STUDIES ON THE NEONATAL DEVELOPMENT OF THE GLUCURONIDE CONJUGATING SYSTEM [1, 2]

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(Submitted for publication August 15, 1957; accepted October 31, 1957)

In jaundice of the newborn, whether "physiologic" or related to hemolytic disease, the bilirubin is known to exhibit the so-called "indirect" (van den Bergh) reaction with diazotized sulfanilic acid. Recently it has been shown that this type of bilirubin is unconjugated bilirubin, in contrast to the water soluble, direct reacting type which is a conjugated form, bilirubin-glucuronide (1–3). Conjugation is believed to occur chiefly in the liver, and bilirubin is excreted in the bile as a glucuronide (4).

Recent studies have demonstrated that an enzyme in the microsomes of liver transfers glucuronic acid from uridine-diphosphate-glucuronic acid (UDPGA) to bilirubin (5–7), a mechanism similar to that previously described for the transfer of glucuronic acid to phenolic (8–11), carboxylic (12), and amine acceptors (13). The reaction would appear to proceed as indicated in the equation:

$$\text{UDPGA} + \underset{\text{(indirect)}}{\text{bilirubin}} \xrightarrow{\text{glucuronyl transferase}} \underset{\text{(direct)}}{\text{bilirubin-glucuronide}} + \text{UDP}$$

It seemed likely that the cause of the indirect hyperbilirubinemia of the newborn might be related to a defect in the ability of the neonatal liver to perform glucuronide conjugation. The reason for such a failure might involve a number of steps in the development of this mechanism (Figure 1). The aim of this study was to define the step or steps at which the synthesizing system might be at fault.

Two enzyme systems involved in glucuronide synthesis were investigated. Since glucuronyl transferase activity would be the ultimate determinant of glucuronide formation, it seemed logical to explore first the glucuronyl transferase activity in newborn liver. Inadequate transferase activity would explain the accumulation of unconjugated bilirubin in the first few days of life even from normal red cell degradation. Gradual development of this system would explain the self-limitation of jaundice of the newborn.

To test this hypothesis, glucuronide synthesis was studied using microsomal fractions from fetal, newborn and adult guinea pig livers as the source of glucuronyl transferase, and bilirubin, ortho-aminophenol and phenolphthalein as the glucuronyl acceptors. In this phase of the study, to isolate the possible defect, pure uridine-diphosphate-glucuronic acid (UDPGA) was added to all reaction mixtures in order to test transferase activity alone in the presence of adequate UDPGA. Thus, specific enzymatic function could be assayed.

In the second phase, study was made of the capacity of the newborn liver to supply its own UDPGA. To investigate the development of this step in glucuronide synthesis, the activity of the enzyme which catalyzes the two-step oxidation of uridine-diphosphate-glucose (UDPG) to uridine-diphosphate-glucuronic acid (UDPGA) (10) was assayed. This enzyme, UDPG dehydrogenase, was first purified from the soluble fraction of liver homogenate by Strominger, Kalckar, Axelrod, and Maxwell (10). Activity of this specific DPN^+ dependent dehydrogenase can be determined from the rate of DPN^+ reduction (14). In this phase of the study of the glucuronide conjugating ability of the newborn, a comparison of UDPG dehydrogenase activity in the soluble fraction of liver homogenate from fetal, newborn and adult guinea pigs was made.

[1] This investigation was supported in part by Public Health Service Grant RG 5105, and by grants from The Children's Leukemia Foundation of Michigan and the Cerebral Palsy Fund, Lambda Tau Delta, Detroit.

[2] Presented in part before The Society for Pediatric Research, 27th Annual Meeting, Carmel, Calif., June, 1957.

1. Uridine triphosphate + α-glucose-1-phosphate $\xrightleftharpoons{\text{uridyl transferase}}$ UDP glucose + pyrophosphate.

