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Bilirubin
developmental
biochemistry
glucuronide
liver
ortho-aminophenol
uridine phosphoglucuronyl-
transferase

Barbiturate Enhancement of Bilirubin Conjugation and Excretion in Young and Adult Animals[35]

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Extract

Glucuronide conjugation of bilirubin in mammalian liver is catalyzed by the microsomal enzyme uridine phosphoglucuronyltransferase (UDPGT). Enzymic activity as measured *in vitro* is low in the young of many species. The present study was designed to determine whether UDPGT activity could be modified in adult, young, and newborn animals. Following intraperitoneal injection of saline and sodium barbital in control and experimental mice, respectively, for three consecutive days, the UDPGT activity in liver homogenates was measured on the fourth day. A significant increase in the enzyme activity was found in all ages. Results (μg bilirubin conjugated/g protein/20 min) include: newborn of dam treated during pregnancy, 96, *vs* control, 38; 4 days of age, treated (3 groups with 3 different levels of barbiturate for 3 days) 240, 362, and 533, *vs* control, 186; adult (treated) 181 *vs* control, 103. These increases were not influenced by adrenalectomy, hypophysectomy, or orchiectomy.

Rabbits also responded with a similar increase in UDPGT activity after pretreatment with phenobarbital given by subcutaneous injection and were utilized in clearance studies. In adult rabbits, following rapid intravenous infusion of bilirubin (8 mg/kg), an enhancement in excretion into bile and an increase in bile flow were observed. Bile flow (μl bile/100 g body weight/min) increased in adult animals following treatment: 2–3 *vs* control of 1–2. Total bilirubin excreted as a percent of the infused load was: treated, 76% *vs* control, 33%. In young rabbits, disappearance of bilirubin from serum was faster in the phenobarbital-pretreated animals than in controls. The excretion of bilirubin (Δ bilirubin mg/100 ml serum) in newborn animals was: treated, 3.01; untreated, 1.13. In 4-day-old animals, the excretion was: treated, 3.89 *vs* controls, 2.61.

It is proposed that several mechanisms may be responsible for the barbiturate effect and they may participate differently at various ages.

Speculation

Barbiturates were found to be effective enhancers of bilirubin conjugation and excretion in animals. A similar approach may have important therapeutic value in the human. Newborn and premature infants with a deficient glucuronide conjugating mechanism and a probable defect in hepatic transport can benefit from this pharamacologic approach. Applicability to patients with hyperbilirubinemia on an inherited metabolic basis also can be considered.

Introduction

During the last decade, research in several laboratories has furthered our knowledge of bilirubin metabolism. The excretion of bilirubin into bile follows the formation of water-soluble metabolites identified as conjugates with glucuronic acid [3, 26]. Conjugation, the last step in a successsion of enzymic-mediated events, involves the transfer of an active glucuronide moiety from uridine diphosphoglucuronic acid (UDPGA) to bilirubin [10]. This transfer is catalyzed by a microsomal enzyme uridine diphosphoglucuronyltransferase (UDP glucuronyl transferase, UDPGT, 2.4.1.17). The activity of this enzyme as measured in liver homogenates in neonates of several species is low when compared to activities obtained in adult animals of the same species [4, 16]. It is possible to increase the activity of this and other microsomal enzymes (assayed *in vitro*) by prior *in vivo* administration of chemical and pharmacologic agents [8, 13]. In the latter report, the authors used ortho-aminophenol as an aglycone acceptor. The present study was designed to determine whether UDPGT activity in adult, young, and newborn animals could be modified *in vitro* and *in vivo*.

Methods

In Vitro Enzymic Activity

All experiments were carried out on male and female mice of the Swiss-Webster strain, adult (3 months old), newborn (less than 24 hours old) and of various ages as required and stated in the results. All animals were maintained on a normal laboratory diet (Purina chow) and tap water *ad libitum*. The newborn and young mice were fed by their mothers. Surgically prepared adult animals [33] (hypophysectomized, orchiectomized, oophorectomized, or adrenalectomized) were used on the 14th postoperative day. They were maintained on a similar standard diet, except that adrenalectomized mice had their drinking water replaced with 0.9 % NaCl solution.

