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PERINATAL RENAL PHARMACOLOGY

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At birth many functions of the kidney are less than would be predicted on the basis of size alone. This does not mean that the kidneys are inadequate in the newborn. Renal function in the perinatal period is adequate to maintain normal homeostasis; however, under conditions of stress (dehydration, infection, marked changes in plasma pH, drug therapy, etc) this immaturity may become manifest as a lack of functional reserve. Of interest to the pharmacologist and toxicologist is the influence of this limited reserve in renal function in the context of exposure of the newborn to drugs and chemicals. Immaturity of renal processes for excretion and metabolism of foreign compounds (or endogenous substrates) may be reflected as a prolonged time of elimination of these compounds from the body. Additionally, because of its relative immature size and function the response of the kidney as a target organ for drugs may be quantitatively or qualitatively different from that of adults.

In the newborn, renal blood flow and glomerular filtration rate (GFR) are low, concentrating and acidifying functions are restricted, and the capacity to secrete organic compounds is limited. These and many other factors may influence the effect and/or disposition of chemicals in the newborn. In the last several years a large body of information has developed concerning renal function in the perinatal period. Such data provide the physiological and biochemical basis on which rational approaches to the use of drugs in premature and full-term newborn may be based. In this review we focus on two areas of research interest: renal hemodynamics and organic

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anion secretion. These subjects have received considerable attention because they are important in total renal function and because they are amenable to quantitative analysis in experimental animals. The final section of this review deals with the action of diuretics in the newborn, a subject of significant clinical importance, which has only recently begun to receive quantitative experimental analysis.

HEMODYNAMICS IN THE DEVELOPING KIDNEY

An increase in renal blood flow with maturation has been described in several species of animals including humans (1), sheep (2), rats (3), and piglets (4). Fetal renal blood flow in near term lambs was reported as 0.96 ml/min/g kidney weight (5). In newborn dogs, Aschinberg et al (6) reported that mean renal blood flow increased from 0.39 ml/min/g kidney weight at 7 days of age to 2.06 ml/min/g kidney weight at 42 days. Jose et al (7) demonstrated that renal blood flow in puppies increased to adult values of 3.5 ml/min/g kidney by 98–112 days of age. Renal blood flow in 17-day-old rats has been reported as 0.25 ml/min/g kidney (3). Flow increased to adult levels (5 ml/min/g kidney) by 60 days of age. Gruskin et al (4) demonstrated that in the first 24 hr of life renal blood flow in the piglet averaged 43 ml/min/m^2. This perfusion rate increased to 760 ml/min/m^2 by 45 days of age. Renal plasma flow in adult swine has been estimated by the clearance of *p*-aminohippurate (PAH) to be approximately 3.85 ml/min/g kidney (8).

The low renal blood flow in the undeveloped kidney may result from increased renal vascular resistance, decreased cardiac output, or a combination of both. In piglets, renal blood flow increased by a factor of 18 between 1 and 45 days of age (4). During this same period of time, cardiac output increased only by a factor of 7 while renal resistance decreased 86%. Thus, the increase in renal blood flow is due both to the increased cardiac output and a decrease in vascular resistance. These data from swine are comparable to the human newborn where the kidney receives 5–6% of the cardiac output in comparison to that of the adult which receives 15–25% (9).

In guinea pigs ranging in age from 1 day to 49 days, significant changes in glomerular capillary pressure have been observed (10). This finding suggests that the age-related increase in glomerular filtration rate (GFR) is also dependent upon a decrease in renal vascular resistance. Kleinman & Lubbe (11) determined blood pressure, renal vascular resistance, renal plasma flow, and glomerular filtration rate in newborn dogs. Renal plasma flow increased from 0.7 ml/min/g kidney at 1 day to 1.8 ml/min/g kidney at 30 days of age. Mean arterial blood pressure increased from 40 mmHg to 80 mmHg over this age range and GFR increased from 0.16 ml/min/g

kidney at birth to 0.35 ml/min/g at 30 days. In these experiments, increases in GFR and renal plasma flow were directly correlated with the increase in mean arterial blood pressure. Mean renal vascular resistance in the newborn puppy was not different from that in adults (11).

