Constitutional Nonhemolytic Hyperbilirubinemia in the Rat: Defect of Bilirubin Conjugation. (22979)

JOHN V. CARBONE AND GEROLD M. GRODSKY
(Introduced by Harold A. Harper)
Department of Medicine, University of California School of Medicine, San Francisco.

Gilbert(1) first described a condition in man in which the blood of an otherwise healthy individual shows a high content of indirect-reacting bilirubin. Since that time other authors have observed similar cases(2-4) and have concluded that a metabolic disturbance in the hepatic mechanism for clearing bilirubin from the blood is responsible(5,6). Several investigators have suggested that an analogous defect might also be responsible for certain frequently fatal forms of hyperbilirubinemia and kernicterus in newborn infants (7,8). A similar constitutional abnormality in bilirubin metabolism has also been observed in a mutant strain of the Wistar albino rat (9). It has been reported that bilirubin is excreted from the body primarily as conjugate of glucuronic acid(10,11). This is supported by recent studies on conjugation of bilirubin in liver and kidney homogenates in the rat(12) and in liver homogenates in man*, in which it was found that the conjugated pigment produced was destroyed by β-glucuronidase, contained one mole of glucuronic acid per mole of azo pigment, and could be assayed by the 1-minute "direct" bilirubin method(13). The following scheme has been proposed for the conjugation mechanism for glucuronides(14): glucose-1-phosphate + uridine triphosphate $\longrightarrow$ uridine diphosphate glucose $\xrightarrow{\text{DPN}}$ uridine diphosphate glucuronic acid (UDPGlu) + aglycone $\longrightarrow$ aglycone glucuronide. Lack of any enzymes or co-factors involved in this mechanism or impaired permeability of the cellular membrane to bilirubin could be responsible for the metabolic defect observed in constitutional nonhemolytic jaundice.

Tissue homogenates were utilized in the present study to investigate the enzymatic mechanisms involved in formation of glucuronides in liver and kidney of rats with constitutional nonhemolytic hyperbilirubinemia (CNH).

Methods. The incubation procedure, a modification of the method of Dutton and Storey(15), was similar to that previously described(12). A homogenate was prepared of 100 mg of liver or kidney tissue from a CNH rat/ml of isotonic potassium chloride containing 3.2 X 10^{-4} M potassium bicarbonate. One ml of this suspension was added to buffer solution containing 0.3 ml of 0.5 M potassium phosphate (pH 7.4) and 0.1 ml of 0.3 M magnesium chloride. To this mixture was added 1 ml of boiled liver extract (15) as source of UDPGlu. Finally, either 40 μM of bilirubin suspended in 1 ml of 0.3% albumin solution (pH 7.4) or 1.4 X 10^{-4} M *o*-aminophenol and 10^{-3} M ascorbic acid in 1 ml of the albumin solution were added to the incubation mixture. Incubations were maintained at 37° for 45 min. The amount of bilirubin conjugate produced was determined after centrifugation by measuring concentration of "direct" reacting azo pigment in the supernatant, using the method of Ducci and Watson(13). *o*-Aminophenol glucuronide was determined by the method of Dutton and Storey(15). Experiments with bilirubin and with *o*-aminophenol were made simultaneously with aliquots from the same homogenate sample. In each experiment a "zero-time" incubation served as control. Parallel experiments were made using tissue from normal rats of the Long-Evans strain of same weight and sex as CNH animals under investigation. All experiments were done in duplicate.

Results. The rats varied from 50 to 210 g in weight and from 4 to 15 weeks in age. The total serum bilirubin levels were 11 to 13 mg/100 ml and the 1-minute bilirubin levels varied from 0 to 0.3 mg/100 ml.

As shown in Table I, liver homogenates from CNH rats did not conjugate bilirubin.

* Unpublished observations.

TABLE I. Amounts of Conjugated Bilirubin and *o*-Aminophenol Produced by Homogenates of Tissue from Normal and CNH Rats (μg/100 mg Tissue).

Material conjugated	Normal liver		CNH liver		Normal kidney with UDPGlu	CNH kidney with UDPGlu
	Without UDPGlu*	With UDPGlu*	Without UDPGlu	With UDPGlu		
Bilirubin						
1	1.5	5.5	.0	.2	1.8	.0
2	2.2	7.3	.1	.0	2.5	.1
3	2.1	6.8	.0	.0		
o-Aminophenol						
1		12.2		.0	.0	.0
2		15.7		.0	.1	.0

* Refers to boiled liver extract as source of uridine diphosphate glucuronic acid.

Addition of boiled liver extract as a source of UDPGlu failed to stimulate conjugation in these tissue preparations, although it did cause a 3-fold increase in conjugative activity in homogenates of normal liver. In like manner, the conjugative activity observed in normal kidney tissue was lacking in kidney tissue from CNH rats.

Liver homogenates from CNH rats also failed to conjugate *o*-aminophenol (Table I). No conjugation of *o*-aminophenol was observed in kidney tissue from normal or CNH rats.

Attempts to inhibit endogenous β-glucuronidase by addition of 10^{-4} or 10^{-3} M concentrations of potassium saccharate failed to influence the conjugative activity in the abnormal tissues studied.