Experimental mice received sodium barbital in an aqueous solution (0.9 % w/v) by intraperitoneal injection in a dose of 300 mg/kg/day for three consecutive days. Control animals were given a similar volume of physiological saline by the same route. Injections were made in the lower quadrant of the abdomen with a microsyringe and a 30-gauge needle. Newborn mice received sodium barbital (50 mg/kg/day) by subcutaneous injection unless otherwise specified. Young mice (10–14 and 20 days of age) were given sodium barbital in a dose of 150 mg/kg/day by intraperitoneal injection. Pregnant mice were handled in a manner similar to adults and were treated for a minimum of four and a maximum of six consecutive days immediately prior to term. The approximate date of conception was determined by timed matings. All animals were sacrificed within 18 to 24 hours following the last injection. After decapitation, livers were excised and gallbladders removed. Livers were homogenized immediately in an isotonic solution of KCl using a Teflon pestle and a glass tube homogenizer. A 10 % homogenate was prepared (w/v). All these procedures were carried out in the cold (4°).

Enzyme Assays

UDPGT activity was assayed in the homogenates of livers or pooled livers from littermates using bilirubin or orthoaminophenol as substrates. The formation of glucuronide conjugates during the time of incubation was measured spectrophotometrically. Both assay systems, which were run in triplicate, followed zero order kinetics.

Bilirubin as Substrate

A modification of the method of GRODSKY and CARBONE [12] was used. It compares the direct diazoreacting bilirubin glucuronides present in the reaction mixture before and after incubation. The bilirubin was protected from light throughout the assay procedure to prevent photooxidation. Crystalline bilirubin [34] was dissolved in chloroform (0.1 mg/100 ml) and extracted with a solution of 0.3 % aqueous bovine albumin brought to pH 9.5 with NH_4OH, and then was adjusted to pH 7.4 with 1 N HCl. The incubation mixture consisted of 30 μmol $MgCl_2$, 200 μmol potassium phosphate buffer (pH 7.4), 0.2 μmol UDPGA [34], 0.07 μmol bilirubin, 0.4 ml of 10 % liver homogenate, and sufficient 0.5 % KCl to make a final volume of 3.5 ml. The nonincubated control flasks were maintained at 0°.

After an incubation period of 20 minutes at 37° in a Dubnoff Shaker, the experimental flasks were chilled and the contents of all flasks (control and experimental) were centrifuged at 0° for 20 minutes at 22,000 × g in a Spinco Model L ultracentrifuge. Three ml of the supernatant were diluted with water to 5 ml. To a 2.5 ml aliquot of this solution, 0.5 ml of freshly prepared diazo reagent was added and mixed. After one minute, the optical density was measured in a Beckman Model DU spectrophotometer at 535 mμ and compared to the blank containing the other 2.5 ml of the sample and 0.5 ml of 0.18 N HCl. The total amount of bilirubin present in the incubation mixture was determined for each experiment and varied by $\pm$10 % from extract to extract.

O-Aminophenol (OAP) as Substrate

The assay procedure to measure the formation of OAP glucuronide was adapted from BROWN *et al.* [4] and LEVVY and STOREY [18]. The incubation mixture consisted of 30 μmol $MgCl_2$, 150 μmol potassium phosphate buffer (pH 7.4), 0.2 μmol UDPGA, 1.32 μmol OAP in an 0.2 % aqueous solution of ascorbic acid, and 0.75 ml of the 10 % liver homogenate, and was brought to a final volume of 3.0 ml with distilled water. The control flasks, containing an identical mixture, were kept at 0°. After an incubation period of 30 minutes at 37° in a Dubnoff metabolic shaker, the reaction was stopped by chilling and protein was precipitated in control and experimental flasks by adding 3.0 ml of an equal mixture of 1.25 TCA and 2 M NaH_2PO_4 (pH 2.1). The contents of the flasks were centrifuged for 20 minutes at 1300 × g at room temperature. Following the procedure for color development, the optical density was measured at 545 mμ in a Beckman spectrophotometer. The amount of OAP glucuronide formed was estimated using an extinction coefficient of 0.224 for a solution with 0.1 μmol/ml and a 1 cm light path [14].

