DEVELOPMENTAL PHARMACOLOGY: A REVIEW OF ITS APPLICATION TO CLINICAL AND BASIC SCIENCE[1] 6547

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Drugs administered to the maturing host may (*a*) be used to alleviate diseases peculiar to his age group, (*b*) undergo alterations in metabolic fate and distribution to influence therapeutic effectiveness as compared with adults, or (*c*) show unexpected adverse effects as a result of systems placed at risk during development. Pharmacologic treatment of neonatal "physiologic" hyperbilirubinemia has been studied, and drugs undoubtedly will be designed for a specific effect on other diseases characterized by a slow rate of enzyme (or transport mechanism) maturation. Many developmental pharmacology studies have considered the effect of host physiology on actions of a drug or, conversely, the effect of a drug on the host. For example, failure in drug efficacy may be secondary to a low conversion of the parent drug to active metabolite (host effect on drug) or structural malformations may be the result of drug action on a developing tissue (drug effect on host). Differences in clinical response or potential toxicity for a drug are demonstrated by comparative studies in the adult versus the young. Of more importance, however, is an explanation for the observed age-related differences in drug action. Such explanations are often broadly applicable to investigations with adults and enhance rational therapeutics in children. Unfortunately, more age-related drug actions have been described than explained. Developmental pharmacologists must now accept the challenge to build programs that encourage observation *and* explanation of drug-host interactions on an age continuum (1).

This review will cite studies to illustrate the three areas of developmental pharmacology outlined above. The effect of enzyme inducing agents on hyperbilirubinemia will be considered as an example of drug use for an illness commonly found in children. The development of liver mixed function oxidase activity as influenced by hormones will illustrate modification of drug fate by age of the host. Brief reference to tetracycline effects on teeth

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and to changes in fetal physiology following drug administration during parturition will demonstrate adverse effects of drugs on the maturing host.

Drugs for a Disorder Commonly Found in Children

Pharmacologic treatment of hyperbilirubinemia.—High blood levels of unconjugated bilirubin are associated with central nervous system dysfunction. The developing organism is particularly susceptible to the encephalopathic effects of hyperbilirubinemia. Most studies indicate that the newborn has a relative hepatic immaturity of bilirubin uptake (2) and conjugation (3). Nonhemoglobin sources of heme (4) and reabsorption of deconjugated bilirubin from the gastrointestinal tract (5, 6) in neonates contribute to the load of bilirubin from hematologic sources presented to the liver for disposal. The not infrequent occurrence of septicemia, autoimmune hemolytic disease, respiratory distress syndrome, or neonatal asphyxia influence the bilirubin binding capacity of albumin. This binding capacity is probably one of the main factors which determines whether or not an infant will show signs of bilirubin encephalopathy (7, 8). Exchange transfusion is used to treat neonatal hyperbilirubinemia, but this procedure is not without risk (9, 10). Several pharmacologic agents have thus been considered for prophylaxis or treatment of hyperbilirubinemia. Drug administration is much easier than an exchange transfusion, but other more important criteria such as efficacy, toxicity (immediate or delayed), onset of action, and overall safety as compared with other forms of treatment should be considered. All too often the simplicity of drug administration prompts widespread use before assessments of toxicity by controlled trials are completed. In the following discussion the pharmacologic treatment of hyperbilirubinemia will be considered with regard to efficacy, toxicity, and alternative forms of therapy. Since some confusion has arisen about possible selective efficacy of phenobarbital, pertinent data on patient selection, etiology of icterus, and patient age are listed in Table 1.

Hyperbilirubinemia from conditions of known or suspected etiology.—Investigations with animals provided evidence that conjugation and excretion of bilirubin are enhanced after phenobarbital treatment (*vide infra*). This prompted a study by Yaffe et al (11) on the effect of phenobarbital in a 39.5 week old patient with congenital nonhemolytic jaundice. Initial and subsequent treatments with phenobarbital produced a lowering of the serum bilirubin level. Indirect assessment of hepatic glucuronide formation was made by administering salicylamide to this patient. Phenobarbital treatment produced an increase in the amount of salicylamide excreted as a glucuronide conjugate and in the maximum excretion rate of salicylamide glucuronide. Phenobarbital induction of hepatic glucuronyl transferase was considered as one factor responsible for the decrease in blood bilirubin and enhanced excretion of salicylamide glucuronide. In a preliminary and then more detailed study, Crigler & Gold (12, 13) investigated the effect of phe-

Subjects	Number	Age	Comment on Treatment	Reference
Hyperbilirubinemia from conditions of known or suspected etiology:				
Congenital nonhemolytic icterus	1	39.5 wk	Lower bilirubin in 3 wk; rose 4th wk after PB discontinued. Bilirubin <3 mg% from 65.5–92 wk on PB.	Yaffe (1966) (11)
Congenital nonhemolytic icterus	1	186 d	Lower bilirubin in 4 wk; rose about 30 d after PB discontinued. Bilirubinabout 8 mg% for 200 d on PB	Crigler & Gold (1969) (13)
Congenital nonhemolytic icterus (siblings)	2	27 yo M	Lower bilirubin in about 5 d.	Kreek (1968) (14)
		31 yo F	Lower bilirubin but rose when dose tapered.	
Congenital nonhemolytic icterus (female siblings)	2	10 yo and 4 yo	Decline in blood bilirubin and loss of icterus which returned when PB discontinued.	Ertel (1969) (15)
Congenital nonhemolytic icterus (Arias Group I)	4	2–16 yo	No effect after 3–32 wk of PB	Arias (1969) (16)
Congenital nonhemolytic icterus (Arias Group II)	9	19–40 yo	Maximum decline in about 20 d and increase in bilirubin level when PB discontinued.	Arias (1969) (16)
Gilbert's syndrome	1	30 yo	Fall in bilirubin in about 4 d and rose to pre-drug level about 3 wk after PB discontinued.	Whelton (1968) (17)
Gilbert's syndrome	13	16–57 yo	Fall in bilirubin within 2–3 d.	Black (1970) (18)
Intrahepatic biliary atresia	1	8 wk	Lower bilirubin (from 8–4 mg%) while PB continued.	Thompson (1970) (19)
Intrahepatic biliary atresia	1	? (Child)	Lower bilirubin; increased bile flow.	Sharp (1970) (20)
Chronic intrahepatic cholestasis	4	Adults	Lower bilirubin level.	Thompson (1967) (21)
Congenital extrahepatic biliary atresia	1	9–11 months	No effect on level of conjugated bilirubin.	Cunningham (1968) (22)
Hyperbilirubinemia from conditions associated with the neonate:				
Neonates of epileptic or pre-eclamptic mothers. (Retrospective study excluding Rh-affected infants)	*BW 2500 g* 98 PB 1469 C	During pregnancy	5.1% with neonatal icterus (expected incidence without PB = 22.6%).	Trolle (1968) (23)
	BW 1500–2500 g 21 PB	During pregnancy	4.8% (1 infant) jaundiced (expected incidence without PB = 50%).	

TABLE 1 (*Continued*)

Subjects	Number	Age	Comment on Treatment	Reference
Neonates (prematures and autoimmune hemolytic disease excluded)	12 PB 16 C	2 wk PTD or longer	Maximum bilirubin level (first 4d): C = 5.9 ± 0.5 mg%. PB = 2.7 ± 0.4 mg%.	Maurer (1968) (24)
Neonates (>2500 g BW with no autoimmune hemolytic disease)	159 C		Bilirubin level <10 mg%: 76.7%	Trolle (1968) (25)
	34 PB	About 1d PTD	73.5%	
	83 PB	>14 d PTD	91.7%	
	452 PB	Infant only, for 5 d	86.3%	
	121 PB	About 1d PTD +infant for 5d	85.1%	
	118 PB	>14 d PTD +infant for 5 d	94.9%	
Neonates with bilirubin >10 mg%	12 PB 12 C	4 d	No effect; possible decrease in duration of hyperbilirubinemia.	McMullin (1968) (28)
Neonates with icterus (bilirubin <12 mg%), fullterm without hemolytic disease.	52 PB 12 C	1 d (avg. 2–3 d)	No effect 24–48 hr after treatment.	Cunningham (1969) (29)
Neonates (36 wk gestation) of treated mothers (double blind)		From 31–33 wk. gestation	Bilirubin of mother lowered after 36 wk. Mean infant bilirubin on 3rd d:	Ramboer (1969) (30)
	24 PB		4.4 ± 2.3 mg%	
	21 C		8.2 ± 2.8 mg%	
Neonates, fullterm		8 hr for 3 d	Mean bilirubin on 3rd d:	Ramboer (1969) (30)
	23 PB		5.9 ± 3.5 mg% NS.	
	23 C		7.8 ± 4.3 mg%	
Neonates, 1.5–2.5 kg	10 PB 10 C	1st d for 6 d	No significant effect.	Ramboer (1969) (30)
Neonates, fullterm (no hemolytic or other disease, no breast-fed infants)		1st d for 4 d	Peak level on d 2. Early and rapid fall. Bilirubin on d 4:	Stern (1970) (32)
	20 PB		4.1 ± 0.5 mg%	
	20 C		6.0 ± 0.8 mg%	
Neonates icteric (fullterm with bilirubin 8–16 mg%)	10 PB 10 C	5th d for 4 d	More rapid fall and lower mean by d 10.	Stern (1970) (32)
Neonates		1st d for 10 d	Increase in salicylamide glucuronide excretion from 5–10 d:	Stern (1970) (32)
	10 PB		59.2%	
	14 C		34.9%	
Grecian neonates (double blind)	99 PB 183 C	36 wk pregnancy	4th d bilirubin less in treated patients (60% PB and 23% C with	Doxiadis (1970) (33)

TABLE 1 (*Continued*)