Discussion. Despite the presence of added bilirubin and UDPGlu, no conjugation was observed in a broken-cell preparation of liver from CNH rats. This evidence suggests that the defect in bilirubin metabolism in this condition is caused, at least in part, by impaired activity of glucuronyl transferase, the enzyme responsible for transfer of glucuronic acid from UDPGlu to the aglycone. This interpretation could be extended to include at lease the following two possibilities: 1) glucuronyl transferase activity may include several unknown enzymatic steps, any of which could be absent, 2) the defect may not be enzymatic, but may be caused instead by the presence of an inhibitor or absence of some co-factor. In addition, the demonstration of existence of one enzymatic defect does not necessarily infer that others further removed in the chain of glucuronide metabolism could not also be involved. A rapid breakdown of formed glucuronide by excess amounts of β-glucuronidase could be responsible for the observed results. Failure of potassium saccharate to influence conjugation, even when added in amounts up to 10 times that which normally inhibits β-glucuronidase(15), makes this possibility highly unlikely.

It is noteworthy that the metabolic defect is not specific to the liver, but is observed in the kidney of CNH rats as well. Conjugative activity for *o*-aminophenol is also absent in the liver of these animals. This observation suggests that an impairment of mechanism of glucuronide conjugation rather than a defect specific to metabolism of bilirubin is involved.

No comparison can be made between rate of conjugation of bilirubin and that of *o*-aminophenol since starting concentrations were different. Furthermore, any such comparison would be complicated by poor solubility of bilirubin and the toxic effects of *o*-aminophenol(15). The toxicity of *o*-aminophenol may explain why we and others(15) failed to find evidence of its conjugation in normal kidneys in spite of the presence of a small amount of conjugative activity in this tissue(16,17).

It is of interest to note that inability of CNH rats to conjugate and excrete bilirubin in a normal manner implies the existence of some as yet unknown alternative metabolic pathway for catabolism of hemoglobin or for removal of bilirubin.

The probable impairment of glucuronyl transferase activity in CNH rats is suggestive

evidence for the occurrence of this same defect in infants and adult man. The finding that a lack of glucuronyl transferase activity exists in the newborn mouse(18) suggests that a similar lack might be a factor in the frequent occurrence of nonhemolytic jaundice in human infants.

Summary. 1. Broken-cell preparations of liver and of kidney from rats with constitutional nonhemolytic hyperbilirubinemia failed to synthesize bilirubin or *o*-aminophenol glucuronides. 2. Inhibition of β-glucuronidase with potassium saccharate failed to influence conjugation of bilirubin in these animals. 3. The evidence presented suggests that in constitutional nonhemolytic hyperbilirubinemia in rats, glucuronyl transferase activity is absent or inhibited.

We are indebted to Dr. W. E. Castle, Department of Genetics, University of California, Berkeley, for supplying the CNH rats, to Dr. J. J. Eiler and Dr. B. McIvor for generous permission to use their facilities.

1. Gilbert, A., and Lereboullet, P., *Gaz. hebd. de méd.*, 1902, v49, 889.

2. Mathewson, F. A. L., and Lederman, J. M., *Canad. M. A. J.*, 1949, v60, 404.

3. Meulengracht, E., *Quart. J. Med.*, 1947, v16, 83.

4. Baroody, W. G., and Shugart, R. T., *Am. J. Med.*, 1956, v20, 314.

5. Blanton, W. B., and Blanton, F. M., *Ann. Int. Med.*, 1955, v43, 598.

6. Dameshek, W., and Singer, K., *Arch. Int. Med.*, 1941, v67, 259.

7. Billing, B. H., Cole, P. G., and Lathe, G. H., *Brit. Med. J.*, 1954, v2, 1263.

8. Meyer, T. C., *Arch. Dis. Child.*, 1956, v31, 75.

9. Malloy, H. T., and Lowenstein, L., *Canad. M.A.J.*, 1940, v42, 122.

10. Billing, B. H., and Lathe, G. H., *Biochem. J.*, 1956, v63, 6P.

11. Schmid, R., *Science*, 1956, v124, 76.

12. Grodsky, G. M., and Carbone, J. V., *J. Biol. Chem.*, in press.

13. Ducci, H., and Watson, C. J., *J. Lab. Clin. Med.*, 1945, v30, 293.

14. Levvy, G. A., *Vitamins and Hormones*, New York, Academic Press, 1956, v14, 267.

15. Dutton, G. J., and Storey, I. D. E., *Biochem. J.*, 1954, v57, 275.

16. Lipschitz, W. L., and Bueding, E., *J. Biol. Chem.*, 1939, v129, 333.

17. Levvy, G. A., and Storey, I. D. E., *Biochem. J.*, 1949, v44, 295.

18. Karunairatnam, M. C., Kerr, L. M. H., and Levvy, G. A., *idem.*, 1949, v45, 496.

Received December 28, 1956. P.S.E.B.M., 1957, v94.

Blood Pressure in Apparently Healthy Aged 65 to 106 Years. (22980)

ARTHUR M. MASTER, RICHARD P. LASSER AND HARRY L. JAFFE
Department of Medicine, Mount Sinai Hospital, New York City

A study of the range of the blood pressure in a large group of apparently healthy white Americans, 65 years of age and over, has just been completed. Little reliable information on this matter has hitherto been available. Previous studies were based on an inadequate number of subjects or covered an unrepresentative sample, of whom an unknown proportion suffered from cardiovascular disease. Little information also has been available on blood pressure in persons over 90 years of age. The study was conducted by means of questionnaires sent to physicians throughout the U.S. It was designed to insure that the population sample would 1) be large enough, 2) be widely distributed throughout the country, 3) cover a proper proportion of rural and urban dwellers, 4) be representative of various ethnic and economic groups, and 5) include only apparently healthy ambulatory people, living in the community, able to take complete care of themselves and without evidence of cardiovascular disease. The blood pressure data which have been obtained, therefore, characterize this select, active, healthy group. As such it is not a random population sample as would be obtained, for example, if every tenth person in the country had been studied.