The method of TALALAY *et al.* [28] was utilized when the substrate used was phenolphthalein. Enzyme protein in all procedures was estimated by the procedure of LOWRY *et al.* [19].

In Vivo Clearance Studies

To simplify surgical procedures and facilitate obtaining serial blood samples in newborn animals, rabbits of the New Zealand strain were selected as the experimental animals. The developmental pattern of UDPGT activity in rabbits [29] and mice follows a similar pattern. Preliminary experiments using sodium phenobarbital as the pretreating agent in adult and newborn rabbits showed a significant increase in the *in vitro* activity of UDPGT. Adult (weighing between 3.8–4.2 kg), newborn (less than 24 hours old), and young (4 days old) rabbits were used. The adult rabbits were maintained on a normal laboratory diet and tap water *ad libitum*. The newborn and young rabbits were fed by their mothers. Control and experimental animals received respectively physiological saline and sodium phenobarbital, 30 mg/kg/day in two divided doses for three consecutive days by subcutaneous injection. Newborn animals received phenobarbital, 15 mg/kg/day by subcutaneous injection. Pregnant rabbits were treated similarly and were injected for a minimum of 3–4 days prior to delivery. All animals were used within 12 hours following the last injection. For clearance, the technique of WEINBREN and BILLING [30] was used. Subcutaneous injections of nembutal (30 mg/kg) supplemented by ether inhalation kept the animals under light anesthesia throughout the experiment. Bile ducts were surgically exposed, the common duct was ligated at its junction with the duodenum, and a polyethylene tube (PE 50) was inserted to obtain the specimens of bile. A single injection of bilirubin-albumin complex was given intravenously in the dose of 8 mg bilirubin/kg of body weight. Aliquots of bile were collected for 10 minutes prior to the injection of bilirubin and for 10-minute periods over a total time of 50 minutes post-injection. The specimens were kept in the dark at 4° throughout the procedure. Samples of blood were obtained at 10-minute intervals in a sequence corresponding to that used for bile collection five minutes after injection. The tubes and syringes were protected from light; the blood was centrifuged immediately and the serum kept in the dark at 4°. Concentrations of bilirubin in serum and bile were measured the same day. Newborn and young animals were anesthetized with an intraperitoneal injection of nembutal, 2–3 mg/kg. The bilirubin-albumin solution was injected into the external jugular vein and blood samples were obtained by cardiac puncture starting at 5 minutes and ending at 45 minutes after the injection. Some additional samples were obtained at 15 minutes and 30 minutes. The animals were kept warm and appeared in good condition at the end of the experiment. No bile duct catheterization was performed in the young animals.

The bilirubin-albumin complex (pH 7.4) was prepared freshly for each experiment according to the method of RUTHVEN as described by ROZDILSKY and OLSZEWSKI [24]. The final concentration of bilirubin in the complex was approximately 365–400 mg % and was determined each time. For the newborn and young rabbits, the concentration of bilirubin in the complex was 100–150 mg %. Concentrations of bilirubin in the complex, serum, and bile samples were assayed by the method of MALLOY and EVELYN [20].

Statistical Analyses

Data are reported as mean and standard error of the mean and were evaluated using the Student's t test for paired observations [27].

Probability values of 0.05 or less were considered significant.

Results

Developmental Curve

The mean values for UDPGT activity at various ages, using bilirubin as substrate, are shown in figure 1. Eight to 25 Swiss-Webster mice of each age group were used in the assay procedure. The values reported as μg bilirubin conjugated/g protein/20 min incubation were as follows: 1 day, 38 ± 9.6; 4 days, 186 ± 22;

Fig. 1. Developmental curve for bilirubin conjugation by liver homogenates of Swiss-Webster mice. The height of each bar represents the mean value for each age expressed in μg of bilirubin conjugated/g liver protein/20 min incubation. Ten to 25 animals of each age were used in the assay procedures. The bracketed vertical lines represent the standard error of the mean (SE).

10 days, 371±17. Values decreased at ages 14 (281±17), 10 (253±17), and 30 (246±13) days. Specific activities for adults were 103±5.7 μg/g protein/20 min. The differences between the newborn, the 4-, 10-, and 14-day-old group, and the adults are statistically significant ($p < 0.01$).

