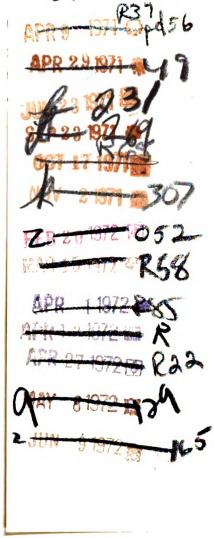
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ABSTRACT

DEVELOPMENT OF RENAL ORGANIC ACID TRANSPORT;
SUBSTRATE STIMULATION BY PENICILLIN AND OTHER COMPOUNDS

by Gerald Herman Hirsch

The objectives of this investigation were: (1) to quantify the maturation of renal organic acid transport in newborn animals; (2) to stimulate the development of organic acid transport by treating either the pregnant female or the newborn with suitable substrates; and (3) to utilize the information gained by these procedures to provide a better understanding of renal organic acid secretion. Organic ion transport was measured by determining the ability of renal cortical slices to accumulate the organic acid, p-aminohippurate (PAH), and the organic bases, tetraethylammonium (TEA) or N-methylnicotinamide (NMN). Accumulation of these compounds by renal cortical slices was expressed as the slice/medium (S/M) ratio. Other studies have demonstrated that accumulation of organic acids and bases by renal cortical slices is closely related to the secretion of these compounds in vivo.

Development of the renal transport system for PAH was determined in young animals of several species. The ability of rat renal cortical slices to accumulate PAH gradually increased from birth until adult levels were reached. PAH S/M ratios increased from birth to 4 weeks of age in rabbits and dogs. Although kidney and body weight continued to increase beyond this age, the PAH S/M ratio began to decline until it reached adult levels.

Administration of PAH to 1 week-old rabbits enhanced PAH accumulation by renal cortical slices. PAH S/M ratios were also increased after treating

2 week-old rabbits with penicillin, but no increase was observed after treatment of 4 week-old rabbits. Substrate stimulation could thus be produced during the developmental period, but not when transport development was complete. Pregnant rabbits were treated with penicillin during the last half of gestation. The offspring were obtained by caesarean section immediately before birth or allowed to deliver normally and used at ages ranging from 1 day to 4 weeks. PAH S/M ratios in renal cortical slices from rabbits whose mothers received penicillin were greater than control values in fetuses and in offspring from 1 day to 2 weeks of age. Thus the presence of penicillin in the fetus stimulated development of tubular secretory processes in fetal and newborn rabbits.

Treatment of nursing rats with penicillin or PAH increased PAH accumulation by renal cortical slices, but had no effect on NMN or TEA accumulation. Administration of triiodothyronine (T_3) to weanling rats caused a marked increase in PAH, but not NMN, S/M ratios. When added directly to the incubation medium, both penicillin and T_3 inhibited PAH accumulation, demonstrating that both of these compounds stimulated organic acid transport by substrate stimulation.

Penicillin treatment of nursing rats increased the kidney weight/body weight ratio. Concomitant treatment of rats with cycloheximide prevented the increase in the PAH S/M ratio induced by penicillin. Renal cortical slices from penicillin-treated rats exhibited an increase in <u>in vitro</u> incorporation of leucine-C^{14} and glutamine-C^{14}. This effect was specific for the renal cortex as was the enhancement of the PAH S/M ratio. Incorporation of orotic acid-C^{14} into RNA was enhanced in penicillin-treated

rats when expressed on the basis of kidney slice weight but not when factored by protein content. The data support the hypothesis that stimulation of the PAH S/M ratio by penicillin was associated with enhanced renal protein synthesis. The effect of penicillin on PAH accumulation was not secondary to a general increase in kidney weight since ammonium chloride enhanced the kidney weight/body weight ratio but not the PAH S/M ratio. Acetate had a similar stimulatory effect on PAH accumulation by renal cortical slices from both penicillin-treated and control rats. Although penicillin treatment of rats enhanced accumulation of PAH by renal cortical slices, the rate of PAH uptake was not significantly increased.

The stimulatory effect of penicillin on PAH accumulation was reversible since the increase in the PAH S/M ratio observed 24 hours after discontinuing penicillin treatment of 2 week-old rabbits was no longer observed when PAH accumulation was determined 8 days after terminating drug treatment. A correlation between histological immaturity of the kidney cortex of various species of young animals and low PAH accumulation was demonstrated. Although normal kidney development could be demonstrated both physiologically and histologically, no histological changes could be seen in kidneys from 2 week-old penicillin-treated rabbits at a time when physiological development (PAH S/M ratio) was accelerated by penicillin. The data suggest that the stimulatory effect of penicillin on PAH accumulation by renal cortical slices is due to specific substrate stimulation of the active concentrating mechanism mediating PAH secretion.

DEVELOPMENT OF RENAL ORGANIC ACID TRANSPORT; SUBSTRATE STIMULATION BY PENICILLIN AND OTHER COMPOUNDS

by

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INTRODUCTION

Functional immaturity of the kidneys of most newborn animals is exemplified by the low activity of the renal transport systems that are responsible for the secretion of organic acids and bases. The ability of the kidneys of the newborn to excrete a concentrated or dilute urine is also significantly less than that of the adult (Falk, 1962). Renal histological immaturity is evidenced by the presence of undifferentiated tissue in the outer cortex and the development of a brush border in the proximal convoluted tubules during the first weeks of life (Wachstein and Bradshaw, 1965).

The development of renal organic acid and base transport in dogs and pigs has been described by Rennick et al. (1961), while New et al. (1959) measured the activity of the organic acid transport system in newborn rabbits. Most investigations concerned with organic ion transport in young animals however, have provided little information on the factors responsible for, or associated with, renal development. The development of other body functions such as carbohydrate metabolism (Tepperman and Tepperman, 1963) and drug metabolism (Conney et al., 1965), can be stimulated by the appropriate substrates. The present investigation was designed to demonstrate that substrate stimulation of the renal organic acid transport system was possible.

The specific objectives of this study were threefold: (1) to quantitate the maturation of renal organic acid transport in newborn animals; (2) to stimulate the development of transport in young animals by treating either the pregnant dam or the newborn with a suitable substrate, and to investigate factors associated with this stimulation; and (3) to utilize the information

gained by these procedures to increase our understanding of organic acid secretion and its normal development to adult levels.

The characteristics of the organic ion secretory systems can be studied by three different approaches: (1) the excretion of organic acids can be studied in the intact animal; (2) the appearance of visible dyes and their approximate concentration in tubular cells or lumina can be observed under the microscope; and (3) the accumulation of actively transported substances by kidney slices in a suitable medium can be measured. The first method is limited to conditions that can be tolerated by the animal, and is difficult to use in very young animals. The second method is difficult to quantify and is limited to colored compounds. The third method described above permits examination of a wide variety of variables and can readily be adapted to studying organic ion accumulation by renal cortical slices from both immature and adult animals. For these reasons the in vitro slice technique developed by Cross and Taggart (1950) was used in the present investigation. The specific compounds whose in vitro accumulation was measured were p-aminohippurate (PAH), which is used as the prototype for organic acids, and N-methylnicotinamide (NMN) and tetraethylammonium (TEA), which are organic bases. Substrate stimulation of organic acid transport was investigated by treating young rats and rabbits with drugs such as penicillin. When compared to kidney slices from control animals, the ability of renal cortical slices from treated animals to accumulate PAH and NMN or TEA provided an indication of the treatment effects.

The following pages are designed to serve as a review of some of the literature pertaining to development in general, renal development, and renal organic acid transport.

Immaturity of Newborn Animals

The newborn animal cannot merely be considered a minature of the adult since the responses of the newborn to a variety of stimuli are often different than what would be expected in terms of size alone (Adolph, 1968). The vulnerability of the developing fetus and newborn infant to pharmacological agents and the resulting incidence of toxic reactions has focused increased attention on the perinate and the factors responsible for the maturation of biochemical and physiological functions to adult levels. In the human, the perinatal period extends from the completion of embryonic differentiation to the end of the first month of postnatal life (Yaffe, 1966). This period represents the time when the most rapid rate of growth and development occurs (Kretchmer et al., 1963). Observation and measurement of developing body functions necessarily preceded study of the factors regulating development. Dawkins (1966) found that most enzymes concerned with homeostatic functions of the liver posses low activity at birth, and that activity increases to adult levels in the neonatal period at varying rates for different functions. Fouts and Adamson (1959) reported that a number of drugs that were normally metabolized by enzymes present in the microsomal fraction from the liver of adult rabbits were not metabolized by livers of newborn rabbits, but by 4 weeks after birth the activity was equal to that of the adult. Similarly, Jondorf et al. (1959) reported that newborn mice and guinea pigs were deficient in various drug metabolizing enzymes normally present in the livers of adult animals.

Maturation of Enzymes

There is some question as to whether an enzyme has adult characteristics when it is first detected, or whether enzymes undergo a process of differentiation and thus become more active as a tissue develops. In the former case, increased enzyme activity during development would be the result of an increase in the amount of enzyme, while the latter situation would involve both increased activity and amount of each enzyme. Two procedures for distinguishing between de novo synthesis and activation involve the use of labeled precursors and inhibitors of protein synthesis. Nemeth (1961) and Nemeth and de la Haba (1962) demonstrated that the developmental increase in tryptophan pyrrolase activity was puromycin sensitive and therefore was associated with de novo synthesis. In contrast, the adaptive increase in tryptophan pyrrolase activity observed after administration of tryptophan was only partially inhibited by puromycin, indicating that both de novo synthesis and activation were involved.

Substrate-Induced Stimulation of Enzymes

Studies by Moog (1952, 1965), Weber (1963) and Greengard and associates (1963, 1967) have provided a basis for investigation of the mechanisms responsible for both the postnatal increase in enzymatic activity and enzyme induction by various compounds. Dawkins (1966) suggested that two factors responsible for the postnatal increase in enzymatic activity are increased adrenocortical activity associated with the stress of birth and changes of environment and induction by accumulation of substrate. Substrate stimulation may involve either acute or chronic

adaptation, depending on the type and duration of stimulus involved. Acute adaptation in immature animals usually involves substrate stimulation of pre-existing enzymes (Weber, 1963). This may involve the release of bound enzymes (Wang, 1968), polymerization of several individual enzyme units into active forms (Moog, 1965), or some other molecular change. In contrast, chronic adaptation usually involves a change in the amount of enzyme. If the metabolic load is increased and kept at a high level for a sufficient period of time, synthesis of new enzyme occurs, resulting in an increase in the amount of active enzyme available (Nemeth, 1963).

Moog (1956) suggested that substrates may have a sequential action whereby exposure of a cell to a substrate may be the triggering event for enhanced enzyme synthesis. Moog indicated that, particularly in immature animals, each substrate may act as an inducer as well as a substrate, so that a local change in the availability of a substrate might have far reaching consequences regarding the metabolic activities of such tissues. Increased accumulation of substrate as animals mature may thus be responsible for the increased activity of many enzymes, and artificially increasing substrate levels may have a marked effect on enzyme activity. Greengard (1963) and Moog (1965) suggested that adaptation to postnatal life might be facilitated if the concentration of certain enzymes were artificially caused to rise before, or shortly after, birth. Stimulation of enzymes involving metabolism of drugs or renal secretion of drugs would be examples of such situations. Induction in the perinatal organism might also serve to determine whether low enzymatic activity is

due to a lack of stimulus for the enzyme or whether the enzyme-synthesizing system itself is incapable of being stimulated.

There are numerous examples in the literature of substrate stimulation. In their review, Knox et al. (1956) presented many examples of substrate-induced adaptations. Tepperman and Tepperman (1958, 1963) demonstrated an increased rate of glucose absorption and increased glucose-6-phosphate dehydrogenase activity after periodic overfeeding or refeeding of glucose and fructose. They suggested that glucose initiated a substrate-induced adaptation, resulting in a "biochemical work hypertrophy" of the glucose transport system. In another study, glucose-6-phosphate was found to act as an inducer of its own active transport system in bacteria (Heppel, 1969). Knox (1951)and Knox and Piras (1967) showed that the catalytic activity of rat liver tryptophan pyrrolase is regulated by the concentration of its substrate L-tryptophan, and that administration of L-tryptophan caused an increased rate of enzyme synthesis. Nachmias (1960) observed that rat brain monoamine oxidase activity was decreased when tyramine levels were reduced by reserpine treatment during development. Evidence has been presented to indicate that steroid hormones are normal body substrates for hepatic drug metabolizing enzymes (Kuntzman et al., 1964; Juchau and Fouts, 1966; Jori et al., 1969). Conney et al. (1965) demonstrated that administration of the same compounds (phenobarbital, phenylbutazone) that stimulate metabolism of drugs also stimulate metabolism of steroid hormones. Thus the induction of hepatic drug metabolizing enzymes and the associated changes in fine structure produced by phenobarbital occur due to substrate-induced activation (Orrenius and Ernster, 1964; Ernster and Orrenius, 1965; Burger and Herdson, 1966). Substrate induction is also possible in

embryonic tissues. Gordon and Roder (1953) adminstered adenosine to chick embryos and observed a marked increase in hepatic adenosine deaminase activity. Administration of tyrosine to pregnant rabbits resulted in an increase in enzymatic conversion of tyrosine to homogentisic acid in the offspring (Mathews, 1968).

Although oxidative drug metabolizing enzymes are localized primarily in the liver, low levels of these enzymes are also present in tissues such as the kidney (Gilman and Conney, 1963), and substrate induction of these enzymes is also possible (Wattenberg and Leong, 1962). Gelboin and Blackburn (1964) also demonstrated the presence of inducible enzymes in the kidney and observed that the substrate-induced increase in enzymatic activity could be blocked by inhibitors of protein synthesis.

Development of Kidney Enzymes

The developmental pattern of a number of kidney enzymes has been determined. Zorzoli (1968) and Zorzoli *et al.* (1969) measured the activity of various gluconeogenic enzymes in mouse, rat and guinea pig kidney cortex. In mice, glucose-6-phosphatase, fructose-1,6-diphosphatase, fumarase and lactic dehydrogenase activities were low at birth but reached adult levels by 28 days of age. Phosphoenolpyruvate carboxykinase activity rose sharply postnatally until it reached a maximum at the end of the second week, after which it declined to adult levels. In the histologically mature guinea pig kidney cortex, glucose synthesis and phosphoenolpyruvate carboxykinase activity were high at birth and subsequently declined to adult values. Phosphoenolpyruvate carboxykinase and glucose-6-phosphatase activities in rats were low at birth, reaching a peak at 7 and 14 days of

age respectively, and then declined to adult levels. Renal carbonic anhydrase, glutaminase and alkaline phosphatase all showed abrupt increases in enzymatic activity between 14 and 21 days of age in rats (Wacker et al., 1961). Whittam (1961) reported that during the first 2 weeks of life in rabbits there is a progressive decrease in the capacity for anaerobic glycolysis and an increase in aerobic glycolysis by kidney tissue.

Histochemical evidence has accumulated indicating that enzymes such as succinic dehydrogenase, cytochrome oxidase and alkaline phosphatase are less active in newborn than adult kidneys (Fisher and Gruhn, 1959; Longley and Fisher, 1956; Pinkstaff et al., 1962). Differentiation of enzymes is related to the differentiation of function (Moog, 1952), and it has been observed that maturation of some oxidative enzymes in the nephrogenic zone of the rat kidney coincides with the time of development of functional ability in this zone (Fisher and Gruhn, 1959). Wachstein and Bradshaw (1965) and Kazimierczak (1963) indicated that enzyme activities were low in the cortical nephrogenic zone of newborn rats and rabbits. The enzymes studied included alkaline phosphatase, glucose-6-phosphatase and succinic dehydrogenase. Cortical tubules demonstrated adult patterns of enzymatic activity at 14 - 16 days in rats and 28 days in rabbits. As more tubules matured, enzyme activity became more widely distributed throughout the cortex until adult distribution was reached. In contrast, the newborn guinea pig possesses adult levels of enzyme activity even in outer cortical tubules.

Histological Development of the Kidney

Using histochemical and conventional staining techniques, a number of investigators have attempted to correlate structure and function of the metanephros in embryonic tissue. Gersh (1937) and Leeson and Baxter (1957) provided histological evidence for the function of the fetal kidneys. These investigators reported that the metanephros, which develops to form the fetal kidney, appears about the fifteenth day of gestation in the rabbit, and becomes functional several days before parturition. At birth there is a distinct zone of maturing glomeruli, the juxta-medullary ones having essentially adult morphology and histochemical properties while those in the cortex are less mature (Leeson and Baxter, 1957). The kidneys of newborn rats and rabbits have a peripheral cortical zone of undifferentiated tissue called the nephrogenic zone (Baxter and Yoffee, 1948; Wachstein and Bradshaw, 1965). Glomeruli, as well as proximal and distal convoluted tubules, are found in the cortical zone of the adult kidney. The inner cortical zone contains no glomeruli and consists mainly of terminal portions of proximal convoluted tubules in addition to ascending loops of Henle and collecting ducts. The glomeruli and tubules in the nephrogenic zone are morphologically and functionally immature (Wachstein and Bradshaw, 1965). They gradually assume the structural characteristics of the mature forms. For example, the brush border, which is found in the proximal convoluted tubules and is associated with absorption, matures at 2 weeks of age in the rat and at 4 to 5 weeks of age in the rabbit. During the first, and most of the second week after birth, nephrogenesis is a marked feature of the kidney in the rat (Baxter and Yoffee, 1948). Early investigations showed that in rats the number of nephrons at birth is more than doubled by 2 weeks of age (Kittelson, 1917; Arataki, 1926).

Development of Kidney Function

The development of kidney function has been investigated using both *in vivo* and *in vitro* techniques. At birth the kidney of the newborn animal assumes the excretory responsibilities which had been previously shared with the placenta. The degree to which this task is successfully performed depends both on the state of development at birth and the duration of time since birth. In rats, rabbits and humans the kidney appears to become functional shortly before birth, although both the fetal and newborn kidney are relatively inefficient by adult standards (McCance and Widdowson, 1960). Dean and McCance (1949) showed that human infants respond poorly to a load of hypertonic saline since their capacity to excrete large loads of either sodium or water is limited. Similarly, Falk (1955) demonstrated that a diuretic response to water was absent in rats at birth and developed gradually with age. The ability of renal tubules to absorb a dye such as trypan blue begins at 12 days of age and reaches a maximum at 28 days in the rat (Baxter and Yoffee, 1948). Renal function in the rabbit as measured by phenolsulfonphtalein excretion increases rapidly during the first 10 days after birth (Williamson and Hiatt, 1947). Several investigators have demonstrated that clearance of PAH is low in the newborn (Levine and Levine, 1958; Alexander and Nixon, 1962). A partial explanation for this low clearance value is the low glomerular filtration rate and the small size of the kidneys in the newborn. Edelmann (1967) indicated that as tubular mass increases and the heterogeneity of the nephrons decreases, tubular function and filtration rate increase. In addition to the decreased rate of clearance however, the transport capacity (Tm_{PAH}) is also less in newborn

animals. Other investigators have shown that both Tm_{PAH} and the extraction of PAH by the newborn kidney are lower than the values observed in adults (Calcagno and Rubin, 1963; Calcagno and Lowe, 1963).

Renal Secretion of Organic Compounds

Many substances are added to the urine by active tubular secretion, which involves transport from the peritubular fluid into the tubular lumen. Marshall and Vickers (1923) and Marshall and Crane (1924) were the first investigators to clearly demonstrate secretion of organic compounds in the mammalian kidney. These workers found that the amount of phenol red appearing in the urine was much greater than what could be attributed to glomerular filtration. Edwards and Marshall (1924), by direct microscopic observation of rat kidneys, found that phenol red was concentrated within the cells of the proximal convoluted tubules. Early studies designed to locate secretory processes by histochemical methods yielded ambiguous results partly because the role of glomerular filtration was not yet fully understood and partly because rapid postmortem changes due to diffusion were not properly considered. After establishing the character of the glomerular filtrate as an ultrafiltrate of plasma, Richards and Walker (1937) analyzed samples of fluid aspirated from the lumen of various regions of nephrons in amphibians (frog and Necturus) and mammals (rat and guinea pig). They suggested that compounds such as phenol red were secreted since their tubular concentration was greater than that in plasma. After it was recognized that inulin could be utilized as a measure of glomerular filtration and water reabsorption, secretion of certain compounds could readily be

demonstrated. Compounds that apprear in the urine at a rate greater than that of inulin are now known to be actively secreted in addition to being filtered. Information concerning the anatomical location of secretory processes has also been obtained by the technique of stop-flow analysis (Wilde and Malvin, 1958; Malvin et al., 1958; Rennick and Moe, 1960). These experiments demonstrated that certain organic acids such as PAH and phenol red, and bases such as TEA, were secreted in the proximal tubules. Secretion of these compounds in proximal tubules has also been demonstrated in nephrons of aglomerular fish (Edwards and Cordorelli, 1928; Shannon, 1938). Isolated flounder, rat and human fetal tubules visibly accumulate phenol red and diodrast only in the proximal segments (Engstrom and Josephson, 1953; Richards and Barnswell, 1927). Similarly, Wedeen and Jernow (1968), utilizing a freeze-dry autoradiographic technique, demonstrated the intracellular accumulation of a hippuric acid derivative in rat proximal tubules.

Clearance experiments have served to identify several independent transport mechanisms, one being responsible for the secretion of organic acids and another for organic bases. The mechanism studied most extensively is that which secretes organic acids such as the phenolsulfophthalein dyes (eg., phenol red), PAH, diodrast, penicillin and a variety of other compounds. The ability of these compounds to compete with each other for transport provides evidence that they share a common secretory mechanism. The transport systems for organic acids and bases appear to be present in all mammals, thus permitting comparative biochemical correlations. Most of the transported compounds are not metabolized so that metabolic reactions unrelated to transport do not interfere with measurement of secretion.

A number of organic cations are actively secreted by the kidney (Sperber, 1948; Beyer et al., 1950) by a mechanism similar to, but distinct from, that mediating secretion of PAH (Rennick and Farah, 1956). Secretion has been shown for NMN (Beyer et al., 1950), TEA (Rennick et al., 1947, 1954), tolazoline (Kandel et al., 1958), and quinine and mecamylamine (Volle et al., 1960). This process is regarded as one of active transport since these compounds inhibit each other's tubular excretion (Kandel and Peters, 1957; Kandel et al., 1958; Volle et al., 1959) and since a transport maximum (Tm) has been demonstrated for some of these compounds (Rennick et al., 1954). Although competitive inhibition exists among organic acids or among organic bases, no mutual interference between members of the two groups has been demonstrated. Rennick et al. (1961) demonstrated that the two transport systems develop independently in the newborn puppy and piglet, suggesting that different factors regulate their development. Quantitative differences in the accumulation of PAH and TEA have also been observed.

In vitro Measurement of Renal Secretion

The secretory mechanisms for organic acids and bases can be studied quantitatively using in vitro preparations as well as the intact kidney. Cross and Taggart (1950) devised an in vitro technique which is widely used to study secretion of organic compounds. Thin slices of renal cortex are incubated in an oxygenated medium and the intracellular accumulation of compounds such as PAH is used as an indication of the activity and capacity of the secretory system. Accumulation of PAH and other organic compounds is expressed as the slice/medium (S/M) ratio. More recently, Burg and

Orloff (1962) described a technique involving the preparation and isolation of individual rabbit tubules. This method, and a modification by Burg et al. (1966) allowing microperfusion of individual renal tubules, permits another in vitro approach to the study of tubular transport.

A number of investigators have utilized the in vitro slice technique to study the development of renal secretion of organic acids and bases. McIsaac (1965) reported that kidney slices from immature cats were not able to accumulate hexamethonium as readily as slices from adult cats. The transport system for TEA in dogs is low at birth and progressively increases until adult levels are reached at 8 weeks of age (Rennick et al., 1961). In the same study, PAH accumulation by dog renal cortical slices was low at birth and reached a maximum at 4 weeks of age, after which it promptly declined to adult levels. Histological studies indicated that the outer surface of the cortex was in an immature form for a period of time that corresponded well with the period of low accumulation of PAH by surface cortical slices.

A number of observations comparing PAH transport in vivo and in vitro indicate the reliability of the slice technique in estimating PAH transport. In vivo studies have shown that the maximum capacity of the rabbit kidney cortex to concentrate PAH is 4-5 μmoles/g tissue, and similar values have been obtained using kidney slices (Foulkes and Miller, 1959a, Mudge and Taggart, 1950). Competitive inhibitors such as penicillin, PAH or carinamide have similar inhibitory actions in vivo and in vitro on the intracellular accumulation of phenol red (Forster and Copenhaver, 1956). Other inhibitors such as dinitrophenol also have similar actions

whether studied *in vivo* or *in vitro*. Mudge and Taggart (1950) demonstrated that succinate and fumarate uniformly depressed PAH transport *in vivo* and *in vitro*, while acetate and lactate had stimulatory effects in both types of experiments. Cross and Taggart (1950) concluded from their results that the accumulation of PAH by kidney slices *in vitro* and the tubular secretion of this compound in the intact animal are closely related phenomena. They indicated that the most obvious difference between the two processes was the lack of a continuous filtration process in the slice, with the resulting disturbance of normal concentration relationships between the cells and tubular urine. Another difference is the ability of intracellulary accumulated PAH to diffuse out of the cortical slices back into the medium, in contrast to the unidirectional movement of PAH in the intact kidney. While these differences between the slice and intact kidney undoubtedly influence certain quantitative aspects of PAH transport, experimental observations suggest that the same biochemical processes operate in both situations. In addition to its reliability in measuring PAH accumulation, the slice technique possesses a number of advantages that do not exist in the intact animal. These include the opportunity to study the action of toxic compounds over a wider range of concentrations and conditions than are possible in intact animal experimentation, the ability to eliminate interfering substances or conditions and the simplicity of the experimental preparation.

The S/M ratio is the net result of active intracellular accumulation, binding within the cell and extrusion out of the cell. Accumulation involves

an active transport process as will be described below. Extrusion of PAH from the cells back into the media, or runout as it is commonly called, is thought to involve passive diffusion to some extent. However, an energy-requiring transport process also may be associated with runout since inhibitors of PAH uptake such as dinitrophenol, octanoate, iodopyracet and probenecid can decrease PAH runout (Kinter and Cline, 1961; Wilbrandt and Rosenberg, 1961). Although the mechanism of efflux is not well understood, it is distinctly different from the process mediating uptake (Farah et al., 1963; Ross et al., 1968b).

The term active transport is applied to those systems in which a substance is transported across a biological membrane against an electrochemical gradient at the expense of energy derived from the metabolism of the cell. The demonstration that a transport mechanism can be inhibited by anoxia or selective enzyme inhibitors provides suggestive evidence that it is active. The active transport system in the kidney for organic compounds exhibits a non-linear relationship between concentration and transport rate. Although the rate of transport increases as substrate concentration increases, saturation of the mechanism eventually occurs. A fixed maximal transport rate has been interpreted to indicate that active transport involves the reversible combination of the transported compound with a cellular carrier that is present in a constant but limited amount. The demonstration of competitive phenomena is another property associated with active transport systems and is consistent with the concept of a cellular carrier of limited capacity.

The tubular secretory mechanisms for organic acids and bases are dependent upon aerobic metabolism since they are blocked by anoxia, by inhibitors of the cytochrome electron transport system, and by inhibitors of the oxidative reactions of the tricarboxylic acid cycle (Taggart and Forster, 1950; Shideman and Rene, 1951; Maxild and Moller, 1969). This dependence on aerobic metabolism appears to be related to the requirement of the transport systems for energy-rich phosphates such as ATP which are generated by these oxidative reactions. Dinitrophenol, which uncouples oxidative phosphorylation, inhibits PAH secretion *in vivo* (Mudge and Taggart, 1950) and *in vitro* (Taggart and Forster, 1950). Huang and Lin (1965) and Berndt and Grote(1968) have reported that dinitrophenol is accumulated by renal cortical cells by a mechanism similar to that of PAH, suggesting that part of its inhibitory effect involves competitive inhibition for transport sites. Nevertheless, uncoupling of oxidative phosphorylation is still believed to be the most important mechanism by which dinitrophenol inhibits PAH transport (Berndt and Grote, 1968) since it also inhibits NMN and TEA transport (Rennick and Farah, 1956; Farah *et al.*, 1959).

Since Cross and Taggart described the use of renal cortical slices for the study of PAH transport, much work has been done in attempts to discover the nature of this carrier system. Transport of many biological substances is considered to be mediated by proteins for several reasons. Transport systems, like enzymes, are quite specific in that a single transport system can catalyze the translocation of a limited number of substrates with similar chemical structures. Proteins could constitute the recognition sites for transported substrates since they have the

required degree of specificity to discriminate between possible substrates. It has been demonstrated that as substrate concentration is increased, a transport maximum is reached, suggesting that the transport enzyme has become saturated (Hook et al., 1970; Pitts, 1968). In studies with bacteria, inhibitors of protein synthesis, such as chloramphenicol, prevent transport systems from being synthesized (Pardee, 1968a). Pardee (1968b), also using bacteria, has crystallized a protein involved in sulfate transport. Using the technique of osmotic shock, Heppel (1969) demonstrated the release of specific proteins involved in glucose transport. That proteins are involved in organic ion transport in mammals is suggested by the data of Ross et al. (1969). These investigators provided evidence that the protein fraction contains the renal carrier of NMN. Since earlier work (Ross et al., 1968a) had demonstrated that dibenamine specifically and irreversibly blocked NMN transport, these workers used the receptor protection technique to label the renal carrier for NMN with C^{14}-labeled dibenamine. Other experiments demonstrated that only the protein fraction was protected by the substrate. To date, similar techniques have not been utilized to identify the carrier for PAH transport, primarily because a specific, irreversible inhibitor of PAH transport is not yet available.