2. UDP glucose + 2 DPN^+ $\xrightarrow{\text{UDPG dehydrogenase}}$ UDP glucuronic acid + 2 DPNH + $2H^+$.

3. UDP glucuronic acid + ROH $\xrightarrow{\text{glucuronyl transferase}}$ R-O-glucuronide + UDP.

FIG. 1. ENZYMATIC STEPS IN GLUCURONIDE SYNTHESIS

MATERIALS AND METHODS

1. Biochemical materials

a. Uridine-diphosphate-glucuronic acid (UDPGA),[3] uridine-diphosphate-glucose (UDPG), and diphosphopyridine nucleotide (DPN^+), were obtained from the Sigma Chemical Co., St. Louis, Mo.

b. Crystalline indirect bilirubin was obtained from Hoffman-LaRoche and Co., Nutley, N. J.

c. Ortho-aminophenol-glucuronide was supplied by Dr. Julius Axelrod, National Institutes of Health, for a reference standard.

2. Preparation of enzyme fractions

a. *Glucuronyl transferase.* Guinea pig fetuses were obtained by caesarean section at approximately four, seven, and eight weeks' gestation. Adult guinea pigs, as well as newborn guinea pigs which ranged in age from 1 to 21 days, were killed by a blow on the head and the livers were immediately excised, chilled and weighed. In a number of instances Sprague-Dawley rats were similarly utilized. The livers were placed in 10 volumes of chilled 0.9 per cent KCl and homogenized in the cold with a Teflon homogenizer. After initial centrifugation at 5,000 × G in an International refrigerated centrifuge to remove nuclei and cellular debris, the supernatant fraction was centrifuged at 80,000 × G for 30 minutes in a Spinco Model E analytical ultracentrifuge. The sediment containing microsomes and mitochondria was washed and resuspended in cold KCl and again centrifuged at 80,000 × G. The sediment was then suspended in phosphate buffer, pH 7.4. This was used as the enzyme fraction containing "glucuronyl transferase."

b. *UDPG dehydrogenase.* For assay of UDPG dehydrogenase activity, the soluble fraction of guinea pig liver was used and fractions of three degrees of purity were assayed. For initial studies, the particle-free supernatant after the initial centrifugation of liver homogenate at 80,000 × G was used as the source of UDPG dehydrogenase. For the next group of studies the enzyme fraction precipitated from homogenized liver between 50 and 70 per cent saturation with ammonium sulfate was prepared by the method outlined by Tomkins (15).

In later studies, to obtain greater activity, the technique of Strominger, Maxwell, Axelrod, and Kalckar (14) was employed. The enzyme fraction by this method was precipitated between 35 and 55 per cent saturation with ammonium sulfate and purified stepwise, except that the final adsorption and elution from calcium phosphate gel was not employed.

3. Assay of glucuronyl transferase activity

a. *Conjugation of bilirubin.* As the glucuronyl acceptor, bilirubin was used initially, since this substance was of immediate interest. In subsequent experiments ortho-aminophenol and phenolphthalein were used as the aglycones to test the same enzyme. Conversion of these substances to the glucuronide can be quantitated more precisely than can the conversion of bilirubin to bilirubin-glucuronide *in vitro*. The reaction mixture for the *in vitro* conversion of bilirubin was prepared according to the method of Schmid, Hammaker, and Axelrod (5) and consisted of the washed microsomal fraction, Tris [tris(hydroxy-methyl) aminomethane] buffer, pure UDPGA, magnesium chloride, and crystalline bilirubin (exact amounts used are summarized in Table I). The bilirubin was dissolved in 0.05 N sodium carbonate immediately before adding it to the reaction mixture. The reaction mixture for the conversion of bilirubin was incubated at 37° C. for 15 minutes under nitrogen in order

TABLE I

Assay systems for conjugation of aglycones

	Bilirubin*	Ortho-amino-phenol	Phenol-phthalein
Micromoles of substrate	3	0.66	0.15
Gm. wet wt. of liver from which microsomal fraction was derived	4†	0.2	0.2
"Tris" buffer‡			
μM	500	100	100
pH	8.5	8.0	8.0
UDPGA (μM)	4.0	0.2	0.2
$MgCl_2$ (μM)	50	50	§
H_2O (ml.)	to 12.0	to 0.8	to 1.0
Incubation (min. at 37° C.)	15 (under nitrogen)	30 (in air)	30 (in air)

* Hoffman-LaRoche crystalline bilirubin.

† In some instances only 2 Gm. of liver was available from fetal or newborn livers, in which cases controls of corresponding amounts of adult livers were used.