Barbital Pretreatment in Adult Mice

Figure 2 shows the results following the injection of barbital (300 mg/kg) for 3 days. In 15 male mice, the mean activity assayed 18–24 hours after the last injection was 181±μg/g protein/20 min. The control mice had a specific activity of 103±3.7 μg/g protein/20 min. This difference was statistically significant ($p < 0.01$). No effect was noted when barbital (increasing amounts up to 0.012 mg/flask) was added *in vitro* to the incubation mixture. In control females, the specific activity of the enzyme was higher than in males (177±11 μg/g protein/20 min), but in contrast to males, the increase following barbital pretreatment was not statistically significant.

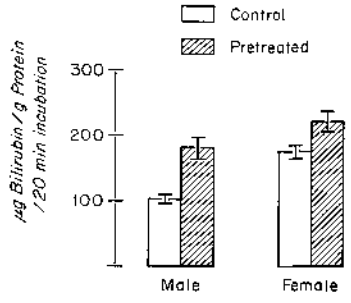

Fig. 2. Effect of barbital pretreatment on bilirubin conjugation by liver homogenates of male and female adult mice. The bars represent mean values for control and experimental animals of each sex, expressed in μg bilirubin conjugated/g protein/20 min incubation. The bracketed vertical lines represent the SE.

Substrates Other Than Bilirubin

The livers of adult male mice were assayed for UDPGT activity with other substrates; the results are shown in figure 3. Six to 12 mice were used for control and barbital-pretreated animals. The mean value in controls for o-aminophenol glucuronide was 563±54 μmol/g protein/30 min incubation and in the experimental mice was 772±41 μmol/g protein/30 min ($p < 0.01$). When phenolphthalein was the substrate used, the mean values for 26 controls and 8 barbital-pretreated mice were 5.05±0.29 μmol glucuronide/g protein/30 min incubation and 5.98±0.56 μmol/g protein/30 min respectively. This difference was not statistically significant ($0 < 0.1$).

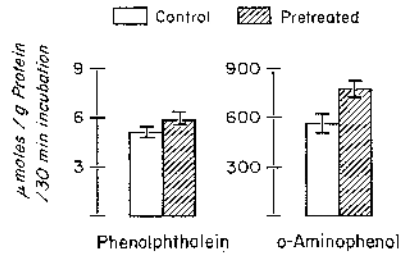

Fig. 3. Orthoaminophenol and phenolphthalein conjugation by liver homogenates of adult male mice, control and barbital-pretreated. The height of the bars represent the mean values expressed in μmol/g protein/30 min incubation, and the bracketed vertical lines the SE.

Hormonal Influence

Figure 4 shows the results obtained following surgical ablation of several endocrine glands. All assays were run between two and three weeks after surgery. Adrenalectomy *per se* did not modify the enzymic activity in male mice (113 μg/g protein/20 min±12). Ten hypophysectomized and eight orchiectomized animals showed a specific activity of 222±19 μg/g protein/20 min and 158±17 μg/g protein/20 min respectively. These increases in activity, when compared to the level of the normal adult, were statistically significant ($p < 0.01$). Following barbital pretreatment of six adrenalectomized mice, an increase in the enzymic activity was found (309±9.5 μg/g protein/20 min). In

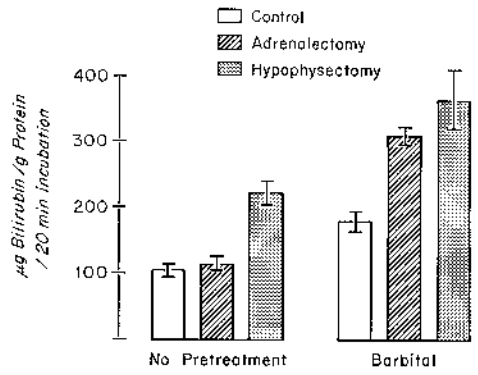

Fig. 4. Bilirubin conjugation by liver homogenates in control, adrenalectomized and hypophysectomized adult male mice. The height of the bars represent mean values after daily intraperitoneal injections of physiological saline or sodium barbital for 3 consecutive days. The bracketed vertical lines represent the SE.

the hypophysectomized mice, a further increase was shown (363 ± 48 μg/g protein/20 min) in eight animals assayed. Both values were statistically significant ($p < 0.01$). Pretreatment of adult mice with ACTH (20, 40, 60, or 100 units/day) for 3 consecutive days, administered subcutaneously, did not change the activity for UDPGT. Simultaneous measurement of glycogen content in liver demonstrated an increment related to the injected dose of ACTH.