Model of the Renal Secretory Mechanism

Organic acid secretion has been extensively studied in fish, and the processes involved have been compared to those believed to function in organic acid secretion in mammals. Phenol red accumulates within the tubular lumen in high concentrations in isolated flounder tubules (Forster, 1948; Forster and Taggart, 1950). No staining of the tubular epithelium

is apparent, and storage within the cells is quantitatively unimportant. It has been proposed that in fish there are two spatially separated transport systems for organic acids within each renal epithelial cell, one which transports compounds from the plasma or bath into the cells and the other from the cells into the lumen (Forster and Hong, 1958; Hong and Forster, 1959). Slices of mammalian renal cortex differ in that accumulation of organic acids appears to be predominantly intracellular. The secretory mechanism in mammalian renal cortical slices is thought to involve two distinct processes, the first involving transport from peritubular fluid into the cell, and the second movement from the cell into the tubular lumen (Taggart, 1958). The step involving movement across the luminal border is downhill and only involves diffusion (Foulkes and Miller, 1959b; Tune et al., 1969). There has been some question as to whether the mechanism responsible for the active accumulation of organic acids (the first step described above) involves a membrane or intracellular carrier mechanism. The active transport step, however, is thought to be the process involving intracellular accumulation (Forster and Copenhaver, 1956; Foulkes and Miller, 1959b; Foulkes, 1963). Foulkes and Miller (1959b) devised the following model (shown in Figure 1) for PAH transport in renal cortical slices: the first step consists of diffusion of PAH from the medium into the extracellular space in the tissue; the second step involves a facilitated diffusion step at the peritubular membrane; the third step causes a build up of a high tissue concentration of PAH within the cell; and finally step four involves diffusion of PAH across the luminal border of the cell.

According to this model, active transport would only be involved in step three. Foulkes and Miller (1959b) further suggested that there were two intracellular fractions of PAH in renal cortical slices. One of these fractions was said to be rapidly diffusible, and rapidly equilibrated with extracellular PAH. The other fraction or pool, in contrast, equilibrated slowly and was associated with the active intracellular accumulation of PAH. Both Foulkes (1963) and Tune et al.(1969) have presented evidence indicating the PAH in the large intracellular pool exists primarily in the free form. Foulkes (1963) found that the fast exchanging portion of cellular PAH contained less than 5% of the total amount of PAH accumulated by renal cortical slices, emphasizing the importance of the active intracellular accumulation step. The concepts outlined above have been utilized in the present investigation to explain the stimulatory effects of a number of compounds such as acetate and penicillin on organic acid transport.

METHODS

Organic Acid and Base Accumulation by Renal Cortical Slices

1. Description of the In Vitro Slice Technique

Animals were killed by a blow on the head and the kidneys removed immediately, weighed, and placed in cold saline. Renal cortical slices weighing about 100 mg were prepared freehand and briefly kept in cold saline until incubated. Slices of renal cortex were incubated in 2.7 ml of the phosphate buffer medium described by Cross and Taggart (1950), which contained 7.4×10^{-5} M PAH and 6.0×10^{-6} M NMN-C^{14} (4.6 mc/mmol) or 1.0×10^{-5} M TEA-C^{14} (1.2 mc/mmol). Incubations were carried out in a Dubnoff metabolic shaker at 25° C under a gas phase of 100% oxygen. Incubation time was 90 minutes, except where the rate of accumulation was determined, when incubation times varied from 2 to 180 minutes. After incubation the slices were quickly removed from the beakers, blotted and weighed. A 2 ml aliquot of medium was taken from each beaker. Three ml of 10% trichloroacetic acid (TCA) were added to graduated cylinders containing tissue or medium, and after the tissue was macerated with a stirring rod, the samples were diluted to 10 ml with water. After centrifuging, two and one-half ml of this final diluted sample were used for the colorimetric assay of PAH as described by Smith et al.(1945). In addition, 1 ml of slice and media homogenate was added to scintillation vials containing 10 ml of modified Bray's solution (6 g of 2,5-diphenyloxazole and 100 g of napthalene per liter of dioxane). Radioactivity was determined using a Beckman LS-100 liquid scintillation counter, employing either internal or external standardization. Results were expressed as slice to medium (S/M) ratio,

where S equals milligrams per gram of tissue or disintegrations per minute per gram of tissue and M equals milligrams per milliliter of medium or disintegrations per minute per milliliter of medium.

2. Effect of Inhibitors

Accumulation of PAH and NMN by renal cortical slices was determined concomitantly in many experiments. Using adult male Dutch Cross rabbits, a series of experiments were done to demonstrate that active transport was required for the intracellular accumulation of these compounds. Incubations were carried out in the presence of dinitrophenol (DNP) and under an atmosphere of nitrogen (N_2) instead of oxygen. In addition, the quaternary base TEA was added to some of the incubation beakers (final concentrations 1×10^{-5} M and 1×10^{-4} M) to demonstrate competitive inhibition of NMN accumulation.

3. Effect of pH

The optimum pH for PAH accumulation was determined in kidney slices from dogs, rabbits and rats. The incubation medium was buffered with sodium phosphate when studying dog renal cortical slices, while 0.1 M 2-amino-2-methyl-1,3-propanediol (propanediol) was used in pH studies involving rabbit and rat kidney slices. The pH of the incubation medium was adjusted at the start of each experiment, and recorded again when incubation was completed. PAH accumulation by renal cortical slices was determined over a final pH range of 7.2 to 8.4.

4. Effect of Acetate and Potassium

The effect of acetate on PAH accumulation by renal cortical slices from 2 and 4 week-old and adult rabbits was determined by adding sodium acetate (final concentration, 10^{-2} M) to the incubation medium.

PAH accumulation by renal cortical slices from 2 and 4 week-old and adult rabbits was determined after varying the potassium concentration of the incubation medium from one-half to double its normal value. The effect on PAH accumulation after replacing potassium with sodium was also determined, using renal cortical slices from adult rabbits.

Development of PAH and NMN Accumulation by Renal Cortical Slices

1. Rabbits and Rats

New Zealand white rabbits and Sprague-Dawley rats were housed and bred in the departmental animal quarters, so that young animals at specific stages of development were available for use. Young animals were left with their mothers until sacrificed. Animals were killed at the desired ages, the kidneys removed, renal cortical slices prepared, and the PAH and NMN S/M ratios determined. Changes in kidney and body weight with increasing age were also recorded and compared with changes in the S/M ratios. The incubation time required to reach maximum PAH S/M ratios was determined in renal cortical slices from animals at various ages. Tissue water content (based on per cent of tissue wet weight) of renal cortical slices from rabbits at different ages was measured and correlated with changes in the PAH S/M ratio.

2. Dogs, Cats and Guinea Pigs

The ability of renal cortical slices from dogs, cats and guinea pigs of various ages to accumulate PAH and NMN was also investigated. Pregnant mongrel dogs and cats were housed in the departmental animal quarters, while male guinea pigs and female guinea pigs with 1 week-old young were purchased and used immediately. PAH and NMN S/M ratios were determined in renal cortical slices from 1 week-old cats and guinea pigs, and in dogs from 1 to 8 weeks of age, as well as in adult animals of these species.

Substrate Stimulation of PAH Transport

1. By PAH

Substrate Stimulation of organic acid transport was studied by treating 1 week-old rabbits with PAH (100 mg/kg, intraperitoneally, twice daily) for 7 days. Beginning at 16 days of age, nursing rats were treated with PAH (100 mg/kg, intraperitoneally, twice daily) for 3 or 7 days, or with 200 mg/kg PAH for 3 days. PAH was dissolved in saline and the solution brought to pH 7.4. Control littermates were injected with saline and all animals were killed 24 hours after the last injection. The ability of renal cortical slices from PAH-treated and control animals to accumulate PAH and NMN was then compared.

2. By Penicillin in Rabbits

Rabbits at 2 and 4 weeks of age were treated subcutaneously twice daily for 3 days with procaine penicillin G (Duracillin, Eli Lilly and Co.) at a dose of 60,000 international units (IU) per injection. Control

littermates received saline. Rabbits were killed 24 hours after the last injection and the ability of renal cortical slices from penicillin-treated and saline-control animals to accumulate PAH was then compared. In some experiments, crystalline potassium penicillin G (E. R. Squibb and Sons) was added directly to incubation beakers (containing PAH medium and renal cortical slices from adult male rats) at concentrations ranging from 10,000 IU to 100,000 IU, and the PAH S/M ratios obtained were compared to control values.

Pregnant rabbits were treated with 60,000 IU penicillin, intramuscularly, twice daily during the latter half of pregnancy (days 16-30). The fetuses were delivered by caesarean section on day 31 of gestation, or allowed to deliver normally. PAH accumulation by renal cortical slices from fetuses or offspring from 1 day to 4 weeks of age was determined and compared to values obtained from rabbits whose mothers received saline.

3. By Penicillin in Nursing Rats

Female Sprague-Dawley rats were bred in the departmental animal quarters or were purchased when 16 - 18 days pregnant. Within 48 hours of birth litter size was reduced to 8, and at 16 days of age ("nursing rats") penicillin treatment was instituted in half of each litter while the 4 remaining rats served as controls. Procaine penicillin G was administered subcutaneously at a dose of 30,000 IU twice daily for 3 or 7 days. Control littermates received saline, and all young rats remained with their mothers until sacrificed. Rats were killed 24 hours after the last injection in all experiments. Body weights and kidney weights were recorded and PAH and

NMN accumulation by renal cortical slices from control and penicillin-treated rats then compared. TEA accumulation by renal cortical slices from penicillin-treated and control rats was also determined in some experiments.

4. By Triiodothyronine (T_3)

DL-Triiodothyroine was dissolved in alkaline saline and injected intraperitoneally into weanling (50-60g) and adult(200-220g) male rats in doses of 200 or 500 µg/kg once daily for 3 or 7 days. Controls received alkaline saline. Forty-eight hours after the last injection the animals were killed and PAH and NMN S/M ratios were determined. The effect of T_3 on PAH and NMN accumulation by rat kidney cortical slices was also determined when added directly to the incubation beakers in concentrations of 10^{-6} M and 10^{-8} M.

Studies on Possible Mechanisms Associated with Substrate Stimulation by Penicillin

1. Effect of Penicillin on Renal Cortical Tissue

The effect of acetate on PAH accumulation by renal cortical slices was measured in slices from both penicillin-treated and control nursing rats. The final concentration of acetate in the incubation medium was 10^{-2} M after the addition of 0.3 ml of 0.1 M sodium acetate. Water (0.3 ml) was added to the incubation medium in beakers not receiving acetate. In some experiments, the equivalent amount of sodium present in 0.3 ml of 0.1 M sodium acetate was added to control beakers as sodium chloride.

The effect of penicillin on the rate of entry of PAH into renal cortical slices from penicillin-treated or saline-control nursing rats

was determined by measuring PAH uptake at incubation times ranging from 2 to 30 minutes. PAH uptake was plotted as a function of incubation time.

Total tissue water was determined by weighing nursing rat kidney cortical slices before and after drying to constant weight in an oven at 100° C. Extracellular water was measured by the inulin space method of Webber and Cairns (1968), using inulin-C^{14} (2 mc/g). Incubations were carried out for 90 minutes using 50 to 100 mg of renal cortical slices and 0.075 µc inulin-C^{14} in 3 ml of Cross and Taggart (1950) medium. Intracellular space as a fraction of wet weight was calculated as the difference between total tissue water and extracellular water.

2. Effect of Penicillin on Renal Protein and RNA Synthesis

a. Increase in kidney weight

The kidney weight : body weight ratio was determined in penicillin-treated and saline-control nursing rats. Kidney weight of nursing rats was also increased by the administration of ammonium chloride. The effect of ammonium chloride on kidney and body weight, as well as PAH and NMN accumulation by renal cortical slices, was determined by injecting 1 M ammonium chloride intragastrically in a dose of 1 ml/100g once daily for 3 days. Control littermates received an equal volume of distilled water, also by stomach tube.

b. Inhibitory effect of cycloheximide

Four nursing rats from each litter were injected subcutaneously with 30,000 IU penicillin twice daily for 3 days, while the remaining 4 rats

received saline. One-half of both the penicillin-treated and saline-control rats were concomitantly injected with 0.1 mg/kg cycloheximide (intraperitoneally), with the remaining rats receiving saline. All rats were killed 24 hours after the last injection.

c. Incorporation of leucine and glutamine by kidney slices

Incorporation of L-leucine-C^{14} (278 mc/mmol) or L-glutamine-C^{14} (212 mc/mmol) into protein was studied using slices of kidney cortex from control or penicillin-treated nursing rats. The ability of cortical and medullary slices prepared from the same kidneys to incorporate L-leucine-C^{14} was determined in some experiments. Ten microliters of L-leucine-C^{14} (0.1 µc) or L-glutamine-C^{14} (0.1 µc) in 0.01N HCl were added to each incubation beaker at the beginning of the incubation period. Incorporation of labeled amino acid into protein was determined using a modification of the method described by Bignall, et al. (1968). After incubating for 90 minutes the slices were removed, blotted on gauze pads, weighed, and homogenized in 10% TCA. The protein precipitate was washed 3 times with 5% TCA containing 0.2% unlabeled amino acid. After the last centrifugation the protein precipitate was dissolved in 2.0 ml of 1 N KOH by warming in a water bath at 50° for 20 minutes. A 0.5 ml aliquot of the resulting solution was then counted in 10 ml modified Brays solution as described above, and protein was determined on another 0.5 ml aliquot by the method of Lowry et al. (1951).

d. Protein content of subcellular fractions

Kidneys from penicillin-treated or control rats were homogenized in 9 ml of 1.15% KCl and centrifuged at 9000 g for 20 minutes. The precipitate

was then washed once with 1.15% KCl and recentrifuged. The supernatant was centrifuged at 105,000 g for 30 minutes, after which both precipitates were resuspended in 5 ml of 0.1 M phosphate buffer. The protein was reprecipitated with 5 ml of 10% TCA, centrifuged, and washed with 5% TCA. The precipitates were resuspended in 10 ml of 0.1 M phosphate buffer, the pH adjusted to 7, and an aliquot taken for protein determination.

e. Incorporation of orotic acid into renal RNA

Nursing rats were treated with penicillin for 3 days, and 24 hours after the last penicillin injection they were injected intraperitoneally with 0.2 μc (0.1 ml) orotic acid-C^{14} (36.5 mc/mmol) per 50 g body weight. Four hours later the rats were killed and the incorporation of label into RNA was determined in cortical slices from one kidney, using a modification of the method described by Hayashi et al. (1968). PAH accumulation was determined in cortical slices from the other kidney as described in an earlier section. To determine orotic acid incorporation, the renal cortical slices were weighed, homogenized in cold 10% TCA and centrifuged for 10 minutes. The precipitate was washed 3 times with 10 ml of 5% TCA and all the supernatants were combined. The radioactivity of this acid-soluble fraction was determined by adding a 1.0 ml aliquot to scintillation vials. Radioactivity in the acid-insoluble fraction was measured by suspending the precipitate in 10 ml of 10% TCA, heating for 15 minutes in a boiling water bath, centrifuging, and adding 1.0 ml of the supernatant to scintillation vials.

In other experiments, subcellular fractionation of kidney homogenates from penicillin-treated and control nursing rats receiving orotic acid-

C^{14} 4 hours before being killed was carried out. Subcellular fractionation was done as described above, and aliquots were taken for protein determinations. The radioactivity of each fraction was determined by adjusting the pH to 2, heating in a boiling water bath for 20 minutes, centrifuging, and adding 1 ml of the supernatant to scintillation vials.

3. Effect of Penicillin on Renal Histological Development

Young rabbits were treated subcutaneously with 60,000 IU penicillin, or saline, twice daily from age day 11 to day 13. PAH accumulation by kidney slices was determined in one-half of the treated and control rabbits 24 hours after the last injection while the same experiment was done in the remaining littermates 8 days after discontinuing treatment.

Histological sections of kidneys from rabbits treated subcutaneously with 60,000 IU penicillin from age day 11 to day 13 were also prepared. Twenty four hours later, one kidney from each rabbit was fixed in Zenker's solution (50 g potassium dichromate, 70 g mercuric chloride, and water to 2,000 ml), while PAH accumulation was determined in renal cortical slices prepared from the other kidney. Fixation involved placing kidney halves in Zenker's solution for 10 - 12 hours, followed by washing the kidneys overnight in cold running water to remove excess mercury salts. The kidneys were then placed in coded vials containing 70% ethanol. A single-blind study was done to determine if histological differences between kidneys from saline and penicillin-treated 2 week-old rabbits could be demonstrated. Histological sections of kidneys from 4 week-old and adult rabbits were also prepared. Kidneys were inbedded in paraffin, and prepared in sections 4 μ thick. All sections were stained with hematoxylin-

eosin and photographed at 640X magnification.

Statistical Analyses

Unless indicated otherwise, all data obtained were subjected to statistical analysis using Student's 't' test, paired or group comparison (Lewis, 1966). Other statistical tests used included Duncan's test following analysis of variance, and linear regression analysis (Steel and Torrie, 1960). The 0.05 level of probability was used as the criterion of significance in all statistical tests.

RESULTS

Organic Acid and Base Accumulation by Renal Cortical Slices

1. Effect of Inhibitors

When renal cortical slices from adult male Dutch Cross rabbits were incubated for 90 minutes in Cross and Taggart medium in 100% oxygen, a PAH S/M ratio of 6.7 ± 1.6 (mean ± S.E.) was obtained (Figure 2). When 10^{-4} M DNP was added to the incubation medium or when the gas phase was nitrogen, the PAH S/M ratios were reduced to less than 2.0. An S/M ratio of about 1 would be the value expected if only passive diffusion of PAH into slices were occurring. The organic base TEA, when present in the incubation medium at a concentration of 1×10^{-4} M, had no effect on the PAH S/M ratio.

In the above experiments, accumulation of NMN by rabbit renal cortical slices was determined concomitantly with that of PAH. An S/M ratio of 1.57 ± .08 was found for NMN in 7 experiments (Table 1). When 10^{-4} M DNP was added to the incubation beakers, the NMN S/M ratio declined to 1.05 ± .06. TEA inhibited the accumulation of NMN in a dose-dependent manner since 10^{-5} M TEA decreased the NMN S/M ratio to 1.33 ± .17 while 10^{-4} M TEA further decreased the value to 1.16 ± .02. When the gas phase was nitrogen, the NMN S/M ratio was 1.03 ± .03.

2. Effect of pH

Using renal cortical slices from adult male rats, mongrel dogs and New Zealand white rabbits, incubations were carried out over a pH range of 7.2 to 8.4 (Table 2). Accumulation of PAH by renal cortical slices

from the three species was found to be dependent on the hydrogen ion concentration of the incubation medium. Optimal PAH S/M ratios were obtained at about pH 8 for rat and rabbit renal cortical slices, while the highest values using dog cortical slices were obtained at pH 7.4.

3. Effect of Acetate and Potassium

The results obtained upon the addition of sodium acetate to the incubation medium or the alteration of the potassium concentration of the medium are shown in Figure 3. Accumulation of PAH by renal cortical slices from rabbits at all ages studied (2 and 4 weeks, and adults) was significantly enhanced in the presence of 10^{-2} M sodium acetate, but the effect was most pronounced in adults. Doubling the concentration of potassium in the medium did not have a significant stimulatory effect on the PAH S/M ratio. PAH accumulation was markedly reduced when potassium was deleted from the medium.

Development of PAH and NMN Accumulation by Renal Cortical Slices

1. Rabbits and Rats

The pattern of development of organic acid transport was determined in young rabbits ranging in age from 1 day to 8 weeks, and in adults (Figure 4). The PAH S/M ratio increased from 2.70 $\pm$ .13 at 1 day of age to a value of 4.30 $\pm$ .46 at 2 weeks. After this age the PAH S/M ratio increased rapidly, reaching a peak value of 14.70 $\pm$ .95 at 4 weeks. The PAH S/M values then gradually declined to adult levels. In contrast, body weight continued to increase rapidly beyond 4 weeks until adult levels were reached. Kidney weight increased in a similar manner to that of

body weight (Figure 5). NMN accumulation by renal cortical slices did not increase with age; instead it was slightly higher at 1 week of age (2.40 ± .20) than at 4 weeks (1.96 ± .30) or in adults (1.56 ± .11). Although the magnitude of PAH accumulation was significantly greater in slices from 4 week rabbits, the incubation time required to achieve the maximum PAH S/M ratio was the same for 2 week, 4 week and adult animals, that is, 60 to 90 minutes (Figure 6).

The total water content of renal cortical slices from 4 week rabbits was significantly less than in slices from 1 or 2 week-old rabbits, but was similar to the values obtained in 6 week-old and adult animals (Figure 7).

The relative maturity of rats at birth is illustrated by the development curve for PAH and NMN accumulation by rat renal cortical slices (Figure 8). By 8 and 12 days of age for example, the PAH S/M ratios reached values of 5.51 ± .80 and 5.76 ± .42 respectively, while the values for NMN were 5.01 ± .62 and 5.94 ± .11 respectively. The PAH and NMN S/M ratios thus were relatively high in young rats and then gradually increased to adult levels. Body weight and kidney weight increased rapidly from 2 to 20 days of age. The PAH S/M ratios obtained for slices from nursing rats (at 20 days) and adult rats were not statistically different at 90 minutes, again indicating the rapid development of PAH transport and kidney function in rats (Table 3).

2. Dogs and Cats

The developmental curve for PAH accumulation by renal cortical slices from dogs was similar to that in rabbits (Figure 9). The PAH S/M ratios

in puppies increased from 4.28 ± .42 at 1 week to 8.82 ± 1.48 at 4 weeks. At 4 weeks of age the S/M ratio appeared to be considerably greater than that seen in any of the older animals, but by 5 weeks the PAH S/M ratio had fallen to the adult level. Due to the marked variation among animals from different litters however, the only statistically significant difference was that seen between puppies 1 and 4 weeks of age.

The relationship between incubation time and PAH and NMN accumulation by renal cortical slices was also examined in 1 week and adult cats (Figure 10). The S/M ratios for PAH and NMN after 90 minutes of incubation in slices from 1 week-old cats (3.67 ± .75 and 1.66 ± .07, respectively) were lower than those from adult animals (6.87 ± .64 and 1.92 ± .28, respectively).

3. Species Comparison of One Week and Adult Animals

Accumulation of PAH by renal cortical slices from 1 week-old dogs, cats, rabbits and guinea pigs was compared to PAH accumulation by slices from adult animals of these species (Figure 11). The low PAH S/M ratios in dogs, cats and rabbits at 1 week relative to the adult values demonstrates the immaturity of the kidneys and the organic acid transport system in young animals of these species. In contrast, the PAH S/M ratios in slices from 1 week-old guinea pigs were higher than adult values.

Substrate Stimulation of PAH Transport

1. By PAH

Treatment of 1 week-old rabbits with 100 mg/kg PAH intraperitoneally, twice daily for 7 days, produced a significant increase in the PAH S/M ratio

(Table 4). However, NMN accumulation measured concomitantly in some of these experiments was not significantly increased. Administration of PAH to nursing rats for 3 or 7 days at either 100 or 200 mg/kg twice daily resulted in stimulation of PAH accumulation by slices from treated animals (Table 5). This stimulation also was specific for organic acid transport, since NMN accumulation was not increased by any of the dosage regimens of PAH

2. By Penicillin in Rabbits

Direct addition of penicillin to incubation beakers significantly depressed the PAH S/M ratio (Table 6). PAH accumulation by renal cortical slices was significantly enhanced after treatment of 2 week-old rabbits with penicillin (60,000 IU, subcutaneously, twice daily for 3 days), as shown by the increase in the PAH S/M ratio from a control value of 4.2 $\pm$ .55 to the treated value of 10.8 $\pm$ .91 (Figure 12). Although penicillin administration produced a significant effect in 2 week-old rabbits, no effect was observed after similar treatment of 4 week-old rabbits. Administration of 120,000 IU penicillin twice daily for 3 days to 6 week-old rabbits had no effect on PAH accumulation by renal cortical slices (Table 7). PAH accumulation by renal cortical slices was determined in kidneys from fetuses obtained by caesarean section on day 31 of gestation after treating the pregnant female with penicillin during the last half of pregnancy (60,000 IU, twice daily). PAH S/M ratios in slices from penicillin-treated fetuses were significantly higher than the values obtained in controls (7.82 $\pm$ .34 vs. 4.97 $\pm$ .14, Table 8). When pregnant

female rabbits were treated with penicillin or saline during the last half of pregnancy and PAH S/M ratios measured in renal cortical slices from the offspring at ages ranging from 1 day to 4 weeks, a stimulatory effect by penicillin was also noted (Figure 13). The PAH S/M ratios in slices from rabbits whose mothers received penicillin were significantly greater than the values obtained from control rabbits (whose mothers received saline) at 1 day, 1 week and 2 weeks of age.

3. By Penicillin in Nursing Rats

Treatment of nursing rats for 3 or 7 days with penicillin (30,000 IU, subcutaneously, twice daily) significantly enhanced PAH accumulation by renal cortical slices (Figure 14). However, NMN accumulation measured concomitantly in some of these animals was not significantly increased (Table 9). In most experiments, the kidney weight : body weight ratio was significantly increased by penicillin treatment (Table 10), although this was not always the case (Table 9). When the kidney weight : body weight ratio was not increased to a significant extent, the body weight of the rats was usually less than the average of 50 g shown earlier in Figure 8. Penicillin administration to nursing rats resulted in a significant enhancement in PAH accumulation, but did not increase accumulation of TEA (Figure 15).

4. By Triiodothyronine (T_3)

Administration of 500 µg/kg of T_3 to weanling rats for 3 or 7 days significantly enhanced the ability of renal cortical slices from these animals to accumulate PAH (Figure 16). Accumulation of NMN, however, was not changed. A lower dose of T_3 (200 µg/kg) stimulated PAH accumulation

only when given for 7 days. Kidneys from T_3-treated weanling rats weighed 80 - 100 mg more than those from control animals. When expressed as a percentage of body weight, the increase in kidney weight became even more apparent (Figure 17) since the T_3-treated rats weighed less than control animals at the time of sacrifice. Treatment of adult rats with 500 µg/kg T_3 for 3 days did not significantly alter either PAH or NMN uptake (Figure 18). When added to renal cortical slices in vitro in concentrations of 1×10^{-6} M or 1×10^{-8} M, T_3 significantly inhibited PAH accumulation (Figure 19). NMN accumulation, measured in the same experiments, was not changed.

Studies on Possible Mechanisms Associated With Substrate Stimulation by Penicillin

1. Effect of penicillin on renal cortical tissue

The addition of sodium acetate to incubation beakers had a similar stimulatory effect on renal cortical slices from both penicillin-treated and control animals (Figure 20). When 10^{-2} M acetate was present in the incubation medium, the control PAH S/M ratios increased from 10.74 ± 1.00 to 15.56 ± 1.81. PAH S/M ratios in renal cortical slices from penicillin-treated rats were increased from 12.57 ± 0.92 to 18.88 ± 2.20 in the presence of 10^{-2} M acetate.

The maximum rate of uptake of PAH by renal cortical slices from penicillin-treated and control nursing rats is demonstrated in Figure 21. Regression analysis indicated that the uptake curves from treated and control animals were linear between 4 and 15 minutes. Although penicillin significantly enhanced PAH accumulation as indicated by the upper curve,

the slopes of the calculated regression lines (not drawn) were not significantly different. The equations for the lines using slices from treated and control animals were: $y = 3.18x + 21.78$; and $y = 2.51x + 17.69$, respectively.

Penicillin treatment of nursing rats did not significantly alter the total water content or the extracellular water content of renal cortical slices (Table 11).

2. Effect of Penicillin on Renal Protein and RNA Synthesis

a. Increase in kidney weight

In addition to stimulating PAH accumulation by renal cortical slices, penicillin treatment of nursing rats consistently produced an increase in kidney weight. When factored by body weight, the resulting kidney weight : body weight ratio was significantly greater in penicillin-treated rats (Table 10).