Subjects	Number	Age	Comment on Treatment	Reference
Neonates from Lesbos (some with ABO incompatibility; double blind study)	220 PB 506 C		Need for exchange transfusion decreased in infants with ABO incompatibility. 15% C and 5% PB showed severe jaundice.	Doxiadis (1970) (33)
Prematures	?	1 d	Earlier peak value (4 d vs 6 d) and lower amount.	Doxiadis (1970) (33)
Chinese infants with icterus (bilirubin 10–20 mg%)	? *ABO incompatibility* PB C *Nonspecific etiology* PB C *G-6-PD deficiency* PB C	2 wk	Exchange transfusion required in: 5% 68% 4% 35% 11% 50%	Yeung & Field (1969) (34)
Neonates with Rh hemolytic disease (not double blind study)	30 PB 30 C	1 d	Number of exchange transfusions per infant reduced except for those requiring it at birth.	McMullin (1970) (35)
Neonates (hemolytic disease excluded, double blind study)	56 PB 60 C	1 d	Number of exchange transfusions decreased. Bilirubin higher in control after 4 d especially in >2500 g infant.	Vest (1970) (38)
Neonates fullterm (double blind; hemolytic disease included)	139 PB 139 C	2–10 hr for 5 d	No effect in Negro female at 5 d. Decreased level in Negro males and Caucasian males and females at 5 d. Race and sex possible influence.	Kokosky (1970) (39)
Low birth weight icteric neonates (retrospective)	101 PB 263 C	During labor for about 3 d	Questionable to slight effect. Toxemia with some effect.	Walker (1969) (40)

C = Controls
PB = Phenobarbital Treated
M = Male, F = Female
BW = Body weight

PTD = Prior To Delivery
NS = Not Statistically Significant
d = day(s)
yo = year old

Age refers to day of life when treatment began. In some studies duration of treatment (for _____ d) is shown.

nobarbital on bilirubin metabolism in another patient with nonhemolytic unconjugated hyperbilirubinemia. Phenobarbital lowered the serum concentration of unconjugated bilirubin, and, when isotopically labeled bilirubin was used, a decrease in the half-life and total body pool of this pigment was noted. In vitro conjugation of p-nitrophenol by liver from this patient was increased and this supported the assumption by Yaffe, et al (11) that phenobarbital promoted a decrease in hyperbilirubinemia via liver enzyme induction.

Several workers described an effect of phenobarbital in siblings with congenital nonhemolytic, unconjugated hyperbilirubinemia. A brother and sister (14) were found to be jaundiced from the first week of life until the time of study when their ages were 27 and 31 years, respectively. It is of interest to note that both siblings were listed as above average in intelligence and neither had a serum bilirubin level recorded above 16 mg%. On one occasion, the serum bilirubin in the male subject was lowered during cholestyramine treatment, and in the female sibling intake of phenobarbital prior to the study was associated with a decrease in jaundice. Previous investigations included a liver biopsy from both patients with the male showing a deficient conjugation of p-nitrophenol and bilirubin. Both subjects showed a decrease in formation of menthol glucuronide as detected in the urine. After about five days of an initial high dose regimen of phenobarbital, a decrease in serum bilirubin levels in both patients was found. Sedation and an allergy to phenobarbital precluded further studies with the male sibling. In the female, however, serum bilirubin fell to 0.3 mg% after 22 days of phenobarbital therapy. Additional treatment for five months produced bilirubin levels near control values. Within a five month period the dose of phenobarbital was tapered to 32 mg per day and a rise in unconjugated levels of serum bilirubin was noted. An increase in the dosage of phenobarbital produced a subsequent fall in serum bilirubin. While phenobarbital was being tapered, the consumption of aspirin produced visible jaundice and, when the salicylates were stopped, the serum bilirubin rose. Urinary excretion of menthol glucuronide was followed during phenobarbital administration in the female subject and appeared to be indirectly correlated with the concentration of serum bilirubin. This finding suggested that glucuronyl transferase activity for bilirubin was increased by pretreatment with phenobarbital. However, Kreek & Sleisenger (14) emphasize that their evidence is indirect and does not exclude the formation of bilirubin metabolites other than the glucuronide. Congenital nonhemolytic hyperbilirubinemia was treated with phenobarbital in two sisters by Ertel & Newton (15). These siblings were jaundiced since the first week of life without any family history for icterus. One sister was ten and the other four years of age when phenobarbital treatment was begun. Prior to therapy, the level of unconjugated bilirubin ranged from 14–35 mg% and from 10–17 mg% in the older and younger sibling, respectively. There was no evidence of kernicterus or mental retardation. Blood levels of bilirubin fell in both subjects following phenobarbital therapy, but it again rose with visible jaundice evident two weeks

after discontinuance of phenobarbital. Chronic therapy with phenobarbital for at least six months produced an anicteric state with blood bilirubin levels less than 5 mg%.

Arias et al (16) presented evidence for genetic heterogeneity and classified patients with chronic nonhemolytic unconjugated hyperbilirubinemia into two groups, Group I and Group II, with respective recessive and probable dominant mode of inheritance. Patients in Group I are more severely affected with hyperbilirubinemia often from birth, whereas patients in Group II show a milder form of icterus. Phenobarbital treatment of Group I patients was without effect on lowering serum bilirubin. Group II patients, however, responded to phenobarbital therapy with a decline in serum bilirubin levels which approached those of controls. Thus, in addition to the genetic pattern of transmittance (16) and clinical severity of icterus in patients with chronic nonhemolytic unconjugated hyperbilirubinemia, the response to phenobarbital may be used as a guide to distinguish Group I from Group II patients.

Phenobarbital has been used to treat patients with Gilbert's syndrome—a mild but prolonged unconjugated hyperbilirubinemia seen in adults. In a 30 year old woman presumed to have Gilbert's syndrome, Whelton et al (17) studied the effect of phenobarbital on serum bilirubin levels and several parameters of liver structure. The patient had been jaundiced since ten days of life and gave no family history of icteric relatives or of hepatic disease or hemolysis. Approximately three weeks after discontinuance of phenobarbital, the serum bilirubin had risen to pretreatment levels. During phenobarbital administration, fecal urobilinogen and urine glucuronic acid excretion was increased. Based on their studies of urobilinogen in the feces, these workers suggest that the lowering of serum bilirubin levels was only in part due to an action of phenobarbital on glucuronyl transferase system for bilirubin. The amount of urobilinogen in feces before and after phenobarbital treatment fell short of the expected excretion of bilirubin as judged from the decline in serum icterus. Black & Sherlock (18) studied 13 patients; all except one were male. Within two to three days after initiation of phenobarbital treatment, the serum level of bilirubin fell as compared with pretreatment values. Approximately half of the patients had normal serum bilirubin levels after two weeks of phenobarbital treatment. In one patient, the level of bilirubin in plasma remained depressed while on chronic phenobarbital treatment for about 60 days. Serum bilirubin again rose to pretreatment levels when phenobarbital was discontinued in this patient. Liver biopsies in three patients revealed a general increase in uridine diphosphate glucuronyltransferase activity for bilirubin while on phenobarbital therapy as compared with values before starting treatment.

Several workers (19, 20) studied the effect of phenobarbital on cholestasis in infants with intrahepatic biliary atresia. In the study by Thompson & Williams (19), an icteric infant with intrahepatic biliary atresia as shown by liver biopsy was treated with phenobarbital at the age of eight weeks.

There was an approximate 50% decrease in the level of serum bilirubin while phenobarbital therapy was continued. Pretreatment levels of bilirubin were again seen, however, when the drug was discontinued. Similar results on the depressant action of phenobarbital on serum bilirubin levels were found in a child studied by Sharp & Mirkin (20) and in adult patients with chronic intrahepatic cholestasis (21). Phenobarbital may have acted in these patients by increasing bile flow, since bilirubin was mainly in the conjugated form. Cunningham and coworkers (22) administered phenobarbital to a nine month old infant with complete extrahepatic congenital biliary atresia and found no effect on the serum level of conjugated bilirubin. These investigators suggest that phenobarbital may be used as a diagnostic agent to distinguish between complete and incomplete biliary obstruction.

Hyperbilirubinemia from conditions associated with the neonate.—Clinical trials have tested the efficacy of phenobarbital on a large number of infants. In a preliminary communication, Trolle (23) reviewed the charts of infants born to mothers treated with phenobarbital for eclampsia or epilepsy. This retrospective study suggested a lower incidence of hyperbilirubinemia among infants from phenobarbital-treated mothers as compared to that expected from infants not exposed to phenobarbital. In a prospective study, Maurer et al (24) determined the level of unconjugated bilirubin in infants delivered of mothers treated with phenobarbital for at least 14 days prior to parturition. The maximum neonatal serum bilirubin level was 5.9 ± 0.5 mg% for controls and 2.7 ± 0.4 mg% for infants of phenobarbital-treated mothers. Serum bilirubin levels of phenobarbital exposed infants were also lower than those of control infants for each of the first four days of life. Breast feeding was more common for infants of phenobarbital-treated mothers (50%) than it was for infants of control mothers (33%). The authors stated, however, that the blood level of unconjugated bilirubin was not related to the feeding regimen although one would expect that breast fed infants would continue to receive phenobarbital secreted in the breast milk postpartum. A word of caution was voiced by these authors with regard to the potential harmful effects of phenobarbital on steroid metabolism and other endogenous substrates. Trolle (25) used a prospective study design to determine blood bilirubin levels in infants exposed to phenobarbital during the latter part of gestation, during the neonatal period, or both. Blood bilirubin levels from untreated control infants born during the previous year were used to compare with bilirubin values from phenobarbital-treated infants. A sample of control infants during the study year showed blood bilirubin values similar to those in infants of the preceding year. Infants from women treated for less than 30 hours with phenobarbital showed no difference in serum bilirubin levels versus those of control infants. Treatment of pregnant women for 14 days or more resulted in more infants with serum bilirubin levels less than 10 mg% as compared with infants from untreated women. Infants who received phenobarbital beginning only at