Young Animals

Less than 24 hours of age. UDPGT activity in littermates of saline- and barbital-injected pregnant mice is shown in figure 5. Liver homogenates of 15 littermates of the pretreated pregnant mice exhibited a marked increase in activity reaching normal adult values (96 ± 13 μg/g protein/20 min). This increase is statistically significant ($p < 0.02$) when compared to control littermates (38 ± 9.6 μg/g protein/20 min). Both groups, control and experimental, were kept with their mothers and assayed between 2 and 20 hours after birth. Liver homogenates of postpartum mice displayed increased activity for this enzyme when compared to normal adult females; a further increase in enzymic activity was found in mice pretreated with barbital prior to delivery. Neither observation was pursued in detail.

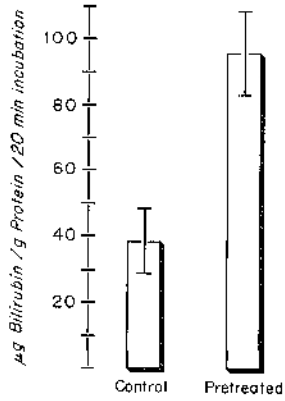

Fig. 5. Bilirubin conjugation by liver homogenates of newborn mice. Pregnant females received a daily intraperitoneal saline or barbital injection for 4 to 6 days prior to delivery. The height of the bars represent the mean values, expressed in μg bilirubin conjugated/g protein/20 min incubation and the bracketed vertical lines the SE.

Four days of age. The mean values for UDPGT activity in three groups of 4-day-old mice, following barbital pretreatment for three consecutive days, are shown in figure 6. Each group of 12 animals received barbital by intraperitoneal injection in doses of 50 mg/kg/day, 50 mg/kg/twice a day, and 100 mg/kg/day respectively. The values for each dose were 240 ± 18, 362 ± 18, and 533 ± 15 μg/g protein/20 min. Control mice of this age exhibited an activity of 186 ± 22 μg/g protein/20 min. When compared with the group receiving 50 mg sodium barbital/kg/day, there was no significant difference ($p < 0.1$), whereas the other two groups showed a significant increase ($p < 0.01$). In every age group tested, the barbital pretreatment caused an increase in the enzymic activity except in 10-day-old mice which, in the developmental curve, had already attained peak activity (371 ± 17 control and 325 ± 13 μg/g protein/20 min barbital-pretreated) ($p < 0.02$).

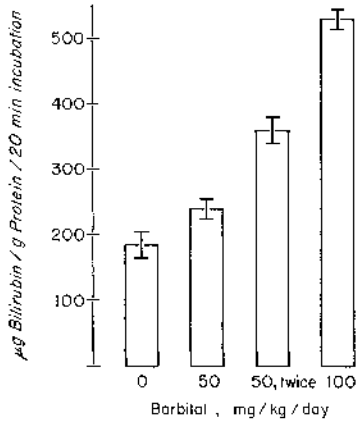

Fig. 6. Bilirubin conjugation by liver homogenates in 4-day-old mice after receiving a daily injection of saline or sodium barbital in varying doses and schedules. The height of the bars represent mean values expressed in μg bilirubin conjugated/g protein/20 min incubation. The vertical lines represent the SE.

In Vivo Studies, Clearance of Bilirubin

Newborn and young rabbits. The differences in concentration of bilirubin in serum (Δ bilirubin) measured 5 and 45 minutes after the intravenous administration of an exogenous load are shown in figure 7. Values obtained in a minimum of 5 newborn animals were averaged for each time and concentrations of bilirubin are expressed in mg bilirubin/100 ml serum. The values for newborn controls were 1.13 ± 0.34 and for pretreated animals were 3.01 ± 0.67 ($p < 0.001$). In the 4-day-old animals, they were 2.61 ± 0.13 and 3.89 ± 0.16 respectively ($p < 0.01$). The disappearance of circulating bilirubin in serum in both groups was accelerated in the phenobartital-pretreated animals. Six to 12 animals were assayed in each group.