Ammonium chloride produced an increase in kidney weight, and when factored by body weight, a significant increase in the kidney weight : body weight ratio was also obtained ($1.04 \pm .02$ vs. $1.20 \pm .03$, Table 12). The increase in the kidney weight : body weight ratio produced by ammonium chloride was greater than that produced by penicillin. Ammonium chloride however, did not stimulate PAH or NMN accumulation by renal cortical slices.

b. Inhibitory effect of cycloheximide

Concomitant administration of 0.1 mg/kg cycloheximide prevented the increase in PAH accumulation produced by penicillin (Figure 22). Cycloheximide treatment alone however, also significantly depressed the PAH S/M ratio.

c. Incorporation of leucine and glutamine by kidney slices

Renal cortical slices from penicillin-treated nursing rats exhibited a significant increase in both C^{14}-leucine incorporation and PAH accumulation (Figure 23) when C^{14}-leucine was added to incubation beakers containing kidney slices and PAH medium. In another series of experiments, the increased incorporation of C^{14}-leucine was shown to be specific to the cortex since it was not observed in medullary slices from the same kidneys (Figure 24). Experiments using nonradioactive leucine showed that this amino acid did not interfere with the uptake of PAH (Table 13). Interestingly, at the higher concentrations, leucine appeared to enhance PAH accumulation. The effect of penicillin was not specific for leucine since the incorporation of C^{14}-glutamine was also significantly increased in renal cortical slices from penicillin-treated rats (Table 14).

d. Protein content of subcellular fractions

The protein content of the 9,000 g precipitate, the 105,000 g precipitate, and the 105,000 g supernatant fractions obtained after homogenizing and centrifuging whole kidneys was determined after treating rats with penicillin or saline (Table 15). The 105,000 g precipitate fraction was the only fraction in which a statistically significant increase in protein content was obtained.

e. Incorporation of orotic acid into RNA

When penicillin-treated and control nursing rats were injected with C^{14}-orotic acid and killed 4 hours later, renal cortical tissue from treated animals contained a significantly greater quantity of label than tissue from

controls (Figure 25). The increased incorporation of C^{14}-orotic acid by kidneys of treated animals was correlated with a significant increase in the PAH S/M ratio. No difference between treated and control values was observed however, when the orotic acid incorporation into RNA in subcellular fractions was factored by the protein content of these fractions (Figure 26).

3. Effect of Penicillin on Renal Histological Development

Penicillin treatment (60,000 IU, subcutaneously) of 11 day-old rabbits twice daily for 3 days resulted in a significant increase in PAH accumulation when measured 24 hours after stopping drug treatment (Figure 27). In other rabbits from the same litters, killed 8 days after penicillin administration was discontinued, there no longer was any difference in PAH accumulation by slices from treated or control animals. The PAH S/M ratio from the 2 week-old treated rabbits was significantly greater than the PAH S/M values obtained in either treated or control rabbits at 3 weeks of age. The PAH S/M ratios observed at 3 weeks of age after penicillin or saline treatment from day 11 - 13 are the same as the values shown on the development curve in Figure 4.

Histological studies indicated that there were no apparent differences, as observed using light microscopy, between kidneys from 2 week-old penicillin-treated or control rabbits (Figure 28). Cortical tubules of kidneys from both treated and control rabbits were small, with crowded nuclei, limited amounts of cytoplasm and inconspicuous tubular lumens. The presence of a nephrogenic zone in the outer cortex was exemplified by the presence of histologically undifferentiated tubular cells. Kidneys from

2 week-old rabbits were also characterized by the presence of small cellular glomeruli with prominent peripheral nuclei. Normal histological development could be detected since distinct differences between kidney sections from 2 and 4 week rabbits, and adults, could readily be observed. Renal cortical tubules were approaching adult size by 4 weeks of age. Rapid kidney growth between 2 and 4 weeks of age was indicated by the increased cell size and increased distance of glomeruli from the cortical surface. The age-related increase in kidney size is evident since all histological sections were photographed at the same magnification. Although normal development of rabbit kidneys could readily be demonstrated both physiologically (Figure 4) and histologically (Figure 28), no histological changes could be observed when physiological development was accelerated by penicillin.

Figure 29 summarizes some of the observations made earlier regarding the development of PAH transport in rabbits and the results obtained after treating rabbits with penicillin. The normal developmental pattern shown in Figure 4, with the addition of the values obtained from fetal kidney slices shown in Table 8, is indicated by the solid line. PAH S/M values, which were $4.97 \pm .14$ in fetal renal cortical slices, decreased to $2.60 \pm .13$ in slices from 1 day-old rabbits and then increased to a maximum value of $14.7 \pm .95$ at 4 weeks. Penicillin treatment of pregnant rabbits during the last half of pregnancy stimulated PAH accumulation by fetal renal cortical slices, as well as by slices from the offspring at ages ranging from 1 day to 2 weeks, but not 4 weeks of age. Treatment of 11 day-old

rabbits with penicillin for 3 days significantly enhanced PAH accumulation by renal cortical slices 24 hours after stopping treatment, but this effect was no longer present when measured in littermates 8 days after discontinuing treatment. Although not shown on Figure 29, treatment of 4 and 6 week-old rabbits with penicillin had no stimulatory effect on PAH accumulation by renal cortical slices.

DISCUSSION

Accumulation of both PAH and NMN by rabbit renal cortical slices was shown to involve active transport since incubation under nitrogen or in the presence of DNP reduced S/M ratios to values that could be attained merely by diffusion (Figure 2, Table 1). This is in agreement with the observations of Nechay and Pardee (1965) who demonstrated that the transport of NMN required the utilization of energy provided by ATP. The ability of TEA to inhibit NMN accumulation in a dose dependent manner indicates that these two organic bases are competing for common carrier transport sites. Similar observations have been made with PAH to indicate that different organic acids compete with PAH for transport. The observation that TEA had no effect on PAH accumulation (Figure 2) provides further evidence that the organic acid and organic base transport systems are distinct.

The studies involving determination of the pH optimum for PAH accumulation by rabbit, rat and dog renal cortical slices confirm the earlier observations made by Copenhaver and Davis (1965) in the rabbit and Ross et al. (1968b) in the dog. The pH optimum in rabbits is about 8 and in dogs it is 7.4 (Table 2), which are the values obtained by the above investigators in separate studies. In addition, the results presented in Table 2 indicate that the pH optimum for PAH accumulation by rat renal cortical slices is 8.0. The different pH optimum in dogs compared to that in rabbits and rats suggests some physical differences between the transport enzymes in these species. Since the normal plasma pH is about 7.4, all other experiments in this investigation were done at this pH.

Taggart et al. (1953) found that kidney slices lose their ability to accumulate PAH in the absence of potassium. They suggested that this may simply reflect the need for potassium in the maintenance of cell integrity. Foulkes and Miller (1960) demonstrated that the addition of potassium to potassium-deficient slices stimulated the uptake of PAH before a significant increase in the intracellular potassium concentration could be observed, suggesting a specific role for potassium at the cell membrane. PAH efflux from potassium-deficient slices was greater than from normal slices, indicating to Foulkes and Miller (1960) that potassium is also required at the intracellular PAH-concentrating mechanism. Thus, these investigators concluded that, in addition to maintaining a normal intracellular environment, potassium plays a specific role in the mechanism involving PAH accumulation by renal cortical slices. The results obtained in the present study (Figure 3) do not provide further information on the specific role of potassium in PAH accumulation, but they do demonstrate that renal cortical slices from immature and adult rabbits possess similar requirements for potassium. When potassium was deleted from the incubation medium, a significant decrease in the PAH S/M ratio was obtained using renal cortical slices from adult rabbits. Potassium was still present in the renal cortical slices, however, so that some PAH accumulation did occur. The potassium concentration (4OmM/l) of the Cross and Taggart incubation medium was near optimal in all cases, since doubling this concentration had only a small stimulatory effect at the three ages (2 week, 4 week, and adult) studied.

The specific mechanism by which acetate stimulates PAH transport *in vivo* and *in vitro* is not established. The hypothesis by Cross and Taggart (1950) that acetate enters the Kreb's cycle and thereby increases energy utilization has neither been confirmed nor disproven. The proposed mechanism is very general and thus far it has not been established that all renal metabolic activities are enhanced by acetate. Schachter *et al.* (1955) indicated that the effect of acetate on PAH transport may be due to the ability of acetate to combine with acylglycines, thereby removing the inhibitory effect of these compounds on PAH transport. According to this theory, endogenous long chain acylglycines inhibit PAH uptake by renal cortical slices but acetylglycine does not. The stimulatory effect of acetate, and lactate which is oxidized to acetate, is said to result from the formation of acetylglycine, thereby decreasing the availabilty of glycine and the consequent synthesis of inhibitory acylglycines. The hypothesis of Schachter et al. (1955) has been criticized by Cohen and Randall (1964) who found that Tm_{PAH} in dogs infused with acetate or lactate remained unchanged or increased at a time when lactate uptake was decreasing. Murdaugh and Elliott (1969) found that excess glycine did not inhibit acetate stimulation of PAH uptake, contrary to what would be expected. The hypothesis by Schachter *et al.* (1955) warrants further investigation, however. Determination of the developmental pattern of the enzyme glycine acetylase in newborn rats and rabbits may provide some indication of the validity of Schachter's proposal. The stimulatory effect of acetate is greater in adult than in immature rabbits (Figure 3). It is not known if this reflects an increased inhibitory effect of the acylglycines in adult animals (which can be removed by acetate) or an increased ability to utilize acetate in energy-requiring reactions.

In their developmental studies, Rennick et al. (1961) observed a correlation between functional and histological maturity in slices of puppy renal cortex. Slices from the inner cortex developed higher PAH S/M ratios and were histologically more mature than slices from the surface of the cortex. The availability of cortical tissue in young rabbits and rats did not permit a comparison of surface and subsurface cortical slices in the present study. Thin slices of all available cortical tissue from kidneys of animals at various ages were prepared and added to incubation beakers, so that the data represent the average capacity of the entire cortex to accumulate PAH.

Renal cortical slices from rabbits and dogs show similar developmental patterns of PAH accumulation (Figure 4, Figure 9), and are qualitatively similar to the values reported by Rennick et al. (1961). In both species, PAH accumulation by renal cortical slices is low at birth and increases to maximum values at 4 weeks, after which the values decline to adult levels. New et al. (1959) measured PAH accumulation by renal cortical slices from rabbits of ages 0 to 9 and 10 to 22 days as well as adults, and obtained values similar to those reported here. These investigators did not measure PAH accumulation by renal cortical slices from rabbits 3 to 4 weeks of age and thus failed to realize that the PAH S/M ratios did not merely increase to adult values.

There are several possible explanations for the abrupt increase and subsequent decline in the PAH S/M ratio at 4 weeks. New et al. (1959) indicated that the difference in PAH accumulation in slices from newborn and adult rabbits was not due to differences in tissue water content.

The data presented here indicate that there was no significant difference in the water content of renal cortical slices obtained from 4 week-old rabbits compared to those obtained from adults, although the value at 4 weeks was significantly less than those observed at 1 or 2 weeks of age (Figure 7). Forrest and Stanier (1964) observed similar changes, showing a significant decrease in water content of renal cortical slices from birth to 3 weeks of age followed by a small rise in adult tissue. Since the S/M ratio is calculated using the slice wet weight, the increase in PAH S/M ratio observed at 4 weeks may be partly due to decreased water content, but the magnitude of the increased S/M ratios at this age suggests that other factors are also involved. Nutritional and hormonal influences (Freedland et al., 1962), and the dietary changes associated with weaning (Wacker et al., 1961) have measurable effects on enzyme activity. A protein-rich diet has been shown to enhance the PAH transport maximum in newborn humans (Calcagno and Lowe, 1963). These factors may be of significance in rabbits at 4 weeks of age when they are beginning to ingest solid foods as well as milk. The observation that the time course for PAH accumulation by renal cortical slices was similar in rabbits of different ages (Figure 6) demonstrates that the comparisons of PAH S/M ratios made at these ages are valid. The appropriateness of such comparisons is supported by the observations of Webber (1970) who found that the overall pattern of amino acid uptake by mature and newborn rat renal cortical tissue was not greatly influenced by size of the tissue slices used.

Newborn rat kidney cortical tissue has a higher concentrating capacity for amino acids than mature tissue (Webber and Cairns, 1968) but the efflux rate is greater in mature cortical tissue (Webber, 1968).

It is possible that the mechanisms responsible for organic acid entry into renal tubular cells are fully developed at 4 weeks of age in rabbits (and dogs), but the mechanisms responsible for efflux develop after this time.

Studies of enzyme development in rat brain indicate that the rate of enzyme development can be influenced by substrate concentration (Nachmias, 1960), and the same principle may be true in the kidney. The levels of organic acids in the blood may increase with age, and the accompanying age-related increase in general kidney and renal tubular mass would then enable the kidney to transport greater quantities of organic acids. Alternatively, increasing substrate levels of organic acids may stimulate activity of the renal tubular transport system as well as stimulate renal growth. As substrate stimulation continued, the kidney eventually would be able to increase organic acid excretion only by increasing proximal tubule mass rather than by stimulating both activity of the organic acid transport system and tissue mass. This hypothesis would be consistent with the observation that kidney weight continues to increase beyond 4 weeks of age (Figure 5) while the PAH S/M ratio declines to adult levels after this time (Figure 4). In a study comparing development of renal cortical cellular function (estimated by determining the PAH S/M ratio) and the development of apparent functional kidney mass (estimated by determining the *in vivo* PAH transport maximum) in dogs, Hook *et al.* (1970) found that these parameters increased with age at different rates. Accumulation of PAH by dog renal cortical slices developed as shown in Figure 9, with a peak at 4 weeks. In contrast, both the PAH transport maximum and PAH clearance were extremely low in puppies at 1 week of age and continually

increased with age until adult levels were reached. A direct correlation between the maximum transport capacity and kidney weight was thus obtained. This supports the suggestion made above that the kidney may increase its capability to excrete organic acids by increasing kidney mass.

Goldberg et al. (1970) demonstrated that oral administration of neomycin prevented the increase in PAH S/M ratio observed after unilateral nephrectomy, but had no effect on the associated increase in kidney weight. Neomycin was thought to prevent the increase in organic acid levels in the blood and the ensuing increase in PAH accumulation by inhibiting intestinal bacteria responsible for the synthesis of most of the organic acids appearing in the urine. Goldberg et al. (1970) suggested that neomycin could therefore be used to distinguish between mechanisms responsible for renal hypertrophy and increased organic acid transport. This technique could also be used in young rabbits to ascertain the importance of increasing substrate levels in stimulating the rapid increase in PAH S/M ratios observed between 2 and 4 weeks of age.

Rat kidneys are relatively more mature shortly after birth than are rabbit kidneys, and are fully developed histologically by about 21 days of age (Baxter and Yoffee, 1948). Adult patterns of a number of enzymes are found at 14 to 16 days after birth (Wachstein and Bradshaw, 1965). There is only a gradual increase in the PAH S/M ratio with age in rats (Figure 8). This suggests that substrate levels of organic acids increase with age in a different pattern than occurs in rabbits, resulting in a different developmental pattern for the PAH S/M ratios. In contrast to the results obtained in rabbits, NMN accumulation increases with age in rats, indicating that the availability of substrates for the organic

base transport system also increases with age. Rat kidney weight and body weight increase with age in a similar fashion to that observed in rabbits and dogs.

Although PAH accumulation by cat renal cortical slices was measured at only 2 ages (1 week and adult), the values obtained (Figure 10) were qualitatively and quantitatively similar to those obtained in rabbits and dogs at these ages. The PAH S/M values obtained in 1 week-old and adult guinea pigs (Figure 11) are different from those obtained in the other species studied. The guinea pig has a long gestation period and is self-sufficient at birth (Zorzoli et al., 1969); in addition the guinea pig kidney is histologically mature at birth (Wachstein and Bradshaw, 1965). Zorzoli (1968) demonstrated that renal phosphoenolpyruvate carboxykinase activity and glucose synthesis from oxaloacetate or succinate were greater in newborn guinea pigs than in adults. Zorzoli et al. (1969) further suggested that in the guinea pig the development of gluconeogenesis starts in utero. The high PAH S/M values observed in 1 week-old guinea pigs (Figure 11) suggests that PAH transport is also well developed in utero.

The suggestion that substrate concentration appears to be important in determining the developmental pattern of PAH accumulation is supported by the results obtained after treating young rats and rabbits with PAH and penicillin. The prolonged presence of these compounds in young animals stimulated the ability of renal cortical slices from rats (Table 5, Figure 14) and rabbits (Table 4, Figure 12) to accumulate PAH. The results suggest that the increased presence of organic acids in the kidney tubular cells during development stimulates maturation or activity of

developing transport sites. The specificity of action also suggests that substrate-induced stimulation is involved. Although PAH and penicillin increased PAH accumulation by renal cortical slices from treated animals, these compounds had no significant effect on NMN accumulation. Transport of organic acids and bases involves different mechanisms (Farah et al., 1959; Rennick et al., 1961) and the lack of stimulation of NMN accumulation by PAH and penicillin indicates that the compounds required to stimulate these systems are different as well. Since penicillin was available as a suspension (procaine penicillin G) and had a longer plasma half-life than PAH, it was used for most studies involving substrate stimulation. Procaine is an organic base and had no effect on PAH transport so that control animals were injected with saline. High S/M ratios are obtained for TEA due to the high affinity of this compound for the base transport system (McIsaac, 1969). It was reasoned that it may be easier to stimulate TEA then NMN transport if penicillin were acting as a nonspecific stimulus to organic ion transport. Although PAH accumulation was significantly increased, TEA S/M ratios were not changed after penicillin treatment of nursing rats (Figure 15), providing further evidence for the specificity of penicillin.

The inability of PAH and penicillin to stimulate NMN or TEA transport may be related to lower substrate levels of organic bases in rats and rabbits. Organic acid transport in the kidney is an important mechanism of homeostasis, and it is a means of excretion of potentially toxic by-products of metabolism (Pitts, 1968). Selleck and Cohen (1965) suggested that the primary function of the PAH transport system is to move specific metabolites of intermediary metabolism including nonesterified fatty acids,

citrate and α-ketoglutarate to sites of dissimilation in the liver and kidney. According to Goldberg et al. (1970) most of the organic acids found in the urine derive from bacterial metabolism in the gut, and include hippuric acids (Asatoor, 1965) and indolic compounds (Milne et al., 1960). While a small portion of hippuric acid is produced endogenously from phenylalanine, the major portion in urine is derived from dietary precursors (Armstrong et al., 1955). Intestinal bacteria play an important role in the production of hippuric acid from dietary precursors by synthesizing aromatic organic acids from non-aromatic precursors (Asatoor, 1965). Benzoic acid and sodium benzoate are two aromatic precursors of hippurates that are present in food. These observations all indicate the relative importance of organic acid transport in normal body function, and it is possible that this system may readily adapt to changes in substrate concentration. Regulatory mechanisms are thought to be at least partially functional in the young, enabling the kidney to respond to alterations in body composition (Edelmann, 1967). This suggests that normal maturation of the organic acid transport system could be correlated with increases in substrate concentration. The response of the organic acid transport system to substrates such as PAH and penicillin demonstrates that this system is capable of responding to changes in organic acid concentration. The importance of the organic base transport system is less apparent, although a number of constituents of plasma and urine are secreted by this system (Peters, 1960). Naturally occurring compounds transported by the organic base system include guanidine, piperidine, NMN, thiamine and choline (Peters, 1960). The organic base secretory mechanism may exist to secrete some unknown substance which, because of high toxicity, must be maintained

in very low concentration (Pitts, 1968). The lesser physiological importance of the cationic transport system, together with the low substrate levels, may be associated with the decreased ability of this system to be stimulated by exogenous or endogenous substrates.

The developing organism is particularly sensitive to drugs (Done, 1966). Significant plasma concentrations of penicillin are maintained for long periods of time in the fetus when this drug is administered to the pregnant female (Moya, 1965). When penicillin was administered to pregnant rabbits during the last half of gestation, PAH accumulation by renal cortical slices from 31 day-old treated fetuses (Table 8), or offspring at ages ranging from 1 day to 2 weeks (Figure 13), was significantly enhanced. These results suggest that the presence of penicillin in the fetus stimulates development of the tubular secretory processes associated with organic acid transport in the fetal and newborn rabbit. Gersh (1937) also concluded that fetal renal tubules could respond to the presence of substrates since he found that the ability of fetal rabbit renal tubules to excrete phenol red was accompanied by structural differentiation. It is possible that the duration of penicillin treatment could be less than the 15 days used in the present study, particularly if the fetal renal tubules become more responsive to substrate concentrations as they mature near term. Enhanced enzyme activity in newborn animals after treatment of the pregnant female has been observed with other systems as well. For example, Pantuck et al. (1968) found that treatment of pregnant rats and rabbits with phenobarbital during the last few days of pregnancy stimulated

the activity of hepatic enzymes that metabolize pentobarbital and meperidine in the newborn.

Thyroxine administration to rats causes an increase in oxygen consumption by kidney slices (Brophy and McEachern, 1949; Pittman et al., 1961) as well as stimulating renal protein synthesis (Michels et al., 1963). Furthermore, thyroidectomy depresses PAH accumulation by rat kidney slices (Farah et al., 1956). The observations that thyroid hormone is important in the maintenance of cellular metabolism (Hamburgh, 1968) and can induce protein synthesis in other tissues (Brodie et al., 1966) further suggested that it may act as a non-specific stimulus to both organic acid and organic base transport when administered to young animals. The results obtained however, indicate that the stimulatory effect of T_3 on the kidney is specific for organic acid transport. The amount of PAH taken up per gram of slice was markedly increased in treated animals while NMN accumulation was not changed (Figure 16). Due to the increase in kidney weight produced by T_3 (Figure 17) the apparent stimulation of renal transport would be even greater if the results were expressed as the amount of PAH taken up per kidney. This suggests that T_3 caused a marked increase in the activity of the specific enzymes responsible for organic acid transport, or that it specifically stimulated the synthesis of new transport enzymes. The ability of T_3 to stimulate PAH transport in weanling but not adult rats also suggests that T_3 stimulated the formation of new organic acid transport sites. Cell division and growth occur more rapidly in young animals and may therefore be easier to stimulate than in adult animals where these processes are occurring at a slower rate. Fouts and Hart (1965) have demonstrated that the hepatic enzymes responsible for the metabolism of

hexobarbital can be activated in the newborn, or their activity can be increased in the adult by the administration of enzyme inducers such as phenobarbital. It is possible that the renal enzymes responsible for organic acid transport can be activated in the newborn or young rat, but once activity is established regulators may act so that transport enzyme activity cannot be further increased in adult animals. Huang and Knoefel (1957) reported that various halogenated tyrosine derivatives are secreted in the kidney and depress Tm_{PAH}, indicating that these compounds are transported by the same system as PAH. In addition, Nepomuceno and Little (1964) suggested that there may be substrate competition for the organic acid transport system between PAH and iodothyronine compounds. The results obtained in the present investigation (Figure 19) also suggest that T_3 is transported as an organic acid since the addition of T_3 *in vitro* inhibited PAH transport but had no effect on NMN accumulation. The results are thus consistent with the hypothesis that T_3 stimulated PAH transport by a mechanism involving substrate stimulation.

A single injection of folic acid causes an increase in kidney weight as well as DNA and RNA synthesis (Threlfall *et al.*, 1966; Taylor *et al.*, 1968). The increase in DNA content and the associated increase in kidney mass after folic acid administration is due to hyperplasia and hypertrophy of the renal tubules (Taylor *et al.*, 1966). The increased renal growth produced by folic acid was also associated with an increased capacity of renal cortical slices from weanling rats to accumulate PAH (Figure 30), but NMN accumulation was not increased in these experiments (Hirsch and Hook, 1969). Since Goresky *et al.* (1963) were unable to

demonstrate secretion of folic acid in dogs, it is possible that folic acid may not be a substrate for the organic acid transport system and that substrate stimulation was not the mechanism involved in the enhancement of the PAH S/M ratio. Taylor et al. (1968) suggested that the renal hypertrophy and hyperplasia produced by folic acid is the result of partial tubular blockage caused by precipitation of folic acid within the tubules. Goresky et al. (1963) also observed concentration and storage of folic acid within the renal tubular cells. Taylor et al. (1968) indicated that the effect of partial tubular blockage is to markedly increase the functional load on the remaining intact tubules, thereby initiating renal tubular hypertrophy and hyperplasia. Thus, although folic acid may not be a substrate for the organic acid transport mechanism, it may indirectly cause specific substrate stimulation of this system by increasing the substrate levels presented to the functioning renal tubules. The lack of concomitant stimulation of the organic base transport system may be due to the possibility that there is insufficient substrate accumulation, even after blocking many of the renal tubules, to elicit substrate stimulation of this system. Liegler et al. (1969) demonstrated that a close structural analogue of folic acid, methotrexate, is secreted in vivo by proximal tubular cells. These investigators suggested that bidirectional transport of methotrexate, involving both secretion and reabsorption, may be occurring. Since only a single injection of folic acid is necessary to stimulate renal growth (Taylor et al., 1968; Baserga et al., 1968) or PAH accumulation by renal cortical slices (Figure 30), it is possible that both secretion and reabsorption of folic acid also occurs. This could

explain both the prolonged action of a single folic acid injection on renal growth and its stimulatory effect on the organic acid transport system.

Phenobarbital administration to 2 week-old rabbits enhanced the capability of renal cortical slices from these animals to accumulate PAH (Figure 31). This effect was not observed after treatment of nursing rats however, even at higher doses. Barbiturates, at plasma concentrations obtained during surgical anesthesia, inhibit PAH uptake (Storen, 1958) and depress oxygen consumption (White, 1957; Despopoulos, 1961) by renal cortical slices. When added in vitro, phenobarbital and a number of other barbiturates have no significant influence on TEA uptake although they inhibit PAH accumulation (Despopoulos, 1961). Inhibition of renal transport can be achieved either by interference with the generation and utilization of cellular energy for transport processes (Cross and Taggart, 1950) or by displacement of the transported substrate from an interaction with its cellular receptor (Despopoulos, 1961). In the latter case, the inhibitor is presumed to react with the same cellular receptor as does the substrate, but transport of the inhibitor is not essential to its action (Beyer, 1950). The renal excretion of barbital and phenobarbital can be accounted for by glomerular filtration and passive diffusion across the renal tubular epithelium (Waddell and Butler, 1957), so that displacement of PAH from its receptor by barbiturates does not appear to involve competition for transport between PAH and inhibitor (Despopoulos, 1961). Uehleke and Greim (1968) demonstrated that phenobarbital treatment significantly increased the concentration of cytochromes and

oxidative drug metabolizing activity in rabbit kidney microsomes, but had no effect on rat kidney microsomes. This may be related to the observation that phenobarbital treatment of young rabbits, but not rats, resulted in an enhanced ability of renal cortical slices to accumulate PAH (Figure 31). If phenobarbital is acting as suggested by Despopoulos (1961) rather than as a substrate of the organic acid transport system, it may be acting by one of the mechanisms postulated earlier for folic acid. Thus phenobarbital may be blocking the secretion of endogenous organic acids, thereby increasing the plasma levels of these substrates. This may explain why phenobarbital can stimulate PAH accumulation by rabbit cortical slices but not in rats where stimulation of PAH transport is somewhat more difficult. It is also possible that both secretion and reabsorption of phenobarbital could be occurring, resulting in stimulation of the organic acid secretory system and a long plasma half-life for phenobarbital. In any case, it appears that phenobarbital is not acting as a "general" inducer of renal transport enzymes, since NMN and TEA accumulation was not changed after phenobarbital administration to rats and rabbits (Figure 31).

The enhanced PAH S/M ratios observed after penicillin treatment could be associated with: (1) altered activity of the renal tubular organic acid transport system; (2) general hypertrophy of the total kidney mass; (3) specific hypertrophy of the proximal tubules associated with the synthesis of new proteins responsible for organic acid transport; or (4) enhanced development of existing tubular transport processes. Altered activity of the transport system may play some role, but the use of short

incubation times demonstrated that the maximum rate of PAH uptake into renal cortical slices was not increased by penicillin. When PAH accumulation by renal cortical slices from penicillin-treated and control nursing rats was compared over short incubation times, the values from treated rats were significantly increased (Figure 21). Nevertheless, the slopes of the calculated regression lines were not significantly different, suggesting that penicillin enhanced the maximum accumulation of PAH but had no effect on the rate of this accumulation. Acetate, on the other hand, has been shown to increase the slope of the uptake curve (Ross and Farah, 1966).

A greater stimulatory effect of acetate on PAH accumulation by renal cortical slices may be expected in slices from penicillin-treated rats if both penicillin and acetate acted on the same process in the PAH transport mechanism. The observation that acetate had a similar stimulatory effect on PAH S/M ratios in renal cortical slices from control and penicillin-treated rats (Figure 20) suggests however, that these compounds enhance PAH transport by different mechanisms. The different effects of acetate and penicillin on the rate of PAH accumulation by renal cortical slices, and the ability of acetate to stimulate PAH transport in mature animals both in vivo (Mudge and Taggart, 1950) and in vitro (Figure 3) support this suggestion. Nevertheless, it is also possible that acetate has its major action on some mechanism distinct from that altered by penicillin while also having a lesser action complementing that of penicillin.

The inability of ammonium chloride to enhance PAH accumulation by renal cortical slices, after producing an increase in kidney weight and a significant increase in the kidney weight : body weight ratio (Table 12),

suggests that the stimulatory effect of penicillin on PAH accumulation was not secondary to a general increase in renal cortical mass. The lack of stimulation of organic base accumulation by penicillin also suggests that a general increase in metabolic activity or renal growth is not the mechanism involved. The specificity of the response to penicillin indicates that the increased PAH S/M ratio is due to specific changes within proximal tubular cells. In addition, the water content and distribution in renal cortical slices was not changed after penicillin treatment of rats (Table 11), indicating that the enhancement of PAH accumulation by penicillin was associated with a specific effect.