‡ Tris (hydroxymethyl) amino-methane.

§ Reaction proceeded adequately without $MgCl_2$.

[3] Pure UDPGA was first obtained through the kindness of Dr. Julius Axelrod at the National Institutes of Health, and subsequently through the generosity of the Sigma Chemical Co., St. Louis, Mo.

to avoid the rapid deterioration of conjugated bilirubin which occurs in air. The mixture was then treated with an excess of diazotized sulfanilic acid and ethanol to convert the bilirubin and the bilirubin-glucuronide to their azo-derivatives. The mixture was shaken with chloroform to remove the protein and the azo-pigments were extracted with butanol. They were then separated and identified by ascending chromatography, following the technique of Schmid (1). Azo-derivatives of direct reacting bilirubin from urine and serum of patients with obstructive jaundice, and of commercial (indirect) bilirubin, were chromatographed as standards for the identification of the two types of bilirubin. The azo-pigments of bilirubin were quantitated spectrophotometrically after elution from the paper.

b. *Conjugation of ortho-aminophenol and phenolphthalein.* The systems for the conjugation of ortho-aminophenol and phenolphthalein were essentially similar to that described for bilirubin. Details of the assay systems are summarized in Table I and are adapted from those previously described (5, 10).

Pure UDPGA was added to the reaction mixture to provide an excess, because of the possibility that there might be a defect in the production of this glucuronyl donor in the newborn.

4. Quantitation of glucuronide formation

Ortho-aminophenol-glucuronide was determined colorimetrically by the method of Levvy and Storey (16). Quantitation of the glucuronide was made possible by using ortho-aminophenol-glucuronide as the standard.

In order to measure the amount of phenolphthalein-glucuronide formed, the free dye concentration was determined before and after incubation by the method of Talalay, Fishman, and Huggins (17). The formation of the glucuronide was represented by the disappearance of free dye. To further demonstrate this, the free dye was extracted with ethyl ether after incubation and the remainder of the incubation mixture which contained phenolphthalein-glucuronide was hydrolyzed by beta-glucuronidase for one hour at 37° C. The amount of phenolphthalein liberated by this specific hydrolysis was then measured.

Glucuronic acid was determined by the carbazole method of Dische (18). Microsome nitrogen content was determined by the micro-Kjeldahl technique.

5. Indirect assay of UDPG dehydrogenase

In the initial step, the particle-free supernatant from liver homogenate, containing the enzyme UDPG dehydrogenase, was incubated with pure UDPG and DPN^+ to catalyze the formation of UDPGA, using the technique described by Strominger, Kalckar, Axelrod, and Maxwell (10). Then glucuronide synthesis was performed as described above, except that no extra UDPGA was added to the UDPGA generated in the initial step. Ortho-aminophenol was the glucuronyl acceptor used. In this group of experiments results were expressed simply as the increase in optical density at 540 mμ after the color reaction for ortho-aminophenol-glucuronide had taken place.

6. Direct assay of UDPG dehydrogenase activity

The UDPG dehydrogenase activity in the two prepared fractions from liver homogenate was measured according to the procedures of Strominger, Maxwell, Axelrod, and Kalckar (14), in which there is specific reduction of DPN^+ by UDPG. The assay system consisted of UDPG (0.2 μM), DPN^+ (0.5 μM) and 0.2 ml. of the enzyme fraction in 0.5 ml. of 0.1 M glycine buffer, pH 8.7. The reaction mixture was placed in a 2 ml. Corex cuvette, light path 0.5 cm. The optical density at 340 mμ was read in a Beckman D.U. spectrophotometer (1 unit of UDPG dehydrogenase equals an increase in optical density of 0.001 per minute). Readings were taken each minute for the first 10 minutes, then at 5 minute intervals for the next 20 minutes.