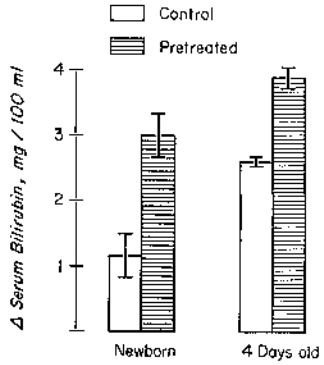

Fig. 7. Δ concentrations (5 to 45 min) of bilirubin in serum in newborn and 4-day-old rabbits, after administration of an exogenous load. The concentrations are expressed in mg/100 ml, the height of the columns represent the mean values, the vertical lines, the SE.

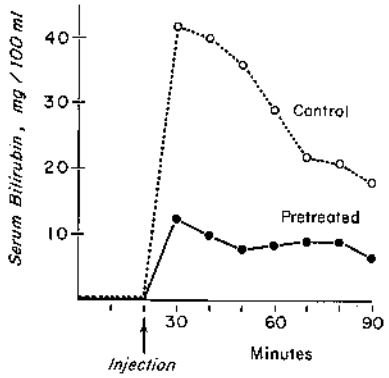

Fig. 8. Bilirubin concentration in serum, expressed in mg/100 ml as function of time in minutes. The 2 curves are representative examples of the ones obtained in control and experimental rabbits after intravenous administration of bilirubin-albumin complex in the dose of 8 mg/kg.

Adult rabbits. The clearance curves depicted in figure 8 are representative examples of the data obtained from a minimum of four experiments. Five minutes after injecting the bilirubin-albumin complex intravenously, the concentration of bilirubin in serum attained in the control animal was twice as high as that attained in the phenobarbital-treated one. By contrast, the Δ bilirubin in serum, between 5 and 60 minutes after injection, was greater in the control rabbit. In control and experimental animals, the results of simultaneous measurements of concentrations of total bilirubin (unconjugated and conjugated) in bile are shown in figure 9. Again, the curves are representative examples of the data obtained in at least four animals. The phenobarbital-treated rabbit showed a marked increase in the

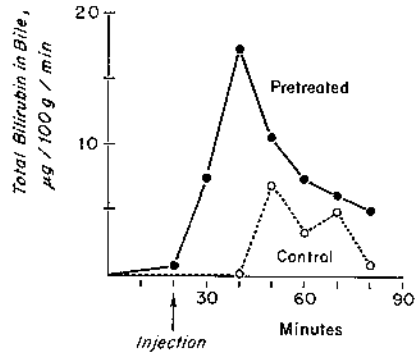

Fig. 9. Total bilirubin concentration in bile, expressed in μg/100 g body weight/min as function of time. The 2 curves are representative examples of the ones obtained in control and experimental rabbits following the intravenous administration of bilirubin-albumin complex.

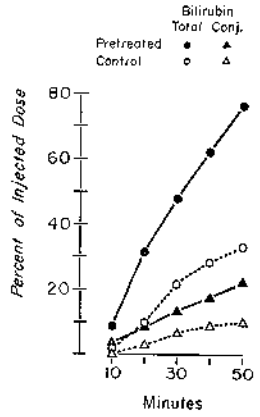

Fig. 10. Total and conjugated bilirubin excreted into bile in control and experimental rabbits, expressed in percent of the total dose given, cumulated each 10-min period for a total of 50 minutes after the intravenous administration of the bilirubin-albumin complex.

excretion of total bilirubin which occurred almost immediately after the bilirubin-albumin complex was administered. The total amount excreted over the period of collection was significantly higher in the barbiturate-pretreated animals. In experimental rabbits, an increase in bile flow was noted throughout the experiment. In the pretreated animals, flow was between 2–3 μl bile/100 g body weight/min; in control rabbits, bile flow was between 1–2 μl/100 g body weight/min. When the results were expressed as μg total bilirubin/μl bile/100 g body weight/min, the increase in concentration of total bilirubin occurred earlier and was greater in bile of experimental rabbits. These events are shown in figure 9.