The increased PAH accumulation produced by penicillin is associated with enhanced protein synthesis. The increase in kidney weight (Table 10) and the ability of cycloheximide to block the stimulatory effect of penicillin (Figure 22) support this statement. In addition to inhibiting the response to penicillin however, cycloheximide also depressed control PAH S/M values. The primary action of cycloheximide is the inhibition of protein synthesis (Ennis and Lubin, 1964; Taber and Vincent, 1969; Young et al., 1963), so that toxicity to young, rapidly growing animals would be anticipated. Preliminary experiments indicated that lower doses of cycloheximide or shorter treatment times than those described earlier did not inhibit the stimulatory effect of penicillin on PAH transport. More definitive results were therefore required to establish that the stimulation of PAH transport produced by penicillin was associated with an increase in protein synthesis. The enhanced incorporation of leucine-C^{14} (Figure 23) and glutamine-C^{14} (Table 14) substantiate the conclusion that increased

protein synthesis was involved. Although the rate of enzyme degradation has not been measured, the increased incorporation of leucine-C^{14} and glutamine-C^{14} by renal cortical slices *in vitro* suggests that increased protein synthesis rather than a decrease in the rate of enzyme degradation is involved. The latter possibility has been suggested by Doyle and Schimke (1969) as a possible mechanism for selectively increasing the concentration of a protein. Increased incorporation of leucine-C^{14} occurred only in renal cortical slices (Figure 24). This is consistent with the suggestion that a correlation exists between increased PAH accumulation and increased protein synthesis in the cortex, since PAH accumulation occurs only in renal cortical slices and not in medullary slices (Cross and Taggart, 1950). Preliminary experiments demonstrated that PAH accumulation did not occur in medullary slices from either penicillin-treated or control nursing rats. Transport systems for amino acids are extremely specific (Wilson and Scriver, 1967) and since leucine did not inhibit PAH accumulation (Table 13), it is unlikely that the increased incorporation of leucine-C^{14} occurred via the organic acid transport system.

After penicillin treatment of nursing rats only the microsomal fraction showed a significant increase in protein content (Table 15). Since the enzymes involved in PAH transport would be expected to be in the membranous fraction, this observation provides suggestive evidence that the synthesis of transport enzymes is stimulated by penicillin. That the protein fraction is involved in transport is supported by the data of Ross *et al.* (1969) who suggested that the renal carrier for NMN is a protein, and by Pardee (1968b) who crystallized a protein involved

in sulfate transport in bacteria. The changes in protein content of the other subcellular fractions observed after penicillin treatment may be due to microsomal contamination, since the techniques used provided only crude cellular fractionation.

The increased incorporation of orotic acid-C^{14} into RNA (Figure 25) in kidneys from penicillin-treated rats apparently can be explained by the increased protein synthesis or functional kidney mass, since no significant incorporation of orotic acid was found after considering the increased protein content in each subcellular fraction (Figure 26). These results are consistent with the observations of Greengard (1963, 1967) who found that substrate-induced stimulation of liver tryptophan pyrrolase was associated with increased protein, but not RNA, synthesis. Greengard (1969) also indicated that an increased rate of synthesis of specific proteins may or may not require concomitant synthesis of RNA. Greengard found that the appearance of enzymes such as tryptophan oxygenase was not inhibited by actinomycin D, suggesting that the necessary RNA species was available for some time before a stimulus triggered the actual accumulation of enzyme. Similarly, Sokoloff *et al.* (1968) found that stimulation of protein synthesis by thyroid hormone was primarily a translational effect. Preliminary experiments in this laboratory indicated that the penicillin-induced enhancement of PAH accumulation by rat renal cortical slices was not blocked by actinomycin D, although it is possible that the low doses used to avoid toxicity were unable to block RNA synthesis. Threlfall and Taylor (1969) showed that folic acid causes a marked and prolonged increase in protein synthesis while RNA synthesis is only transiently increased.

Although the stimulation of PAH accumulation by penicillin is associated with increased protein synthesis, it is not possible to determine if penicillin induces the synthesis of specific proteins responsible for renal organic acid transport. Penicillin may also be acting by enhancing the development of existing tubular transport processes. This effect could be associated with the synthesis of specific renal transport proteins, but it could also involve the synthesis of other renal proteins. Enhanced development of existing transport sites may also involve conversion of an inactive site or protein precursor to an active form, a process not necessarily involving protein synthesis.

A correlation between histological maturity and cellular function was demonstrated by the results presented in Figure 11. The kidneys of guinea pigs are histologically mature at birth, while rabbit, dog and cat kidneys are immature at birth. Renal cortical slices from 1 week-old rabbits, dogs and cats accumulated PAH to a lesser extent than slices from adult animals, while the values from 1 week-old guinea pigs were higher than those obtained in adults. It was therefore reasoned that the stimulatory effect of penicillin on PAH accumulation by renal cortical slices may be associated with enhanced histological maturation of young rabbit and rat kidneys. One way of approaching this question was to treat young rabbits from age 11 to 13 days with penicillin and to measure PAH accumulation by renal cortical slices from treated and control littermates 1 and 8 days after discontinuing treatment. The stimulatory effect of penicillin on PAH accumulation appears to be reversible, since the increased PAH S/M ratio observed at 2 weeks of age was no longer present in the 3 week-old

littermates 8 days after stopping penicillin treatment (Figure 27). These observations suggest that the action of penicillin cannot be explained by enhancing histological development of the immature kidney since a decrease from the 2 week PAH S/M value would not then be expected.

The PAH S/M ratios obtained in both 3 week treated and control rabbits (Figure 27) are the same as those obtained at 3 weeks while determining the normal developmental pattern of PAH transport in rabbits (Figure 4). The data indicate that the presence of penicillin acts as an increase in functional load in the 2 week-old rabbits, producing an increase in the transport capacity. When penicillin treatment was discontinued, the stimulus for increased transport was removed, and the transport capacity decreased to the normal level observed at 3 weeks of age. Thus penicillin appears to enhance development of secretory transport processes, but not renal histological maturation. These observations provide additional evidence for the specificity of the penicillin-induced substrate stimulation of PAH transport, suggesting the transport capability is closely related to substrate availability.

Reversal of substrate stimulation was also observed by Gordon and Roder (1953) in measuring the age-related increase in adenosine deaminase activity of chick embryos. Enzyme activity progressively increased from days 7 to 14 of incubation, and injection of the embryos with adenosine on day 7, 9 or 11 resulted in enhanced adenosine deaminase activity for about 2 days, after which the enzyme activity fell to the new control level. Using weanling rats (35 - 45 g), Conney et al. (1960) found that zoxazolamine hydroxylase activity in control rats gradually increased during the period when the enzyme activity in phenobarbital-treated

markedly increased. Enzyme activity returned to the new control values within several days after stopping drug administration. The results of histological studies (Figure 28) confirm the observations made above. Penicillin did not produce any observable histological changes in kidneys from 2 week-old rabbits, suggesting that its stimulatory effect on PAH transport involves specific subcellular changes in renal structure and function. In this regard, Zorzoli (1968) found that although increases in gluconeogenic enzyme activity occurred during the period of morphological change in mouse kidneys, there was not a strict parallelism between biochemical and anatomical differentiation. Since the kidney has other specific functions in addition to secretion of organic acids, the lack of a direct correlation between physiological and anatomical changes in the present study is not unreasonable. Significant changes in tubular size and volume of cytoplasm were observed in kidneys from 2 and 4 week-old rabbits, so that normal histological development could readily be detected. Inasmuch as precise changes such as those associated with renal growth during tubular regeneration can be detected using electron microscopy (Kempczinski and Caulfield, 1968), such studies may provide specific information regarding the possible renal histological effects produced by penicillin. The results of the present investigation suggest that the stimulatory effect of penicillin on PAH accumulation by renal cortical slices is primarily associated with enhanced development of existing tubular transport processes.

SUMMARY

The pattern of development of organic acid transport was determined in young rabbits, ranging in age from 1 day before birth to 8 weeks, by measuring the ability of renal cortical slices from these animals to accumulate PAH. PAH S/M ratios increased slowly from birth to 2 weeks and then increased rapidly until peak values were obtained at 4 weeks of age. Although kidney and body weight continued to increase beyond 4 weeks, the PAH S/M ratios gradually declined to adult values. It is suggested that prior to 4 weeks of age secretion of organic acids increases with age by a combination of increased activity of the organic acid secretory system and increased functional tubular mass. Transport activity reaches a maximum at 4 weeks of age so that further increases in organic acid secretion to adult levels are accomplished only by increasing kidney weight.

Substrate stimulation of the organic acid transport system was demonstrated in young rats and rabbits by the administration of PAH, penicillin and T_3. Treatment of 1 week-old rabbits with PAH twice daily for 7 days resulted in a significant increase in the PAH S/M ratio, but had no effect on NMN accumulation. Similarly, accumulation of PAH by renal cortical slices was significantly enhanced after treatment of 2 week-old rabbits with penicillin for 3 days. No stimulation was observed after penicillin treatment of 4 week-old rabbits however, suggesting that penicillin can stimulate organic acid transport during the developmental period, but has no effect when transport development is complete. Treatment of pregnant rabbits during the last half of gestation significantly enhanced

the ability of renal cortical slices from 31 day-old fetuses (delivered by caesarean section) to accumulate PAH. When pregnant rabbits were treated with penicillin during the last half of pregnancy, and PAH accumulation measured in renal cortical slices from the offspring, the PAH S/M ratios were significantly enhanced when compared to controls at ages ranging from 1 day to 2 weeks, but not at 4 weeks.

Treatment of nursing rats with PAH twice daily for 3 or 7 days resulted in stimulation of PAH, but not NMN, accumulation by renal cortical slices. Administration of penicillin to nursing rats also significantly enhanced PAH accumulation by renal cortical slices without stimulation of NMN or TEA transport. Addition of penicillin to incubation medium containing renal cortical slices from adult rats significantly depressed the PAH S/M ratios, demonstrating that penicillin is a substrate for the organic acid secretory system. The in vitro addition of T_3 had a similar inhibitory effect. Administration of T_3 to weanling rats for 3 or 7 days significantly enhanced the PAH S/M ratios, but no effect was observed after treating adult rats with T_3. Kidney weight was increased after treating weanling rats with T_3, but NMN accumulation was not changed. Although folic acid and phenobarbital are not rapidly secreted in the same manner as PAH, penicillin or T_3, the administration of folic acid to weanling rats and phenobarbital to young rabbits enhanced PAH, but not NMN, accumulation by renal cortical slices. This may be due to enhanced levels of endogenous organic acids occurring after the administration of folic acid and phenobarbital in association with their ability to block organic acid secretion.

Acetate had a similar stimulatory effect on PAH accumulation by renal cortical slices from penicillin-treated or control nursing rats, suggesting that acetate and penicillin act at different steps in the secretory process, or by different mechanisms. The effect of penicillin on the rate of PAH accumulation by renal cortical slices was determined over short incubation times. Although penicillin significantly enhanced PAH accumulation, the slopes of the calculated regression lines between 4 and 15 minutes of incubation were not significantly different. Since it has been suggested that the slope of the uptake curve is a measure of the rate of uptake, this indicates that the stimulatory effect of penicillin on the PAH S/M ratio is not due to an enhanced rate of uptake.

A number of observations suggest that the stimulatory effect of penicillin on PAH accumulation by renal cortical slices was associated with increased renal protein synthesis. Penicillin administration to nursing rats resulted in a significant increase in the kidney weight : body weight ratio. Concomitant administration of cycloheximide prevented the increase in PAH accumulation produced by penicillin. Renal cortical slices from penicillin-treated rats exhibited a significant increase in incorporation of both C^{14}-leucine and C^{14}-glutamine. The increased incorporation of C^{14}-leucine was specific to the cortex since it was not observed in medullary slices from the same kidneys. This is analogous to the uptake of PAH which occurs only in the cortex. Although the incorporation of C^{14}-orotic acid was significantly increased in kidneys from penicillin-treated rats, no difference between values from treated or control rats was observed when factored by the protein content.

The stimulatory effect of penicillin on PAH accumulation did not appear to be secondary to the increase in kidney weight that it produced, since the increased kidney weight resulting after ammonium chloride administration was not associated with an increase in the PAH S/M ratio. Specificity of the penicillin effect was further indicated by the observation that kidney tissue water content and distribution were not changed after penicillin administration.

Low PAH S/M ratios were obtained in renal cortical slices from 1 week-old rabbits, cats and dogs, all of which have histologically immature kidneys at birth, while high PAH S/M values were obtained in slices from adult animals of these species. Renal cortical slices from 1 week-old guinea pigs, which have histologically mature kidneys at birth, developed higher PAH S/M ratios than those obtained in adult guinea pigs. These observations demonstrated a correlation between histological maturity and the ability of renal cortical slices to accumulate PAH. The stimulatory effect of penicillin on PAH accumulation by renal cortical slices however, was not associated with an increased rate of renal histological maturation, since sections of kidneys from 2 week-old treated and control rabbits exhibited no histological differences. Normal histological development could be detected since differences between kidneys from 2 week, 4 week, and adult rabbits were readily apparent. Treatment of rabbits with penicillin from 11 to 13 days of age produced a significant increase in the PAH S/M ratio when determined at 14 days of age, but this high ratio declined to the normal 3 week value when measured 8 days after discontinuing drug treatment. The stimulatory effect of penicillin on PAH accumulation was

therefore reversible, also indicating that histological changes were not involved. The data suggest instead, that penicillin administration acts as an increase in functional load in the 2 week-old rabbits, resulting in an increased transport capacity (S/M ratio) which decreases shortly after stoppage of penicillin treatment. Transport capability is thus closely related to substrate availability. These results demonstrate that penicillin exerts its substrate stimulatory action by initiating specific subcellular changes in the organic acid secretory mechanism.

TABLE 1

Effect of various inhibitors on NMN accumulation (S/M ratios) by renal cortical slices from adult male Dutch Cross rabbits

Control	10^{-4}M DNP	10^{-5}M TEA	10^{-4} TEA	N_2
1.49[a]				
1.58	0.95	1.32	1.16	
1.72				1.09
1.94	1.16	1.69	1.36	
1.34	1.03			0.99
1.40		1.10	0.94	
1.51				1.01
1.57(.08)[b]	1.05(.06)[c]	1.33(.17)	1.16(.02)[c]	1.03(.03)[c]

[a]Each value represents the mean of 3 determinations from individual rabbits. Values in the same rows were obtained from the same rabbits.

[b]Mean ±(S.E.)

[c]Significantly different from control ($P < .05$), group comparison.

TABLE 2

Effect of pH on PAH accumulation (S/M ratio) by renal cortical slices[a]

Species/pH	7.2	7.4	7.7	7.8	8.0	8.1	8.3
Rabbit	4.14[b] (.84)	4.62 (.57)		8.98 (.78)	9.39 (.86)	8.33 (.87)	5.50 (.87)
Rat		4.15 (.61)	6.68 (.62)	9.22 (.62)	12.63 (.38)	7.20 (.40)	2.82 (.57)
Dog	3.73 (.52)	6.54 (.44)	4.18 (.47)	3.77 (1.18)		3.80 (.91)	3.34 (1.09)

[a]Renal cortical slices from each animal were randomly distributed among incubation beakers containing phosphate buffer medium. The initial pH varied from 7.0 to 9.0 in different experiments. After incubating for 90 minutes, the pH of the medium was measured, and the PAH S/M ratio determined in the usual manner.

[b]Each value represents the mean PAH S/M ratio ±(S.E.), determined in 3 to 6 experiments.

TABLE 3

Relationship between incubation time and PAH and NMN S/M ratios in nursing and adult rats[a]

Incubation Time (min)	2	5	15	30	60	90	180	N
PAH S/M								
Nursing Rats	1.1(0.3)	2.2(0.4)	3.6(0.3)	5.3(0.4)	8.4(0.2)	9.7(0.9)	10.0(2.1)	6
Adult Rats	1.6(0.2)	2.5(0.3)	3.7(0.2)	5.0(0.2)	8.5(0.5)	8.6(0.8)	8.0(1.2)	4
NMN S/M								
Nursing Rats	1.0(0.1)	1.9(0.1)	3.5(0.1)	4.7(0.1)	6.2(0.1)	6.9(0.2)	7.9(0.1)	3
Adult Rats	1.1(0.1)	1.7(0.1)	3.2(0.2)	4.9(0.1)	7.0(0.2)	7.4(0.7)	8.1(0.1)	4

[a]Each value represents the mean PAH or NMN S/M ratio ±(S.E.) obtained in 3 to 6 experiments, in which renal cortical slices from 1 litter of nursing rats or 2 adult rats were randomly distributed in 7 beakers and incubated for 2 to 180 minutes.

TABLE 4

PAH and NMN accumulation (S/M ratio) by renal cortical slices after treatment of rabbits with PAH[a]

	PAH S/M		NMN S/M	
Litter	Saline-Control	PAH-Treated	Saline-Control	PAH-Treated
A	3.40[b]	6.20		
B	4.15	5.95		
C	4.00	5.45	1.90	1.67
D	2.53	4.02	1.81	2.15
E	3.16	6.35	2.33	2.12
F	5.73	6.19	1.95	1.90
Mean ±(S.E.)	3.83 (0.41)	5.69[c] (0.41)	2.00 (0.11)	1.96 (0.11)

[a]Beginning at 7 days of age, rabbits were treated intraperitoneally with saline or 100 mg/kg PAH twice daily for 7 days.

[b]Each value represents the mean obtained from 2-4 animals in each litter.

[c]Significant different from control ($P < .05$), paired comparison.

TABLE 5

PAH and NMN accumulation (S/M ratio) by renal cortical slices after treatment of nursing rats with PAH[a]

PAH Treatment mg/kg/day	Duration days	N[b]	PAH S/M		NMN S/M	
			Saline-Control	PAH-Treated	Saline-Control	PAH-Treated
200	3	10	6.70 (0.42)	8.16 (0.50)[c]	6.30 (0.21)	6.83 (0.22)
400	3	5	6.09 (0.47)	8.45 (0.80)[c]	6.95 (0.47)	7.35 (0.17)
200	7	7	5.45 (0.49)	7.81 (0.83)[c]	5.99 (0.44)	6.01 (0.32)

[a]Nursing rats were treated, beginning at age day 16, with PAH (100 or 200 mg/kg, twice daily, i.p. for 3 or 7 days) or saline. PAH and NMN uptake were determined 24 hours after the last injection. Each value represents the mean ±(S.E.).

[b]N, number of control and treated rats.

[c]Significantly different from control ($P < .05$), group comparison.

TABLE 6

Effect of *in vitro* penicillin on PAH accumulation (S/M ratio) by rat renal cortical slices [a]

Addition	PAH S/M
Control Medium	10.84 (0.61)[b]
Pottasium Control	11.13 (1.24)
10,000 IU	9.23 (0.48)
50,000 IU	4.24 (0.39)[c]
100,000 IU	2.16 (0.03)[c]

[a]The indicated number of units of potassium penicillin G (0.3 ml) were added to incubation beakers containing Cross and Taggart PAH medium and kidney cortical slices from adult male rats. The controls used were Cross and Taggart medium without PAH (0.3 ml) and potassium chloride (0.3 ml) equivalent to the amount of potassium in 100,000 IU potassium penicillin G.

[b]Each value represents the mean ±(S.E.) from duplicate determinations in 5 experiments.

[c]Significantly less than control ($P < .05$), group comparison.

TABLE 7

PAH accumulation (S/M ratio) by rabbit renal cortical slices after treatment of 6 week-old rabbits with penicillin[a]

Litter	Saline-Control	Penicillin-Treated
A	12.75[b]	14.44
B	14.74	11.90
C	14.38	12.47
D	10.14	11.06
Mean ±(S.E.)	13.00 (1.04)	12.47 (.72)

[a]Six week-old rabbits were treated subcutaneously with 120,000 IU procaine penicillin G twice daily for 3 days.

[b]Values represent the means obtained from triplicate determinations in 2 saline-control and 2 penicillin-treated rabbits per litter.

TABLE 8

PAH accumulation (S/M ratio) by renal cortical slices from 31 day rabbit fetuses after maternal administration of penicillin [a]

Litter	PAH S/M Control	Litter	PAH S/M Treated
A	4.91[b]	E	7.19
B	4.63	F	7.92
C	5.08	G	8.34
D	5.28		
Mean ±(S.E.)	4.97 (0.14)		7.82[c] (0.34)

[a]Pregnant rabbits were treated with saline or penicillin (60,000 IU, twice daily) during the last half of pregnancy.

[b]Each value is the mean from 3 fetuses.

[c]Significantly different from control ($P < .05$), group comparison.

TABLE 9

PAH accumulation by renal cortical slices (S/M ratio) after penicillin treatment of nursing rats[a]

	Saline-Control (N = 6)	Penicillin-Treated (N = 7)
Body Weight (g)	34.5 (3.3)[b]	36.6 (1.3)
Kidney Weight (g)	0.408 (0.046)	0.465 (0.041)
Kidney Weight/Body Weight x 100	1.18 (0.04)	1.27 (0.09)
NMN S/M	5.94 (0.20)	6.73 (0.28)
PAH S/M	7.11 (0.70)	10.68 (1.35)[c]

[a]Beginning at 16 days of age nursing rats were treated subcutaneously twice daily for 3 days with 30,000 IU of procaine penicillin G or saline.

[b]Values represent means ±(S.E.) from six control and seven treated animals.

[c]Significantly different from control ($P < .05$), group comparison.

TABLE 10

Effect of penicillin on kidney weight and body weight of nursing rats[a]

	Saline-Control	Penicillin-Treated
Kidney Weight (g)	0.53 (0.01)[b]	0.57 (0.01)
Body Weight (g)	53 (0.5)	52 (1.8)
Kidney Weight/Body Weight x 100	1.00 (0.02)	1.10 (0.02)[c]
PAH S/M	8.77 (1.20)	11.67 (1.60)[c]

[a]Beginning at 16 days of age nursing rats were treated subcutaneously twice daily for 3 days with 30,000 IU of procain penicillin G or saline.

[b]Values represent means ±(S.E.) from 4 litters.

[c]Significantly different from control ($P < .05$) paired comparison.

TABLE 11

Effect of penicillin on water distribution in kidney cortical slices from nursing rats[a]

	Extracellular Space	Intracellular Space	Total H_2O
		% of Wet Weight	
Saline-Control	27.8 (0.7)[b]	52.3 (0.6)	80.1 (0.4)
Penicillin-Treated	26.5 (0.9)	53.3 (0.6)	79.7 (0.5)

[a]Beginning at 16 days of age nursing rats were treated subcutaneously twice daily for 3 days with 30,000 IU of procaine penicillin G or saline.

[b]Values represent means ±(S.E.) from 5 litters.

TABLE 12

Effect of ammonium chloride on nursing rat kidney weight and accumulation of PAH and NMN[a]

	Saline-Control	Ammonium Chloride-Treated
Kidney Weight (g)	0.539 (0.03)[b]	0.610 (0.04)
Body Weight (g)	51 (2.7)	54 (3.4)
Kidney Weight/Body Weight x 100	1.04 (0.02)	1.20 (0.03)[c]
PAH S/M ratio	9.70 (1.20)	9.45 (0.68)
NMN S/M ratio	7.30 (0.23)	7.56 (0.16)

[a]Beginning at 16 days of age nursing rats were treated with 1M ammonium chloride (1 ml/100g, orally, once daily for 3 days). Controls received an equal volume of saline.

[b]Values represent means ±(S.E.) from 5 litters.

[c]Significantly different from control ($P < .05$), paired comparison.

TABLE 13

Effect of L-leucine on PAH accumulation (S/M ratio) by adult rat renal cortical slices[a]

Leucine concentration	PAH S/M
0	11.80 ± .62[b]
2.6×10^{-6}M	11.92 ± .86
2.6×10^{-5}M	11.00 ± .35
2.6×10^{-4}M	14.66 ± 1.17
2.6×10^{-3}M	20.58 ± 1.80

[a]Unlabeled L-leucine was added to beakers containing PAH media and renal cortical slices from adult male rats.

[b]Values represent means ±(S.E.) from triplicate determinations in 4 experiments.

TABLE 14

Effect of penicillin treatment of nursing rats on incorporation of L-glutamine-C^{14} by renal cortical slices[a]

	Saline-Control	Penicillin-Treated	Difference
DPM/100 mg slice	3871[b]	4422	551 ± 178[c]
DPM/mg protein	209	255	46 ± 7.5[c]
PAH S/M ratio	7.75	9.27	1.52 ± 0.4[c]

[a]Beginning at 16 days of age nursing rats were treated subcutaneously twice daily for 3 days with 30,000 IU of procaine penicillin G or saline. The concentration of L-glutamine-C^{14} in each beaker was 1.2×10^{-4}M.

[b]Values represent the means obtained from 5 litters.

[c]Significant difference ($P < .05$), paired comparison.

TABLE 15

Effect of penicillin on protein content of nursing rat kidneys[a]

Fraction	Units	Saline-Control	Penicillin-Treated	Difference
9,000g ppt	mg protein/fraction	2.27[b]	2.64	.37(0.14)
105,000g ppt	mg protein/fraction	150	176	26.5 (3.6)[c]
105,000g supnt	mg protein/fraction	8.62	10.43	1.96(0.77)

[a]Beginning at 16 days of age nursing rats were treated subcutaneously twice daily for 3 days with 30,000 IU of procaine penicillin G or saline. Twenty-four hours after the last injection the rats were killed, the kidneys removed, homogenized in 1.15% KCl and the indicated fractions obtained by differential centrifugation.

[b]Values represent the means obtained from 4 litters.

[c]Significantly different from control ($P < .05$), paired comparison.

Figure 1: Model of the PAH transport mechanism (proposed by Foulkes and Miller, 1959b). The symbols and abbreviations used are as follows: ISF (interstitial fluid), ICF (intracellular fluid), M (PAH in the medium), E (PAH in the interstitial fluid), pah (rapidly diffusible fraction of PAH, and PAH (slowly equilibrating intracellular fraction of PAH resulting from the active concentrating mechanism of step 3).

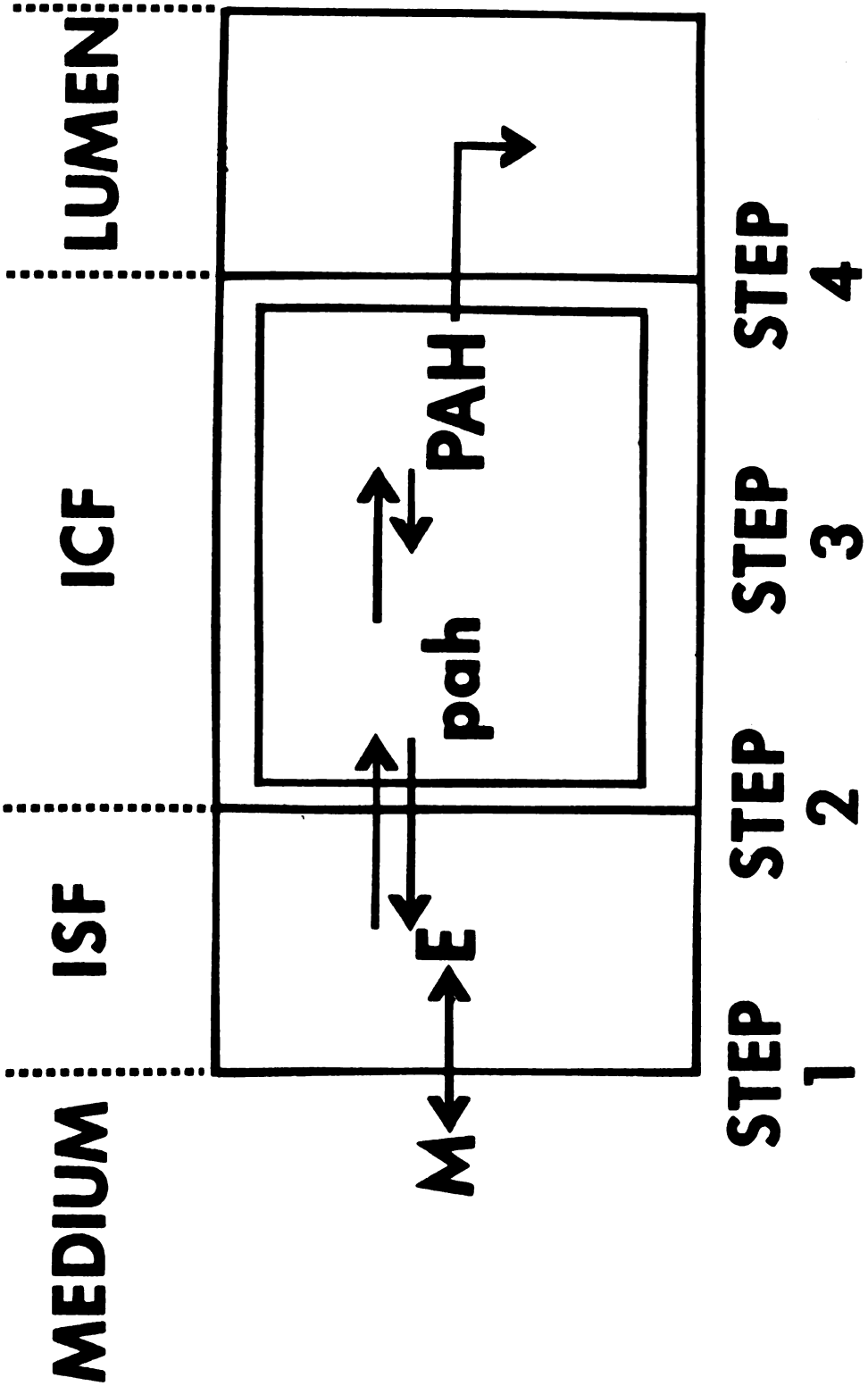

Figure 1

Figure 2: Effect of various inhibitors on PAH accumulation (S/M ratio) by renal cortical slices from adult male Dutch Cross rabbits. Each bar represents the mean ±(S.E.) of triplicate determinations using the number of rabbits indicated in parentheses. The PAH S/M ratios obtained in the presence of dinitrophenol (DNP) or nitrogen (N_2) are significantly less than control ($P < .05$, group comparison).