Developmental changes in renal hemodynamics have also been correlated with structural changes. Nephrogenesis begins with the juxtamedullary nephrons and maturation proceeds outward to the superficial cortical nephrons (12). Spitzer & Brandis (13) noted in the first 2 weeks of guinea pig development that the whole kidney GFR increased by an average of 0.96 nl/min per nephron, while single nephron filtration (SNGFR) of the superficial cortical nephrons increased only 0.17 nl/min. Clearly, a more marked increase in single nephron GFR occurred in the juxtamedullary nephrons. In contrast, the increase in SNGFR during the third and fourth week of neonatal development could be attributed almost entirely to changes in the outer cortex. In 21- to 69-day-old puppies, Horster & Valtin (14) demonstrated a 7-fold increase in SNGFR of outer cortical glomeruli while whole kidney GFR increased 4.5-fold. Similar data were obtained by Solomon in rats (15).

It may be postulated from these observations that at birth an uneven distribution of blood flow exists within the kidney, and this distribution changes with increasing age. The distribution of renal blood flow in the neonate has been documented primarily in the canine puppy. Olbing et al (16) showed that between 5 and 36 hr after birth in dogs, inner cortical glomeruli were perfused at a rate 5 times that of the outer cortical glomeruli. At 6 weeks of age, however, flow to outer cortical glomeruli exceeded flow to juxtamedullary glomeruli. Aschinberg et al (6) showed that during the first week of life the renal cortex was homogeneously perfused at a rate of 0.88 ml/min/g kidney. At 2 weeks, the outer cortex received only 15% of the total renal blood flow while the inner cortex and medulla received 53%. Outer cortical flow then increased with age reaching adult values of about 40% of total renal blood flow at 6–10 weeks of age. These data indicate that a shift in distribution of blood flow may have occurred between the first and second week of life in the canine kidney. Jose et al (7), utilizing the xenon-133 washout technique and autoradiography in dogs, showed that flow to the outer cortex increased 16% from 6 weeks to 16 weeks of age. Kleinman & Reuter (17) determined that the inner cortical to outer cortical flow ratio decreased linearly between 1 and 14 days of age in the newborn dog. The decrease in the ratio was due primarily to an increase in outer cortical blood flow. Furthermore, these authors demonstrated a correlation between the decrease in inner cortical to outer cortical flow ratio and mean systemic blood pressure. Since this was a direct correlation, it was concluded that the transient change in blood pressure with age may be a factor in increasing

outer cortical blood flow, but the maturation and development of outer cortical glomeruli also significantly contribute to the distribution of renal blood flow.

It has been postulated that the high renal resistance and low outer cortical blood flow in the newborn reflect a mature and active sympathetic nervous system (18). Gootman et al (19) demonstrated that in the newborn piglet the central vasomotor regulatory centers are functional at birth but are not fully mature. Reddy et al (20) reported that low frequency sciatic nerve stimulation in newborn pigs decreased renal blood flow, whereas high frequency stimulation increased renal flow. In anesthetized piglets 1–22 days of age, severe hypercapnic acidosis and hemorrhage increased renal blood flow (20, 21). Alward et al (22) demonstrated the effects of asphyxia on renal hemodynamics in unanesthetized newborn piglets, 6–96 hr of age. Increasing the respiratory dead space with resultant hypoxemia, hypercarbia, and acidosis resulted in little change in glomerular filtration rate while renal vascular resistance increased threefold and total renal blood flow decreased. There was no change in the intrarenal distribution of blood flow as measured by radioactive microspheres.

Jose et al (23) reported that epinephrine, 10–19 ng/g kidney weight/min, i.v., decreased renal cortical blood flow in the canine puppy while not affecting cortical flow in the adult. Furthermore, 21–31 ng/g/min reduced renal blood flow by 92% in the puppy and only 27% in the adult. In the puppy, cortical blood flow was redistributed to the inner cortex. This evidence suggests that the neonatal kidney possesses an increased sensitivity to circulating catecholamines and these hormones may maintain low renal blood flow in the puppy.

The low outer cortical blood flow in the newborn may be responsible for the observation that both infants and piglets are unable to excrete a sodium load rapidly (24). Kleinman & Reuter (17) reported that both puppies and adult dogs increase their glomerular filtration rate after infusion of a saline load. The adults, however, excreted an average of 6.4% of the filtered sodium load, while the 1–30-day-old puppies excreted 2.4% of the filtered sodium. Following saline expansion in the puppy there was a significant decrease in the inner cortical to outer cortical flow ratio, which was attributed to an increase in blood flow to the outer cortex. In these experiments the saline infusion decreased hematocrit in the puppies by 22% while only decreasing hematocrit by 14% in the adult. It was concluded from these experiments that puppies do not respond as well to a saline load as do adults. Although there was an increase in outer cortical blood flow in response to a saline load, this increase in flow was probably due to a change in extracellular fluid volume rather than causally related to sodium excretion (17).