Cumulative percent of bilirubin excreted. The total and conjugated bilirubin measured in the aliquots of bile obtained at 10-minute intervals, expressed as percent of the total amount of bilirubin administered and added over a total 50-minute period, is shown in figure 10. Total bilirubin excreted in control rabbits was 33 %, in contrast to 76 % in experimental animals. A difference also existed for conjugated (direct-reacting) bilirubin, 9.5 % *vs* 22 %. As seen in figure 11, the ratio of conjugated to total bilirubin in bile was determined before and after the injection of the bilirubin-albumin complex; no differences were noted between the control and experimental groups. In both groups, the relative amount of conjugated bilirubin in bile declined following administration of the exogenous load.

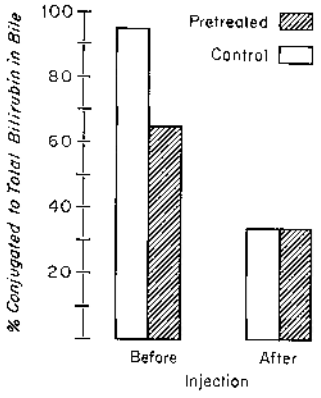

Fig. 11. Ratio of conjugated to total bilirubin in control and experimental rabbits before and after the administration of the bilirubin-albumin complex. The height of the bars expresses the percent of conjugated to total bilirubin in bile.

Discussion

Renewed interest and further research in the metabolism of bilirubin has been prompted by the development of modern laboratory tools and techniques. The formation of bilirubin and transport in the blood, its uptake by the liver, and formation of conjugated and excretion of end products have been investigated [1, 5, 17]. Conjugation of bilirubin with glucuronic acid in the liver cell is enzymically mediated and involves the conversion of unconjugated bilirubin into water-soluble bilirubin glucuronides. The enzyme responsible (UDPGT) is deficient in activity not only in the fetus but also during the first days of life. In the human fetus, unconjugated bilirubin is cleared by the mother through the placenta, whereas in the neonate, a temporary increase in the concentration of unconjugated bilirubin is found in the blood (physiologic jaundice) [5, 25].

In guinea pigs, the *in vitro* measurement of UDPGT activity in the liver using OAP as substrate demonstrated a progressive 'developmental increase' from birth, attaining maximum values by 3 weeks of age and remainnig unchanged thereafter [4]. In mice, the 'developmental increase' followed a different pattern not only in the Swiss-Webster strain, but also in other strains (C_{57}Bl, Balb C) [7]. It is difficult to explain the peak activity at 10 days of age followed by a continuous decrease to adult values. In mice, other hepatic enzymes, when assayed *in vitro*, exhibited peaks in activity, but the maximum values are attained at other ages. For example, the peak for hexobarbital-metabolizing enzyme occurs at 21 days of age [6] and for phenolsulfotransferase at 28 days of age [32]. The reasons for these sudden increases in activity are not fully understood. The presence of activators and inhibitors should be investigated, although it would be difficult to explain their selective action on several enzymes at different stages of development. The same could be considered with respect to hormonal influences. Administration of ACTH to pregnant, newborn, and adult mice does not modify the activity of UDPGT in liver. The possibility must be considered that the enzyme protein changes with age, for such changes have been demonstrated for other enzymes [2, 15, 22, 31, 32].

Following the administration of foreign compounds (carcinogenic hydrocarbons, steroids, barbiturates, etc.), an 'adaptive or induced' increase of several microsomal enzymes in liver has been described. A recent extensive review deals with this subject [9]. Inscoe and Axelrod [13] demonstrated an increase in oAP glucuronide formation in rats pretreated with benzpyrene, but no response was found in the newborn animal when the drug was injected into the pregnant female prior to delivery. Different results are

obtained using barbital as the inducing agent and when the formation of glucuronides is assayed in mice with several substrates (bilirubin, oAP, and phenolphthalein).