birth showed a tendency toward lower serum bilirubin levels. When both the mother and infant were treated with phenobarbital, a greater reduction in the level of unconjugated bilirubin was seen in blood from infants delivered of mothers treated with phenobarbital for at least three days with continuance of infant treatment during the neonatal period. These treatment regimens revealed that when phenobarbital was given for more than one day prior to delivery and then continued in the neonate, a greater percentage of these infants had serum bilirubin levels of less than 10 mg% (94.9 percent of phenobarbital-treated subjects as compared with 76.7 percent of control subjects). It was pointed out that, except for treatment of both pregnant female and infant, the other treatment regimens did not influence the percentage of infants with serum bilirubin levels higher than 18 mg%. Acute toxicity was assessed by examining the effect of phenobarbital on binding of bilirubin to albumin. Elution of bilirubin-albumin on a Sephadex column and determination of albumin reserve binding capacity did not suggest that phenobarbital displaced bilirubin from albumin. Absence of disturbances in infant behavior and utilization of low doses of phenobarbital were used in an attempt to exclude adverse reactions to this form of therapy. In a subsequent report, Trolle (*26*) indicated that phenobarbital might have a beneficial effect on mortality during the first week of life. Robinson's (*27*) comments on this observation are of interest. Examination of Trolle's data (by Robinson) showed that the control group contained more infants in the lower birth-weight category and thus unduly influenced the mortality figures. Robinson's recalculations showed that no differences in mortality were apparent between control and phenobarbital-treated infants from 1000 to 1500 grams or from 1500 to 2500 grams body weight.

McMullin (*28*) reported on a pilot study in which phenobarbital was administered to four-day old infants who showed a blood bilirubin above 10 mg%. Phenobarbital was without effect on the late phase of jaundice in these neonates, but he suggested that this form of treatment may decrease the duration of hyperbilirubinemia. Further doubt was cast upon the efficacy of phenobarbital for hyperbilirubinemia by results of Cunningham et al (*29*). In their study, infants were not treated with phenobarbital until observance of icterus after the first day of life. All infants were healthy and over 2500 grams birth-weight. This study is not too dissimilar to the one by McMullin (*28*) because most of the infants were not treated until two to three days of age. These infants exhibited mild icterus and the blood bilirubin level did not exceed 12 mg%. Administration of phenobarbital twice a day for three days did not produce a decrease in the level of serum bilirubin. Cunningham and coworkers (*29*) suggested that, once jaundice had appeared, phenobarbital treatment was without effect in ameliorating the level of serum bilirubin. In the study by Trolle (*25*) (*vide supra*) infants who showed a severe degree of jaundice (bilirubin levels above 18 mg%) failed to respond to phenobarbital given during the neonatal period. The question of effectiveness of phenobarbital after appearance of jaundice or in cases of

severe or a rapidly rising hyperbilirubinemia was raised by these studies.

Phenobarbital treatment of mothers during the last trimester of pregnancy and its effect on hyperbilirubinemia during the first days of life was studied by Ramboer et al (30). Pregnant females were treated from about 32 weeks gestation with phenobarbital in a double-blind manner. All newborns showed gestational age of greater than 36 weeks duration. It is of interest that maternal blood levels of bilirubin were lowered in the phenobarbital-treated group after the 36th week of pregnancy. No significant difference was found in the mean cord blood bilirubin in infants from control or phenobarbital-treated
phenobarbital-treated mothers had a mean bilirubin level of 4.4 mg%, whereas those from control mothers had a level of 8.2 mg%. In another part of the study, full-term infants were given phenobarbital for three days beginning within eight hours of life. No significant difference in blood bilirubin levels could be demonstrated for control or treated infants on the third day of life. Low birth weight infants treated with phenobarbital from the first to the seventh day of age also failed to show a statistically significant difference in serum bilirubin values on the fifth and seventh day of life. These workers (30) reported the effect of phenobarbital on two pairs of twins with each of the phenobarbital-treated member of the pair showing a lower serum bilirubin peak value than his respective untreated twin. No statement is made about the identical or fraternal origin of these twins. It is of interest, however, that a third pair of twins both received phenobarbital and had identical peak levels of plasma bilirubin. Similar results for drug metabolizing enzymes were found for identical twins by Vesell et al (31). Thus, the study by Ramboer et al (30) suggests that treatment of fullterm or low birth weight infants with phenobarbital after birth produces very little effect on hyperbilirubinemia, but treatment of the mothers does reduce the level of bilirubin on the third day of life. This is in agreement with the study by Trolle et al (25) who found that an effective therapy regimen for phenobarbital-induced lowering of bilirubin levels was treatment of the pregnant woman.

Studies on neonatal hyperbilirubinemia and phenobarbital have raised two questions: (*a*) Would phenobarbital administration reduce icterus after it had appeared in the neonate and (*b*) how did the effect of phenobarbital on liver glucuronide forming capacity correlate with a reduction in serum bilirubin levels? These questions were considered by Stern et al (32). Infants received phenobarbital within the first 24 hours of life in one part of the study or, in another part, infants received the barbiturate on the fourth day of life if they had a minimum serum bilirubin level of 8 mg%. Measurement of salicylamide glucuronide in the urine of phenobarbital-treated infants served as an estimate of hepatic glucuronide forming enzyme activity. In newborns treated with phenobarbital from the first day of life, a reduction in the serum level of bilirubin was seen on days four and five. Phenobarbital-treated infants also showed an earlier plateau of serum bilirubin levels at two days as

compared with four days on the control group. After three days of phenobarbital treatment, the absolute level of serum bilirubin was lower and the rate of fall was greater than in control infants. In the control group, the level of serum indirect bilirubin on day four was 6 ± 1.6 for females and 6 ± 0.9 for males. Icteric infants treated with phenobarbital from the fifth to tenth day of life showed a reduction in serum bilirubin levels by day ten. These infants also showed a greater rate of decline in the level of serum bilirubin as compared with control infants. In infants treated with phenobarbital for the first ten days of life, urine salicylamide glucuronide increased 59.2% from the fifth to tenth day of life as compared with 34.9% in the control group. At five days of age the percent of salicylamide glucuronide in urine was indirectly correlated with serum bilirubin levels in both phenobarbital and control infants. Salicylamide glucuronide excretion in the urine thus appeared to be appropriate for a gross estimate of hepatic glucuronide-forming activity and to serve indirectly as a monitor for hepatic capacity to conjugate indirect bilirubin. These workers offered supporting evidence for an effect of phenobarbital on the bilirubin glucuronide forming system and also showed that this drug was effective in lowering serum bilirubin levels in icteric infants. Like other studies, however, their results again revealed a delay of approximately three days before a significant reduction in serum bilirubin levels were found. Although phenobarbital may not be useful for lowering of serum bilirubin levels on an acute basis, it was shown that the rate of decline in serum bilirubin is enhanced by prior treatment with phenobarbital beginning at birth. As pointed out by Stern and coworkers (32) studies on the correlation between bilirubin levels in serum and excretion of salicylamide glucuronide again emphasize the hepatic immaturity as a critical factor in the production of neonatal icterus.

Neonatal hyperbilirubinemia of specific etiology has been studied with regard to possible alterations in the effect expected from phenobarbital. Doxiadis (33) studied the effect of phenobarbital on neonatal jaundice associated with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and with geographical differences in the incidence of neonatal icterus. He observed that, although the frequency of G-6-PD deficiency in southern Greece was 2.92%, in Rhodes, 12.5%, and in Lesbos, 4.93%, children both with and without this deficiency in Lesbos had a higher incidence of severe neonatal jaundice. In all three regions the incidence of neonatal jaundice (bilirubin level above or equal to 16 mg%) was higher in infants who also had G-6-PD deficiency. Furthermore, infants with G-6-PD deficiency showed a hyperbilirubinemia associated with red cell hemolysis. Occurrence of neonatal icterus without availability of exchange transfusion prompted a study on efficacy of phenobarbital for hyperbilirubinemia in these Grecian infants. When a double blind study design was used, phenobarbital treatment of women approximately 36 weeks pregnant produced lower cord blood bilirubin values. More infants in the phenobarbital-treated group had blood bilirubin less than 4 mg% and less than 16 mg% on the fourth day of life as

compared with control infants. Gradual elimination of bilirubin in the stools of phenobarbital-treated infants was found. This study included only women who took at least 1 gram of phenobarbital. In another part of the study by Doxiadis (33) women 32 to 38 weeks pregnant from the island of Lesbos were used. Some of the infants in this study showed evidence of ABO blood group incompatibility. Phenobarbital treatment of the mothers reduced the number of exchange transfusions required in infants with or without ABO incompatibility and the severity of jaundice was similarly reduced. In additional studies, Grecian premature infants were given phenobarbital from the day of birth and showed a lower peak level of blood bilirubin. Not unlike the study by Stern et al (32), an earlier peak level of serum bilirubin was found in these infants treated with phenobarbital after birth. Based on his experience with a high-risk population, Doxiadis suggests that phenobarbital should be used prophylactically for pregnant women on the island of Lesbos and that it may be considered for women suspected of bearing children of low birth weight or with excessive hemolysis.