While it has been suggested that the resistance of the newborn kidney to the diuresis induced by volume expansion is secondary to high circulating levels of aldosterone (25), the data of Horster & Larsson (26) are also of interest. Their finding that the hydraulic hydrostatic conductance of the newborn rabbit proximal tubule is eight times greater than that of the adult suggests that changes in pressure gradients during saline loading are apt to affect the immature proximal tubule more than the adult tubule. Because of the low blood flow to the outer cortex of the newborn kidney, changes in protein oncotic pressure may have less influence on fluid reabsorption. On the other hand, changes in oncotic pressure may be more effective, because the paracellular shunt is not fully developed (26).

In addition to sodium reabsorption, other functions of the proximal tubule, such as secretion of organic anions, may be related to changes in renal hemodynamics. Horster & Lewy (27) have suggested that at least some of the developmental increase in PAH extraction can be explained by the increase in blood flow to the superficial cortex and an increase in tubular load of PAH.

Intrarenal Vasoactive Hormones

Since developmental changes in renal hemodynamics appear to be causally related to changes in renal vascular resistance, the importance of the intrarenal vasoactive hormones must be considered. The relationship of catecholamines and renal blood flow has been discussed. The renin-angiotensin system has also been implicated in both intrarenal and extrarenal homeostatic control mechanisms. As reviewed recently by Thurau & Boylan (28), angiotensin II may be involved in the local regulation of nephron filtration rate. As an extrarenal hormone, angiotensin II appears to be involved in the maintenance of blood pressure under conditions of sodium depletion and reduced extracellular fluid volume (29, 30). In addition to possible direct pressor effects on vascular smooth muscle, the actions of angiotensin on renal prostaglandins may indirectly modify renal function. Angiotensin II stimulates release of renal prostaglandins (31), and prostaglandin synthetase inhibitors blunt renin secretion (32).

RENIN-ANGIOTENSIN SYSTEM The existence of a functional renin-angiotensin system in the newborn has been demonstrated in rats (33), sheep (34), dogs (35), piglets (22), and human infants (36). It was originally reported that juxtaglomerular granulated cells do not appear until 14 days postpartum in the rat, but a progressive rise in total juxtaglomerular index occurs from the fourteenth through the sixtieth day of life (37, 38). In mice, juxtaglomerular granulation was not observed until the second to fourth week of life (39). An absence of juxtaglomerular granulation in 1-day-old

piglets has also been reported (40). However, Schmidt et al (41) have recently isolated juxtaglomerular granules by differential centrifugation from rat kidney homogenates, 5–6 hr of age. These homogenates contained pressor activities 7.4 times higher than adult rat homogenates. Electron micrographs demonstrated the presence of granulated epitheloid cells in the juxtamedullary juxtaglomerular apparatus of these rats.

The systemic plasma renin concentration (PRC) in neonatal dogs (12 hr and 48 hr) has been reported to be 25.6 ng/ml compared to 1 ng/ml in adult dogs (35). At 8–15 days of age, PRC remained elevated, averaging 23.8 ng/ml. Pipkin et al (34) determined the PRC in fetal lambs, 9 ng/ml (123–138 days of gestation), newborn lambs 22 ng/ml (0–10 days old), and older lambs 7 ng/ml (6–8 weeks). Angiotensin II–like concentration was determined by bioassay as 315 pg/ml in fetal lambs, less than 123 pg/ml in newborn lambs delivered by caesarean section, 839 pg/ml in newborn lambs delivered vaginally, and less than 111 in lambs 6–8 weeks old. These results suggest that stress induced by labor may elevate circulating angiotensin levels in the blood. Pohlova & Jelinek (33) observed that 1-day-old rat plasma generated 9.82 ng AII/ml/hr and this decreased to adult values by 80 days of age (3.37 ng AII/ml/hr). Plasma concentration of renin substrate decreased from 11 ng AI/mg plasma protein at day 1 to 6 ng AI/mg plasma protein at day 80. Renal angiotensinase activity increased slightly from 10.53 min to 7.57 min (angiotensinase activity expressed as AII half-time in minutes) over this age range. Wallace et al reported an increase in the activity of pulmonary angiotensin I converting enzyme in rat lung homogenates between 1 and 40 days of age (42). Such developmental changes in converting enzyme could result in the modulation of circulating concentration of AII during early postnatal life.