Barbital was selected in the present experiments because it undergoes minimal changes in the liver, 60–90 % of the drug being recovered unmodified in the urine, and only small quantities of phenobarbital and barbital are bound to plasma proteins [11]. At all ages, except in 10-day-old animals which have peak enzyme activity, a statistically significant increase in UDPGT activity follows barbital pretreatment for two of the three substrates used. The reason for this difference in substrate response is unknown but it has been suggested that more than one glucuronide-forming enzyme system exists, each with different specificities and responses to inducers [29]. The lack of barbital effect at the time of maximum peak activity suggests that a common mechanism operated for both developmental and induced increases. The barbital effect is not mediated by adrenal, pituitary, or sex hormones, as surgical ablation of endocrine glands does not prevent the increase in activity of the enzyme. Important to note is the positive effect on one-day-old animals when barbital is injected into pregnant mice. Barbital crosses the placenta and therefore can exert an effect on the fetus as well as on the pregnant animal.

The mechanism by which this induction occurs is still unknown. Several types of inducers are recognized by their specific effects on several pathways of drug metabolism in the liver [21]. A different mechanism of action can be attributed to each one. In this case, an effect is obtained by barbital pretreatment, but there is none following ACTH administration. It is of some interest that in several newborn litters from barbital-treated mothers tested in the first two hours postdelivery (results not included), UDPGT activity was equivalent to the normal control for age. The lack of induction might be attributed to the multiple environmental changes occurring during delivery such as hypothermia and hypotension, which may in themselves decrease enzymic activity.

The clearance studies were carried out in order to assess whether the *in vitro* increase in enzymic activity correlated with measureable *in vivo* effects. In young animals, the fall in concentration of bilirubin in serum was significantly greater in the pretreated group than in the control group. In both groups, serum concentrations at five minutes were equal. In adult rabbits, the fall in concentration of bilirubin in serum was greater in the control group, but concentrations at five minutes were twice as high as those in the pretreated animals. The increased excretion of total bilirubin in bile in the phenobarbital-pretreated group was noted almost immediately after the administration of the bilirubin-albumin complex. This, coupled to some increase in bile flow, explains the ability of this pretreated group to eliminate a greater amount of bilirubin in a given time. The increase in UDPGT activity by the administration of barbiturates, therefore, is not the only mechanism for a greater clearance.

The lower concentration of bilirubin in serum at five minutes after the administration of an exogenous load may be due to an enhanced uptake by the liver. Although no measurement was made of total bilirubin content in liver, it has been shown by ROBERTS and PLAA [23] to be augmented under these conditions. Simultaneously, a facilitated transport of bilirubin through the liver (or an increased permeability) may explain the greater concentration of total bilirubin in aliquots of bile obtained in the first 20 minutes of the experiment. But the increased activity of UDPGT does not appear to play a role, since the ratio of conjugated to total bilirubin is not different in either group. These apparently contradictory data may be explained if we consider the 'phenobarbital effect' to be multifactorial, reflecting increased microsomal enzymic activities, cell or membrane permeability, and transport maxima through liver cells. In the young, the phenobarbital pretreatment would include a higher enzymic activity followed by an increased formation of glucuronides which, therefore, would accelerate the total bilirubin disappearance from the serum. In the adult without impairment of glucuronide formation, pretreatment with phenobarbital would increase transport maxima and/or cell permeability, resulting in a faster and more immediate excretion of total and conjugated bilirubin into the bile. Therefore, the measured increase in the activity of UDPGT would not appear to have an important role in the enhanced clearance.

Summary

UDPGT activity of liver homogenates obtained from mice or rabbits was enhanced by prior *in vivo* administration of barbital or phenobarbital. Bilirubin glucuronide-forming enzyme activity was increased in the newborn by administration of the inducing agent either during late gestation or in the early postnatal period. The effect of barbiturate administration on bilirubin disposition *in vivo* was studied after intravenous injection of a bilirubin load. It was observed that in comparison with control animals, in young rabbits there was a quicker fall in the concentration of bilirubin in serum; in adult rabbits, excretion of bilirubin into bile was both more rapid and greater in amount.

These results are consistent with the hypothesis that several pharmacological agents are capable of inducing increased enzyme activity for the conjugation of bilirubin.

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