RESULTS

1. Glucuronyl transferase activity

When the microsomal fraction from adult guinea pig liver was used as the source of glucuronyl transferase, bilirubin, in the presence of UDPGA, was conjugated as bilirubin-glucuronide. No bilirubin-glucuronide formation could be demonstrated when the reaction mixture was incubated with microsomes from fetal or newborn guinea pig liver. When no UDPGA was added, bilirubin-glucuronide formation did not take place either in the adult or newborn system. Figure 2 demonstrates the identification of the unconjugated and the conjugated bilirubins by paper chromatography. The faster component (Rf 0.47) is the azo-pigment of unconjugated bilirubin. Only in the adult system was the slower component (Rf 0.39) seen. The Rfs of these two pigments compared well with the values established in this laboratory for the azo-pigment of commercial (unconjugated bilirubin) (0.44 to 0.48) and for that of the azo-pigment of direct bilirubin obtained from urine and serum of patients with obstructive jaundice (0.36 to 0.40). The slow component was shown to be the azo-pigment of bilirubin-glucuronide by the method of Schmid (1). Each azo-pigment was eluted, the bilirubin measured spectrophotometrically, and then specific hydrolysis with beta-glucuronidase at 37° C., pH 6.2, was carried out. After hydrolysis, glucuronic acid was determined. No glucuronic acid was found in the fast component or in the commercial bilirubin used in these experiments. In the eluate from the slow

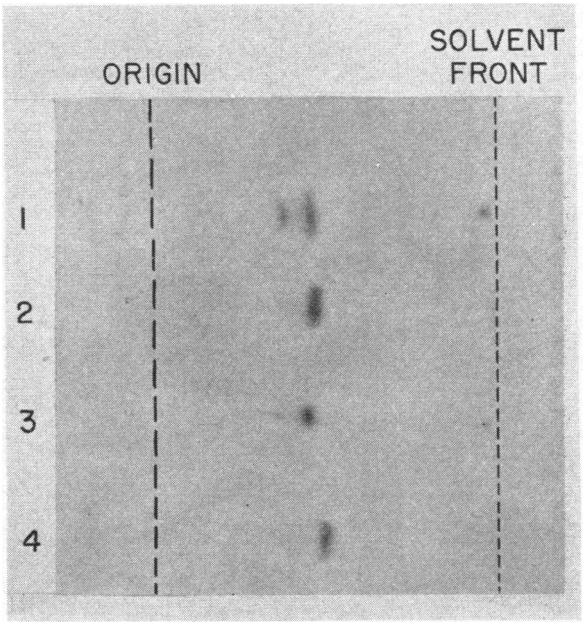

FIG. 2. RESULTS OF *IN VITRO* CONJUGATION OF BILIRUBIN

Paper chromatography demonstrating the separation of bilirubin and bilirubin-glucuronide after extraction of pigments from reaction mixtures. 1. Adult microsomal fraction (from 4 Gm. wet weight of liver), bilirubin, and UDPGA. 2. Adult microsomal fraction (from 4 Gm. wet weight of liver), bilirubin, and no UDPGA. 3. Adult microsomal fraction (from 2 Gm. wet weight of liver), bilirubin, and UDPGA. 4. Young (four days) microsomal fraction (from 2 Gm. wet weight of liver), bilirubin, and UDPGA.

Fast component indicates azo-pigment of unconjugated bilirubin. Slow component in 1 and 3 indicates azo-pigment of conjugated bilirubin. The faint (green) spot near the solvent front for 1 and 3 was presumably biliverdin.

moving spot, the molar relationship of the azo-pigment to glucuronic acid was found to be 1 : 1, indicating that bilirubin-glucuronide was present. Having demonstrated a qualitative defect in the ability of the fetal and neonatal liver to conjugate bilirubin, quantitative assays of glucuronyl transferase activity were undertaken using ortho-aminophenol and phenolphthalein as the aglycones.

Figure 3 shows a comparison of the amount of phenolphthalein conjugated when the microsomal fractions from 200 mg. of liver from fetal, newborn and adult guinea pigs were used as a source of glucuronyl transferase. The data in the figures represent the values for the disappearance of free phenolphthalein. In each instance an equal amount of phenolphthalein was recovered after beta-glucuronidase hydrolysis, indicating that the phenolphthalein had been conjugated in the reaction mixture. It is evident that at birth the guinea pig liver possesses only a slight degree of activity and that there is a gradual, steady increase in the ability to conjugate phenolphthalein during the postnatal period.

