1925, v26, 215.
3. Stead, E. A., Wallace, J. M., *Tr. A. Am. Physicians,* 1957, v70, 275.
4. Neumann, C., Cohn, A. E., Burch, G. E., *Am. J. Physiol.,* 1942, v136, 448.
5. Burch, G. E., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 400.
6. Milnor, W. R., Jose, A. D., McGaff, C. J., *Circulation,* 1960, v22, 130.
7. Ross, J., Braunwald, E., Morrow, A. G., *ibid.,* 1960, v22, 929.
8. Braunwald, E., Brockenbrough, E. C., Frahm, C. J., Ross, J., *ibid.,* 1961, v24, 267.
9. Hyman, A. L., Myers, W. S., *ibid.,* 1962, v26, 735.
10. Mayer, A., *Sitzungsberichte d. Kais. Akad.,* 1876, v74, 281.
11. Rapaport, E., Severinghaus, J., *Blood Vessels and Lymphatics,* Academic Press, New York, 1962, 306.
12. Burch, G. E., Cohn, A. E., Neumann, C., *Am. J. Physiol.,* 1942, v136, 433.
13. Kuramoto, K., Robard, S., *Circulation Res.,* 1962, v11, 240.
14. Burch, G. E., Romney, R. B., *Am. Heart J.,* 1954, v47, 58.
15. Little, R. C., *Circulation Res.,* 1960, v8, 594.

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Effect of Several Drugs and Chemicals on Hepatic Glucuronide Formation in Newborn Rats.* (28246)

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The administration of various drugs to rats and guinea pigs increases the activity *in vitro* of several hepatic microsomal enzymes which metabolize drugs(1-10) and, *in vivo,* the duration of drug action is decreased by pretreatment with these same agents(7,8). In addition, some drugs increase the activity of uridine diphosphate glucose (UDPG) dehydrogenase which is not a microsomal enzyme(11, 12). Although the mechanism of these effects is uncertain, several studies suggest that the enzyme synthesis is induced(1,4,12-14).

The transient unconjugated hyperbilirubinemia of newborn infants is currently believed to result from a normally occurring delayed development of the hepatic glucuronide conjugating system(15,16). Although hepatic UDPG dehydrogenase activity is reduced in newborns as compared with adults, the major limitation in glucuronide formation in neonates is in glucuronyl transferase. This microsomal enzyme catalyzes the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to bilirubin forming bilirubin glucuronide which is subsequently excreted in the bile.

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Inscoe and Axelrod(17) treated neonatal rats and guinea pigs with 3,4 benzpyrene and observed an increase in hepatic glucuronyl transferase activity *in vitro.* These observations prompted a study of the effect of various drugs and chemicals on hepatic glucuronide formation in newborn rats and on the course of hyperbilirubinemia in human neonates.

Methods. Wistar rats in late pregnancy were obtained from the Camm Research Institute, Inc., Wayne, N. J. The estimated time of parturition is accurate to within 12 hours. Pregnant rats were fed mouse breeder chow up to the time of sacrifice and newborn rats were nursed by their mothers.

3,4 Benzpyrene; 1-(4-chloro-benzhydryl)-4-methylpiperazine dihydrochloride (chlorcyclizine); 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline (chloroquine), and 8-(4 - diethylamino - 1 - methylbutylamino) - 6-methoxy quinoline (pamaquine) were separately administered to pregnant and newborn rats in various doses.

O-aminophenol (OAP) glucuronide and direct-reacting bilirubin formation were estimated in liver homogenates with the addition of optimal amounts of UDPGA(18,19).