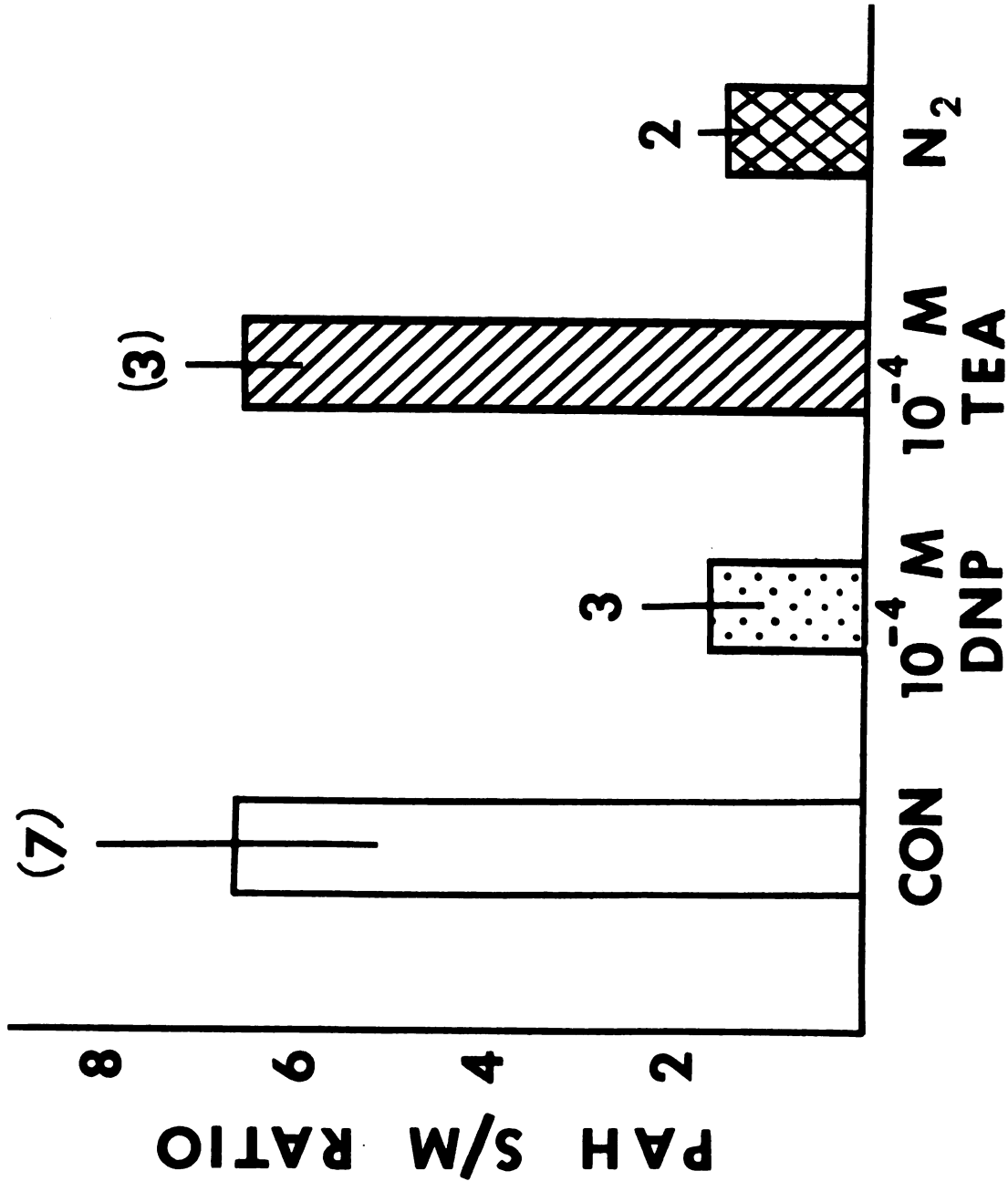

Figure 2

Figure 3: Effect of acetate and potassium on PAH accumulation (S/M ratio) by renal cortical slices from 2 week, 4 week and adult rabbits. Each bar represents the mean ±(S.E.) of duplicate determinations from 4 rabbits at each age. The effect of acetate is significantly greater than its respective control in the 4 week and adult rabbits.

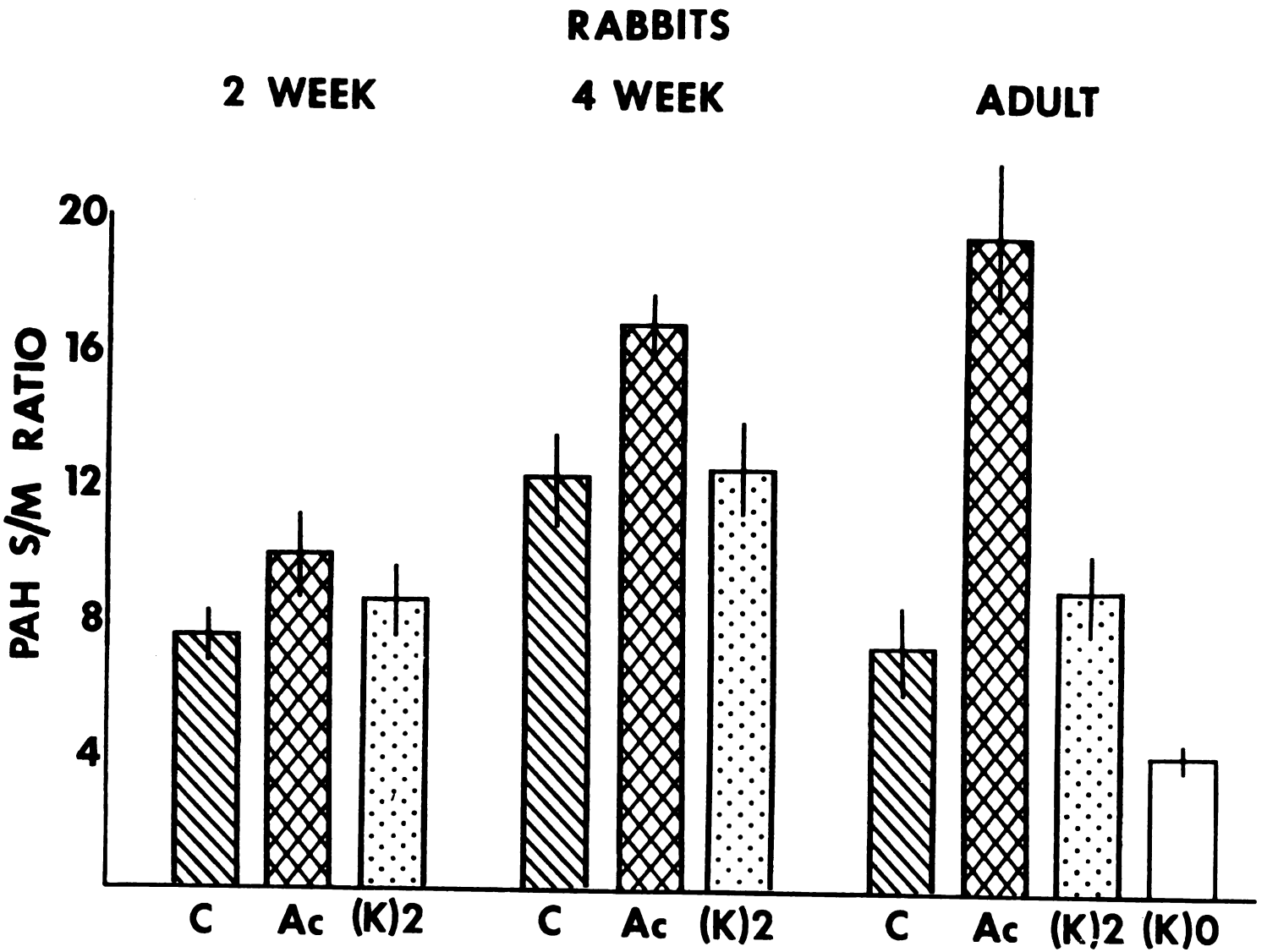
RABBITS
2 WEEK
4 WEEK
ADULT
PAH S/M RATIO
20
16
12
8
4
C
Ac
(K)2
C
Ac
(K)2
C
Ac
(K)2
(K)0

Figure 3

Figure 4: Relationship between age and PAH accumulation (S/M ratio) by rabbit renal cortical slices. PAH S/M ratios are represented by the solid line, while the increase in body weight with age is demonstrated by the dotted line. Each point represents the mean ±(S.E.) determined in 6 to 10 rabbits. Where no vertical line is shown the S.E. is less than the radius of the point.

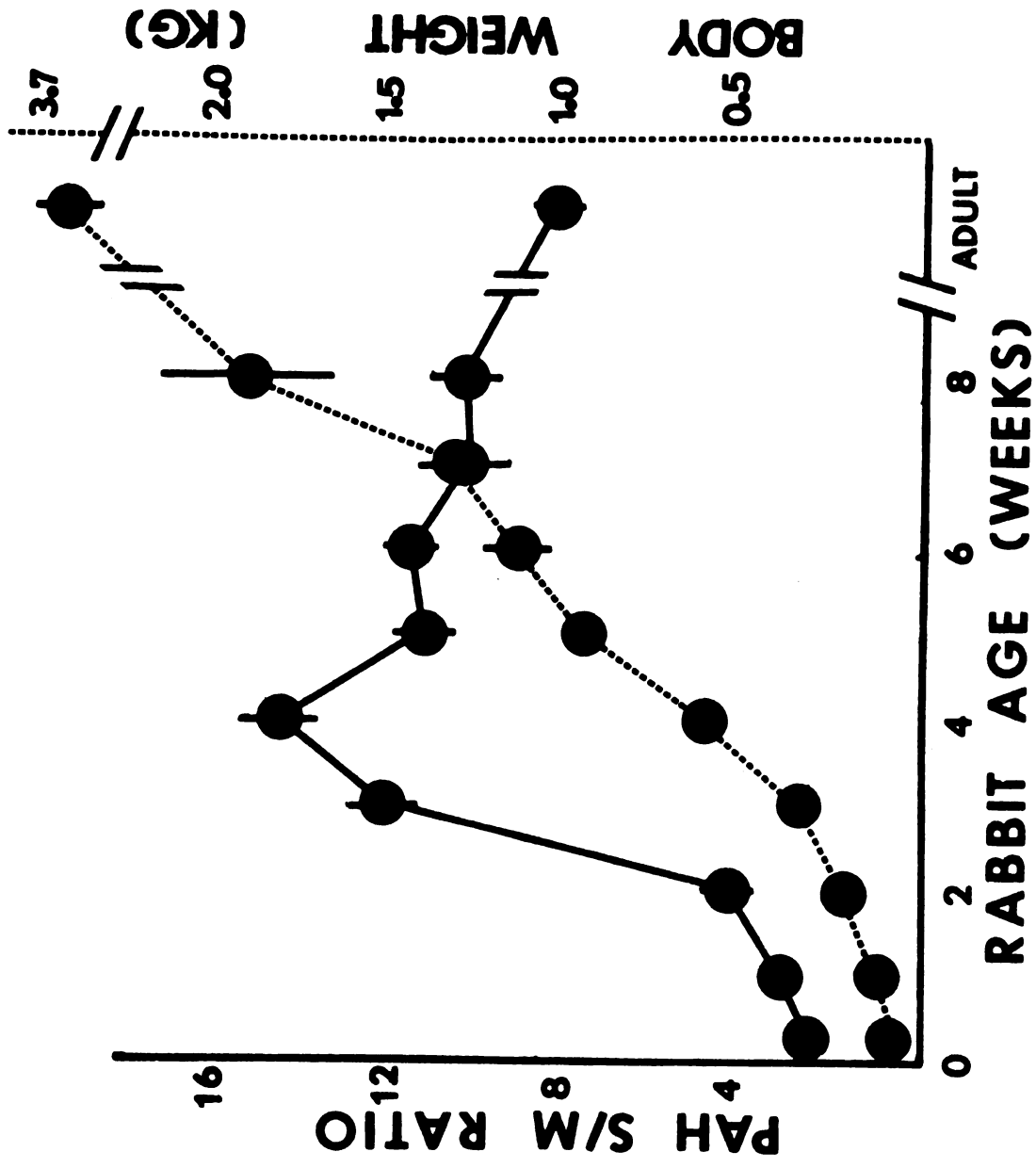
BODY WEIGHT (KG)
3.7
2.0
1.5
1.0
0.5
ADULT
RABBIT AGE (WEEKS)
0
2
4
6
8
PAH S/M RATIO
16
12
8
4

Figure 4

Figure 5: Relationship between age and NMN accumulation (S/M ratio) by rabbit renal cortical slices. NMN S/M ratios are represented by the dotted line, while the increase in kidney weight with age is demonstrated by the solid line. Each point represents the mean ±(S.E.) determined in 3 to 9 rabbits. Where no vertical line is shown the S.E. is less than the radius of the point.

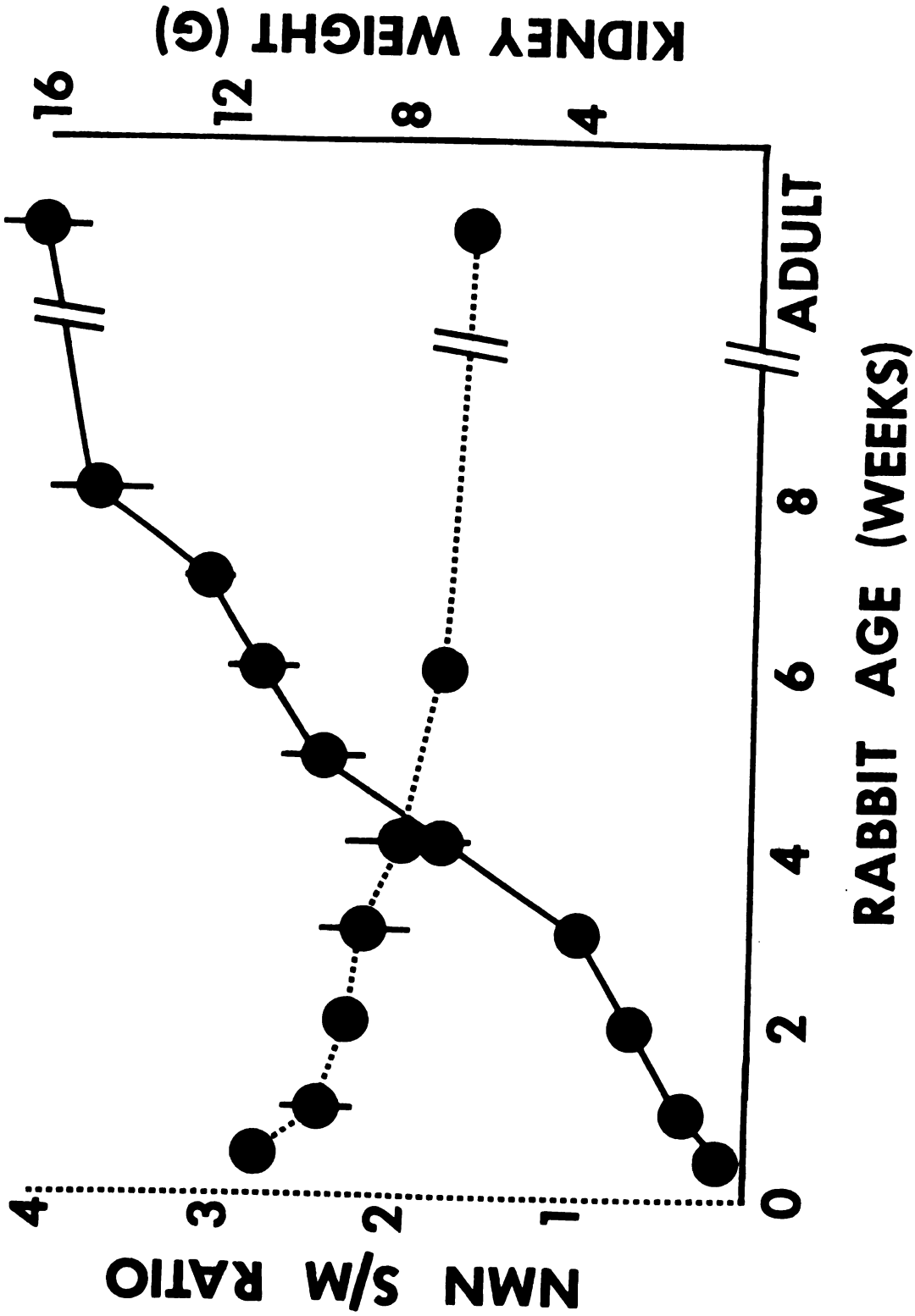
NMN S/M RATIO
4
3
2
1
0
2
4
6
8
ADULT
RABBIT AGE (WEEKS)
16
12
8
4
KIDNEY WEIGHT (G)

Figure 5

Figure 6: Relationship between incubation time and PAH accumulation (S/M ratio) by renal cortical slices from rabbits at various ages. Each point represents the mean ±(S.E.) determined using 3 to 6 rabbits. Renal cortical slices from each rabbit were randomly distributed among 6 beakers which were incubated for times ranging from 5 to 180 minutes. Where no vertical line is shown the S.E. is less than the radius of the point.

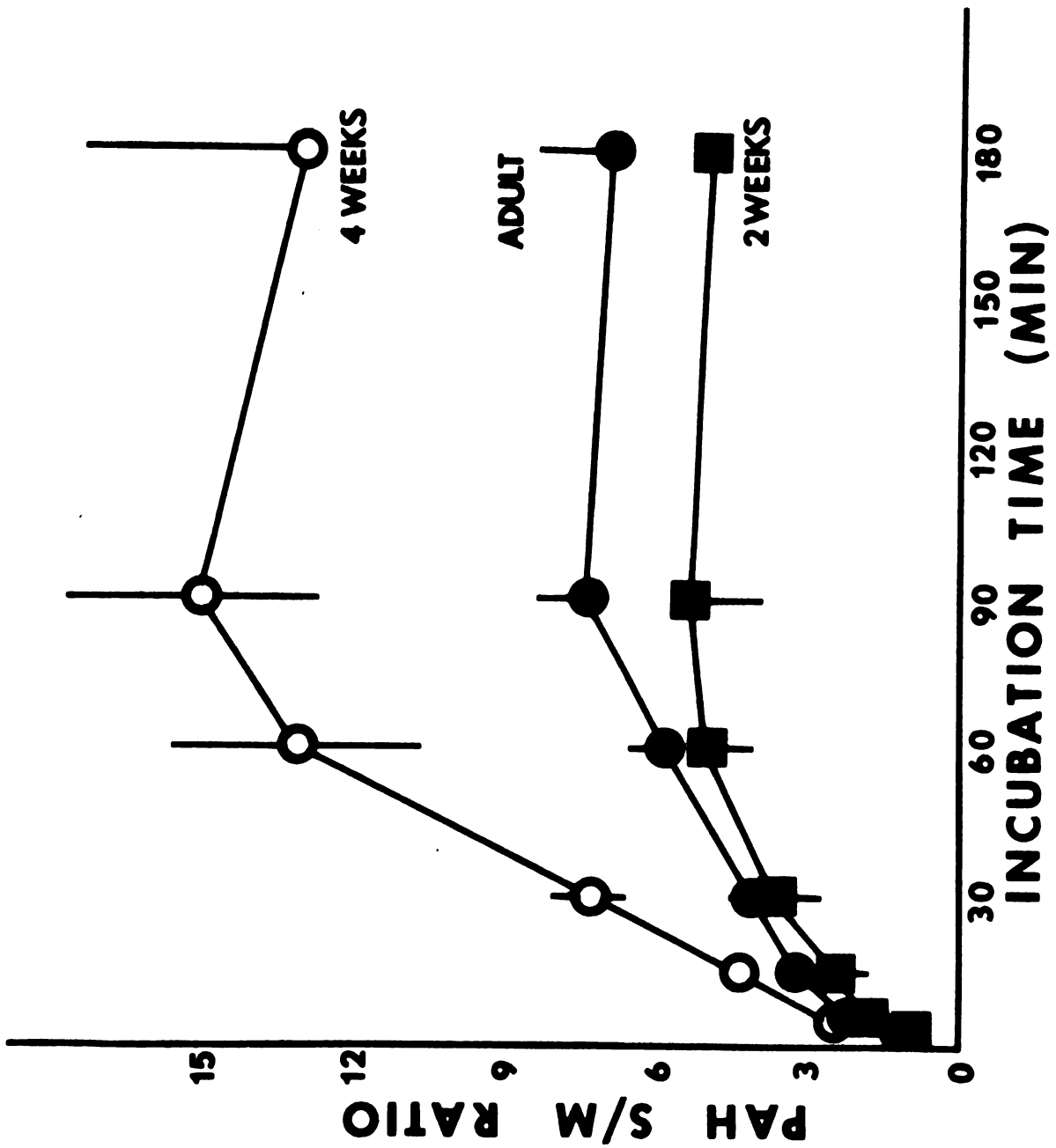
PAH S/M RATIO
15
12
9
6
3
0
30
60
90
120
150
180
INCUBATION TIME (MIN)
4 WEEKS
ADULT
2 WEEKS

Figure 6

Figure 7: Relationship between age and water content of rabbit renal cortical slices. Each bar represents the mean ±(S.E.) determined in 6 to 14 rabbits. Any bars overscored by the same line are not statistically different.

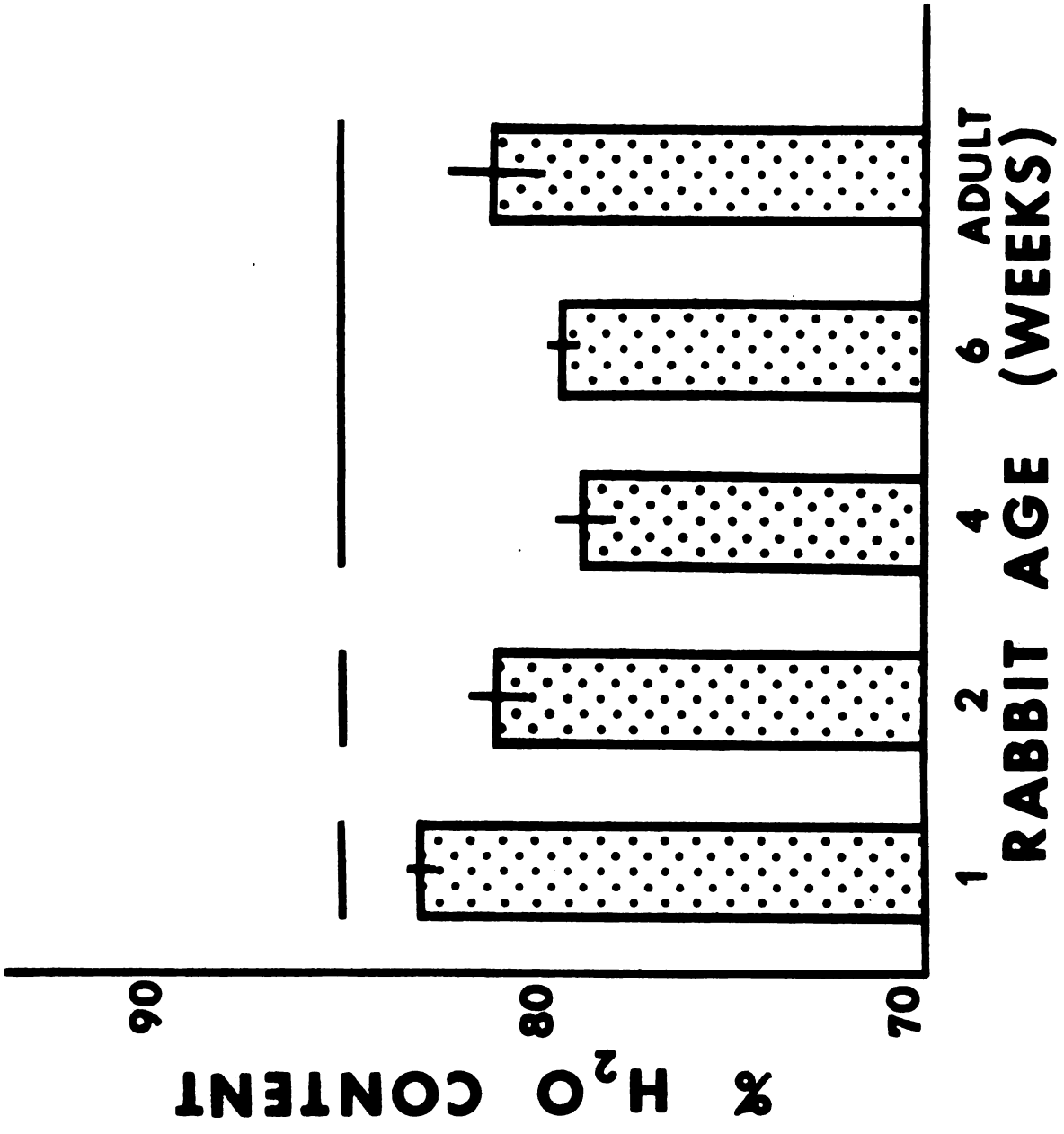

Figure 7

Figure 8: Relationship between age and PAH and NMN accumulation (S/M ratio) in rats. The upper panel demonstrates the increase in PAH S/M ratios (solid line) and body weight (dotted line) with increasing rat age. The bottom panel demonstrates the increase in NMN S/M ratios (solid line) and kidney weight (dotted line) with increasing rat age. Each point represents the mean $\pm$(S.E.) obtained using 8 to 10 rats. Where no vertical line is shown the S.E. is less than the radius of the point.

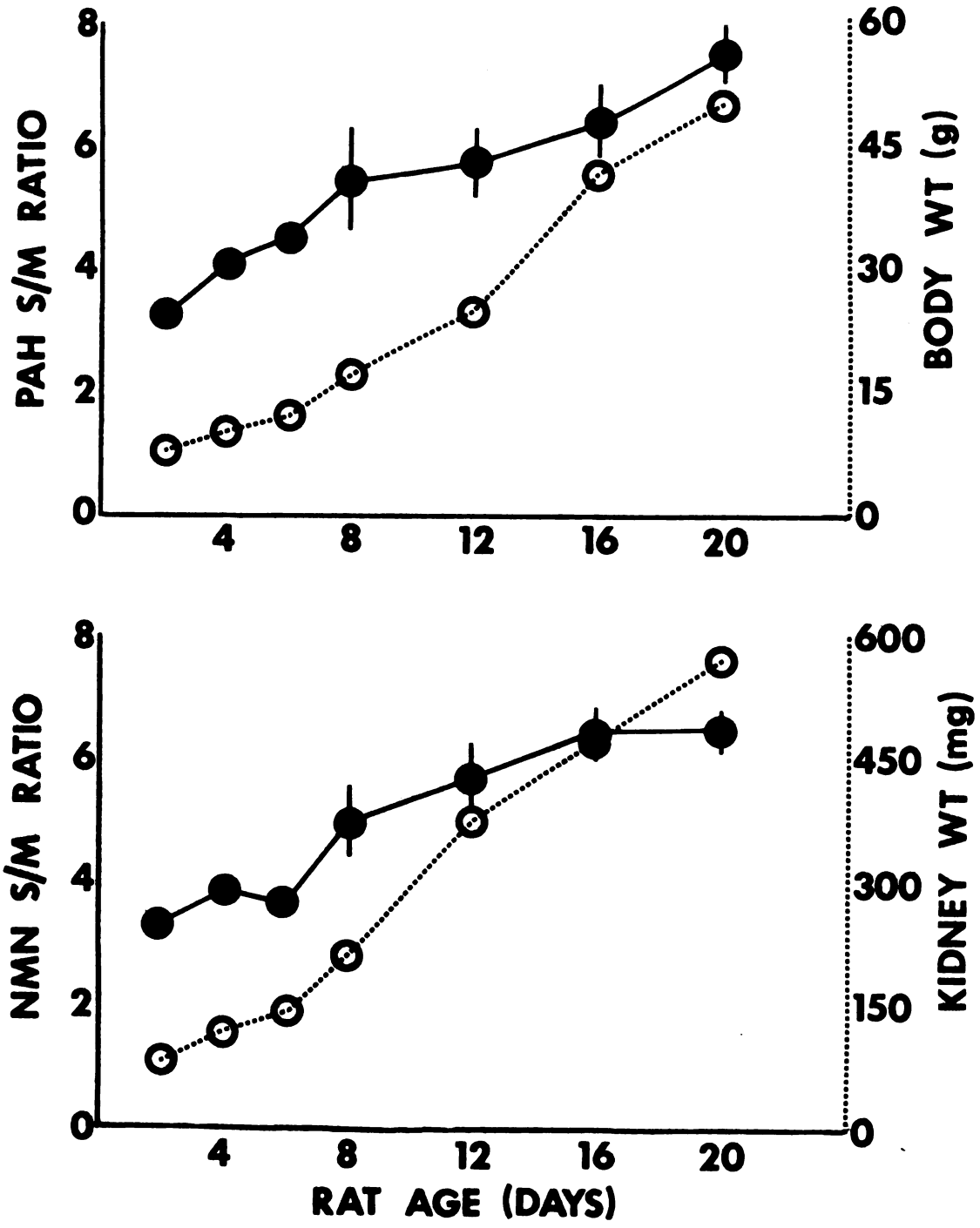
PAH S/M RATIO
BODY WT (g)
NMN S/M RATIO
KIDNEY WT (mg)
RAT AGE (DAYS)
0
2
4
6
8
15
30
45
60
150
300
450
600
4
8
12
16
20

Figure 8

Figure 9: Relationship between age and PAH accumulation (S/M ratio) by renal cortical slices from puppies and adult dogs. Each point represents the mean ±(S.E.) obtained using 4 to 8 animals.