Entirely comparable results were obtained in corresponding experiments with ortho-aminophenol (Figure 4). In the fetus no activity was evident until close to term (about eight and one-half weeks in the guinea pig). To verify the apparent absence of this enzyme activity in the fetal liver, several experiments were performed in which the enzyme fraction from 1,000 mg. instead of 200 mg. of fetal liver was used. In some of the seven week fetuses there was a proportional increase in the amount of glucuronide formed, whereas the amount formed by the four week fetus was not affected (Table II).

In order to ascertain that the amounts of liver tissue used in the assays of fetal and adult mi-

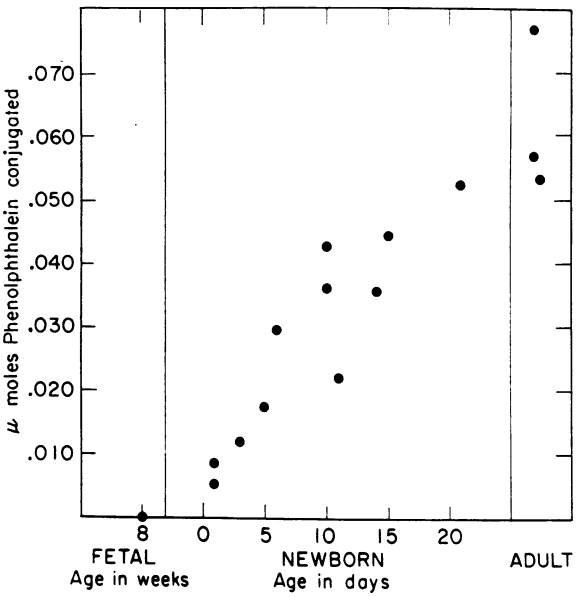

FIG. 3. COMPARISON OF THE AMOUNT OF PHENOLPHTHALEIN CONJUGATED BY FETAL, NEWBORN AND ADULT GUINEA PIG LIVER

Microsomal fraction from 200 mg. liver, with added UDPGA, incubated with 0.15 μM phenolphthalein for 30 minutes at 37° C.

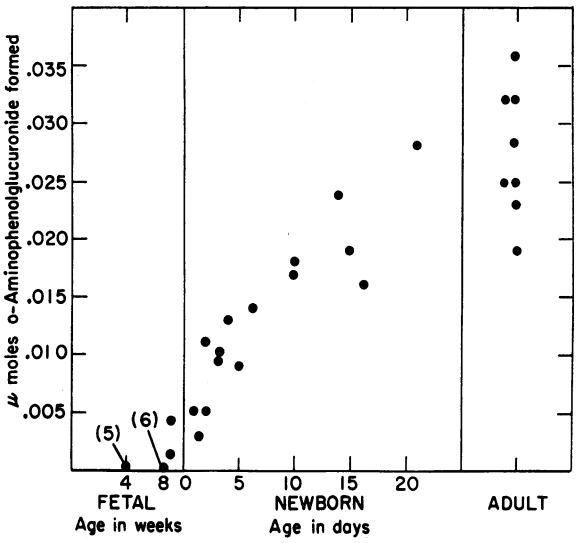

FIG. 4. COMPARISON OF THE AMOUNT OF ORTHO-AMINOPHENOL-GLUCURONIDE FORMED BY FETAL, NEWBORN AND ADULT GUINEA PIG LIVER

Microsomal fraction from 200 mg. liver, with added UDPGA, incubated with 0.66 μM ortho-aminophenol, for 30 minutes at 37° C. The parenthetical numbers 5 and 6 represent numbers of experiments performed at four and eight weeks.

crosomal activity were comparable, nitrogen determinations were performed on some of the washed microsomal preparations. The nitrogen content of the enzyme fractions are given in Table III.