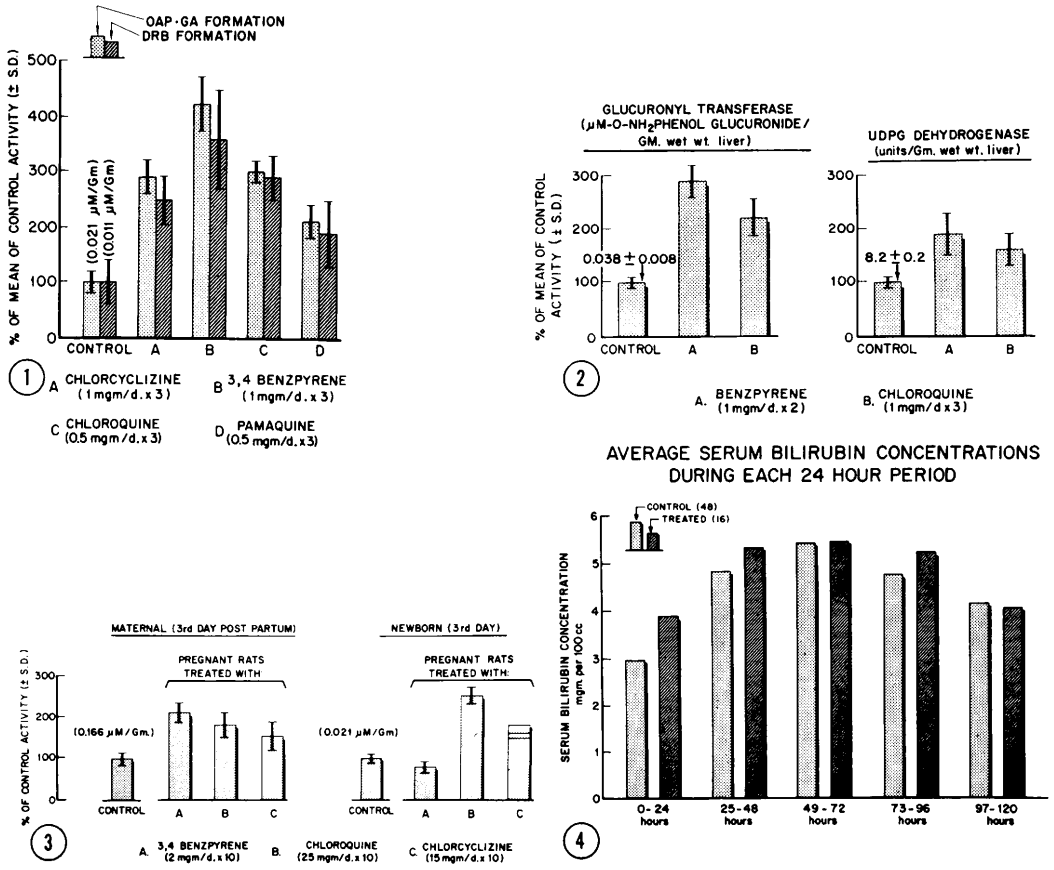

FIG. 1. Effect of drugs on O-aminophenol glucuronide and direct-reacting bilirubin formation by homogenates of liver from 3-day-old Wistar rats.

FIG. 2. Effect of drugs on hepatic glucuronide formation in 3-day-old Wistar rats *in vitro*.

FIG. 3. Effect of drug administration to pregnant rats on glucuronide formation by homogenates of maternal and newborn liver.

FIG. 4. Effect of administration of chloroquine to pregnant women on the course of hyperbilirubinemia in their infants.

Hepatic microsomal and soluble fractions were prepared according to Hogeboom(20). Glucuronyl transferase activity was estimated in the microsomal fraction using OAP as a glucuronide receptor(18) and UDPG dehydrogenase activity was estimated in the soluble fraction(21).

Women were selected at random from the prenatal clinic of Bronx Municipal Hospital Center. Forty-eight women served as controls. Sixteen women received 50 mg chloroquine per week for from one to 8 weeks prior to delivery. All babies were born at the Bronx Municipal Hospital Center. Serum bilirubin concentration was estimated daily for the first 5 days of life in infants in the control and treated groups(22). Chloroquine was administered to pregnant women after information was obtained from the World Health Organization regarding the safety of administering the drug insofar as the health of fetus and newborn is concerned. The records of the World Health Organization did not reveal any deleterious effect of chloroquine in the doses used on pregnancy, parturition or neonatal health.