Yeung & Field (34) reported on the effect of phenobarbital on hyperbilirubinemia in icteric Chinese infants. These neonates had a serum bilirubin of 10–20 mg% and were less than two weeks of age when placed on the study. These authors pointed out that, in control infants with hyperbilirubinemia from all causes, 45% required an exchange transfusion whereas this procedure was performed in only 4% of phenobarbital-treated subjects. In most cases, the exchange transfusion was performed within 24 hours after initiation of drug therapy. Infants with hyperbilirubinemia due to ABO incompatibility showed lower levels of bilirubin in the phenobarbital-treated group from days three to five as compared with untreated controls. Only one infant in the phenobarbital-treated group required exchange transfusion. Infants with G-6-PD deficiency and hyperbilirubinemia showed a 50% and 11% incidence of exchange transfusion for controls and phenobarbital treated, respectively. The effect of phenobarbital in infants with hyperbilirubinemia associated with cephalohematoma or immaturity was also examined, but the number of cases in the study was too low to draw any definite conclusions. Phenobarbital treatment of hyperbilirubinemia in infants from nonspecific causes showed that only 4% of these infants required exchange transfusion as compared with about 35% of control infants. Phenobarbital treatment of these infants also lowered the mean level of blood bilirubin during the course of treatment. The data presented by these workers is somewhat difficult to interpret because little information is given about the number of icteric infants who entered this study at a particular age in either the control or phenobarbital-treated group. As emphasized in their study, however, phenobarbital did appear to reduce the number of exchange transfusions for hyperbilirubinemia of varied etiology. Another interesting aspect of their study is that an effect of phenobarbital was noted in icteric infants. This does not agree with the results of McMullin (28) or Cunningham et al (29), but does support the

findings by Stern et al (32) with regard to an effect of phenobarbital after appearance of jaundice. A difference in phenobarbital effects in icteric infants may be partly explained by race, since Chinese infants do not usually show clinical icterus until the third day of life. Thus, in the absence of anemia or a severe hemolytic disorder, there may be relative differences in maturation of liver function between Caucasian and Chinese infants. This in turn may modify their response to phenobarbital, but, until more information is available on the number of infants selected for study at each postnatal age (34) this point will not be resolved.

The effect of phenobarbital on neonatal hyperbilirubinemia of specific etiology, namely, Rhesus hemolytic disease, was studied by McMullin et al (35). The mean gestational age, birth weight, and cord bilirubin level of control and treated infants were similar. If need for exchange transfusion is taken as an index of efficacy of phenobarbital treatment, then infants in the treated group required fewer exchange transfusions per infant. However, severely affected infants requiring an exchange transfusion at birth showed no effect on the number of exchange transfusions per infant in control versus phenobarbital-treated groups. If transfusions required at birth were excluded, then the number of exchange transfusions per infant in the treated group for all infants or for infants requiring the exchange transfusion at birth were decreased as compared with the nonphenobarbital-treated group. A lack of information about the level of blood bilirubin, duration of phenobarbital treatment, or time of exchange transfusion following initiation of phenobarbital treatment presents some difficulty with interpretation of these results (36, 37). Frequency of exchange transfusion and level of hyperbilirubinemia following phenobarbital administration to fullterm and low birth weight infants was investigated further by Vest et al (38). They excluded infants with hemolytic disease, and the study was conducted in a double blind fashion. Phenobarbital was started on the first day of life with dosage varying according to the weight category of the infant. Mean body weight for treated infants was 2070 grams and for control infants, 2203 grams. When all infants were considered without regard to body weight, the frequency of exchange transfusion was reduced in phenobarbital-treated infants. On the fourth day of life, the serum bilirubin level in phenobarbital-treated infants remained lower than that of the control group and this difference was exaggerated when only fullterm infants (greater than 2500 grams body weight) were considered. In infants less than 2500 grams body weight there was a significant difference in serum bilirubin levels between placebo and phenobarbital-treated infants on the seventh day of life as compared with a significant difference found with fullterm infants noted on the third day of life.

Factors other than hemolytic disease have been shown to modify the level of serum bilirubin and a response to phenobarbital. Efficacy of phenobarbital for hyperbilirubinemia with regard to race and sex of the infant was examined by Kokosky et al (39). Infants were treated with phenobarbital soon

after birth in a double blind study. An extra water-treated control was provided for the ethanol vehicle of phenobarbital elixir. Ethanol was found to have no effect on serum bilirubin level. On the third and fifth day of life the serum bilirubin level showed some variation with regard to race and sex. Caucasian males showed a greater mean difference (controls minus phenobarbital-treated) in blood bilirubin levels on the third and fifth day of life than did Negro males. A similar finding was noted with Caucasian females versus Negro females. In general, females showed a lower mean difference than did males of both races, a finding not confirmed by Stern et al (32). Phenobarbital-treated Negro females failed to show a statistically significant decrease in the level of serum bilirubin on either the third or fifth day of life. Phenobarbital pretreatment of Caucasian females produced a decrease in the level of serum bilirubin on the fifth day but not on the third day of life. When the effect of sex on the level of serum bilirubin was examined, Caucasian or Negro male controls had a higher serum bilirubin level than female controls on the third and fifth day of life, respectively. No significant differences on the third and fifth day of life in the serum bilirubin level were found in either Caucasian or Negro phenobarbital-treated males versus females. The effect of race was also examined. Untreated Caucasian males had higher levels of serum bilirubin on days three and five as compared with untreated Negro males. Similar findings were observed for phenobarbital-treated Caucasian males versus Negro males. No race difference was demonstrated for phenobarbital-treated Caucasian females versus similarly treated Negro females. In general, when examined on the third and fifth day of life, control Caucasian males showed the highest level of serum bilirubin. This group of infants also showed the greatest mg% fall in serum bilirubin following treatment with phenobarbital for three to five days. Conversely, control Negro females had the lowest level of serum bilirubin at age three and five days and also exhibited the least amount of response to phenobarbital pretreatment. Examination of differences in serum bilirubin levels with regard to race indicates that race influences the response to phenobarbital in the male. These workers pointed out that when all their data in control versus phenobarbital-treated infants were compared without regard to race or sex, a significant reduction in serum bilirubin levels on days three and five were observed. No explanation is available for the observed differences with regard to race and sex, especially in the Negro female.

Walker et al (40) studied hyperbilirubinemia in the low birth weight infant in relation to phenobarbital and toxemia of pregnancy. Barbiturate-treated women who showed no evidence of toxemia produced infants in whom the level of serum bilirubin did not differ significantly from nontoxemic mothers without barbiturate treatment. No control or barbiturate-treated women who were toxemic showed infants with a blood bilirubin level of above 15 mg%. When all mothers were considered without regard to toxemia, non-barbiturate-treated mothers produced infants 11% of whom had blood bilirubin levels greater than 15 mg% whereas those mothers treated with the

barbiturate showed 2–6% of their infants with this level of bilirubin. Dr. Walker and coworkers suggested that toxemia of pregnancy had an ameliorating effect on hyperbilirubinemia which was exerted through a process dissimilar to that found with barbiturates.

Waltman et al (41) gave an infusion of ethanol to pregnant women shortly before delivery as a prophylactic measure for neonatal hyperbilirubinemia (Table 2). From one to five days after birth the infants from ethanol-treated women showed lower serum bilirubin values as compared with infants from untreated control mothers. Treatment of mothers with ethanol at least two hours before delivery yielded infants who had a maximum serum bilirubin level of approximately one-half of that of infants from control mothers. In a subsequent report, Waltman et al (42) found that treatment of women from 3 to 96 hours prior to delivery produced infants with lower serum bilirubin levels from three to five days of life and a lower mean maximum level as compared with untreated controls. Infants from women who received ethanol (less than 100 grams) more than 96 hours or less than 3 hours prior to delivery showed no difference in their serum bilirubin levels as compared with untreated controls. These workers found no acute toxic effects in infants delivered of ethanol-treated mothers, and they suggest that microsomal enzyme induction, which may have occurred as a result of ethanol infusion, is not regarded as a damaging or toxic effect. Comments on enzyme induction by Meldolesi (43) would not support this assumption. Safety of ethanol relative to phenobarbital was considered by Waltman et al (42) because of rapid elimination of the former compound. Potential toxicity of ethanol, however, probably shares some characteristics that were explored for phenobarbital (44). Isaac & Dundee (45) raise an additional question about adverse effects in mothers who were given ethanol. A rapid infusion of 8% ethanol was associated with a higher incidence of thrombosis and phlebitis. They also mentioned in vitro studies wherein the ethanol in saline solution produced red cell hemolysis. Flock, et al (46) showed that an acute dose of ethanol in the rat altered brain utilization of glucose. Although glucose is probably not the only source of nutrients for the neonatal brain, administration of a drug that may interfere with glucose utilization (if these rat studies can be extended to man) may place the infant at risk for neurological damage.

If ethanol acted by inducing hepatic bilirubin glucuronyl transferase, then it is puzzling that no effect was seen in mothers treated with ethanol 96 hours or more before delivery. Furthermore, the metabolism of ethanol, and its possible inducing effect on hepatic microsomal drug metabolizing enzymes, show some differences as compared with other enzyme inducing agents. Participation of the classical mixed function oxidase system in the hepatic microsomal oxidation of ethanol has been questioned by Khanna et al (47). Ethanol metabolism as measured in vivo (48) or in vitro (49) did not respond in the expected manner to agents that either inhibit or induce mixed function oxidase activity. A role for NADPH oxidase and catalase

TABLE 2. DRUG TREATMENT OF HYPERBILIRUBINEMIA

Subjects	Number	Age	Comment on Treatment	Reference
Ethanol Treatment:				
Neonates without hemolytic disease		1–2 d PTD	Lower bilirubin from 2–5 d of life. Mean maximum:	Waltman (1969) (41)
	10 EtOH		4.6±0.4 mg%	
	10 C		8.0±0.8 mg%	
	15 EtOH	3–96 hr PTD	Mean peak=4.8 mg%	Waltman
			Daily mean less from d 3–5	(1969) (42)
	11 EtOH	<3, >96 hr PTD	No effect	
	18 C		Mean peak=7.5 mg%	
Diethylnicotinamide Treatment:				
Congenital nonhemolytic icterus (female siblings)	2	10 yr	Some decrease in serum bilirubin.	Ertel
		4 yr	More rapid rise when drug discontinued.	(1969) (15)
Neonates (twins without hemolytic disease)	10 treated	2 d for 7 d	Bilirubin lowered by 6th d of life.	Sereni
	10 C			(1967) (55)
Glutethimide Treatment:				
Gilbert's syndrome	1	18 yo M	Fall in bilirubin in about 6 d to approximately normal level while on drug.	Black (1970) (18)

C = Controls
PB = Phenobarbital Treated
M = Male, F = Female
BW = Body weight

PTD = Prior To Delivery
NS = Not Statistically Significant
d = day(s)
yo = year old

Age refers to day of life when treatment began. In some studies duration of treatment (for _____ d) is shown.

activity with regard to hepatic microsomal oxidation of ethanol was suggested by Tephly et al (48) whereas Lieber & DeCarli (50) presented evidence for participation of the mixed function oxidase system not associated with generation of peroxide. Studies by Rubin et al (51, 52) showed inhibition of mixed function oxidase activity by ethanol. Tolerance to ethanol after barbiturate treatment may result from an increase in liver acetaldehyde dehydrogenase activity, but in the mouse, pretreatment with ethanol did not enhance this enzyme activity (53). The effect of ethanol on conjugation reactions depends upon substrates used in the study. Amsel & Levy (54) found that salicylurate formed from salicylate was not affected by ethanol administration in man, but formation of the glycine conjugate of benzoate (hippuric acid) was decreased after an acute dose of ethanol.