Kotchen et al (36) reported that fewer than 6 hr after birth plasma renin activity (PRA) in infants averaged 2.4 ng/ml/hr, increasing to 8.8 ng/ml/hr within 24 hr after birth, 11.6 ng/ml/hr at 3–6 days, and 2.5 ng/ml/hr by 3–6 weeks of age. The values at 6 hr, 24 hr, and 3–6 days were significantly greater than adult control values (0.7 ng/ml/hr). Maternal PRA during labor was elevated (4.6 ng/ml/hr) above control values, and umbilical cord PRA was significantly greater than maternal levels (12.7 ng/ml/hr). Furthermore, it was noted that infant renin substrate concentrations were significantly elevated over control values at 24 hr, 3–6 days, and 3–6 weeks of age. The control of renin release in the newborn appears to be under some of the same influences as in the adult (43). However, experiments to evaluate the individual influences of the macula densa or baroreceptor mechanisms have not been undertaken [for review of control of renin secretion in adults see Davis & Freeman (44)].

The significance of the renin-angiotensin system in the newborn is unclear. Both plasma renin activity and aldosterone concentration are elevated

in the newborn (45, 46). Studies in developing animals suggest that many of the factors controlling renin secretion are intact (35, 43, 47). Jose et al (48) reported that the angiotensin II antagonist, 1-Sar, 8-Ala angiotensin II, modified both glomerular filtration rate and clearance of PAH in newborn animals. Such data suggest a role for the renin-angiotensin system in the control of renal hemodynamics in the young animal.

PROSTAGLANDINS In addition to the renin-angiotensin system, renal prostaglandins may be important mediators in the control of intrarenal hemodynamics. At the present time there is a large and growing literature on this subject and it is not reviewed in detail here. Control of prostaglandin synthesis in the newborn kidney has been studied by only a few investigators. Day et al (49) isolated prostaglandins A_2, E_2, and $F_{2\alpha}$ from fetal kidneys. While the significance of prostaglandin A_2 may be questionable, the other two forms are probably significant. Pace-Asciak (50) demonstrated prostaglandin-catabolizing enzymes in kidneys during development. He suggested a possible relationship between the ability to catabolize prostaglandins and nephrogenesis. Recently, Terragno et al in two preliminary reports characterized the prostaglandins produced by bovine fetal and maternal blood vessels in vitro (51) and studied the prostaglandin biosynthetic capacity of fetal renal cortex (52). The fetal vascular tissue released tenfold more PGI_2 as measured by the stable metabolite 6 keto $PGF_{1\alpha}$ than PGE_2 and also released more PGI_2 than adult vessels (51). It was also reported that fetal pig renal cortical slices possess a higher prostaglandin biosynthetic capacity than adults (52). An endogenous prostaglandin synthesis inhibitor has been demonstrated in adult mammalian plasma (53) as well as in adult pig kidneys (52). Adult hog renal cortical slices released a material that inhibited prostaglandin synthesis in both fetal and adult kidneys (52). Thus, the lower net prostaglandin biosynthetic capacity observed in adult cortical slices may be due to the presence of an endogenous prostaglandin inhibitor. The apparent lack of inhibitor synthesis in the fetal kidney suggests that PGI_2 may act as a regulator of renal function in the fetus. In view of the significant relationship between the renin-angiotensin system and the prostaglandin system in adult animals, further studies in newborns should be undertaken.

ORGANIC ANION TRANSPORT IN THE DEVELOPING KIDNEY

The kidneys of newborn humans and animals are markedly undeveloped in their ability to excrete foreign organic anions such as penicillin (54) and phenolsulfonphthalein (55). Undoubtedly the anatomic and hemodynamic immaturity of the neonatal kidney is partially responsible for this phenome-

non. However, the reduced extraction of organic anions by the kidney suggests that the transport capacity (e.g. secretion) of proximal tubule cells is immature as well (56).