Results. Fig. 1 presents the effect of intraperitoneal administration of chlorcyclizine (1 mg/d × 3); 3,4 benzpyrene (1 mg/d × 3); chloroquine (0.5 mg/d × 3), and pamaquine (0.5 mg/d × 3) on OAP glucuronide and direct-reacting bilirubin formation by homogenates of liver from groups of six 3-day-old Wistar rats. OAP glucuronide and di-

rect-reacting bilirubin formation were increased approximately 2 to 4 times by drug administration.

Fig. 2 presents the effect of intraperitoneal administration of 3,4 benzpyrene (1 mg/d × 2) and chloroquine (1 mg/d × 3) on hepatic microsomal glucuronyl transferase and soluble fraction UDPG dehydrogenase activities in groups of six 3-day-old Wistar rats. Administration of either 3,4 benzpyrene or chloroquine increased glucuronyl transferase activity approximately 2 to 3 times and UDPG dehydrogenase activity was increased approximately 1.5 to 2 times above that observed in subcellular fractions similarly obtained from untreated newborn rats.

Fig. 3 presents OAP glucuronide formation by homogenates of 6 maternal and 20 newborn rat livers following intraperitoneal administration of 3,4 benzpyrene (2 mg/d), chloroquine (25 mg/d) or chlorcyclizine (15 mg/d) to pregnant Wistar rats for approximately the last 10 days of gestation. No drugs were administered after parturition. When the newborn rats were 3 days old, they and their mothers were sacrificed and OAP glucuronide formation was estimated in homogenates of liver. In the control group of 6 animals, mean glucuronide formation by homogenates of newborn liver was approximately 15% of that observed in maternal liver. Administration of 3,4 benzpyrene resulted in a 2-fold increase in glucuronide formation by maternal liver but did not significantly affect glucuronide formation by neonatal liver. Administration of chloroquine or chlorcyclizine to pregnant rats increased glucuronide formation by homogenates of maternal rat liver approximately 1.5 times. Glucuronide formation by homogenates of neonatal rat liver was increased from 1.5 to 2.5 times that observed in controls.

Fig. 4 presents the average daily serum total bilirubin concentrations in infants in the control and treated groups. The direct-reacting fraction did not exceed 1.5 mg% in any infant. At none of the time intervals is the difference between control and treated groups statistically significant. Average maximum serum total bilirubin concentration for the control infants was 6.07 mg% and was 6.60 mg% in the treated infants.

Discussion. 3,4 Benzpyrene was used in this study because of the observations of Inscoe and Axelrod(17). Chlorcyclizine was studied because Conney *et al.*(11) have observed that the drug stimulates microsomal enzymes which metabolize various drugs. Chloroquine and pamaquine were observed in our laboratory to stimulate glucuronide formation by homogenates of adult guinea pig liver *in vitro*.

Inscoe and Axelrod(17) treated pregnant rats with 3,4 benzpyrene and observed an increase in glucuronide formation by maternal liver but not by neonatal liver. It is not known if 3,4 benzpyrene crosses the rat's placenta. Chloroquine readily crosses the rat and human placenta and, in rats has been demonstrated to accumulate in fetal as well as maternal liver.

In this study the prepartum administration of chloroquine to humans was without effect on the duration or severity of neonatal unconjugated hyperbilirubinemia. Interpretation of these results is difficult because induction of enzymes which metabolize drugs has not been demonstrated in man and it is not known if chloroquine administration enhances hepatic glucuronide formation in man.

Summary. 1. These studies confirm the observations of Inscoe and Axelrod that 3,4 benzpyrene administration to newborn rats enhances hepatic glucuronide formation. 2. Administration of chlorcyclizine or chloroquine to rats in late pregnancy increased glucuronide formation by homogenates of neonatal rat liver. 3. In rats and humans, chloroquine crosses the placenta and in rats chloroquine is concentrated in maternal and fetal liver. Administration of chloroquine, in the doses used to suppress malaria, to women in late pregnancy was without effect on the duration and magnitude of the hyperbilirubinemia observed in their infants. Further studies of the mechanism of these drug effects and their possible application to man are indicated.