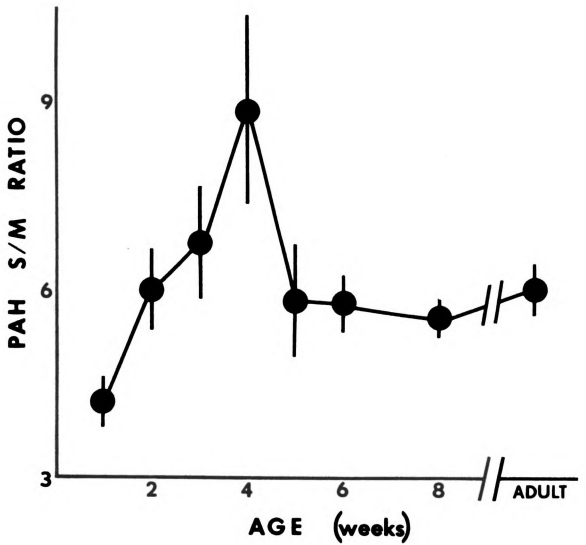

Figure 9

Figure 10: Relationship between incubation time and PAH and NMN accumulation (S/M ratio) by renal cortical slices from 1 week-old and adult cats. Each point represents the mean ±(S.E.) obtained using 2 to 5 cats. Renal cortical slices from each animal were randomly distributed among 6 beakers which were incubated for times ranging from 5 to 180 minutes. Where no vertical line is shown the S.E. is less than the radius of the point.

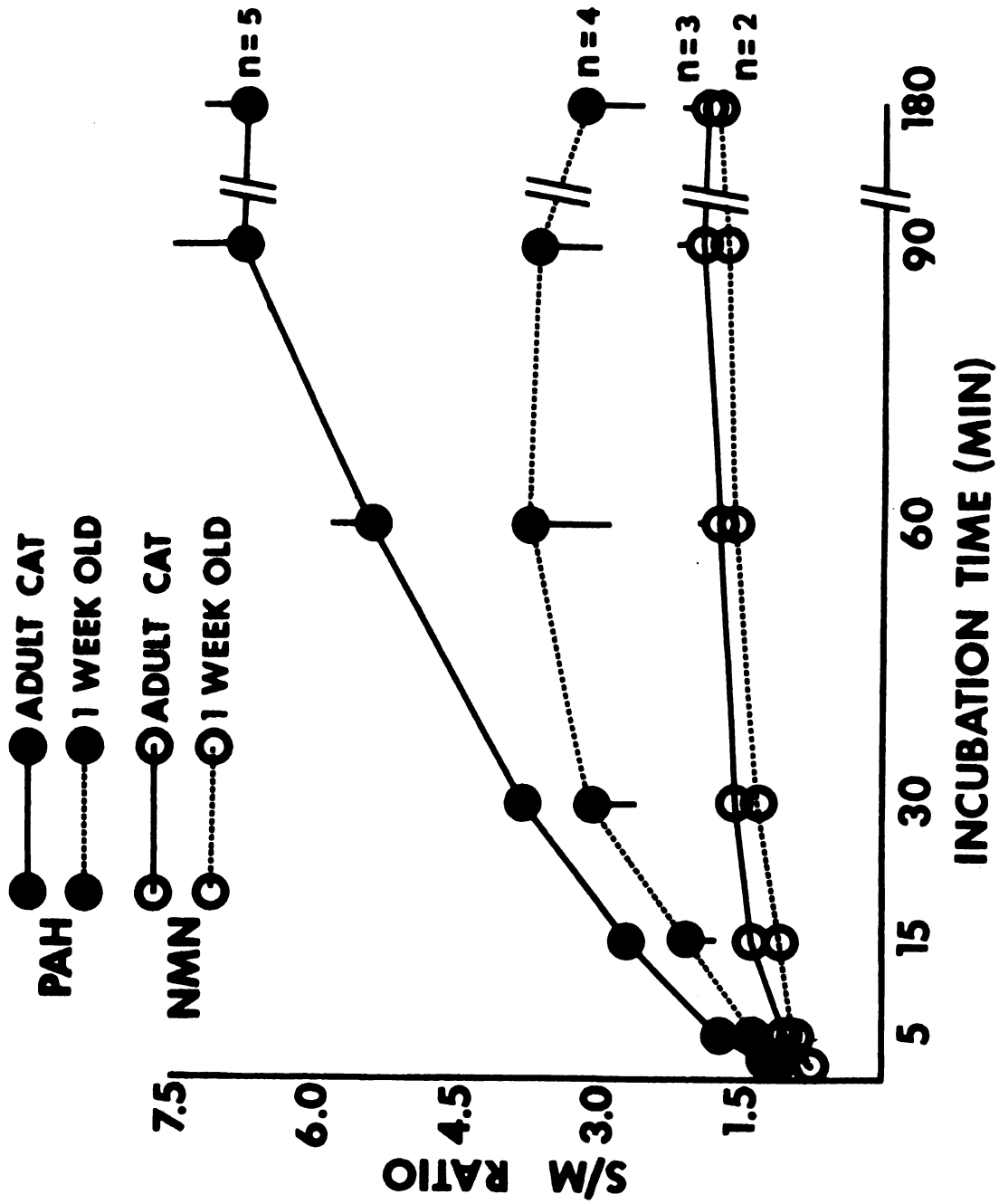
PAH
ADULT CAT
1 WEEK OLD
NMN
ADULT CAT
1 WEEK OLD
n=5
n=4
n=3
n=2
S/M RATIO
7.5
6.0
4.5
3.0
1.5
5
15
30
60
90
180
INCUBATION TIME (MIN)

Figure 10

Figure 11: Accumulation of PAH (S/M ratio) by renal cortical slices from 1 week old and adult dogs, cats, rabbits and guinea pigs. Each bar represents the mean ±(S.E.) of triplicate determinations from the number of animals indicated in parentheses. The adult PAH S/M ratios for dogs (D), cats (C) and rabbits (R) are significantly higher than the respective values at 1 week, while the reverse is true for guinea pigs (GP) ($P < .05$, group comparison).

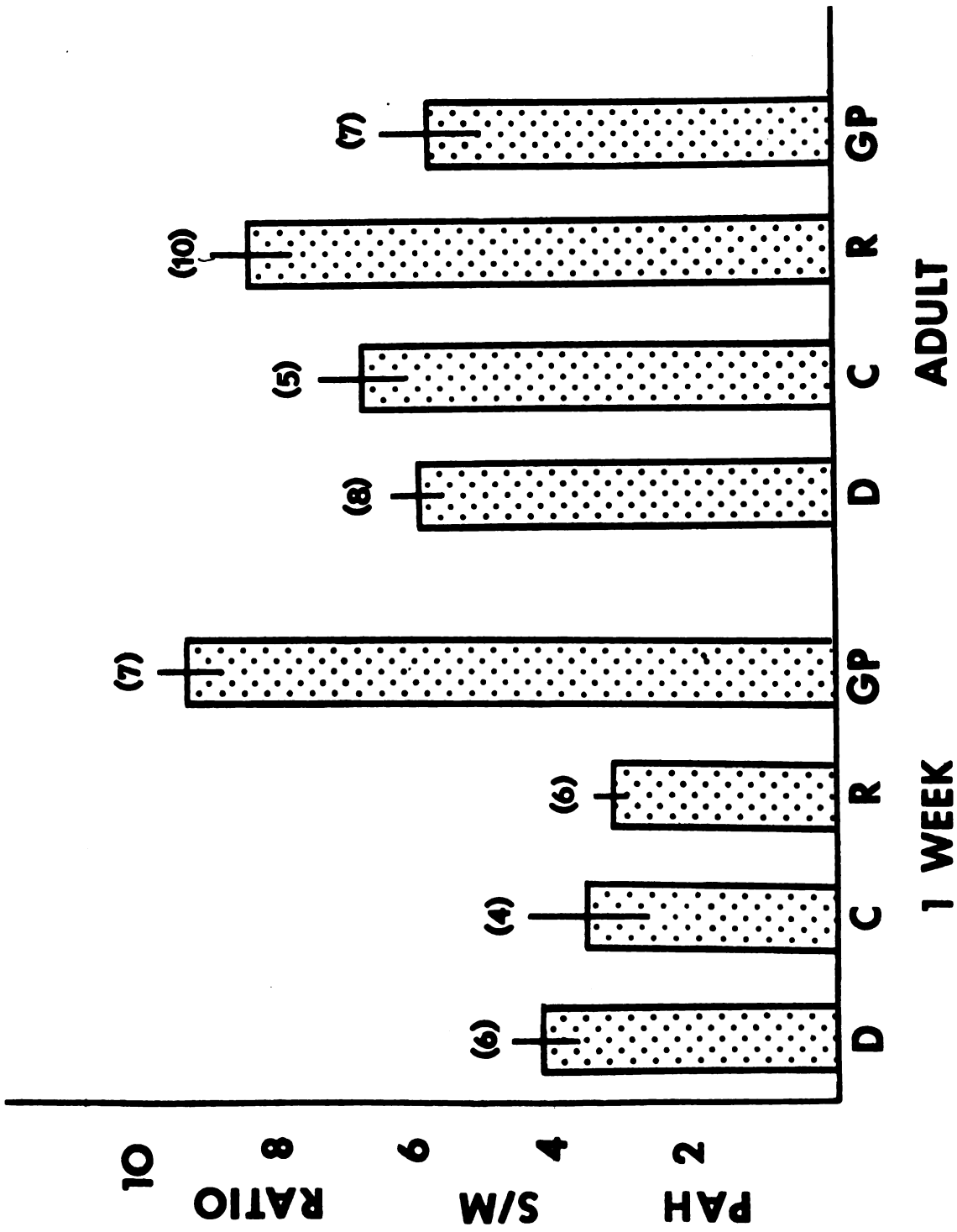

Figure 11

Figure 12: PAH accumulation (S/M ratio) by rabbit renal cortical slices after treatment of 2 and 4 week old rabbits with penicillin. Rabbits were treated with saline or 60,000 IU procaine penicillin G, subcutaneously, twice daily for 3 days. Each bar represents the mean ±(S.E.) determined in 5 to 7 rabbits. The asterisk indicates a significant difference from control ($P < .05$, paired comparison).

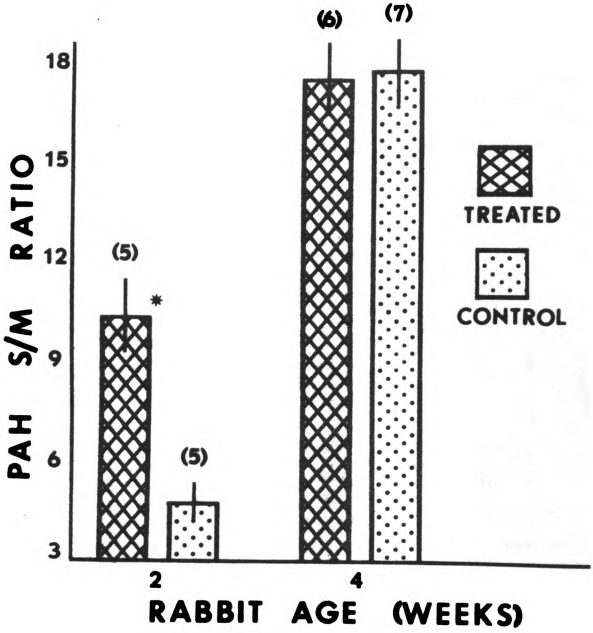
(6)
(7)
18
15
12
9
6
3
PAH S/M RATIO
(5)
*
(5)
TREATED
CONTROL
2
4
RABBIT AGE (WEEKS)

Figure 12

Figure 13: PAH S/M ratios in renal cortical slices from young rabbits after treating pregnant dams with penicillin. Pregnant rabbits were treated daily during the last half of pregnancy with 60,000 IU procaine penicillin G, or saline, and PAH accumulation by renal cortical slices from the offspring was studied at 1 day and 1, 2 and 4 weeks of age. Each bar represents the mean $\pm$(S.E.) determined in the number of rabbits shown in parentheses. The asterisks indicate those values that are significantly different from their respective controls ($P < .05$, group comparison).

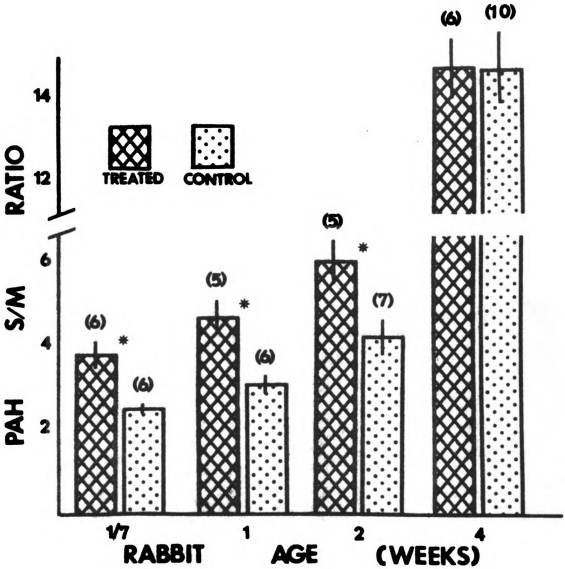
PAH S/M RATIO
14
12
6
4
2
TREATED
CONTROL
(6)
(10)
(5)
(7)
(6)
(5)
(6)
*
*
*
1/7
1
2
4
RABBIT AGE (WEEKS)

Figure 13

Figure 14: PAH S/M ratios in renal cortical slices from nursing rats after penicillin treatment. Rats were injected twice daily, subcutaneously, with 30,000 IU procaine penicillin G. Each bar represents the mean ±(S.E.) obtained after treating 16 rats (from 4 different litters) for 3 days and 6 rats (from 3 different litters) for 7 days with penicillin or saline. Significant effects were obtained after treating for both 3 and 7 days ($P < .05$, group comparison).

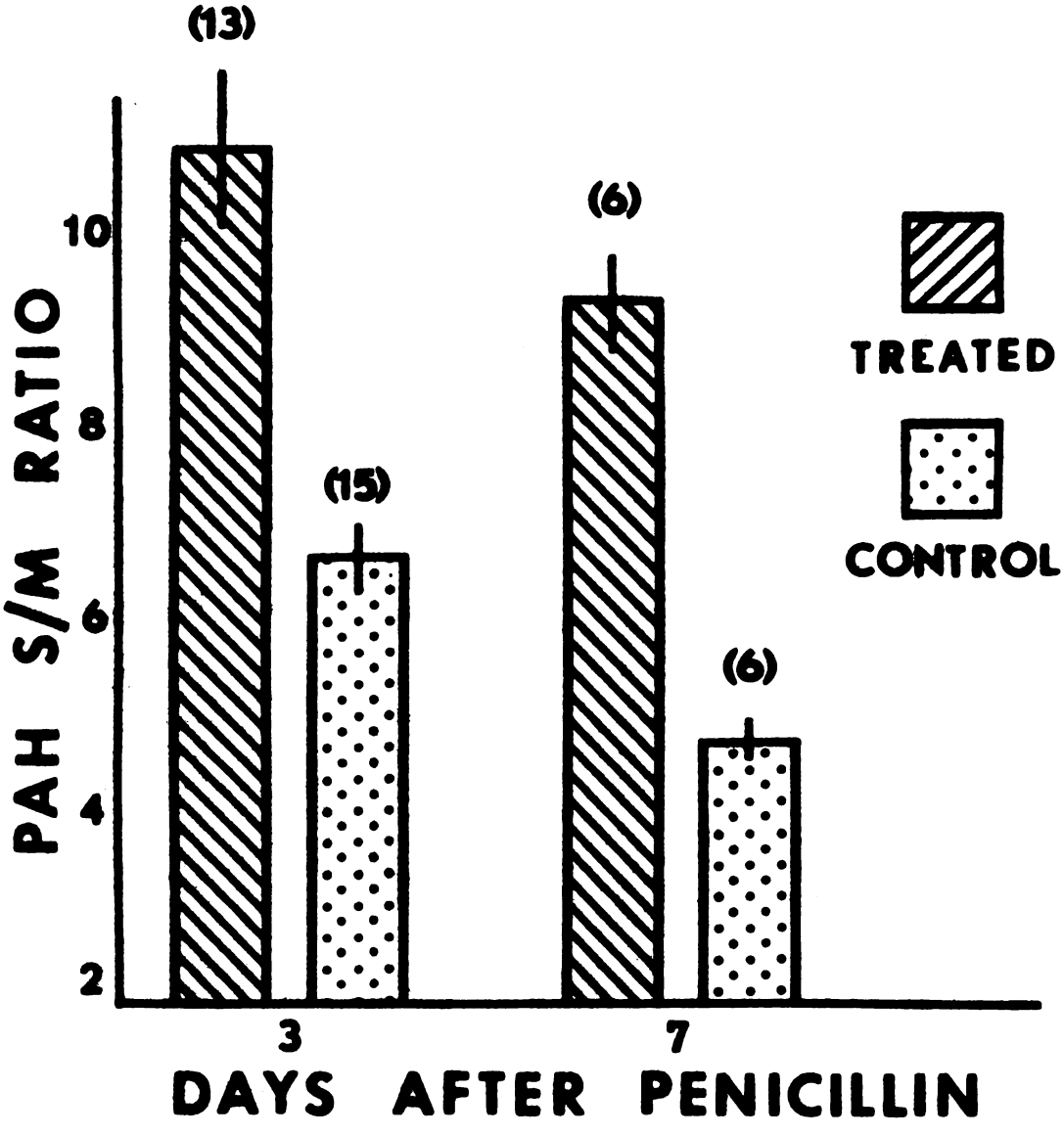
(13)
(15)
(6)
(6)
10
8
6
4
2
PAH S/M RATIO
TREATED
CONTROL
3
7
DAYS AFTER PENICILLIN

Figure 14

Figure 15: PAH and TEA S/M ratios in renal cortical slices from nursing rats after penicillin treatment. Procaine penicillin G (30,000 IU) was administered subcutaneously twice daily for 3 days. Each bar represents the mean ±(S.E.) obtained from 6 litters. The asterisk indicates that the PAH S/M ratio was significantly enhanced after penicillin treatment ($P < .05$, paired comparison). C represents control values while PEN represents values from penicillin-treated rats.

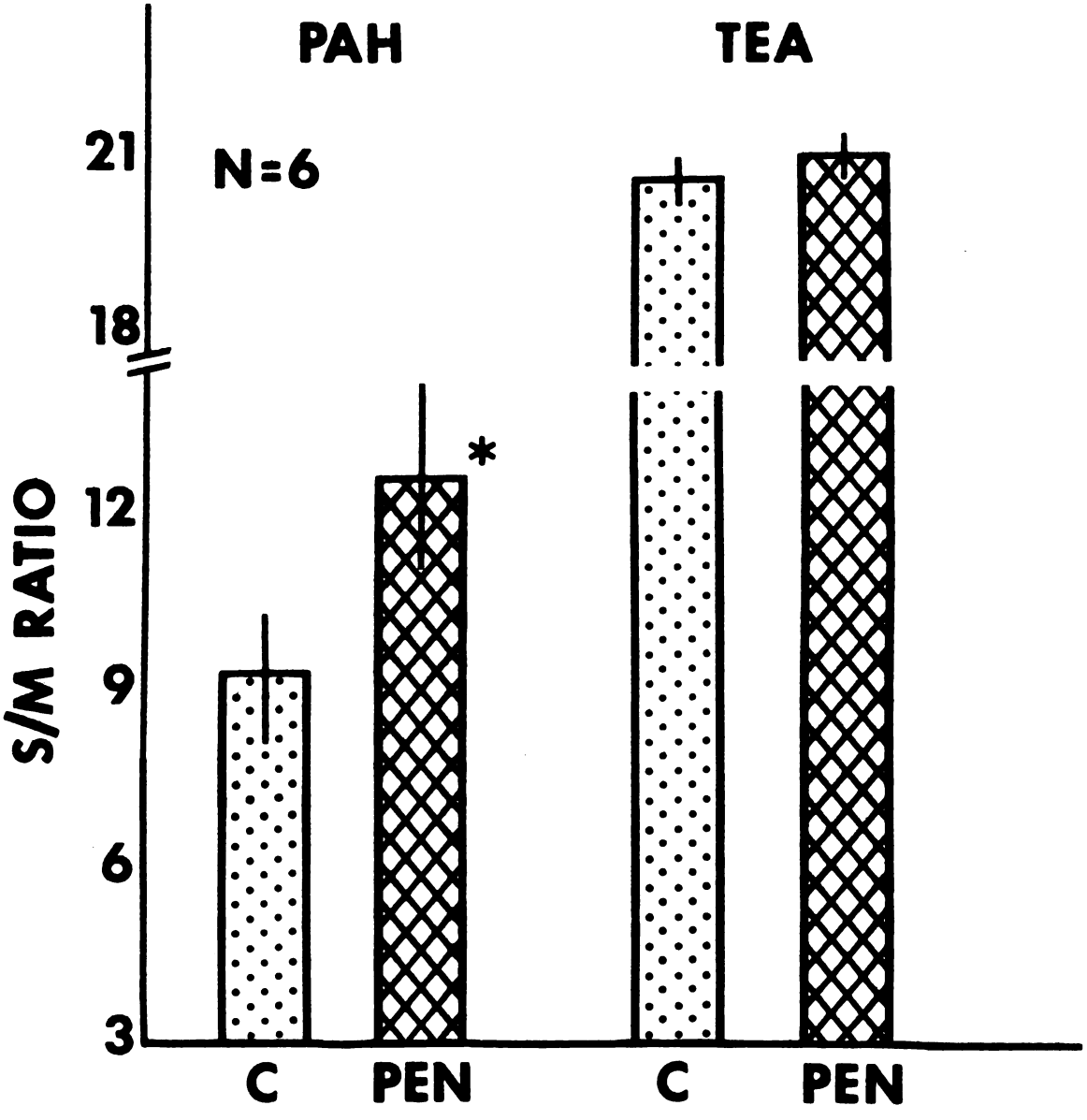

Figure 15

Figure 16: PAH and NMN S/M ratios in renal cortical slices from weanling rats after treatment with T_3. Rats were injected intraperitoneally with T_3 once daily, for 3 or 7 days. Control rats received alkaline saline, and all animals were killed 48 hours after the last injection. Each bar represents the mean PAH or NMN S/M ratio ±(S.E.) obtained from duplicate determinations using the number of animals indicated in parentheses. The asterisks indicate those values that are significantly different from their respective controls ($P < .05$, group comparison).

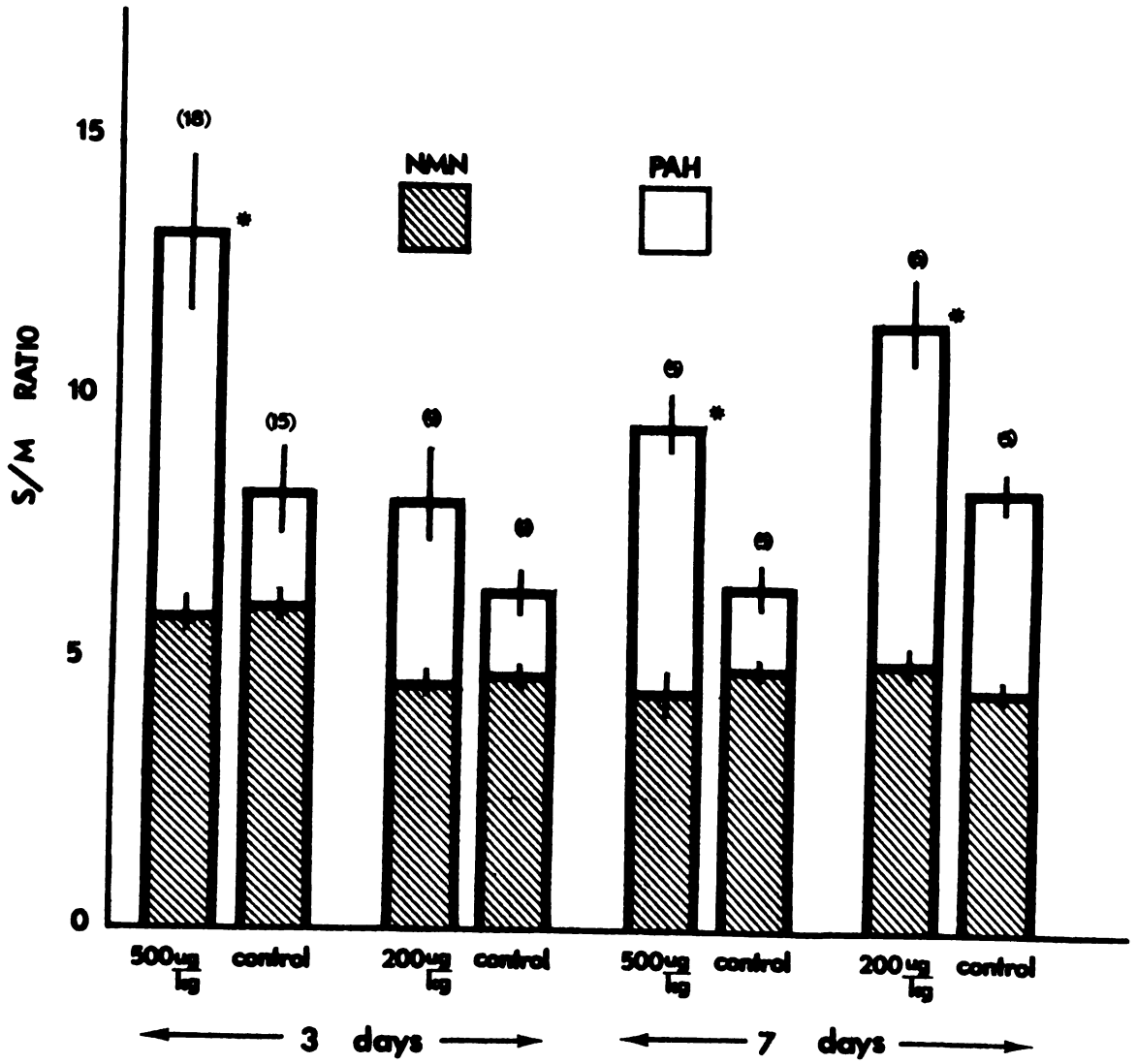
S/M RATIO
15
10
5
0
NMN
PAH
(18)
(15)
500µg/kg
control
200µg/kg
control
500µg/kg
control
200µg/kg
control
3 days
7 days

Figure 16

Figure 17: Effect of T_3 administration on the kidney weight/body weight ratio in weanling rats. Rats were injected intraperitoneally with 200 or 500 µg/kg of T_3 once daily for 3 or 7 days, while control rats received alkaline saline. All animals were killed 48 hours after the last injection. Each bar represents the mean percentage of kidney weight/ body weight ±(S.E.) using the number of animals indicated in parentheses. As indicated by the asterisks, T_3 treatment caused a significant increase in kidney weight in all cases ($P < .05$, group comparison).