2. *Indirect assay of UDPG dehydrogenase activity*

When soluble fraction of liver was used as the source of UDPG dehydrogenase in a system that included UDPG, DPN^+, and liver microsomal fractions, with ortho-aminophenol as the glucuronyl acceptor, glucuronide synthesis occurred. After incubation of the mixture for 30 minutes at 37° C., protein was removed and the color reaction for ortho-aminophenol-glucuronide was carried out. The ortho-aminophenol-glucuronide formation in this experiment is indicated only by the increase in transmittance at 540 mμ. Table IV summarizes the results of these experiments. It is known that synthesis of glucuronides in cell-free preparations of liver requires the presence of UDPGA (8, 9, 19). Since no UDPGA was added to the test system, glucuronic acid coupling

TABLE II

Ortho-aminophenol conjugation with varying amounts of liver microsomes from fetal and adult guinea pigs

Age of animal	Weight of animal	Wet weight of whole liver	Ortho-aminophenol conjugated* (wet weight of liver used for microsomes)	
			0.2 Gm.	1.0 Gm.
	(*Gm.*)	(*Gm.*)	(*μM*)	(*μM*)
Adult	1,074.0	44.6	0.020	0.079
Adult	845.0	39.7	0.030	0.096
Fetus 7 weeks	71.4	6.1	0	0.002
Fetus 7 weeks	97.4	6.8	0	0.002
Fetus 7 weeks	89.6	7.2	0	0
Fetus 7 weeks	47.6	3.5	0	0
Fetus 7 weeks	57.6	3.8	0	0
Fetus 4 weeks	68.2	3.0	0	0
Fetus 4 weeks	59.3	3.1	0	0

* Ortho-aminophenol-glucuronide obtained from Dr. Julius Axelrod was used as a reference standard.

TABLE III

Nitrogen content of liver microsomal fraction in fetal, newborn and adult guinea pigs

Age of guinea pig	Nitrogen content of microsomal fractions* (*mg.*)
Fetus (weeks)	
4	9.3
8	9.6
8	9.8
Newborn (days)	
1	9.7
2	9.5
3	9.2
4	8.7
5	10.0
10	9.4
10	9.8
11	9.6
11	9.3
13	9.4
14	9.1
Adult	10.5
Adult	10.0
Adult	10.7
Adult	10.2
Adult	10.3
Adult	10.0
Adult	10.2
Adult	9.7
Adult	10.2
Adult	10.1

* In each instance samples of microsomal fraction equivalent to 200 mg. wet weight of liver were taken.

under these circumstances could occur only if UDPGA were formed in the system by the action of UDPG dehydrogenase.

When both enzyme fractions, the glucuronyl transferase, and the UDPG dehydrogenase, used in the incubation system, were from adult liver, glucuronide synthesis proceeded readily when UDPG was also added, indicating that UDPG dehydrogenase had oxidized UDPG to UDPGA. Slight activity in the absence of UDPG probably represented the presence of some UDPG or UDPGA introduced into the system with the adult soluble fraction. By contrast, when a soluble fraction of newborn liver served as the source of the dehydrogenase, only minimal glucuronide synthesis proceeded even when the glucuronyl transferase was supplied by adult microsomes. Thus a marked decrease in newborn UDPG dehydrogenase as compared to that of the adult was demonstrated. As expected, when the newborn liver microsomal fraction was used as the source of glucuronyl transferase, there was virtually no demonstrable glucuronide synthesis, whether adult or newborn soluble fraction served as the source of dehydrogenase, indicating again the lack of transferase activity of newborn liver as previously shown.

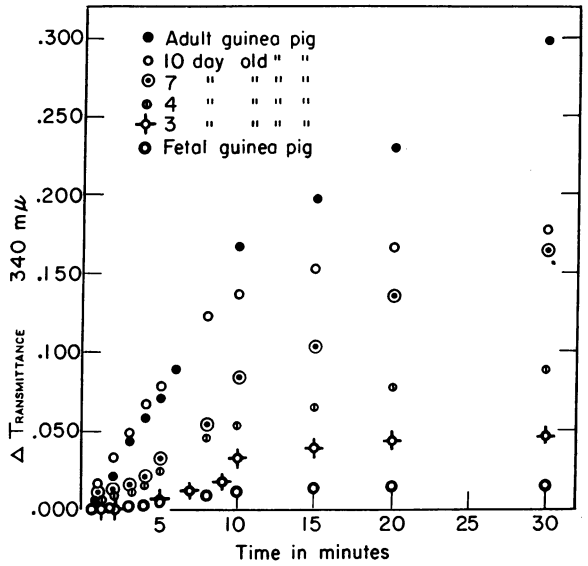

FIG. 5. REDUCTION OF DPN+ BY CRUDE PREPARATION OF UDPG DEHYDROGENASE IN SOLUBLE FRACTIONS FROM FETAL, NEWBORN AND ADULT GUINEA PIG LIVER

Activity during 30 minute period is indicated by change in optical density at 340 mμ.