1. Conney, A. H., Miller, E. C., Miller, J. A., *Cancer Res.*, 1956, v16, 450.

2. ———, *J. Biol. Chem.*, 1957, v228, 753.
3. Cramer, J. W., Miller, J. A., Miller, E. C., *ibid.*, 1960, v235, 250.
4. Von Der Decken, A., Hultin, T., *Arch. Biochem. Biophys.*, 1960, v90, 201.
5. Gelboin, H. V., Miller, J. A., Miller, E. C., *Cancer Res.*, 1959, v19, 975.
6. Conney, A. H., Gilette, J. R., Inscoe, J. K., Trams, E. R., Posner, H. S., *Science*, 1959, v130, 1478.
7. Conney, A. H., Burns, J. J., *Ann. N. Y. Acad. Sci.*, 1960, v86, 167.
8. ———, *Nature*, 1959, v184, 363.
9. Conney, A. H., Michaelson, I. A., Burns, J. J., *J. Pharm. and Exp. Therap.*, 1961, v132, 202.
10. Touster, O., Hollman, S., *Proc. Int. Biochem. Congr.*, Moscow, 1961.
11. Conney, A. H., Bray, G. A., Evans, C., Burns, J. J., *Ann. N. Y. Acad. Sci.*, 1961, v92, 115.
12. Touster, O., Hester, R. W., Siler, R. A., *Biochem. and Biophys. Res. Com.*, 1960, v3, 248.
13. Gelboin, H. V., Sokoloff, L., *Science*, 1961, v134, 611.
14. Conney, A. H., Miller, E. C., Miller, J. A., *Cancer Res.*, 1956, v16, 450.
15. Brown, A. K., Zuelzer, W. W., *J. Clin. Invest.*, 1958, v37, 332.
16. Lathe, G. H., Walker, M., *Biochem. J.*, 1957, v67, 9P.
17. Inscoe, J., Axelrod, J., *J. Pharm. and Exp. Therap.*, 1960, v129, 128.
18. Levy, G. A., Storey, I. D. E., *Biochem. J.*, 1949, v44, 295.
19. Lathe, G. J., Walker, M., *ibid.*, 1958, v70, 705.
20. Hogeboom, G., in Colowick and Kaplan, Academic Press, N. Y., 1955, v1, 16.
21. Strominger, J. L., Maxwell, E. S., Axelrod, J., Kalckar, H. M., *J. Biol. Chem.*, 1957, v224, 79.
22. Malloy, H. T., Evelyn, K. A., *ibid.*, 1937, v119, 481.

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Contributions to Characterization and Classification of Animal Viruses. (28247)

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The virus "explosion" of the past two decades has emphasized the need for systematic organization of the known viruses. Such information is of importance not only from the standpoint of taxonomy and identification, but to provide inferential knowledge of properties of viruses based on known qualities of related agents.

From the purely taxonomic standpoint, viruses, like other microbes, might be classified best according to their chemical and physical properties such as size, shape, chemical composition, and the like. Certain of these qualities, however, may not be easy to measure and may be of little use for identification in the routine laboratory. Classification systems, therefore, which embrace easily measured basic properties provide a bonus in being useful in the practical sense.

The ECHO 28 - rhinovirus - coryzavirus (ERC) group(1-3) of agents associated etiologically with the common cold(4) is included presently in the Picornavirus family and shares with the enterovirus subgroup (poliovirus, Coxsackie A and B, ECHO) the properties of small size, RNA type, and ether stability. During studies with the ERC agents in our laboratory(1,2), it was found that measurement of lability at pH 3.0 provided a simple and reliable means for separating these agents from the enteroviruses. Further studies have shown that the quality of acid lability or stability is an apparently stable property of viruses which is measured easily and is of very great value as a criterion for secondary separation of virus groups. Tests for nucleic acid type, for stability or instability to lipid solvents (ether or chloroform) and for acid stability or lability were found to provide a simple and easy means for primary subgrouping of viruses. Further subgrouping into families generally required an

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