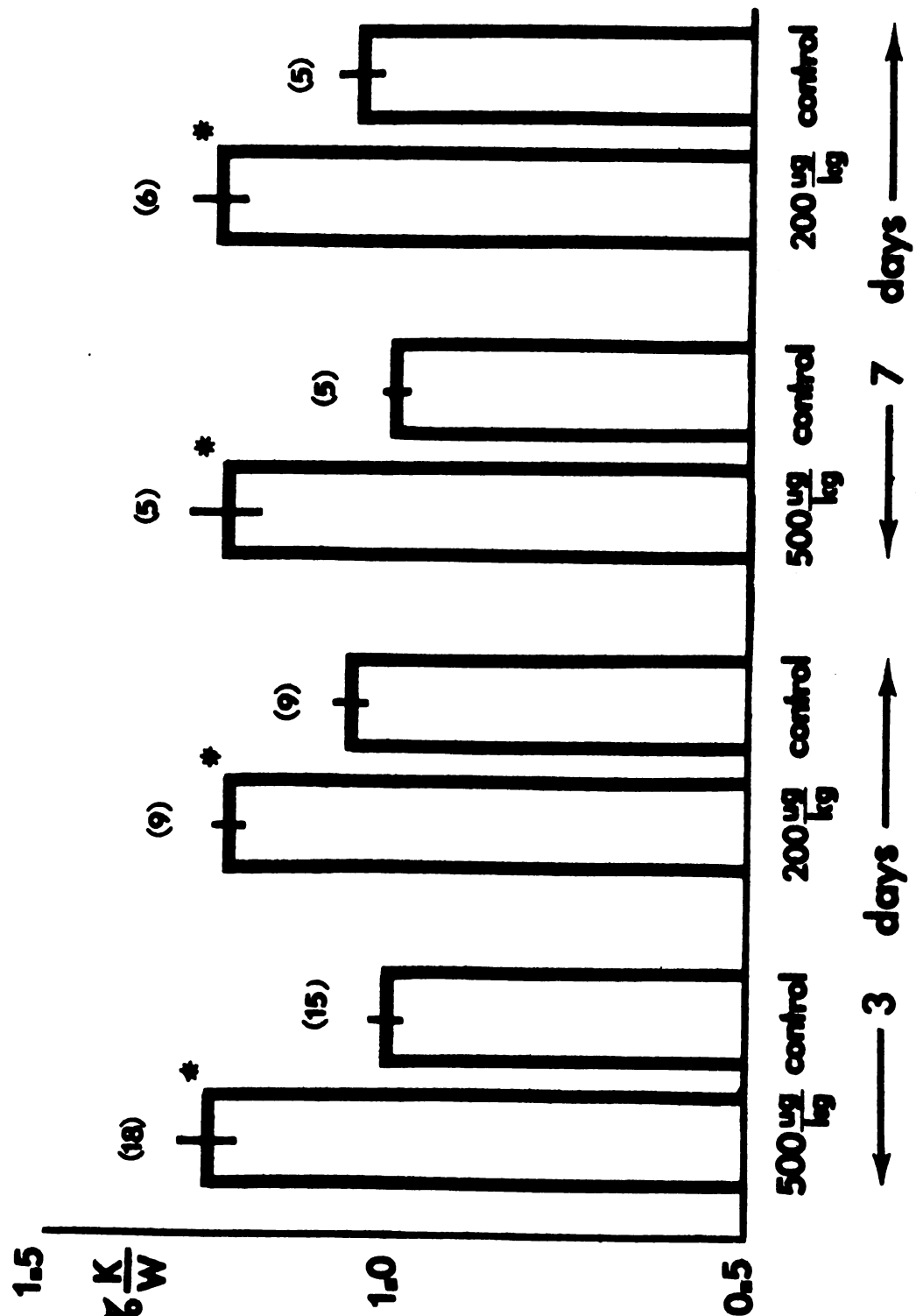
1.5
% K/W
1.0
0.5
(18)
(15)
(9)
(9)
(5)
(5)
(6)
(5)
*
*
*
*
500 µg/kg
control
200 µg/kg
control
500 µg/kg
control
200 µg/kg
control
3 days
7 days

Figure 17

Figure 18: Effect of T_3 administration to adult rats on PAH and NMN accumulation (S/M ratio) by renal cortical slices, and on kidney weight. Adult male rats were injected intraperitoneally with 500 µg/kg of T_3 once daily for 3 days. Controls received alkaline saline and all animals were killed 48 hours after the last injection. In the left panel each bar represents the mean PAH or NMN S/M ratio ±(S.E.) while the results in the right panel are expressed as the percentage of kidney weight/body weight ±(S.E.). The numbers in parentheses indicate the number of animals in each group. There was no significant difference between treated and control effects in any case ($P > .05$, group comparison).

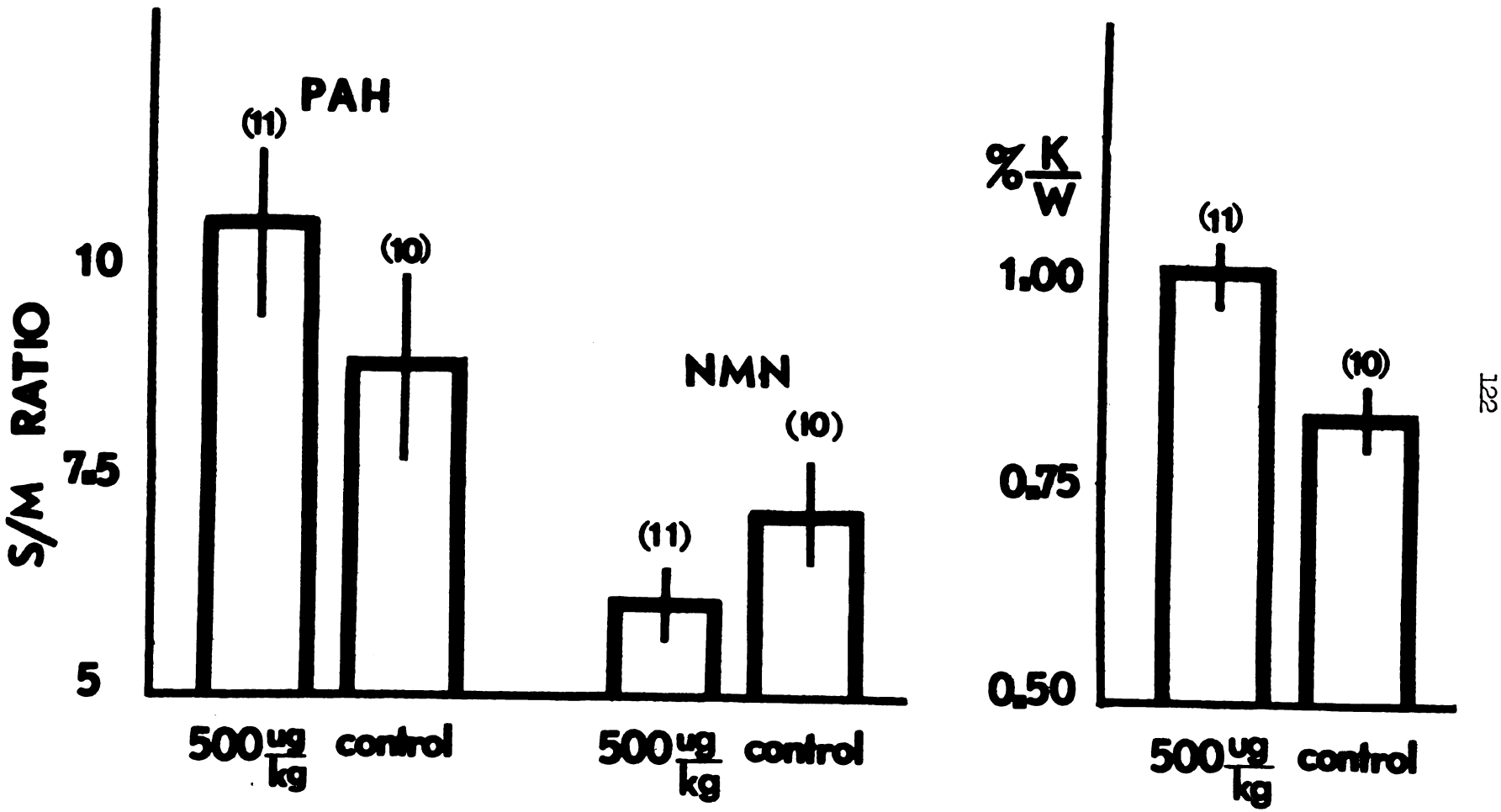
PAH
(11)
(10)
NMN
(11)
(10)
S/M RATIO
10
7.5
5
500 ug/kg
control
500 ug/kg
control
%K/W
1.00
0.75
0.50
(11)
(10)
500 ug/kg
control

Figure 18

Figure 19: PAH and NMN accumulation (S/M ratio) by rat renal cortical slices after the addition of T_3 to the incubation medium. The final concentration of T_3 was 10^{-6}M or 10^{-8}M. Values represent the mean S/M ratio ±(S.E.). The numbers in parentheses indicate the number of animals in each group, while the asterisks indicate values significantly different from control ($P < .05$, paired comparison).

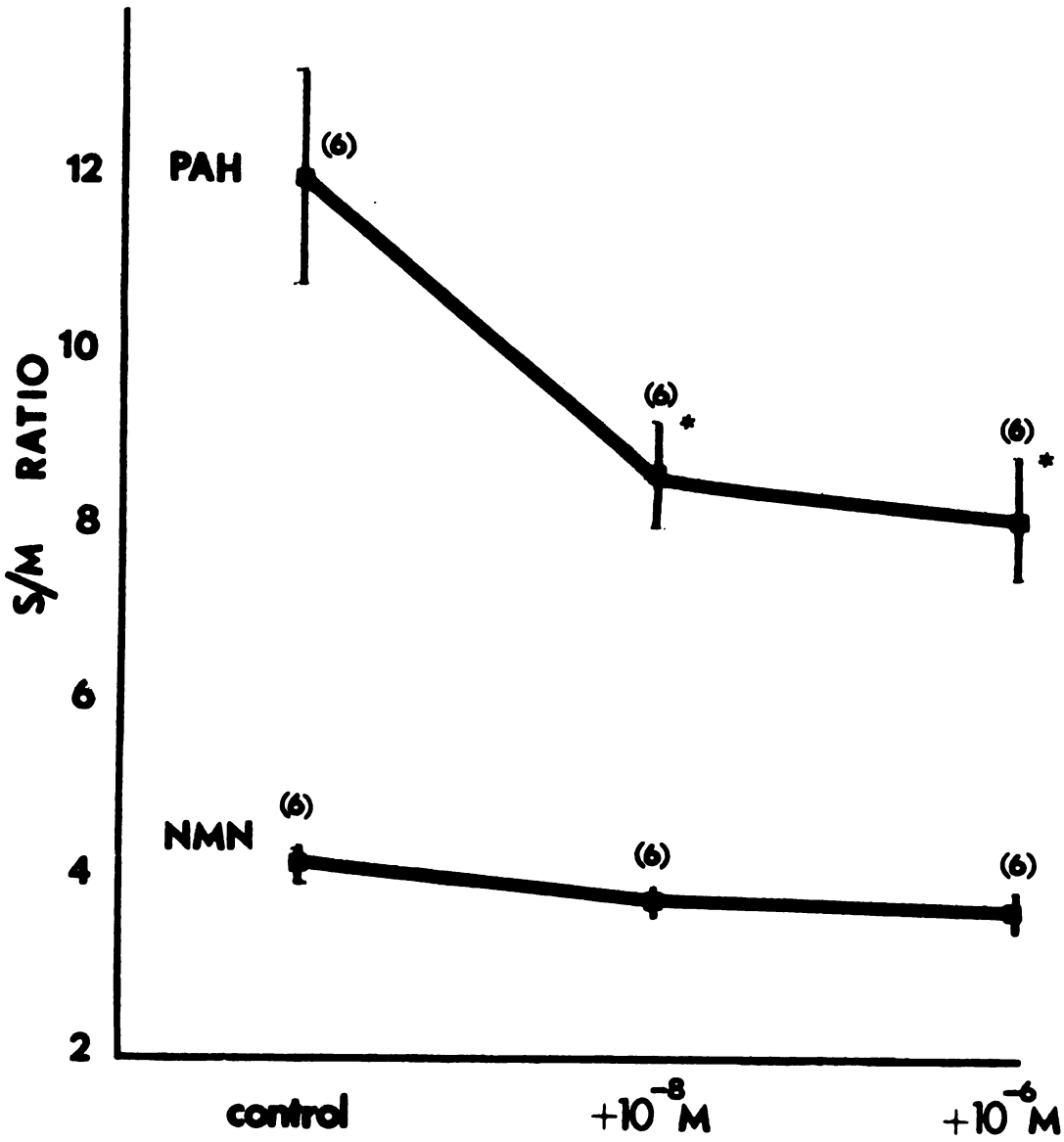

Figure 19

Figure 20: Effect of *in vivo* penicillin treatment and *in vitro* acetate addition on PAH accumulation (S/M ratio) by rat renal cortical slices. Nursing rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Sodium acetate (final concentration 10^{-2}M) was added to one-half of the beakers containing renal cortical slices from control or treated rats. Each bar represents the mean ±(S.E.) obtained using 6 litters, and is significantly different from all other values ($P < .05$). Data were analyzed by Duncan's test following analysis of variance. Coefficient of variability = 13.65.

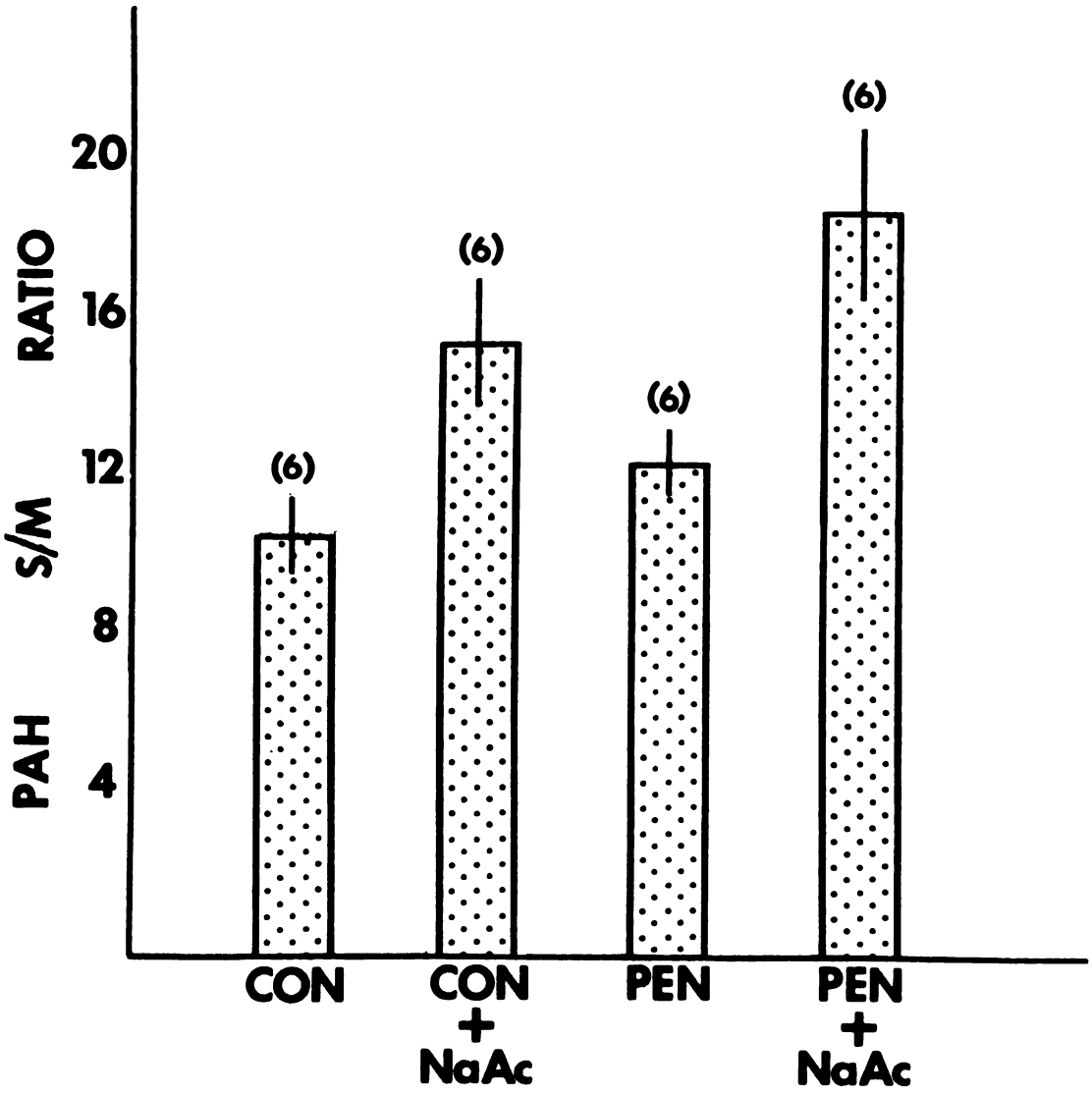

Figure 20

Figure 21: Effect of penicillin treatment of nursing rats on initial rate of PAH uptake by renal cortical slices. Nursing rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Slices from treated animals (closed circles) from within a given litter were randomly distributed among 6 beakers which were incubated for times ranging from 2 to 30 minutes. Tissue from control littermates (open circles) was treated similarly. Points on the graph represent the means $\pm$(S.E.) obtained from 5 litters. Regression lines from 4 to 15 minutes (not drawn) were linear (control: $y=2.51x+17.69$; treated: $y=3.18x+21.78$), but the slopes were not significantly different ($P > .05$).

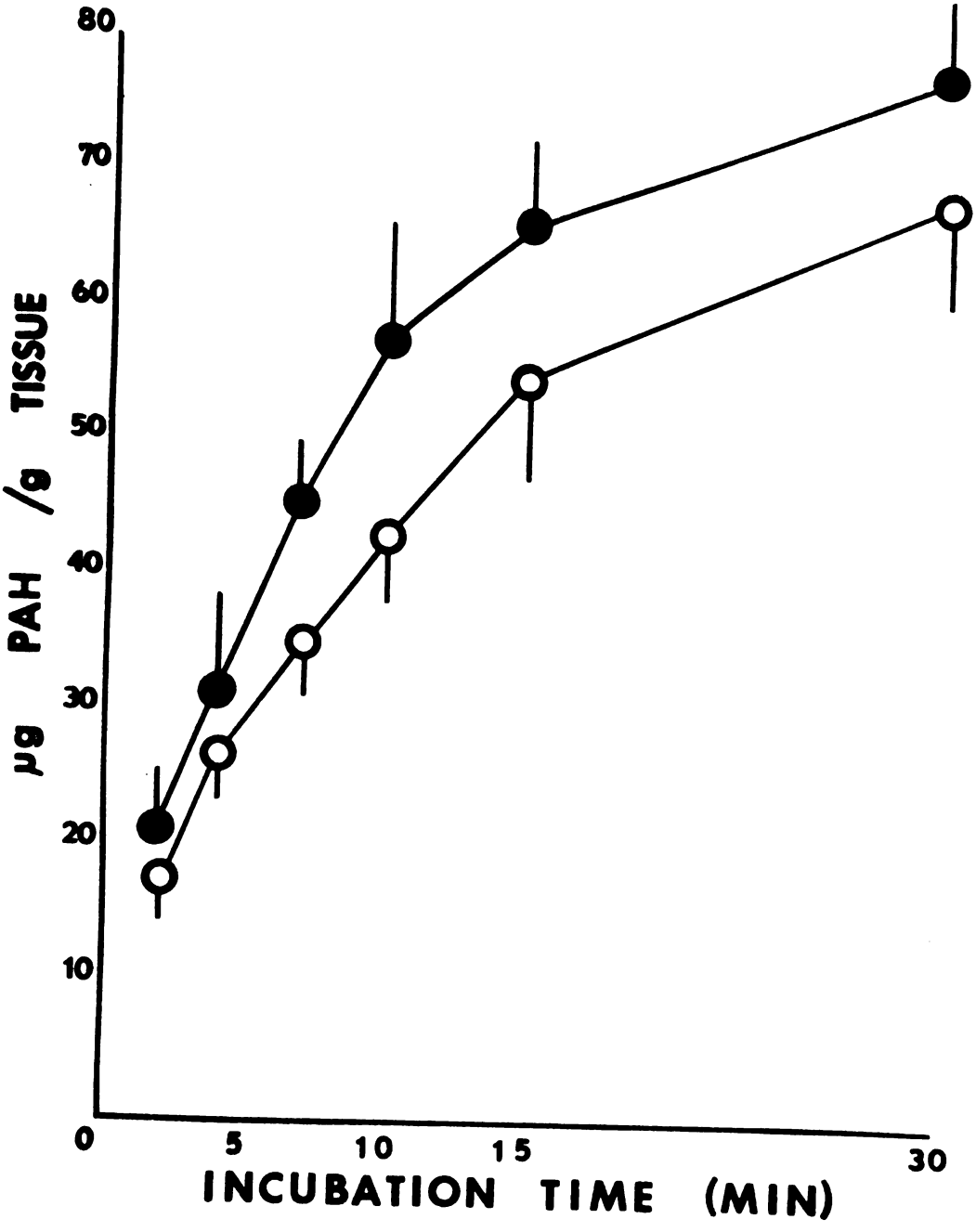
80
70
60
50
40
30
20
10
0
µg PAH /g TISSUE
5
10
15
30
INCUBATION TIME (MIN)

Figure 21

Figure 22: Effect of cycloheximide on the penicillin-induced enhancement of PAH accumulation (S/M ratio) by rat renal cortical slices. Nursing rats were treated subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Half of the penicillin-treated and half of the saline-control rats received 0.1 mg/kg cycloheximide intraperitoneally for the same length of time. Each bar represents the mean PAH S/M ratio obtained from 6 litters. Any bars overscored by the same line are not statistically different ($P < .05$). Data were analysed using Duncan's test following analysis of variance. Coefficient of variability = 14.90.

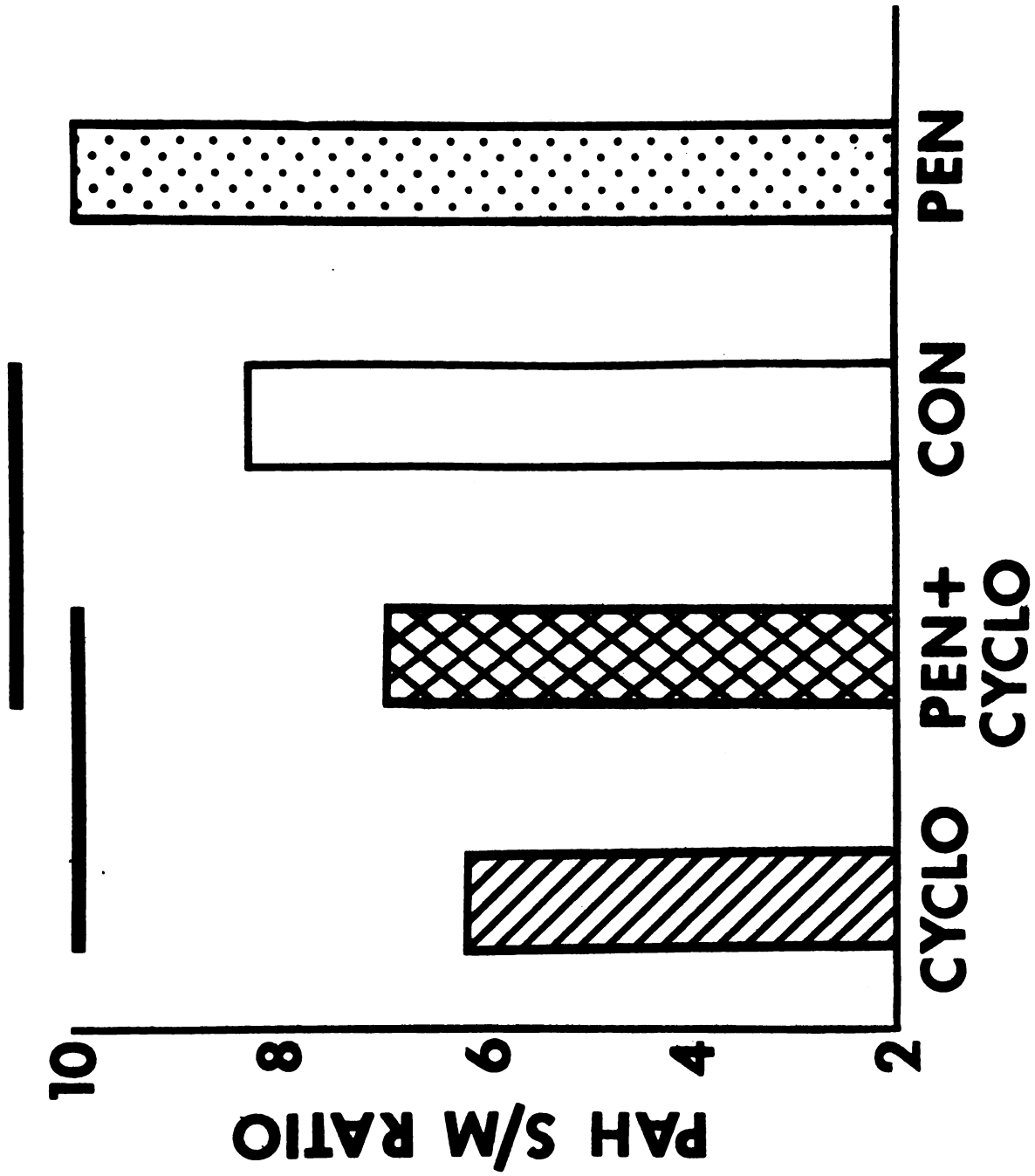

Figure 22

Figure 23: Accumulation of PAH (S/M ratio) and incorporation of L-leucine-C^{14} by renal cortical slices from nursing rats. Rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Ten microliters of L-leucine-C^{14}(.01 μc) were added to incubation beakers containing PAH medium and renal cortical slices from penicillin-treated or control nursing rats. The final leucine concentration was 2.7×10^{-4}M. Each bar represents the mean ±(S.E.) obtained from 6 litters. The asterisks indicate values significantly different from control ($P < .05$, paired comparison). C represents control values; T represents values from penicillin-treated rats.

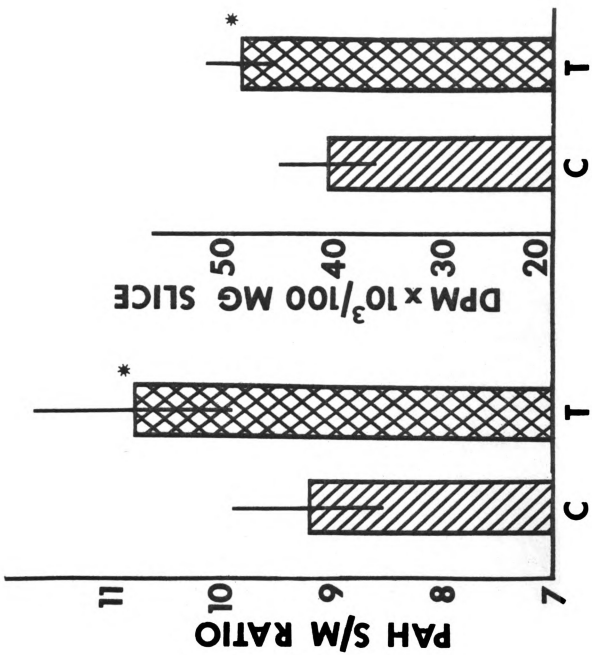

Figure 23

Figure 24: Effect of penicillin treatment of nursing rats on incorporation of L-leucine-C^{14} by renal cortical and renal medullary slices. Nursing rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Ten microliters of L-leucine-C^{14} (.01 μc) were added to incubation beakers containing PAH media and renal cortical slices from penicillin-treated or control nursing rats. The final leucine concentration was 2.7×10^{-4}M Each bar represents the mean ±(S.E.) obtained from 5 litters. The asterisk indicates significant difference from control ($P < .05$, paired comparison). C represents control values; T represents values from penicillin-treated rats.