Additional drugs have been evaluated for treatment of hyperbilirubinemia (Table 2). Ertel & Newton (15) used diethylnicotinamide (Coramine) to reduce the level of serum bilirubin in two sisters with congenital nonhemolytic jaundice. The level of bilirubin was lowered in these diethylnicotinamide-treated subjects, and, on discontinuance of therapy, the serum bilirubin rose within two weeks. In this condition, diethylnicotinamide was less effective than phenobarbital treatment such that the siblings were placed in chronic phenobarbital therapy for alleviation of their jaundice. Sereni et al (55) evaluated the effect of diethylnicotinamide on serum bilirubin levels in neonatal twins. One twin of each pair was injected with diethylnicotinamide from the second to the ninth day of life. Both mono- and dichorionic twins showed a decrease in blood bilirubin values at 96 and 192 hours of age when treated with diethylnicotinamide. It is of interest to note that monochorionic twins who were not treated had similar serum bilirubin values during the first ten days of life. Although diethylnicotinamide did have an effect on neonatal serum bilirubin values, the effect, not unlike that of phenobarbital, was not evident until three days of treatment. Thus, the acute use of this agent for the rapid lowering of serum bilirubin levels is not recommended. Glutethimide was used in one male subject with Gilbert's syndrome in order to note any effects of this drug on persistent unconjugated hyperbilirubinemia (18). Within about six days after starting treatment, serum bilirubin levels fell to approximate those found in healthy control patients. These levels were maintained for 19 days of treatment with glutethimide.

Since the use of phenobarbital and other drugs for hyperbilirubinemia represents a new form of therapy for this condition, critical comments about study design and potential toxicity were prompted by some of the articles cited above. The reader is referred to analyses by Robinson (56), Wilson (44,, 57), Davies (58), McMullin (37, 59), Walker (36), Behrman & Fisher (60), Thaler & Schmid (61) for further information on the clinical use of phenobarbital to alleviate icterus. Precautions with chemical therapeutic agents for neonatal hyperbilirubinemia emphasized in these papers should not be neglected when future studies are designed or

when follow-up examinations are performed on those infants already treated with phenobarbital. Another treatment regimen, in addition to phenobarbital or exchange transfusion, is phototherapy. For a discussion of phototherapy in hyperbilirubinemia, please refer to work by Lucey et al (62), Giunta & Rath (63), Callahan et al (64) and Patel et al (65) on its clinical effectiveness; to studies by Silberberg et al (66) and Glauser et al (67) on photodegradation of bilirubin; to toxicity studies by Sisson et al (68) and Kopelman et al (69); and to general reviews by Behrman & Hsia (70) and by Lester & Troxler (71).

Animal studies.—Clinical trials with phenobarbital to reduce hyperbilirubinemia were undertaken because animal studies showed an effect of this barbiturate on bilirubin glucuronyl transferase activity and bile flow. In the maturing mouse, injection of phenobarbital for three days produced an increase in bilirubin glucuronyl transferase activity in liver homogenates. This increase was not dependent on or influenced by adrenalectomy, hypophysectomy, or gonadectomy. Rabbits responded in a similar manner and showed excretion of exogenous bilirubin in the bile. Bile flow was increased and the clearance of bilirubin after phenobarbital treatment of young rabbits was enhanced as compared with controls. Pretreatment of pregnant does for three to four days prior to delivery produced young rabbits with a greater capacity to rid the serum of an exogenous bilirubin load (72). Adult rats showed an increase in bilirubin conjugation observed by the third day after phenobarbital treatment (73). Pregnancy lowered the ability of female rats to conjugate bilirubin, but conjugation returned to control levels three days after delivery. Treatment of pregnant rats with phenobarbital during the last week of gestation increased bilirubin conjugating activity in the dam as well as the newborn. An increase in morbidity and mortality with young from phenobarbital-pretreated dams was noted. The Gunn rat, in the homozygous recessive form, exhibits high levels of unconjugated bilirubin in serum and a deficiency of bilirubin glucuronyl transferase in the liver. DeLeon and coworkers (74) were unable to enhance bilirubin conjugation by administration of phenobarbital to these young rats. These studies with Gunn rats and with patients who showed a deficiency of bilirubin conjugation as well as a failure in response to phenobarbital suggested that complete deficiency of bilirubin glucuronyl transferase would preclude an action of phenobarbital on this system (16). In contrast to the homozygous Gunn rat, Robinson (75) produced an increase in excretion of conjugated bilirubin after phenobarbital treatment of heterozygous Gunn rats. The maximum excretion of bilirubin in homozygous Gunn rats treated with phenobarbital did not change as compared with control homozygous rats. Bile flow was increased in homozygous as well as heterozygous strains of rats by phenobarbital treatment. Thus, in the Gunn rat, ability to form the bilirubin glucuronide rather than a defect in bile flow appeared to account for the phenobarbital effect. Potrepka & Sprat (76)

cast doubt on the enhanced bilirubin glucuronyl transferase activity as the sole explanation for rapid clearance of bilirubin in phenobarbital-treated subjects. As opposed to the study of Catz & Yaffe (72), these workers found no increase in specific activity (micromoles bilirubin conjugated per milligram protein) after phenobarbital pretreatment of guinea pigs. The effect of phenobarbital may be species specific and call for more direct studies in man before enzyme inducing effects of this agent are accepted as the primary explanation for its mechanism of action on the hepatic clearance of bilirubin.

Hepatic uptake and transport of bilirubin may be enhanced by phenobarbital, as suggested by Roberts & Plaa (77). The relation of bile flow to liver microsomal enzyme induction was considered by Klaassen (78). As compared with other agents such as 3–4-benzpyrene and 3-methylcholanthrene, phenobarbital was the only drug that increased bile flow ***and*** microsomal enzyme activity. Liver weight gain after pretreatment with phenobarbital was not correlated with an increase in bile flow in the rat. Changes in plasma levels of bilirubin in relation to bile flow or liver conjugating activity were not determined, and leave open the question of relative importance of each factor to bilirubin clearance following phenobarbital treatment. In a subsequent study, Klaassen (79) found that pretreatment with phenobarbital enhanced the biliary excretion of drugs that were not conjugated prior to excretion. For most agents studied, a correlation was noted between biliary excretion and disappearance of the drug from plasma. This study suggested that an increase in bile flow provoked by phenobarbital could explain the enhanced clearance of some but not all drugs that were excreted without undergoing conjugation. Results with indocyanin green disappearance from plasma in phenobarbital-treated rats suggested that this drug was stored in the liver rather than excreted in the bile. Enhanced excretion of phenol red or succinyl sulphathiozole was not observed even with an increase in bile flow. Levine et al (80) showed that transport of conjugated metabolites out of the liver cell was unaffected by phenobarbital treatment. Phenobarbital had no effect on the biliary excretion of nonmetabolizable compounds such as stilbestrol monoglucuronide or phenophthalein glucuronide, although there was an increase in bile flow. These workers postulated that phenobarbital is without effect on the hepatic system responsible for transport of certain metabolized compounds to bile. Excretion of compounds that require prior metabolism, however, was increased by phenobarbital pretreatment. These articles by Klaassen (79) and by Levine et al (80) suggest that the primary action of phenobarbital is at the level of uptake and metabolism by the hepatic cell rather than export of the metabolite, and that enhancement of bile flow contributes to the hepatic clearance of some drugs and possibly of bilirubin.

Anion binding proteins have been proposed as factors responsible for uptake of bilirubin and other drugs by liver of various species (81). These anion accepting proteins have been named Y and Z. Y was found to in-

crease with age and following administration of phenobarbital. In the monkey, the decline in the level of serum bilirubin was correlated with the rise in hepatic concentration of Y protein as the animal matured (81). A prolonged disappearance of BSP in plasma of these young monkeys was ascribed to a defect in hepatic uptake of the dye. Concentrations of Z protein at birth were similar to those in the adult. Liver bilirubin binding proteins were also studied in the rat by Grodsky et al (82). Binding proteins were preferentially located in the liver and found to be low in the young and fetal rat as compared with the adult. This maturational change in binding protein was not found in kidney, brain, spleen, or muscle. BSP competed with bilirubin for the binding protein. Thus, substances that require uptake by the liver cell but do not require conjugation with glucuronic acid (e.g. BSP) may lower bilirubin clearance by competition for the uptake mechanism. Reyes and coworkers (83) administered phenobarbital to rats and found an increase in Y protein. After phenobarbital pretreatment there was an increase in the first order rate constant for removal of BSP from plasma. This rate constant for BSP and the amount of Y protein returned to normal within about nine days after discontinuance of phenobarbital. Phenobarbital did not increase the amount of Z protein. Phenobarbital may thus operate through several mechanisms to promote the plasma clearance of bilirubin. These mechanisms include enhanced uptake and conjugation of bilirubin with an additional effect on bile flow. Contribution of each action in patients with unconjugated hyperbilirubinemia during the newborn period remains to be clarified. Multifaceted causes of icterus in newborns suggest that icterus lowering effects of phenobarbital may utilize several mechanisms of action to produce the desired effect. This subject was recently reviewed by Berk et al (84).