The renal clearance and extraction of PAH by the newborn has been reported to be low in human subjects (57), puppies (14, 58), rats (27), rabbits (59), and sheep (60). In addition, slices of renal cortex from newborn animals of several species exhibit reduced transport capacity for PAH when compared to adult or fetal tissue (61–65). Maturation of tissue transport capacity proceeds in a characteristic pattern. For instance, in rabbit renal cortical slices accumulation of PAH increases from birth to two weeks and then declines to an intermediate value (66). Since kidney weight develops more linearly, these data suggest that increased accumulation of PAH represents more than simple organ growth and development. That is, there appears to be specific maturation of the transport system.

A question arises as to the "trigger" responsible for initiating the maturation of PAH transport capacity. The decreased transport capacity observed in renal tissue from the newborn compared to fetal rabbits could be interpreted as an indication that the functional load presented to the kidney at birth exceeded secretory capacity (65). At birth, the sudden change in supply of energy-yielding substrates from maternal blood glucose to a milk diet high in lipid (including medium- and long-chain fatty acids) would increase the concentration of organic anion substrates in plasma (67–69). These changes, along with the increasing renal blood flow, would serve, presumably, to increase the functional load imposed upon the transport system. This increased load could be the stimulus to maturation.

Substrate Stimulation of Organic Anion Transport

The functional capacity of many developing enzyme systems increases in response to an increased load (70). Similarly, after treatment of newborn rabbits prior to maturation with large exogenous loads of penicillin the ability of renal tissue to accumulate PAH was more than doubled (64–66, 71). However, upon reaching maturation (i.e. four weeks), exogenous substrate loads produced no effect (64). Substrate stimulation of PAH transport has been produced with a variety of secreted organic anions. In addition to penicillin, transport was stimulated with PAH and triiodothyronine (66, 72). Substrate stimulation has also been observed in species other than rabbit, including rat and dog (65, 71, 73).

IN VITRO STUDIES Schwartz et al (74) used the isolated perfused tubule to estimate PAH transport in control and penicillin-treated rabbits. The intrinsic transport capacity for PAH in tubules from control animals increased nearly fivefold between 8 and 19 days of age. Tubular length simi-

larly increased, but only about twofold. Tubules from 9–13-day-old animals treated with procaine penicillin over 3 days demonstrated a 90% increase in intrinsic transport capacity compared to age-matched untreated controls. Penicillin produced no change in tubular geometry. This led to the conclusion that substrate stimulation augments the rate of maturation of the intrinsic transport mechanism without altering the age-related change in length of the proximal tubule.

The enhanced PAH transport capacity following administration of penicillin occurred without any change in organic cation transport (65) and did not result from an alteration in the morphologic (75) or ultrastructural (76) characteristics of the proximal tubule cell. Although substrate stimulation enhanced the ability of renal tissue to accumulate PAH, it did not alter the oxygen consumption of proximal tubules (77). In addition, penicillin and acetate stimulated PAH accumulation via separate mechanisms, since the acetate-induced stimulation of PAH accumulation was observed in renal tissue from treated neonates (65, 78).

The degree of stimulation produced by penicillin pretreatment was dose-dependent (78). Maximal stimulation of PAH transport capacity was observed 24 hr after the last of four injections of 90,000 IU of procaine penicillin G was administered to young rabbits at 12-hr intervals (78). Penicillin excretion was essentially completed during the intervening 24-hr period (78). Therefore, the enhanced PAH transport capacity could not be attributed to a mechanism (e.g. counter-transport) dependent upon the immediate presence of penicillin in kidney tissue during estimation of PAH accumulation. Indeed, when penicillin was added directly to incubation beakers containing PAH and renal cortical slices, PAH accumulation was markedly depressed (65). If estimation of transport capacity in rabbit tissue was delayed more than 36 hr after the final penicillin injection, no stimulation of PAH accumulation could be detected (65, 78). The transitory nature of this effect suggests that the proximal tubule cells responded to the increased load produced through penicillin injection by increasing the number of specific transport proteins available for anion transport (71, 75). This hypothesis is tenable since both normal (79) and penicillin-induced (76) maturation of transport capacity was associated with an increase in the apparent maximal velocity (V_{max}) for PAH accumulation. This point is far from established, however. When a slightly different technique for estimating kinetic constants was utilized, the data suggested that penicillin treatment altered K_m rather than V_{max} (80).

