menced their estrous cycles. In addition to the type of controls used in the two experiments, there may have been differences in stages of the estrous cycle at the time of killing of the rats.

Summary. Increasing the litter size of rats from 0 to 2, 6, or 12 pups progressively decreased ($p<0.05$) pituitary growth hormone (GH) content, whereas mammary development (DNA) and metabolic activity (RNA and litter weight gain) progressively increased ($p<0.01$). Nursing intensity did not significantly alter ($p>0.05$) LH content of the pituitary, although LH tended to decrease in the lactating rats with increasing nursing intensity. All non-nursed and one-half of the 2-pup nursing intensity group began their estrous cycles before day 16 of lactation, but rats nursing 6 or 12 pups did not.

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Bilirubin Production in Endotoxin-Treated or Tumor-Bearing Rats (33375)

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Treatment of rats with endotoxins has been used in this laboratory as a model system for studying abnormalities in the metabolism of iron similar to those that occur during tumor growth and various infections (1–3). These investigations have indicated that the decrease in the concentration of plasma iron after the injection of endotoxin is primarily due to an inhibited return of iron to the plasma from recently destroyed erythrocytes. Such a block in the release of iron from the reticuloendothelial system (RES) has been suggested to contribute to the decrease in plasma iron observed in dogs with sterile inflammations (4) and in patients with cancer (5).

The mechanism by which iron from recently destroyed erythrocytes was prevented from returning to the plasma is not known. If the block occurs at some point prior to the release of iron from heme, then this iron might also be prevented from entering the ferritin storage pool. Although endotoxin has been shown to favor the movement of iron from

plasma to ferritin (6), transfer of iron from erythrocytes to ferritin under similar circumstances has not been clearly established. Treatment of rats with endotoxin resulted in an inhibition of the clearance of heat-damaged red cells as well as several other substances (1, 7). During chronic disorders, or in experimental animals given endotoxin, there appeared to be a decrease in the life span of erythrocytes (8). This raised the question of whether effects of endotoxin might be different on endogenous red cells as compared to those that had been damaged by heating. Since bilirubin arises predominantly as a catabolic product of hemoglobin degradation, measurement of its production by rats treated with endotoxin appeared to be a suitable approach toward answering these questions.

Methods. Female Holtzman rats weighing approximately 200 g were used. Endotoxin from *Escherichia coli* 055:B5 (Difco Laboratories, Detroit, Michigan) was suspended in water, and 100 μg was injected intraperitoneally in a 1-ml vol; control animals received 1 ml of water intraperitoneally. Ligation of the bile duct was accomplished under ether anesthesia 2 or 4 hr before sacrifice. The duodenal loop was drawn through an abdominal incision, and the bile duct was tied off with 3/0 surgical silk at ¼–½ in from its junction with the duodenum. Wounds were closed with stainless-steel wound clips. At sacrifice, blood was taken by cardiac puncture into a heparinized syringe, and plasma was recovered by pipet after centrifugation of the blood. Any samples in which obvious hemolysis had occurred were discarded.

The plasma iron concentration was measured by the 2, 2′, 2″-terpyridine method described by Schade *et al.* (9). Slight modifications of the procedure of Fister (10) were employed for the determination of both total and direct-reacting bilirubin by reaction with diazotized sulfanilic acid. The plasma was diluted 1:5 (rather than 1:10) prior to taking aliquots for assay. Absorbency of direct-reacting bilirubin samples was read within 1–5 min after mixing all reactants (no increase in absorbency was observed after an additional 5-min period). Bilirubin (Eastman Organic Chemicals, Rochester, New York) was used as a standard.

The Walker carcinoma 256 was transplanted into the *rectus femoris* as described previously (11). Bile duct ligation and plasma assays were performed 7 days later, at which time the tumors ranged from 11.3–13.3% of the body weight (234–245 g).

Results. Ordinarily bilirubin is not present in the plasma of rats in detectable amounts. To measure its production under various conditions, we ligated the bile duct so bile constituents would regurgitate into the plasma. Measurement of the rate of accumulation of bilirubin in plasma as a consequence of the experimentally induced jaundice is indicative of its rate of production provided other excretory mechanisms (e.g., renal) are not operative during the period of time selected for measurement. Figure 1 shows the observed increase in concentration of both total and conjugated (direct-reacting) bilirubin in the plasma of normal rats for 4 hr after ligation of the bile duct. The concentration at 4 hr is approximately twice the concentration at 2 hr indicating that alternate excretory pathways have not come into play during the period involved. Assuming the rate of production of bilirubin to be linear during the 4 hr, extrapolation of the curves in Fig. 1 shows that this pigment first appears in the plasma approximately 15 min after ligation of the bile duct. Plasma iron determinations in these animals revealed no alteration from normal levels as a consequence of the surgical procedures or induced jaundice. In view of the above results in normal rats, a period of 2 hr postligation was selected for measuring plasma bilirubin in subsequent experiments.