In summary, phenobarbital effectively lowered serum levels of unconjugated bilirubin in most fullterm infants without severe jaundice or in patients with moderate jaundice of varied etiology. Observance of an effect in the neonate required treatment while in utero or from the first day of life if normal peak levels of hyperbilirubinemia in the first week of life were to be altered. An effect of phenobarbital solely on glucuronyl transferase, bile flow, or hepatic uptake of bilirubin cannot be used to explain the anicteric action of this drug. Rather, an interaction of these phenobarbital-mediated factors with situations that produce the endogenous load of bilirubin must be considered. Since phenobarbital induction of enzyme activity or anion-binding protein probably requires about three days to approach a maximum, it is not surprising that an effective reduction of serum bilirubin levels requires at least three days of prior administration of phenobarbital. This lag period places it low on the list for the treatment of hyperbilirubinemia on an acute basis. Most of the reports cited here echo caution about the use of phenobarbital or other serum bilirubin lowering drugs in newborn infants because of their lack of specificity and potential toxicity unrelated to acute effects on the bilirubin metabolic system.

Host Maturation and Fate of Drugs

Numerous studies have demonstrated a lower level of hepatic mixed function oxidase activity in young versus adult animals. After birth there is a normal age-dependent maturation of this enzyme system. Biochemical and physiologic regulation of the developmental pattern for drug metabolism remains unknown, but the influence of hormonal changes that occur during the first days and weeks of life has been emphasized recently. Both steroid and polypeptide hormones may affect the normal postnatal development of hepatic microsomal drug metabolic enzyme activity. Identification of such hormonal or other factors as determinants of the normal maturation of this system may have the additional advantage of providing probes for biochemical studies on rate limiting steps in drug metabolism.

Soyka and coworkers (85, 86) suggested that high serum levels of progesterone inhibit liver mixed function oxidase activity. Some inhibition of p-nitroanisole o-demethylation was noted with certain metabolites of progesterone, but one active metabolite, pregnanolone, did not inhibit NADPH cytochrome-c reductase activity. Increased resistance to the toxicity of both progesterone and pregnanolone were found as the newborn rat matured. Significant conversion of pregnanolone to pregnanediol was demonstrated in three-day old rats. A major portion of pregnanolone toxicity resulted from increased brain sensitivity rather than from higher brain uptake or decreased hepatic metabolism of this steroid. Feuer & Liscio (87) proposed a role for steroid inhibition of mixed function oxidase activity in the rat. Early weaning produced rat pups with an enhanced 3-methylcoumarin-3-hydroxylase activity and a decrease in sleeping time to pentobarbital. This evidence is suggestive but inconclusive, since drug metabolism was not compared with blood levels of endogenous or exogenous specific steroids. It was also assumed that steroids transferred to the young animal via placenta or milk would act solely on the liver and not influence the function of other endocrine organs such as the pituitary. In a study by Henderson (87a), prolongation of weaning did not affect the development of aminopyrine demethylation. If liver drug metabolic enzyme activity were inhibited by one or more maternal steroids, then this phenomenon should be demonstrable in preparations of pup or lactating dam plasma. As seen in Figure 1, no in vitro inhibition of hexobarbital or aniline metabolism was observed after the addition of various amounts of plasma from ten-day old suckling rats or lactating females. This study assumes that plasma protein binding of the steroid did not prevent detection of an inhibitor. It is unlikely that the amount of protein-bound steroid changes with age such that the presumed high concentration of a steroid inhibitor would not be detected (88). Davies et al (89) did not find an endogenous inhibitor of male rat liver drug metabolism in liver preparations from female rats. Thus, no in vitro inhibitor was detected in plasma from lactating females or in liver of mature females.

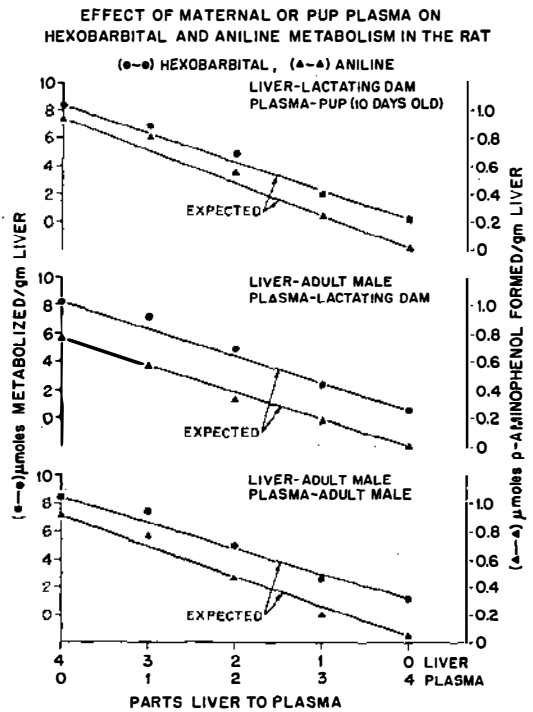

FIG. 1. Effect of Maternal or Pup Plasma on Hexobarbital and Aniline Metabolism in the Rat. Male (250g) or lactating Female (180g) Fischer rats were used. The reaction mixture contained 0.25 ml 9000 × g supernatant fraction (equivalent to 1/12 g liver), substrate (hexobarbital 1.5 μmoles or aniline 5 μmoles) and cofactors (in μmoles/2.5 ml): glucose-6-phosphate, 9; NADP, 2.08; and $MgSO_4$, 24.2. Potassium phosphate buffer 0.1M pH 7.35 was added to adjust the mixture volume to 2.5 ml. The reaction mixture was incubated under oxygen at 37° for 20 min. For the purpose of calculation, plasma was considered as "liver equivalent."

Androgenic and estrogenic steroids influenced the level of drug metabolism when these hormones were administered in vivo and sex differences in this metabolism are found in some animals (90, 91). Davies et al (89) showed that the apparent Michaelis constant (Km) of ethylmorphine metabolism for immature male and female rats was similar, but the Km for mature male animals was lower than that of females. Castration of male animals did not affect the Km but it did lower the rate of metabolism (Vmax) to a level approaching that of the female. Castration of the female rat had no effect on either the Km or Vmax. These workers suggest that testosterone may affect the amount of drug metabolic enzyme protein but not the apparent Km. Treatment of female or castrated male rats with

androgenic or male rats with estrogenic steroids raised and lowered, respectively, the metabolism of estradiol and hexobarbital (91, 92). Gram et al (93) examined the effect of age and sex steroids on the apparent Km and Vmax of ethylmorphine and aniline metabolism by rat liver microsomes. By four to five weeks of age, the Vmax for ethylmorphine increased whereas that for aniline metabolism increased as early as one week of age. The apparent Km for aniline slowly increased to six weeks of age, but that of ethylmorphine was low at one week, higher between weeks two and three, and then decreased to lower levels after four weeks of age. No correlation of these parameters with hepatic cytochrome P-450 content was found. It is of interest that others have found little relation between the level of drug metabolism and ability to synthesize heme (94) or the level of liver cytochrome P-450 (95) in samples of immature liver. Gram et al (93) questioned the role of testosterone as a primary determinant of the sex difference in drug metabolism in the rat. Although liver ethylmorphine metabolism increased at around three to four weeks of age, a maximum increase in the weight of testis and seminal vesicle was not seen until six and eight to nine weeks, respectively. Testosterone concentration in the blood of neonatal animals is high, decreases until about 30 days of age and then slowly increases to reach adult levels in 60 to 90 day old rats (96). Mature male or female rats "programmed" (97–99) with one injection of testosterone as neonates showed no change in the biotransformation of estrogen to water-soluble products (92). Sex steroids undoubtedly influence the level of drug metabolic enzyme activity in some animals, but their role in the postnatal maturation of this activity may be more passive than primary and subject to interaction with other humoral factors.

Studies with growth hormone indicate that hormonal factors can influence liver drug metabolism to produce age-related changes in the fate of drugs. Growth hormone, although found in the pituitary of the 15-week old fetus (100) and present, possibly in high levels, in blood of the human newborn (101–101c), is apparently not required for fetal growth (102–105). The primary role for an action of this hormone in the young infant remains obscure. Several studies, however, suggest a causal relationship between high blood levels of growth hormone and the capacity of the neonate's liver to metabolize drugs. Implantation of a growth hormone secreting pituitary tumor into young rats prevented the normal postnatal development of the hepatic microsomal system responsible for hexobarbital metabolism (106). In fact, hexobarbital metabolism in the adult was decreased to a level normally seen in the 20 day old rat. In a subsequent study, the administration of growth hormone to immature rats also impaired drug metabolism and prevented its age-related development (107). Thus, the fact that growth hormone could markedly impair hepatic drug metabolism in both immature and adult rats suggests that it may indeed play an essential role in the regulation of hepatic microsomal drug metabolizing enzyme activity. Prelimi-

nary measurements show higher serum levels of immunoreactive growth hormone associated with low hepatic mixed function oxidase activity in one- to ten-day old rats as compared to 30-day old animals (108). These and other investigations in progress suggest that high blood levels of growth hormone, or a growth hormone-like substance [e.g., human placental lactogen (109, 110)], or both, modulate the level and postnatal maturation of liver microsomal drug metabolism.