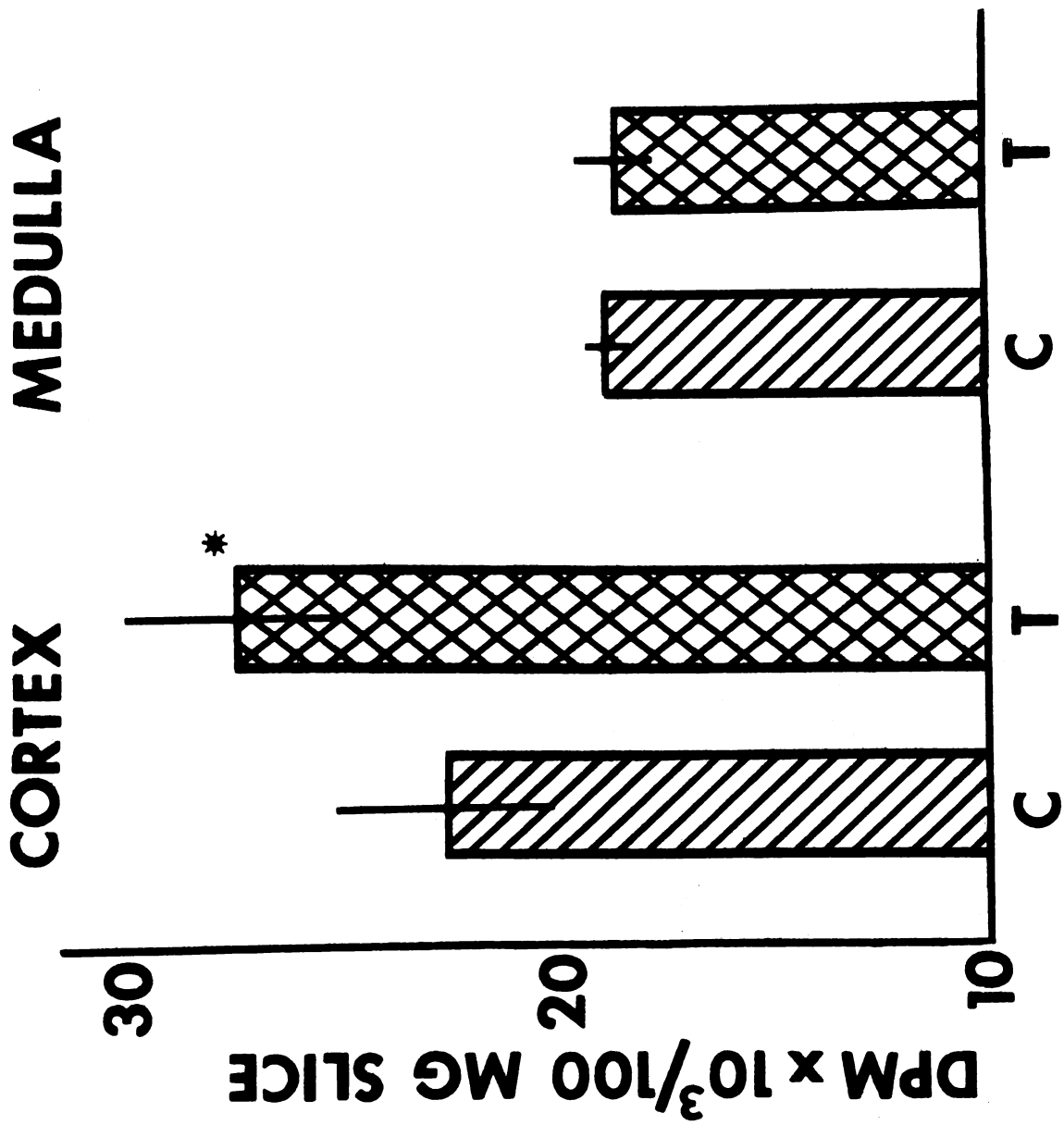

Figure 24

Figure 25: Effect of penicillin treatment of nursing rats on accumulation of PAH and incorporation of orotic acid-C^{14} by renal cortical tissue. Nursing rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Twenty-four hours after the last penicillin or saline injection the rats were injected with 0.2 µc/50g orotic acid-C^{14} intraperitoneally and killed 4 hours later. The quantity of orotic acid-C^{14} was determined in cortical tissue from one kidney of each rat while PAH accumulation (S/M ratio) was measured in cortical slices from the other kidney. Each bar represents the mean ±(S.E.) obtained from 4 litters. The asterisks indicate significant difference from control ($P < .05$, paired comparison). C represents control values; T represents values from penicillin-treated rats.

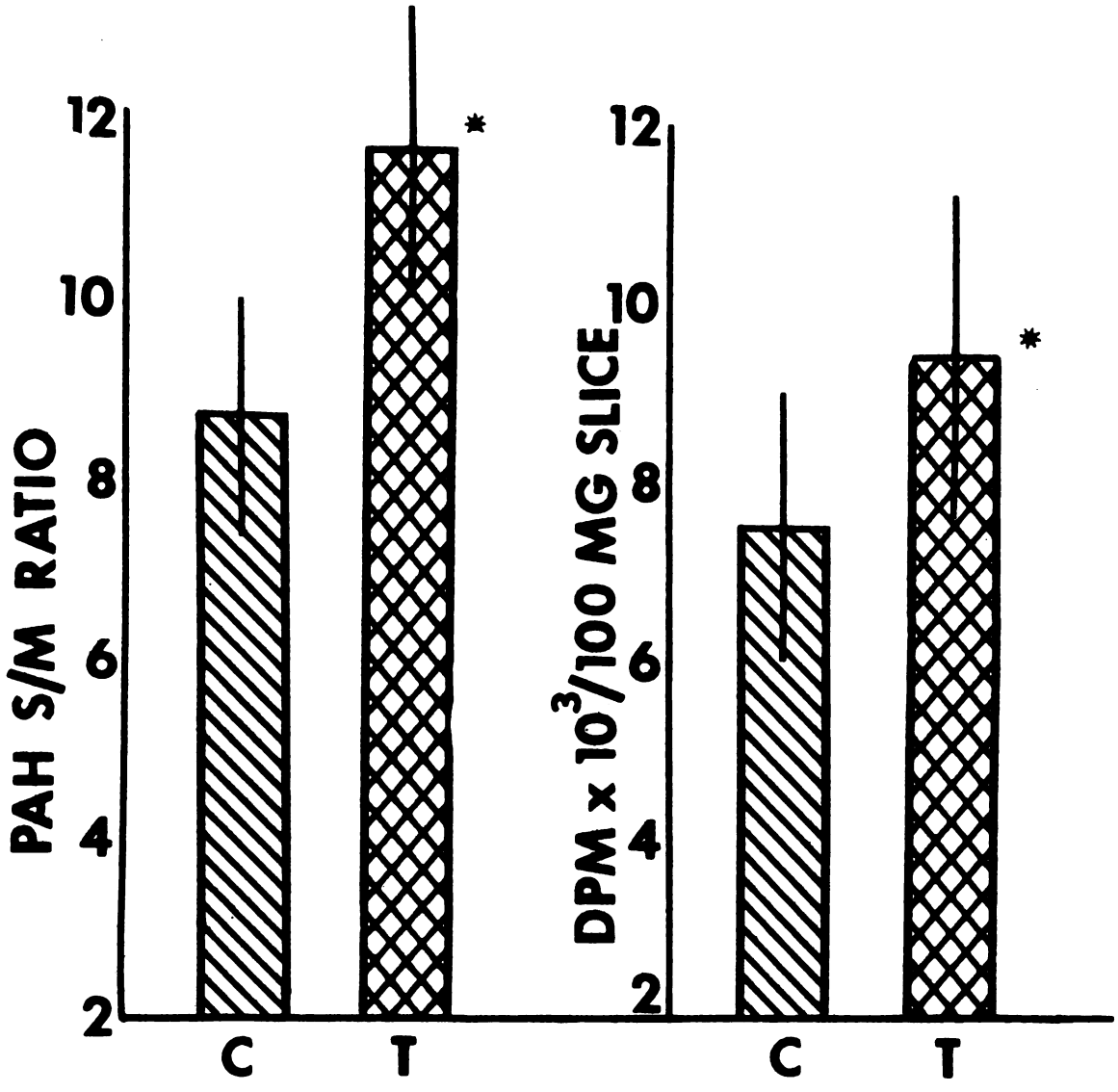

Figure 25

Figure 26: Incorporation of orotic acid-C^{14} into renal tissue after penicillin treatment of nursing rats. Nursing rats were injected subcutaneously with 30,000 IU procaine penicillin G, or saline, twice daily for 3 days. Twenty-four hours after the last penicillin or saline injection the rats were injected with 0.2 µc/50g orotic acid-C^{14} intraperitoneally and killed 4 hours later. The quantity of orotic acid-C^{14} was determined in each subcellular fraction after homogenization and centrifugation of both kidneys from each rat. Each bar represents the mean ±(S.E.) obtained from 4 litters. C represents control values; T represents values from penicillin-treated rats.

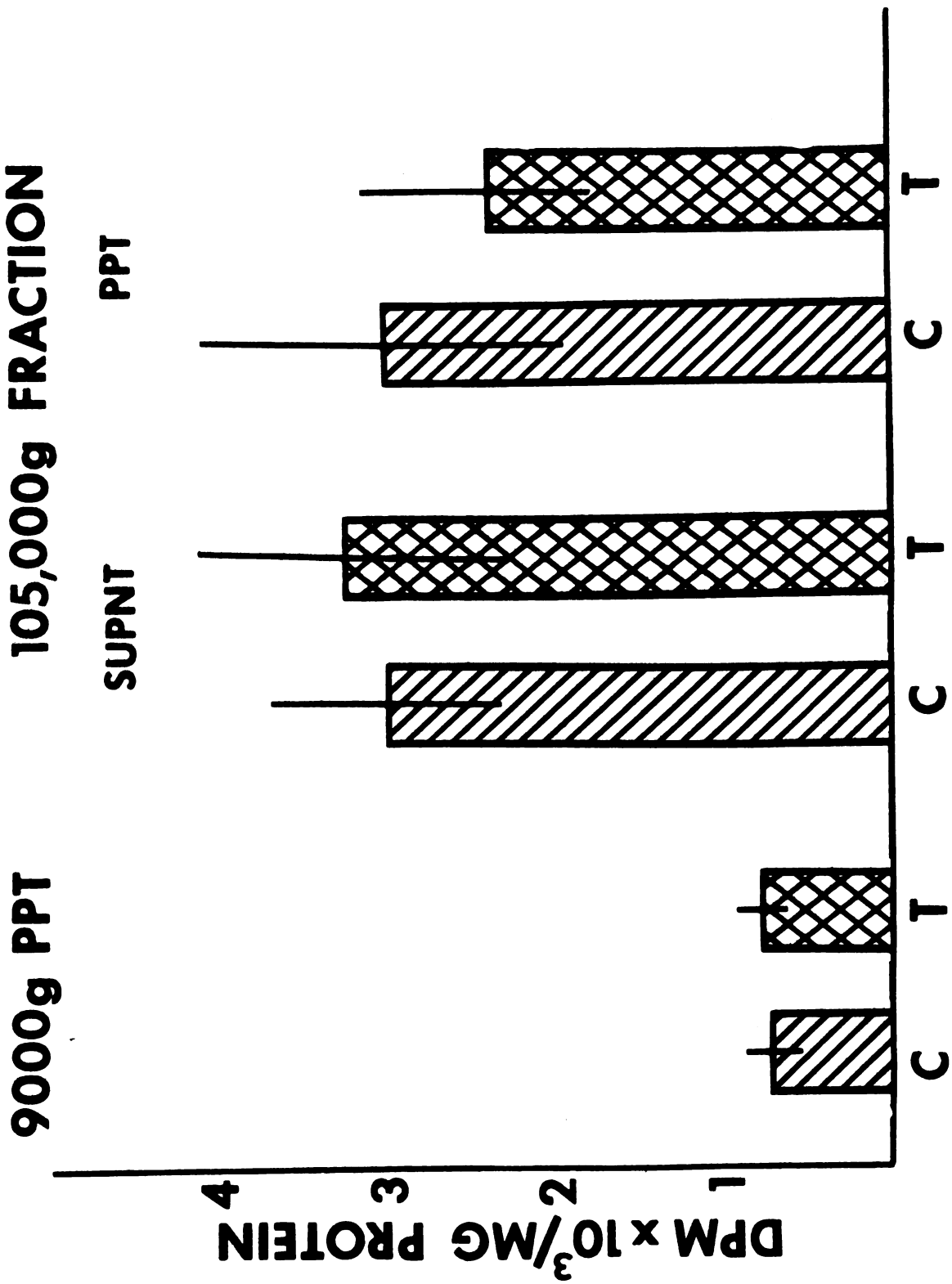
DPM x 10³/MG PROTEIN
4
3
2
1
9000g PPT
105,000g FRACTION
SUPNT
PPT
C
T
C
T
C
T
C
T

Figure 26

Figure 27: PAH accumulation (S/M ratio) by rabbit renal cortical slices 1 and 8 days after penicillin treatment. Rabbits were treated subcutaneously with 60,000 IU procaine penicillin G, or saline, twice daily from 11 to 13 days of age. One half of the treated and control rabbits from each litter were killed 24 hours after the last injection, while the remaining littermates were killed 8 days after treatment was discontinued. Each bar represents the mean ±(S.E.) obtained from 4 litters. The 14 day treated value is significantly greater than the 14 day control value, and is also significantly greater than both the 21 day treated and control values ($P < .05$, paired comparison).

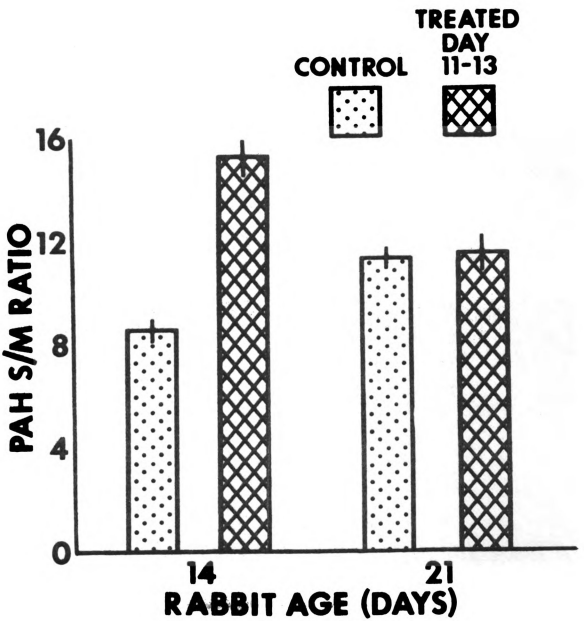
TREATED
DAY
11-13
CONTROL
16
12
8
4
0
PAH S/M RATIO
14
21
RABBIT AGE (DAYS)

Figure 27

Figure 28: Histological sections of kidneys from 2 week-old penicillin-treated and saline-control rabbits, 4 week-old, and adult rabbits.

A. Kidney from typical 2 week-old saline-control rabbit, X640. Hematoxylin-eosin stain. The glomerulus is small and densely cellular with prominent peripheral nuclei. Cortical tubules are small with crowded nuclei, limited amounts of cytoplasm and inconspicuous tubular lumens. Measured PAH S/M ratio: 6.62.
B. Kidney from typical 2 week-old penicillin-treated rabbit, X640. Hematoxylin-eosin stain. Tubules and glomerulus are indistinguishable from those in A. Measured PAH S/M ratio: 12.98.
C. Kidney from typical 4 week-old rabbit, X640. Hematoxylin-eosin stain. Cortical tubules are almost adult size and contain ample cytoplasm. Insert: Glomerulus greatly expanded and relatively less cellular than in kidney from 2 week-old rabbit. Measured PAH S/M ratio: 14.51.
D. Kidney from typical adult rabbit, X640. Hematoxylin-eosin stain. Cortical tubules are full sized. Insert: Fully developed, normally cellular glomerulus with only the occasional peripheral nucleus. Measured PAH S/M ratio: 8.98.

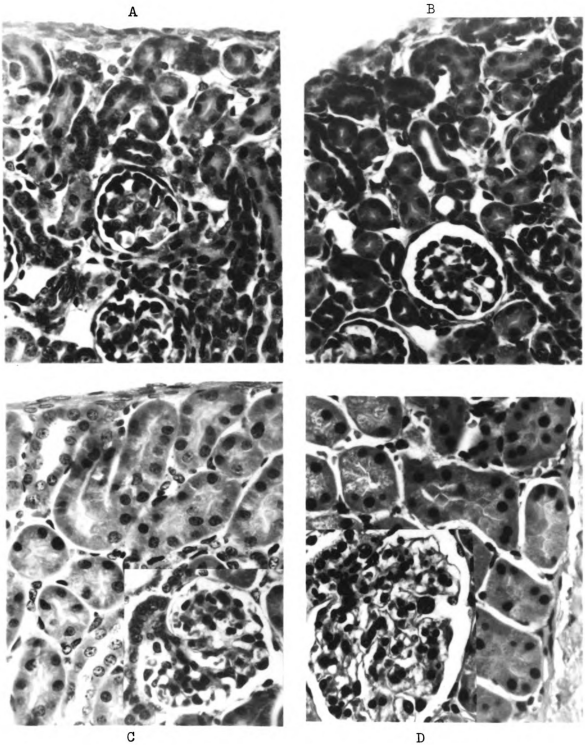

Figure 28

Figure 29: Accumulation of PAH (S/M ratio) by renal cortical slices after treatment of pregnant or newborn rabbits with penicillin. Pregnant rabbits were treated intramuscularly with 60,000 IU procaine penicillin G twice daily during the last half of pregnancy. PAH accumulation by renal cortical slices from 31 day old fetuses (half-filled circle) or offspring from 1 day to 4 weeks (dashed line) was measured. Rabbits were also treated subcutaneously twice daily with 60,000 IU procaine penicillin G from 11 to 13 days of age , and PAH S/M ratios determined 1 and 8 days after discontinuing treatment (broken line). The solid line represents the normal PAH S/M ratio developmental pattern presented in Figure 4.

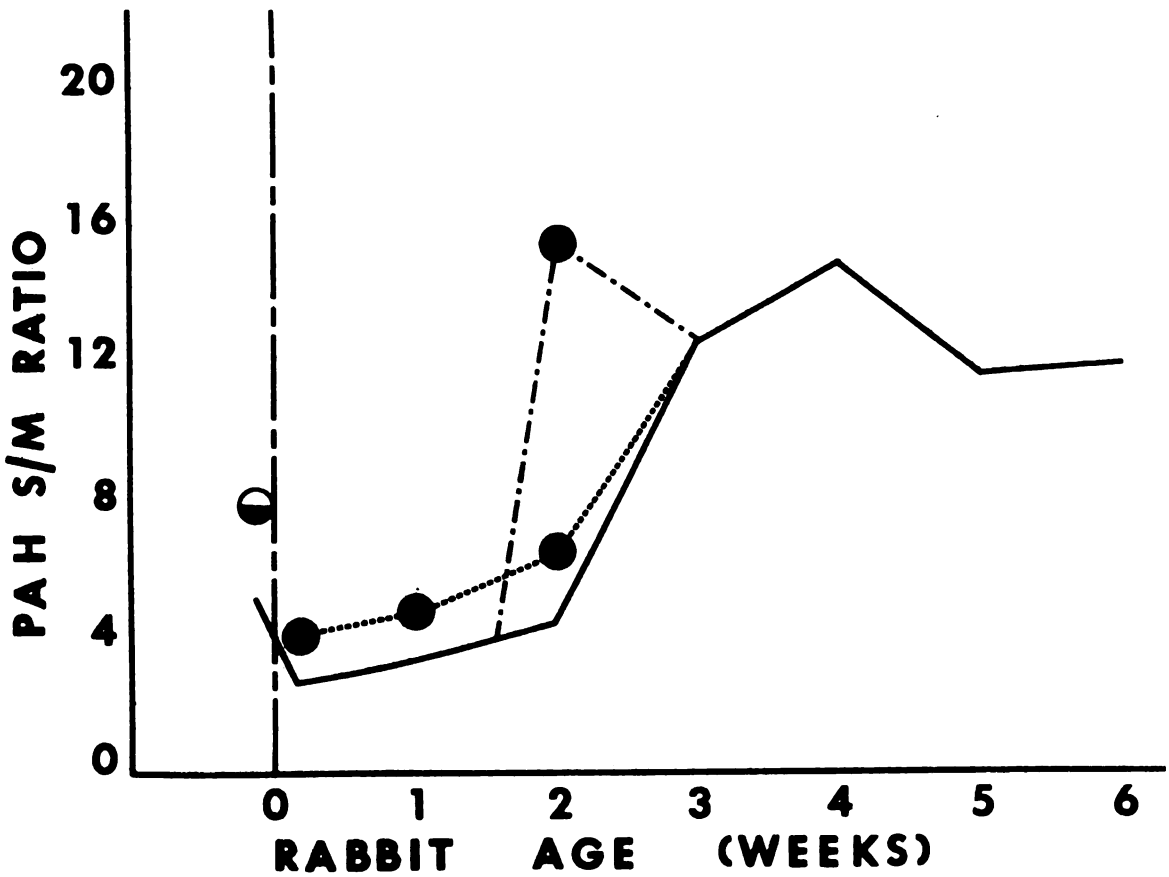

Figure 29

Figure 30: PAH accumulation (S/M ratio) by renal cortical slices after treatment of weanling rats with folic acid. Weanling rats received a single intraperitoneal injection of 125 mg/kg or 250 mg/kg of folic acid dissolved in 0.01M sodium carbonate. Controls received an equal volume of 0.01M sodium carbonate, and all animals were killed 2, 3 or 4 days after injection. Each bar represents the mean ±(S.E.) obtained using the number of weanling rats indicated in parentheses. Values from treated animals are significantly greater than their respective controls in all cases ($P < .05$, group comparison).

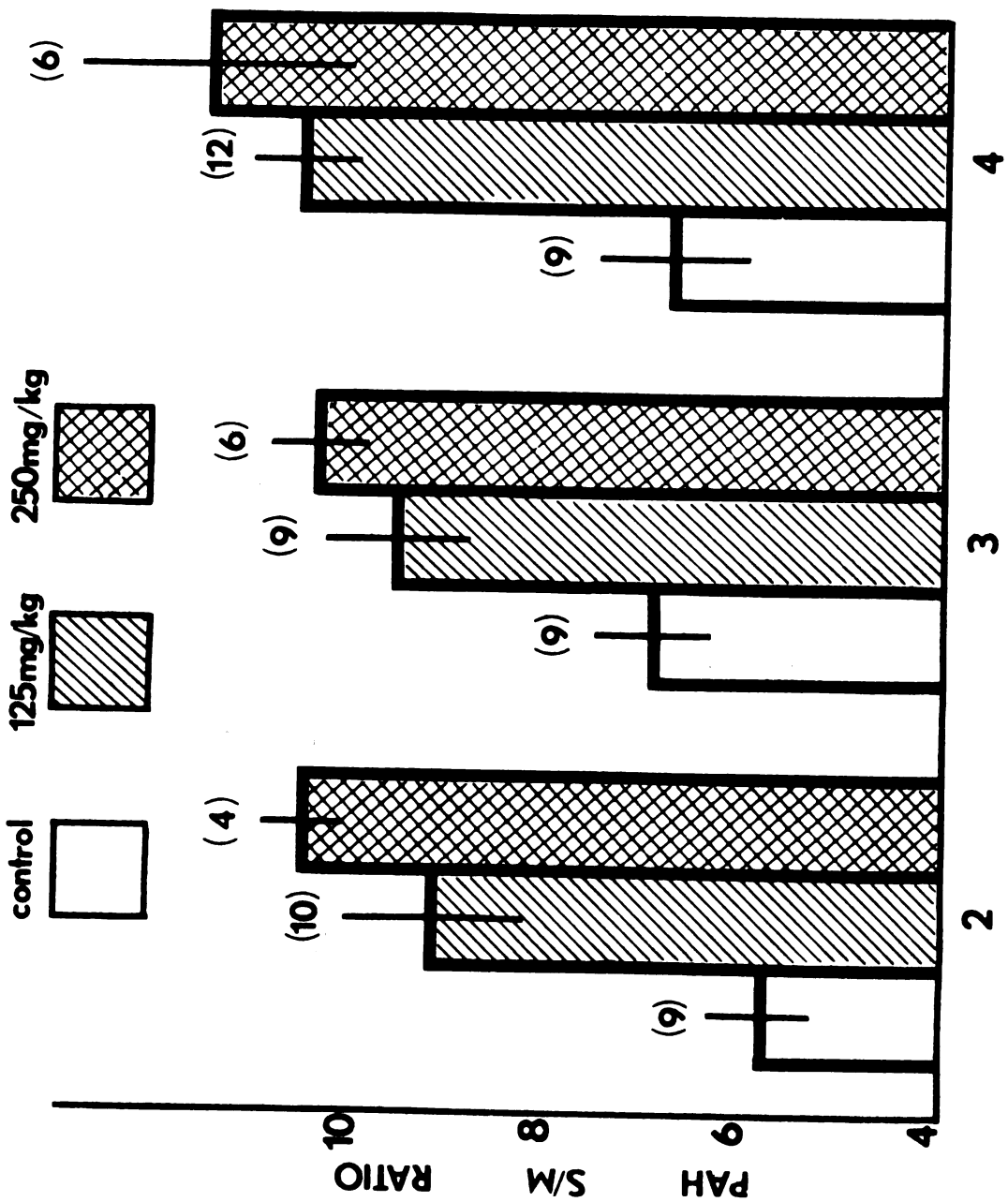
control
125mg/kg
250mg/kg
PAH S/M RATIO
10
8
6
4
2
3
4
DAYS AFTER FOLIC ACID
(4)
(10)
(9)
(6)
(9)
(9)
(6)
(12)
(9)
(6)

Figure 30

Figure 31: Effect of phenobarbital on PAH, TEA and NMN accumulation (S/M ratio) by rabbit and rat renal cortical slices. Rabbits were treated intraperitoneally with 15 mg/kg phenobarbital twice daily from 11-13 days of age, while 16 day old rats were similarly treated with 40 mg/kg phenobarbital for 3 days. Control animals received saline and all animals were killed 24 hours after the last injection. Each bar represents the mean S/M ratio ±(S.E.) obtained from 4 litters of rabbits and rats, each containing 6 to 8 animals. The PAH S/M ratio in slices from phenobarbital-treated rabbits is significantly different from control ($P < .05$, paired comparison).

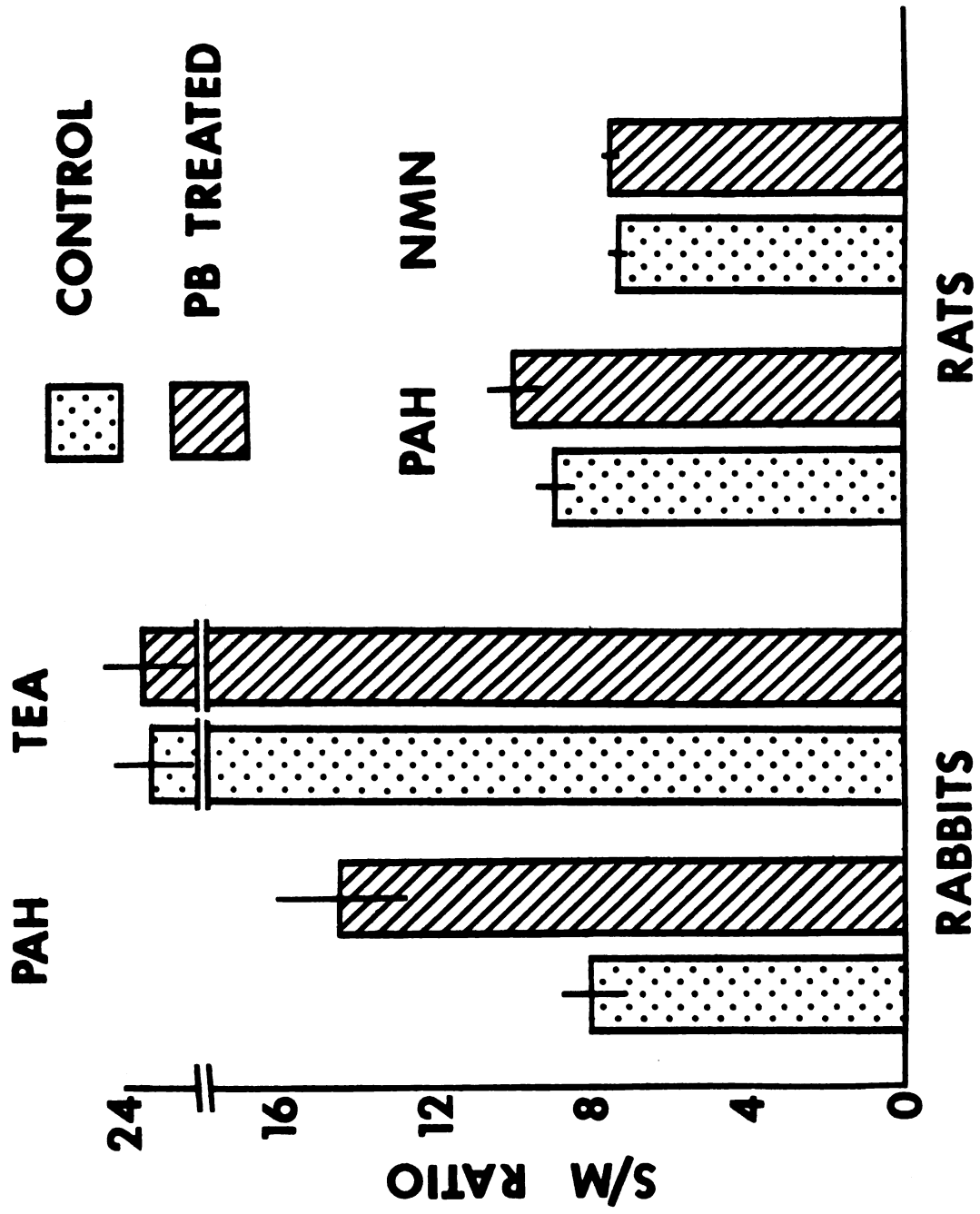
CONTROL
PB TREATED
S/M RATIO
24
16
12
8
4
0
PAH
TEA
PAH
NMN
RABBITS
RATS

Figure 31

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BIBLIOGRAPHY

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