In Fig. 2 are presented the plasma concentrations of total bilirubin and bound iron 2 hr after ligation of the bile duct as measured at various times after the administration of 100 μg of endotoxin. The changes in plasma iron are similar to those previously reported (1). The production of bilirubin is not affected by the endotoxin during the first 2 hr, after which a rapid rise occurs, reaching a maximum by 8 hr. This maximum represents approximately a twofold increase in the rate

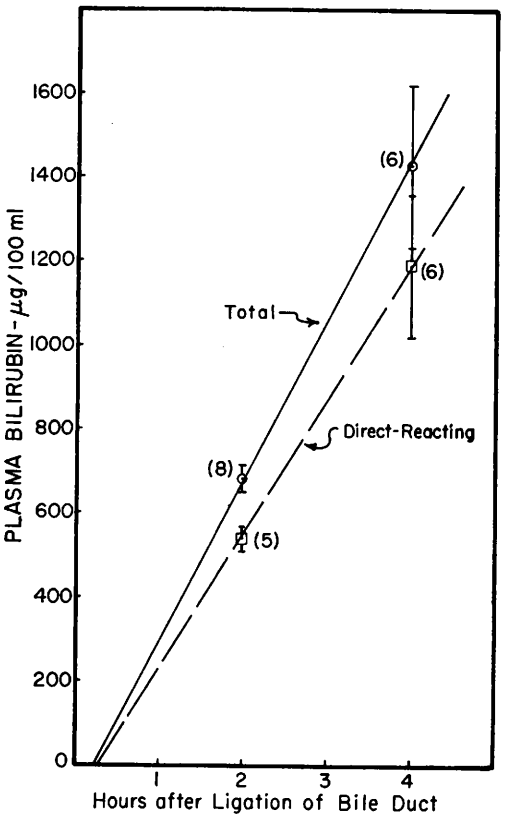

FIG. 1. Bilirubin concentration in plasma after ligation of the bile duct in normal rats. Data presented as mean ± standard error (SE). Values in parentheses indicate the number of animals used.

of production of bilirubin as compared to the control animals, and is maintained until at least 24 hr after injecting the endotoxin. The 2-hour accumulation of bilirubin then decreases to control levels by 36–48 hr after treatment. No change in bilirubin production occurs in control rats 18 hr after injecting 1 ml of water.

Measurement of the direct-reacting (conjugated) bilirubin provides a means of assessing the functional ability of the liver to conjugate bilirubin, since this reaction occurs predominantly in this tissue (12). The amounts of direct-reacting bilirubin accumulated in the plasma 2 hr after ligation of the bile duct in rats treated with endotoxin are given in Table I. It can readily be seen that the changes in production of conjugated bilirubin are similar to those of total bilirubin after injection of endotoxin, the percentage of the total remaining essentially unaltered. The amounts of unconjugated bilirubin (by difference) must also increase, then return to normal in the plasma after treatment with endotoxin.

TABLE I. Direct-Reacting Bilirubin in Plasma 2 Hr after Ligation of the Bile Duct in Rats Treated with Endotoxin.

Hr after endotoxin	No. of animals	Direct-reacting bilirubin ± SE	
		μg/100 ml plasma	% of total
0[a]	5	536 ± 31	77.8 ± 4.3
2	7	524 ± 40	75.9 ± 1.4
4	8	768 ± 46	77.4 ± 1.9
8	8	1026 ± 62	75.9 ± 1.4
18	8	1048 ± 76	74.2 ± 0.9
24	6	1103 ± 92	78.5 ± 1.4
36	4	590 ± 44	77.2 ± 1.1
48	4	444 ± 20	76.3 ± 0.9
72	4	442 ± 10	73.3 ± 1.7

[a] Normal controls.

The Walker carcinoma was selected as an example of a tumor that results in anemia (decreased hemoglobin concentration) and a decreased level of plasma iron in the host animal (11). Alterations in levels of plasma iron and in bilirubin production in rats 7 days after tumor implantation are presented

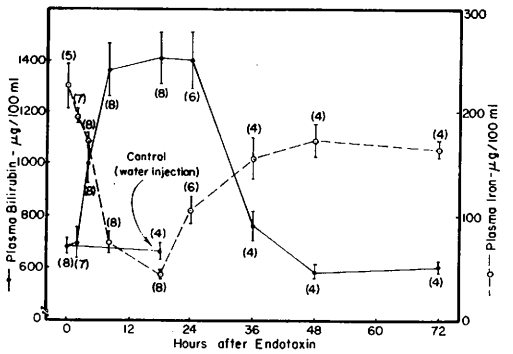

FIG. 2. Plasma iron and bilirubin concentration 2 hr after ligation of the bile duct in rats pretreated with endotoxin (100 μg, intraperitoneally). Data presented as mean ± SE. Values in parentheses indicate the number of animals used.