Substrate stimulation and renal protein synthesis An intact protein synthetic mechanism appears to be required for substrate stimulation since concomitant administration of cycloheximide prevented penicillin enhance-

ment of PAH transport capacity (71, 78). Substrate stimulation was associated with increased uptake of ^{14}C-L-leucine and ^{14}C-L-glutamine into the trichloroacetic acid insoluble fractions of rat renal cortical homogenates (71). However, no effect was observed on amino acid uptake into the corresponding fraction from rat renal medulla. In addition, a nonspecific stimulus of renal protein synthesis (chronic NH_4Cl acidosis) increased labeled amino acid uptake but had no stimulating effect on PAH transport (71). Therefore, the effect of penicillin is probably not the result of nonspecific increases in protein content. From these observations, Hirsch & Hook (71) concluded that the stimulatory effect of penicillin on PAH transport was the result of increased synthesis of specific transport proteins.

Thus, a great deal of data strongly support the hypothesis that substrate stimulation of organic anion transport is associated with a selective increase in a protein or proteins closely associated with the organic anion secretory process. However, definitive data related to specific proteins or even the specific cellular location of these proteins are not yet available.

Substrate stimulation and renal lipid metabolism Hewitt & Hook approached the study of substrate stimulation of organic anion transport from a somewhat different perspective (81). They reasoned that penicillin stimulation of anion transport might not induce a specific transport protein, but might bring about a rather subtle change in metabolism within the proximal tubular cell that could secondarily influence PAH transport when measured in vitro or in vivo. The renal organic anion transport system has been linked to the selective extraction of nonesterified fatty acids (NEFA) from arterial blood. Consequently, PAH and certain fatty acids such as palmitate could compete for a common intracellular binding site or possibly are handled by a common enzymatic pathway. For instance, substrates of the PAH transport system such as probenecid, chlorothiazide, or furosemide have been shown to inhibit the in vivo uptake of nonesterified fatty acids (NEFA) by dog kidney (82, 83). Barac-Nieto (84) suggested that the enhancement of PAH transport in vitro produced by carnitine was secondary to reduction of intracellular NEFA concentration. Heinemann et al (85) observed that experimental maneuvers that depress PAH accumulation routinely altered the degree of oxidation and/or esterification of palmitate without altering total slice palmitate uptake. Hewitt & Hook (81) observed that penicillin treatment of immature rabbits increased PAH accumulation by suspensions of proximal tubules and altered distribution of incorporated palmitate C^{14} within lipid classes. Penicillin treatment increased esterification of palmitate to triglycerides and decreased the fraction of palmitate recovered as NEFA. The data were interpreted to suggest that palmitate and PAH share a common intracellular binding site and that penicillin enhanced PAH accu-

mulation by removing endogenous inhibitors, that is, NEFA. Subsequent studies demonstrated that the effect of penicillin on palmitate esterification is restricted to the proximal tubule and does not occur in glomeruli (W. E. Stroo and J. B. Hook, unpublished observations), again suggesting that the changes in palmitate metabolism are associated with organic anion transport. However, these changes in lipid metabolism are difficult to quantify and even more difficult to correlate with the magnitude of changes in anion transport.

Substrate stimulation and renal microsomal enzymes Hirsch & Hook (71) observed that the increased incorporation of leucine and glutamine into renal cortical protein after penicillin occurred mostly in the 100,000 X g (microsomal) pellet. Holohan et al reported a PAH-specific binding protein in the microsomal fraction of dog kidneys which they believe to be involved in organic ion transport (86). Possibly the penicillin treatment increased the quantity or activity of the membrane component and facilitated PAH transport into the renal tubular cell. The classical inducers of hepatic microsomal enzyme activity, phenobarbital and 3-methylcholanthrene (3MC), also increased PAH accumulation by renal cortical tissue from young rabbits (87), suggesting that organic acid transport might be dependent in some way on membrane-bound enzyme systems that could be stimulated nonspecifically by any number of chemicals. Penicillin, phenobarbital, and 3MC had no effect on PAH transport in slices from adult rabbits but significantly increased PAH transport in slices from 14-day-old rabbits. Aryl hydrocarbon hydroxylase activity was enhanced in both adult and two-week rabbit kidneys by 3MC while biphenyl-4-hydroxylase activity was enhanced in two-week and adult rabbit kidneys by phenobarbital. Epoxide hydratase was unaffected by all three treatments. 3MC increased glutathione-S-transferase (ligandin) activity in two-week but not adult kidneys. Thus, since enzyme activity was increased in both immature and adult kidneys after phenobarbital and 3MC and yet anion transport was stimulated by these agents only in the newborn, induction of PAH transport does not seem to be absolutely linked to microsomal drug-metabolizing enzymes. The lack of any effect of penicillin on enzyme activity in two-week-old rabbits at a time when transport was markedly increased suggests that neither microsomal enzymes nor ligandin was involved in the penicillin-induced stimulation of transport (87).