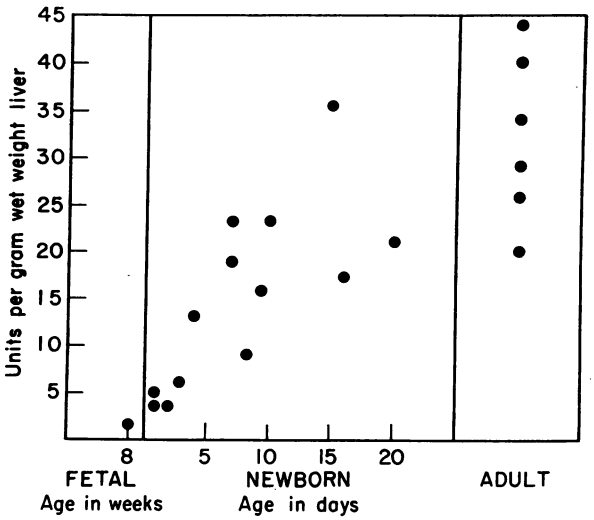

FIG. 6. COMPARISON OF UNITS OF UDPG DEHYDROGENASE ACTIVITY IN FETAL, NEWBORN AND ADULT GUINEA PIG LIVER

The enzyme fraction from liver homogenate precipitated between 50 and 70 per cent ammonium sulfate saturation was used. One unit of UDPG dehydrogenase activity equals change in transmittance at 340 mμ of 0.001 per minute.

3. Direct assay of UDPG dehydrogenase activity

Direct measurements of the UDPG dehydrogenase activity, based on reduction of DPN^+, were undertaken and confirmed the conclusions of the first experiments. Typical reaction curves of the dehydrogenase activity are illustrated in Figure 5.

UDPG dehydrogenase activity was virtually absent in the fetus. The same fraction from newborn livers showed very slight activity. Thereafter, as in the case of glucuronyl transferase, comparison of the enzymatic activity of different animals at various ages indicated the development of the enzyme system in the postnatal period. Levels of activity comparable to those found in the adult animal were evident by 15 to 20 days of age (Figure 6).

To evaluate further the quantitative difference between the adult and newborn enzyme activity, more purified preparations were made of the UDPG dehydrogenase contained in the soluble fraction of liver. When the fraction, precipitated between 35 and 55 per cent ammonium sulfate saturation, was used, the activity of the enzyme was markedly increased. When this more purified preparation of UDPG dehydrogenase was used,

TABLE IV

UDPG dehydrogenase activity in adult and newborn guinea pig liver *

Composition of reaction mixtures†	Condition	Final transmittance‡ at 540 mμ
DPN + adult soluble fraction + adult microsomes	Incubated	0.080
UDPG + DPN + adult soluble fraction + adult microsomes	Not incubated	0.015
UDPG + DPN + adult soluble fraction + adult microsomes	Incubated	0.418
UDPG + DPN + newborn soluble fraction + adult microsomes	Incubated	0.120
UDPG + DPN + newborn soluble fraction + newborn microsomes	Incubated	0.035
UDPG + DPN + adult soluble fraction + newborn microsomes	Incubated	0.040

* Using crude supernatant soluble fraction of liver homogenate.
† In addition to variables listed, all mixtures contained 0.66 μM ortho-aminophenol.
‡ Increase in transmittance at 540 mμ represents formation of ortho-aminophenol-glucuronide.

the activity (per unit weight of liver) in the two day old guinea pig liver was demonstrated to be about half that of the adult guinea pig liver (Table V).

TABLE V

Activity of purified UDPG dehydrogenase in adult and young guinea pig liver*

Age of animal	UDPG dehydrogenase activity (*units/Gm. wet wt. liver*)
2 days (pooled livers of 4 animals)	156
6 days (pooled livers of 3 animals)	231
12 days (pooled livers of 2 animals)	306
25 days (pooled livers of 2 animals)	330
Adult	
Single animal	318
Single animal	388

* Fraction precipitated between 35 and 55 per cent ammonium sulfate saturation.