Drug Effects on the Developing Host

Effect on morphogenesis.—Organ system maturation predisposes the fetus and young child to drug-mediated disturbances in organ development. For example, tooth texture can be modified by early ingestion of tetracyclines. Previous workers (111, 112) have noted an association between tetracycline exposure and brownish discoloration of the teeth in children and adults. Recent studies have emphasized the relation of time, dose, and frequency of tetracycline administration to the development of changes in tooth texture. Anthony (113) described an infant born to a woman who received a course of tetracycline for two weeks and then another six-week treatment regimen beginning about the seventh or eighth month of gestation. Deciduous teeth showed a yellowish-brown discoloration, which darkened with time. At the age of seven years the child showed four permanent incisors that were normal in texture. Failure to find tetracycline effects on the secondary teeth were probably related to time of drug administration during pregnancy. Enamel deposition for secondary teeth begins about the time of birth and continues until the age of about eight years. In contrast, enamel formation in the primary teeth can be seen from about four to six months of gestation until the child is 12 months old. Time of tetracycline exposure is thus an important factor in predicting the involvement of primary or secondary tooth discoloration. In another study, Grossman et al (114) examined this subject in relation to the dose of tetracycline administered. Differences in tooth shade were determined without knowledge of previous drug exposure. Children in this study were 6 to 12 years of age and had been treated previously with D-methylchlortetracycline or tetracycline. The number of courses of tetracycline was directly correlated with darkening of teeth. However, little cosmetic effect was noted with as many as five courses of tetracycline during the course of tooth formation. Incisor discoloration was correlated with tetracycline administration during the period of crown calcification (before four and one-half years of age). Little difference in tooth discoloration was found on comparison of children with exposure during the first year to those exposed after three years. Emphasis in this study was on the color of permanent incisors, but other teeth, whose calcification is not complete until after five years of age, may be of cosmetic importance. In summary, alterations in tooth texture from tetracycline may be expected to occur with an increased incidence if a high or repetitive dose

of the drug is given during a time in fetal or child life when tooth mineralization is in progress.

Effect on fetal physiology.—The maternal-fetal relationship often places the unborn child at risk from drug exposure. Changes in fetal physiology are commonly noted at the time of birth when a large number of analgesic, tranquilizing, and sedative compounds are used. Unexpected drug effects on this two-life system are now being encountered with increased frequency due to technological improvements in fetal monitoring systems. Considerations of an enhanced susceptibility of the intrauterine patient to adverse drug effects include: (*a*) fetal homeostasis as influenced by drug-mediated changes in maternal physiology, (*b*) indirect dosing of the fetus by drugs administered to the mother, and (*c*) dependence on maternal metabolic and excretory parameters for drug elimination. A few recent studies will demonstrate some of these drug effects on fetal physiology.

Anesthesia is given during labor and delivery for maternal indications, but this treatment is not without effects on the fetus. Lidocaine, propitocaine, or mepivacaine for paracervical block anesthesia was studied by Shnider et al (115). In some cases fetal distress and neonatal depression were noted. In a subsequent study Asling et al (116) found fetal acidosis and bradycardia in association with fetal blood levels of mepivacaine higher than those of the mother. Direct absorption of the anesthetic agent from the paracervical region into fetal blood was postulated. A similar interpretation was offered to explain perinatal deaths after mepivacaine paracervical block as described by Rosefsky et al (117). In their study, the finding of mepivacaine in a urine sample of the newborn infant suggested an inadequate neonatal capacity to metabolize the drug. Exchange transfusion has been used successfully to treat a critically ill infant who had measurable blood levels of mepivacaine following use of this drug for paracervical block anesthesia (118). Murphy et al (119) recorded fetal bradycardia and two fetal deaths associated with bupivacaine paracervical block. Similar deleterious effects of mepivacaine on the neonate were noted after direct injection of this drug into the fetus (120). Vasopressors have been used to correct adverse effects of maternal anesthetic agents, and the question of fetal risk from these corrective measures has arisen. In pregnant sheep near term, Shnider et al (121) found fetal hypoxia and acidosis in association with spinal hypotension. Correction of maternal hypotension with ephedrine stopped the progession of changes in fetal acid-base parameters. However, in hypotensive sheep given methoxamine as the corrective agent, the condition of the fetus worsened, possibly due to underperfusion of the placenta (122). Analgesic agents administered as premedication during labor are not without effects on the fetus or neonate. For example, premedication with narcotics produced neonatal depression and changes in acid-base parameters even with addition of a narcotic antagonist (123). Neonatal depression was also found

after administration of large doses of barbiturates to mothers during labor (124). Behavioral and EEG pattern changes in the neonate as related to maternal medication during labor have been noted (125). More detailed reviews of metabolic changes in the intrauterine patient as influenced by maternal drug administration have appeared recently (126, 127).

LITERATURE CITED

1. Wilson, J. T. 1971. *J. Med. Educ.* 46:449–55
2. Levi, A. J., Gatmaitan, Z., Arias, I. M. 1970. *Metabolism* 19:246–52
3. Lathe, G. H., Walker, M. 1958. *Biochem. J.* 70:705–11
4. Schmid, R., Marver, H. S. Hammaker, L. 1966. *Biochem. Biophys. Res. Commun.* 24:319
5. Poland, R. L., Odell, G. B. 1971. *New Eng. J. Med.* 284:1–6
6. Brodersen, R., Hermann, L. S. 1963. *Lancet* I:1242
7. Odell, G. B. 1970. *Pediatrics* 46:16–24
8. Odell, G. B., Storey, G. N., Rosenberg, L. A. 1970. *J. Pediat.* 76:12–21
9. Weldon, V. V., Odell, G. B. 1968. *Pediatrics* 41:797–801
10. Boggs, T. R., Westphal, M. C. 1960. *Pediatrics* 26:745–55
11. Yaffe, S. J., Levy, G., Matsuzawa, T., Baliah, T. 1966. *New Eng. J. Med.* 275:1461–66
12. Crigler, J. F., Jr., Gold, N. I. 1966. *J. Clin. Invest.* 45:998–99
13. Crigler, J. F., Gold, N. I. 1969. *J. Clin. Invest.* 48:42–55
14. Kreek, M. J., Sleisenger, M. H. 1968. *Lancet* II:73–77
15. Ertel, I. J., Newton, W. A., Jr. 1969. *Pediatrics* 44:43–47
16. Arias, I. M., Gartner, L. M., Cohen, M., Ben Ezzer, J., Levi, A. J. 1969. *Am. J. Med.* 47:395–409
17. Whelton, M. J., Krustev, L. P., Billing, B. H. 1969. *Am. J. Med.* 45:160–63
18. Black, M., Sherlock, S. 1970. *Lancet* I:1359–62
19. Thompson, R. P. H., Williams, R. 1970. *Lancet* II:466
20. Sharp, H. L., Mirkin, B. L. 1970. *Clin. Res.* 18:344
21. Thompson, R. P. H., Williams, R. 1967. *Lancet* II:646–48
22. Cunningham, M. D., Kelley, L. R., Peters, E. R. 1968. *Lancet* I:1089
23. Trolle, D. 1968. *Lancet* I:251–52
24. Maurer, H. M., Wolff, J. A., Finster, M., Poppers, P. J., Pantuck, E., Kuntzman, R., Conney, A. H. 1968. *Lancet* II:122–24
25. Trolle, D. 1968. *Lancet* II:705–08
26. Trolle, D. 1968. *Lancet* II:1123–24
27. Robinson, R. J. 1968. *Lancet* II:1243–44
28. McMullin, G. P. 1968. *Lancet* II: 978–79
29. Cunningham, M. D., Mace, J. W., Peters, E. R. 1969. *Lancet* I:550–51
30. Ramboer, C., Thompson, R. P. H., Williams, R. 1969. *Lancet* I:966–68
31. Vesell, E. S., Page, J. 1969. *J. Clin. Invest.* 48:2202
32. Stern, L., Khanna, N. N., Levy, G., Yaffe, S. J., 1970. *Am. J. Dis. Child.* 120:26–31
33. Doxiadis, S. 1970. *Hosp. Pract.* 5: 115–24
34. Yeung, C. Y., Field, C. E. 1969. *Lancet* II:135–39
35. McMullin, G. P., Hayes, M. F., Arora, S. C. 1970. *Lancet* II:949–50
36. Walker, W. 1970. *Lancet* II:1089–90
37. McMullin, G. P. 1970. *Lancet* II: 1194–95
38. Vest, M., Signer, E., Weisser, K., Olafsson, A. 1970. *Acta Paediat. Scand.* 59:681–84
39. Kokosky, M. J., Falconer, H. A., Weiss, L. 1970. *Henry Ford Hosp. Med. J.* 18:275–82
40. Walker, W., Hughes, M. I., Barton, M. 1969. *Lancet* I:548–50
41. Waltman, R., Bonura, F., Nigrin, G., Pipat, C. 1969. *Lancet* II:108
42. Waltman, R., Nigrin, G., Bonura, F., Pipat, C. 1969. *Lancet* II:1265–67
43. Meldolesi, J. 1967. *Biochem. Pharmacol.* 16:125–29
44. Wilson, J. T. 1969. *Pediatrics* 43: 324–27
45. Isaac, M., Dundee, J. W. 1970. *Lancet* I:37–38
46. Flock, E. V., Tyce, G. M., Owen, C. A., Jr. 1970. *Proc. Soc. Exp. Biol. Med.* 135:325–33