IN VIVO STUDIES Substrate stimulation of renal organic anion transport has also been quantified in vivo. Renal extraction of PAH in 14-day-old rabbits was increased from 37–52% by pretreatment with penicillin (59). Penicillin-treated canine puppies excreted a single intravenous bolus of

PAH more rapidly than control animals with the half-time decreased and the constant of elimination increased (88). The extraction of PAH by kidneys of penicillin-treated animals was 165% that of control (88). A recent report of Schwartz et al (89) suggested that the phenomenon of substrate stimulation of anion transport also occurs in human neonates. These investigators were treating a human neonate with oxacillin and phenobarbital. Subsequently, they were unable to obtain therapeutic concentrations of dicloxacillin even with supratherapeutic doses. Intestinal absorption appeared to be high, but plasma levels were low and were correlated with a high urinary excretion rate. Therefore, these investigators postulated that the renal tubular transport of dicloxacillin was stimulated by the administration of oxacillin (and/or phenobarbital).

Wold and colleagues had observed that the well-known nephrotoxicity to cephaloridine was less in newborn rabbits than in adults (90). They demonstrated that substrate stimulation of the anionic transport system with penicillin increased the nephrotoxicity of this antibiotic. The ability of renal cortical tissue to accumulate cephaloridine increased from birth to adult levels and pretreatment of newborn with procaine penicillin G enhanced the ability of the kidney to accumulate cephaloridine in vitro and in vivo, thus documenting that enhanced toxicity to the antibiotic was due to stimulation of uptake by renal tissue (91).

SUBSTRATE STIMULATION AS A PHARMACOLOGICAL TOOL Hewitt et al (92) quantified the pattern of development of the transport capacity for several organic anions in newborn rabbit kidney slices. The patterns of development and the response of the newborn animals to substrate stimulation were used to test the hypothesis that more than one transport system for organic anions exists in the kidney. The anions were tentatively placed into three groups based on similar patterns of maturation: PAH, penicillin G, and phenolsulfonphthalein comprised group I; urate, sulfisoxazole, and acetylsalicylate were designated group II; chenodeoxycholate was relegated to a third group. In theory, pretreatment with a substrate from one transport system should enhance accumulation of that anion group only. Penicillin pretreatment markedly enhanced maturation of group I anion accumulation without altering maturation of slice urate, salicylate, or chenodeoxycholate accumulation (an increase in sulfisoxazone accumulation was attributed to overlapping with the group I system). Pretreatment of neonatal rabbits with group II or group III anions did not alter group I (PAH) accumulation. Acetylsalicylate in high doses did stimulate PAH accumulation, but this was attributed to a marked toxicity observed after salicylate. Groups II and III anion administration had no effect on maturation of their respective transport systems.

DIURETICS IN THE DEVELOPING KIDNEY

The immature state of the newborn kidney, including low glomerular filtration rate, reduced ability to excrete a sodium load, and diminished rate of transport of organic anions, might suggest that the response to diuretics would be quantitatively different from that in adults. In 1975, Loggie and colleagues reviewed diuretic therapy in infants and children with emphasis on the developmental aspects of renal function (93–95). As pointed out at that time, most of the literature dealing with diuretic drugs involved studies in adults; very few studies were available concerning children, especially in the immediate newborn period. Because of ethical considerations, diuretics are given to newborn humans only in response to therapeutic requirements and dosages are established empirically by response. Consequently, few quantitative data on the effects of diuretics are available from newborn humans.

Because of its predictability and efficacy of action, furosemide appears to be the most widely used diuretic in pediatrics. In 1972, Repetto et al described the response to furosemide in children with reduced creatinine and inulin clearances secondary to acute glomerulonephritis or congestive heart failure (96). Following 1 mg/kg furosemide, i.v., both groups had a 10-fold increase in urinary flow rate. The patients with glomerulonephritis had a 13-fold increase in sodium excretion, and those with congestive heart failure a 30-fold increase. Unfortunately, the age of the children studied was not stated. Pruitt & Boles (97) administered furosemide orally and intravenously to patients 8–15 years of age with acute poststreptococcal glomerulonephritis. They reported an adequate natriuretic response to an intravenous dose of 1 mg/kg and noted that oral doses above 2 mg/kg were required for a similar response. They found that the plasma half-life varied from 2.3 to 4.4 hr and was not related to the level of blood urea nitrogen. They did not compare responses in children of different ages. Najjar and colleagues (98) demonstrated in three infants between 13 and 16 months of age that furosemide would also reduce serum calcium and cause a calciuresis similar to the response in adults.