To make certain that we were dealing with an enzyme that specifically oxidizes UDPG to UDPGA, the product of oxidation was adsorbed on charcoal and eluted with 50 per cent ethanol according to the technique of Strominger, Kalckar, Axelrod, and Maxwell (10). A positive carbazole reaction was obtained with the eluted product, indicating that oxidation of UDPG to UDPGA had taken place by the action of UDPG dehydrogenase.

DISCUSSION

The enzyme systems in the liver of mammals involved in the formation of glucuronides have been shown to be of importance in the *in vitro* transformation of bilirubin to the excretable form, bilirubin-glucuronide (5–7). If a similar mechanism exists in the human, a defect in the system in the newborn could be responsible for accumulation of unconjugated bilirubin in the plasma. We have demonstrated that in newborn guinea pigs and rats there is very limited activity in the enzyme responsible for the transfer of the glucuronide moiety to an acceptor. Results indicate a gradual increase in activity of this enzyme, glucuronyl transferase, during the neonatal period. This defect was shown qualitatively for formation of bilirubin-glucuronide and quantitatively for formation of ortho-aminophenol-glucuronide and phenolphthalein-glucuronide, using *in vitro* assay systems in the presence of excess UDPGA.

The fetal liver in mid-gestation possesses virtually no glucuronide conjugating activity; the newborn seems to have about one-fifth the activity of the adult (per unit weight of liver used). At about 15 to 20 days of life, the enzyme system has an activity comparable to that of the adult.

These findings could be explained by a gradual, perhaps "adaptive," development of the enzyme system or the gradual disappearance of tissue inhibitors of the enzyme. Though beta-glucuronidase activity in the liver of newborn animals is greater than in the adult (20), it is unlikely that hydrolysis of the formed glucuronides explains the findings, since it has been found in work with liver slices that even with inhibition of beta-glucuronidase by saccharic acid there is a discrepancy between the glucuronide synthesizing ability of adult and newborn animals (20).

The data further indicate that the enzyme UDPG dehydrogenase required in the synthesis

of the physiologic glucuronic acid donor (UDPGA) is deficient. This enzyme activity also increases rapidly in the newborn period in approximate parallelism with the glucuronyl transferase.

The apparent rapidity of development of these enzyme systems is a challenging finding and the factors influencing their activity deserve attention. It is probable that in the newborn, there are enzymatic defects at other intermediate levels linking glucuronide synthesis to glycogen metabolism. Investigation of these steps as well as of means of augmenting development of enzyme activity in the newborn is under way.

Data directly relating these findings to the problem in the human newborn are difficult to obtain; however, indirect evidence based on total glucuronide excretion, presented elsewhere (21), supports the concept that the human newborn has an ineffective mechanism for the conjugation of glucuronides. This inefficiency is likely to be based on an enzymatic defect like that demonstrated *in vitro* in young mammals. It is also known that sulfo-bromophthalein sodium (BSP) is poorly handled by the neonatal liver (22). Preliminary observations in this laboratory suggest that this may also be indicative of a defect in glucuronide synthesis in the newborn. Coupling with glucuronic acid is one of the major detoxification mechanisms used for exogenous substances (23), as well as for endogenous substances including corticosteroids (11). Apart from furnishing a likely explanation for hyperbilirubinemia of the newborn the inadequacy of this mechanism must be of fundamental importance in newborn physiology.

SUMMARY

Defects have been demonstrated in two enzymatic steps in the glucuronide synthesizing system in the fetal and newborn guinea pig. The glucuronyl transferase activity as well as the UDPG dehydrogenase activity are markedly deficient in the fetus and gradually increase during the first few days of life.

These enzymes are important in the formation of glucuronides, including bilirubin-glucuronide. The data support the hypothesis that the excess of "indirect" reacting, unconjugated bilirubin, regularly found in the human neonate, is due to inadequate development of the glucuronide conjugating mechanism.

ACKNOWLEDGMENTS

We are indebted to Drs. Rudi Schmid, Julius Axelrod, Kurt Isselbacher and Gordon Tomkins for their many helpful suggestions while this work was in progress, and to Mr. Dan Broida of the Sigma Chemical Co., for his help and generosity in supplying UDPG and UDPGA.

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