47. Khanna, J. M., Kalant, H., Lin, G. 1970. *Biochem. Pharmacol.* 19: 2493–99
48. Tephly, T. R., Tinelli, F., Watkins, W. D. 1969. *Science* 166:627–28
49. Klaassen, C. D. 1969. *Proc. Soc. Exp. Biol. Med.* 132:1099–1102
50. Lieber, C. S., DeCarli, L. M. 1970. *Science* 170:78–80
51. Rubin, E., Bacchin, P., Gang, H., Lieber, C. S. 1970. *Lab. Invest.* 22:569–80
52. Rubin, E., Gang, H., Misra, P. S., Lieber, C. S. 1970. *Am. J. Med.* 49:801–06
53. Redmond, G., Cohen, G. 1971. *Science* 171:387
54. Amsel, L. P., Levy, G. 1970. *Proc. Soc. Exp. Biol. Med.* 135:813–16
55. Sereni, F., Perletti, L., Marini, A. 1967. *Pediatrics* 40:446–49
56. Robinson, R. J. 1968. *Lancet* II: 1243–44
57. Wilson, J. T. 1969. *Lancet* II:214
58. Davies, P. A. 1969. *Lancet* II:273–74
59. McMullin, G. P. 1969. *Lancet* II: 385–86
60. Behrman, R. E., Fisher, D. E. 1970. *J. Pediat.* 76:945–48
61. Thaler, M. M., Schmid, R. 1971. *Pediatrics* 47:807–10
62. Lucey, J., Ferreiro, M., Hewitt, J. 1968. *Pediatrics* 41:1047–54
63. Giunta, F., Rath, J. 1969. *Pediatrics* 44:162–67
64. Callahan, E. W., Jr., Thaler, M. M., Karon, M., Bauer, K., Schmid, R. 1970. *Pediatrics* 46:841–48
65. Patel, D. A., Phildes, R. S., Behrman, R. E. 1970. *J. Pediat.* 77: 1048–51
66. Silberberg, D. H., Johnson, L., Schutta, H., Ritter, L. 1970. *J. Pediat.* 77:613–18
67. Glauser, S. C., Lombard, S. A., Glauser, E. M., Sisson, T. R. C. 1971. *Proc. Soc. Exp. Biol. Med.* 136:518–19
68. Sisson, T. R. C., Glauser, S. C., Glauser, E. M., Tasman, W., Kuwabara, T. 1970. *J. Pediat.* 77:221–27
69. Kopelman, A. E., Brown, R. S., Odell, G. B. 1971. *APS-SPR Abstracts,* p. 3
70. Behrman, R. E., Hsia, D. Y. Y. 1969. *J. Pediat.* 75:718–26
71. Lester, R., Troxler, R. F. 1969. *New Eng. J. Med.* 280:779–80
72. Catz, C., Yaffe, S. J. 1968. *Pediat. Res.* 2:361–70
73. Halac, E., Jr., Sicignano, C. 1969. *J. Lab. Clin. Med.* 73:677–85
74. DeLeon, A., Gartner, L. M., Arias, I. M. 1967. *J. Lab. Clin. Med.* 70:273–78
75. Robinson, S. H. 1969. *Nature* 222: 990–91
76. Potrepka, R. F., Spratt, J. L. 1971. *Biochem. Pharmacol.* 20:861–67
77. Roberts, R. J., Plaa, G. L. 1967. *Biochem. Pharmacol.* 16:827–35
78. Klaassen, C. D. 1969. *J. Pharmacol. Exp. Therap.* 168:218–23
79. Klaassen, C. D. 1970. *J. Pharmacol. Exp. Therap.* 175:289–300
80. Levine, W. G., Milburn, P., Smith, R. L., Williams, R. T. 1970. *Biochem. Pharmacol.* 19:235–44
81. Levi, A. J., Gatmaitan, Z., Arias, I. M. 1969. *J. Clin. Invest.* 48: 2156–67
82. Grodsky, G. M., Kolb, H. J., Fanska, R. E., Nemechek, C. 1970. *Metabolism* 19:246–52
83. Reyes, H., Levi, A. J., Gatmaitan, Z., Arias, I. M. 1969. *Proc. Nat. Acad. Sci.* 64:168–70
84. Berk, P., Bloomer, J. R., Berlin, N. I. 1970. *New Eng. J. Med.* 283:1166–67
85. Soyka, L. F., Long, R. J. 1971. *Fed. Proc.* 30:281 (Abstr.)
86. Soyka, L. F., Gyermek, L., Campbell, P. 1970. *J. Pharmacol. Exp. Therap.* 175:276–82
87. Feuer, G., Liscio, A. 1969. *Nature* 223:68–70
87a. Henderson, P. Th. 1971. *Biochem. Pharmacol.* 20:1225–32
88. Ramaley, J. A. 1970. *Steroids* 16:611–30
89. Davies, D. S., Gigon, P. L., Gillette, J. R. 1968. *Biochem. Pharmacol.* 17:1865–72
90. Castro, J. A., Gillette, J. R. 1967. *Biochem. Biophys. Res. Commun.* 28:426–29
91. Quinn, G. P., Axelrod, J., Brodie, B. B. 1958. *Biochem. Pharmacol.* 1:152–59
92. Jellinck, P. H., Lucieer, I. 1965. *J. Endocrinol.* 32:91–98
93. Gram, T. E., Guarino, A. M., Schroeder, D. H., Gillette, J. R. 1969. *Biochem. J.* 113:681–85
94. Woods, J. S., Dixon, R. L. 1970. *Biochem. Pharmacol.* 19:1951–54
95. Yaffe, S. J., Rane, A., Sjoqvist, F., Boreus, L.-O., Orrenius, S. 1970. *Life Sci.* 9:1189–1200
96. Resko, J. A., Feder, H. H., Goy,

R. W. 1968. *J. Endocrinol.* 40:485–91
97. Edwards, D. A. 1969. *Physiol. Behav.* 4:333–38
98. Bronson, F. H., Desjardins, C. 1970. *Gen. Comp. Endocrinol.* 15:320–25
99. Denef, C., DeMoor, P. 1969. *Endocrinology* 85:259–69
100. Kaplan, S. L., Grumbach, M. M. 1962. *Am. J. Dis. Child.* 104:528
101. Cornblath, M., Parker, M. L., Reisner, S. H., Forbes, A. E., Daughaday, W. H. 1962. *J. Clin. Endocrinol.* 25:209–18
101a. Greenwood, F. C., Hunter, W. M., Marrian, V. J. 1964. *Brit. Med. J.* 1:25–26
101b. Laron, Z., Mannheimer, S., Nitzan, M., Goldman, J. 1967. *Arch. Dis. Child.* 42:24–28
101c. Shaywitz, B. A., Finkelstein, J., Hellman, L., Weitzman, E. D. 1971. *Pediatrics* 48:103–09
102. Seckel, H. P. G. 1960. *Am. J. Dis. Child.* 99:349–79
103. Kiyono, H. 1925. *Arch. Pathol. Anat.* 257:441
104. Wright, S., Wagner, K. 1934. *Am. J. Anat.* 54:383–447
105. Jost, A. 1954. *Cold Spring Harbor Symp. Quant. Biol.* 19:167
106. Wilson, J. T. 1968. *Pediat. Res.* 2: 514–18
107. Wilson, J. T. 1970. *Nature* 225:861–63
108. Wilson, J. T., Frohman, L. A. Unpublished observations
109. Burr, I., Grumbach, M. M., Kaplan, S. L. 1967. In Program, *49th Ann. Meet. Endocrine Soc.,* Bal Harbour, Fla., p. 39
110. Selenkow, H. A., Saxena, B. N., Dana, C. L., Emerson, K., Jr. 1969. *The Feoto-Placental Unit,* Excerpta Medica Foundation: Amsterdam, 425 pp.
111. Schwachman, H., Fekete, E., Kulczycki, L. L., Foley, G. E. 1958–59. *Antibiotic Annuals,* p. 692
112. Douglas, A. C. 1963. *Brit. J. Dis. Chest* 57:44–47
113. Anthony, J. R. 1970. *Postgrad. Med.* 48:165–68
114. Grossman, E. R., Walchek, A., Freedman, H. 1971. *Pediatrics* 47:567–70
115. Shnider, S. M., Asling, J. H., Holl, J. W., Margolis, A. J. 1970. *Am. J. Obstet. Gynecol.* 107:619–25
116. Asling, J. H., Shnider, S. M., Margolis, A. J., Wilkinson, G. L., Way, E. L. 1970. *Am. J. Obstet. Gynecol.* 107:626–34
117. Rosefsky, J. B., Petersiel, M. E. 1968. *New Eng. J. Med.* 278:530–33
118. O'Meara, O. P. 1968. *New Eng. J. Med.* 278:1127
119. Murphy, P. J., Wright, J. D., Fitzgerald, T. B. 1970. *Brit. Med. J.* 1:526–29
120. Sinclair, J. C., Fox, H. A., Lentz, J. F., Fuld, G. L., Murphy, J. 1965. *New Eng. J. Med.* 273:1173–77
121. Shnider, S. M., DeLorimier, A. A., Chapler, F. K., Morishima, H. O. 1968. *Am. J. Obstet. Gynecol.* 102:911–19
122. Shnider, S. M., DeLorimier, A. A., Asling, J. H., Morishima, H. O. 1970. *Am. J. Obstet. Gynecol.* 106:680–86
123. Clark, R. B., Cooper, J. O., Stephens, S. R., Brown, W. E. 1969. *Obstet. Gynecol.* 33:30–34
124. Batt, B. 1968. *Am. J. Obstet. Gynecol.* 102:591–96
125. Borgstedt, A. D., Rosen, M. G. 1968. *Am. J. Dis. Child.* 115:21–24
126. Goodlin, S. N. 1969. *Obstet. Gynecol.* 34:109–12
127. Cohen, S. N., Olson, W. A. 1970. *Pediat. Clin. N. Am.* 17:835–50