Engle et al (99) treated a large number of children both above and below one year of age with furosemide, 1 mg/kg parenterally and 2 mg/kg orally. These children were edematous secondary to either cardiac or renal disease. Of interest in this study is that the response to the diuretic was similar in infants less than three months of age when compared to those over three months. In addition, the results suggest that children with renal and cardiac disease respond equally well to furosemide.

In two recent studies furosemide has been evaluated in newborns and low birth weight infants. Ross and colleagues (100) utilized the diuretic in a dose

of 1 mg/kg in 6 infants with a mean gestational age of 30.7 weeks who were between 10 and 57 days old at the time of treatment. These infants showed an average fourfold increase in urinary output and a sixfold increase in osmolar clearance. The creatinine clearance during the first hour following drug administration was approximately double that seen during control periods. The diuretic produced an increase in both absolute and fractional sodium and potassium excretion and a decrease in urinary pH. These data suggest that furosemide can be used effectively in low birth weight infants and that the drug causes both quantitatively and qualitatively similar responses to that seen in older infants and adults. A study by Woo and colleagues (101) confirms that furosemide produces a rapid increase in urine volume, free water clearance, and excretion of sodium, potassium, and chloride in newborn infants. However, it was suggested that the children who had been asphyxiated in the immediate newborn period may have had a decreased response to furosemide compared to those infants not under this stress. They also suggested that the diuretic response was prolonged when compared to adults.

The prolonged diuretic response may be related in part to an increased plasma half-life of the drug in newborn. Aranda et al (102) quantified the pharmacokinetic disposition of furosemide in premature and full-term neonates with fluid overload. They found that the plasma clearance of furosemide was very low in the newborn, the half-time of disappearance from plasma being eight times that of the adult. They also found that the volume of distribution of the drug was four times greater than in the adult, which they related to decreased protein binding of the drug in the newborn.

Furosemide is bound to plasma proteins and is handled by the kidney as an organic anion, i.e. filtered and secreted (103). The binding of drugs to serum albumin and the number of binding sites available are particularly significant in the newborn where binding of bilirubin is an important protective mechanism. At normal physiological concentrations of serum albumin, most of the furosemide that appears in the tubular fluid and urine of adults will arise primarily from secretion since its binding to albumin minimizes filtration (103). Wennberg et al (104) studied displacement of bilirubin from albumin by several diuretic agents including furosemide and ethacrynic acid. Although furosemide did displace bilirubin from albumin, the authors concluded that at the usual recommended dosage of 1 mg/kg, with resultant plasma concentrations of 50–100 μM, the diuretic would not produce an increase of any significant quality in free bilirubin because of the existence of multiple binding sites on the albumin. However, they pointed out that in infants who already have compromised binding for bilirubin it may be possible to have significant further elevations in free bilirubin following diuretics.

Therefore, at least for highly efficacious diuretics, like furosemide, it appears that there are only minimal qualitative and quantitative differences in response at various ages. However, the studies appearing in the literature to date have been primarily clinical and the drug has been given infants who were sick at the time of study. Only recently have a few quantitative studies on the response of newborn animals to diuretics begun to appear. These studies provide a surprising lack of age-related differences in diuretic responsiveness. Banks & Kleinman (105) observed that the increase in fractional sodium excretion after amiloride was somewhat greater in puppies than in adults and this difference was magnified by volume expansion. Potassium excretion by puppies and adult dogs was decreased markedly and to a similar degree prior to volume expansion. Noordewier et al (106) constructed dose-response curves to constant infusions of furosemide, ethacrynic acid, hydrochlorothiazide, and amiloride in 5- to 10-day old unanesthetized piglets and concluded that the response to the diuretics was similar to that expected in adults. Hydrochlorothiazide produced a milder natriuresis than furosemide or ethacrynic acid but all three drugs had similar kaliuretic activity. Amiloride, the least natriuretic of the drugs, was not antikaliuretic when given alone and abruptly decreased the potassium excretion during hydrochlorothiazide infusion.

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