TABLE II. Plasma Iron and Bilirubin 2 Hr after Ligation of the Bile Duct in Rats Bearing the Walker Carcinoma 256 (7 days after transplant).

Parameter	Mean ± SE	
	Normal controls[a]	Tumor bearers[a]
Plasma Fe (μg/100 ml)	225 ± 22 (5)	130 ± 9 (4)
Total bilirubin (μg/100 ml)	683 ± 31 (8)	1000 ±112 (4)
Direct-reacting bilirubin (μg/100 ml)	536 ± 31 (5)	795 ± 73 (4)
% of total	78 ± 4 (5)	80 ± 3 (4)

[a] Values in parentheses indicate the number of animals used.

in Table II. Concomitant with a decrease in plasma iron there is an increase of approximately 50% in bilirubin production in the tumor bearers as compared to normal rats. The similar percentage of direct-reacting bilirubin in both normal and tumor-bearing rats indicates that the carcinoma does not affect the conjugating ability of the host's liver.

Discussion. The rate of accumulation of bilirubin in the plasma of normal rats after ligation of the bile duct (Fig. 1) is quantitatively similar to that observed in serum by Krstulovic *et al.* (13) during the first 12 hr after duct ligation. The time of the first appearance of bilirubin in the plasma is consistent with the measurements of Barber-Riley (14) showing that regurgitation of bile commences about 9 min after duct obstruction and becomes equal to the secretory rate after 15 min. These observations are consonant with the assumption that plasma levels of bilirubin 2 hr after duct ligation are indicative of the rate of bilirubin production.

The doubling of the rate of bilirubin production in rats treated with endotoxin (Fig. 2) suggests an increased rate of destruction of endogenous red cells by the RES under these conditions. Whether the influence of the endotoxin is on the erythrocyte or on the RES cannot be decided on the basis of these experiments, but previous studies on the inhibition of phagocytosis of various substances in endotoxin-treated rats (7) would tend to rule out direct enhancement of RES activity. The apparent discrepancy between the effects of endotoxin on endogenous versus heat-damaged erythrocytes may simply mean that destruction of red cells altered by endotoxin takes precedence over those damaged by heat. An alternate, but less likely, possibility is that the increased bilirubin production seen after treatment with endotoxin arises from nonhemoglobin sources such as myoglobin, cytochromes, or catalase. Such sources normally account for only about 16% of total bilirubin production in the rat (15). Little investigation has been made on the effects of endotoxin on heme-containing compounds other than hemoglobin. Liver catalase activity is depressed by endotoxin (16), but concentrations 13-fold higher than that used in these experiments were required to result in a 50% depression. Although it seems unlikely that nonhemoglobin sources could quantitatively account for the increase in bilirubin production reported here, this possibility cannot be rigorously excluded. On the other hand, the estimation of erythrocyte life span from bilirubin turnover in normal humans and in patients with hemolytic anemias has been shown to correlate well with conventional isotopic methods (17).

The block in re-utilization of iron from destroyed red cells that is observed during chronic disorders is usually accompanied by a somewhat shortened erythrocyte survival time (8). If this block in iron metabolism occurs at some point after the separation of iron from heme by the RES, then one might expect to find moderately increased rates of production of bilirubin. Such an increase was observed by us in rats bearing the Walker carcinoma 256 (Table II). A later step in heme catabolism, the conjugation of bilirubin with glucuronic acid, appears to be functioning normally in both the tumor-bearing (Table II) and the endotoxin-treated (Table I) animals. The observed percentage of bilirubin that was of the direct-reacting type was of the same magnitude as that found shortly after injecting unconjugated ^{14}C-bilirubin into rats with obstructive jaundice (18). Regardless of the initial source of hemes giving

rise to increased bilirubin production reported here, the block in reutilization of iron must occur at some point after the release of iron from heme since catabolism of the porphyrin moiety proceeded normally.

Summary. Bilirubin production was found to increase twofold during the first 24 hr after administering 100 μg endotoxin (intraperitoneally) to rats. A 50% increase in bilirubin production was observed in host rats 7 days after transplant of the Walker carcinoma 256 (intramuscularly). Ability of the liver to conjugate bilirubin was normal in both types of animals. The results obtained are consistent with the view that the concomitant block in re-utilization of iron occurs at some point after its release from heme.

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Proliferation and Resistance of Epidermis in Response to Harmful Stimuli* (33376)

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Proliferation and hyperplasia of cells are phenomena observed widely in various biologic systems. In such effects of overgrowth, it is obvious that the dimensions of the involved tissue exceed those established by normal controls on growth. Very little, however, is understood about this, even as to initiating circumstances (1–3).

One phenomenon that might be explored for possible significance in proliferative overgrowth is the biologic reaction of repair, that is, regeneration after loss of part of a given tissue. In other words, it may be asked

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