized
rats treated with thyroxine. This difference may result from
alterations of liver cell composition or increased availability
of UDPG resulting from hypothyroidism. Thus, Freedland
(9, 25) observed that thyroxine administration to normal
rats reduced activity of UDPG dehydrogenase, the cyto-
plasmic enzyme which converts UDPG to UDPGA.

In the course of these studies, an additional method for
estimation of glucuronyltransferase activity, using bilirubin
as the glucuronide receptor, became available (15). This
technique involved addition of digitonin to homogenates
and increased hepatic glucuronyltransferase activity approx-
imately 10-fold without increasing UDPGA require-
ments (Figs. 12 and 13). Using this method, hypophyse-
tomy doubled glucuronyltransferase activity. The relative
insensitivity of the earlier transferase method may have
masked this effect of hypophysectomy.

Formation of ortho aminophenol glucuronide (an ethereal
conjugate) in vitro by liver was markedly decreased follow-
ing hypophysectomy or thyroidectomy. The time required
for maximal reduction in OAP glucuronide formation ac-

tivity in both groups of animals was similar and paralleled
the time period required for maximal reduction in hepatic
excretion in thyroidectomized animals (4 weeks). Hepatic
excretion in hypophysectomized rats reached its lowest
point in approximately 1 week. Following combined surgi-
cal removal of both the thyroid and pituitary glands, OAP

glucuronide formation decreased more precipitously (1–2
weeks), suggesting a possible synergistic effect between
thyroid and pituitary secretions. Thyroxine administration
in physiologic doses to hypophysectomized animals restored
OAP glucuronide formation to normal in approximately 3
weeks in hypophysectomized rats and to supernormal en-
zyme activity in 2 weeks in thyroidectomized rats. The time
course for restoration of activity was similar to that of the
decline following gland ablation.

The disparate effects of hypophysectomy and thyroidoe-
tomy on bilirubin and OAP glucuronide formation raise
questions about the nature of the enzyme(s) and the mecha-


nism of action of thyroid hormone. There is uncertainty

about the existence of more than one glucuronyltransferase
(20). The divergent effect of hypophysectomy or thyroide-
tomy on formation of the two glucuronides suggests that
there may be two glucuronyltransferase enzymes. However,
reduction in OAP glucuronide formation may not result
from decreased enzyme protein, but from reversible altera-
tion in the enzyme structure which diminishes its affinity for
OAP, but increases it for bilirubin. Thus, Mowat and Arias
(21) have demonstrated that diethylnitrosamine added in
vitro increased the formation of OAP glucuronide by liver
from hypophysectomized and thyroidectomized rats to nor-
mal. These observations on the differences in enzyme re-

cponse to hypophysectomy, depending upon the substrate
used, point up once again the risks of drawing conclusions
regarding the etiology of hyperbilirubinemia when a sub-
strate other than bilirubin is used to assay glucuronyltrans-
erase activity.

Histologic examination of liver cells of hypophysectomized
rats shows no alterations under light microscopy, but elec-
tron microscopic examination has demonstrated a decrease
in cytoplasmic volume, decreased rough and smooth endo-

ploasma reticulum, swelling of mitochondria, and loss of

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particulate glycogen (3). These observations suggest that protein synthesis may be deficient following hypophysectomy and that the excretion of bilirubin by the liver cell may be dependent on the presence of adequate quantities of a transport protein elaborated by the endoplasmic reticulum. The observed increase in bilirubin glucuronoyltransferase activity does not correlate with the reported decrease in smooth endoplasmic reticulum, however, although the reduction in ortho-aminophenol glucuronoyltransferase does. Thus, the changes in the endoplasmic reticulum may be selective.

In conclusion, thyroxine is the major hormonal influence on the capacity of the liver to excrete conjugated bilirubin into bile in the rat. Pituitary thyroid stimulating hormone, through its control of thyroid secretion, is also involved. A pituitary hormone, possibly growth hormone, may act synergistically with thyroxine. Hypophysectomy and thyroidec- tomy do not reduce the capacity of the liver to conjugate bilirubin as estimated in vivo, incease bilirubin glucuronide formation in vitro, and decrease ortho-aminophenol glucuronide formation in vitro.

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