DEVELOPMENT OF RENAL ORGANIC ION TRANSPORT IN THE NEWBORN RAT

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ABSTRACT

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TRANSPORT IN THE NEWBORN RAT

By

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Development of organic ion transport was determined in renal cortical slices from newborn rats. The ability of renal cortical slices to accumulate the organic ions, p-aminohippurate (PAH) and N-methylnicotinamide (NMN), was used as an index of the transport capacity in adult and newborn rats, 2 to 20 days old. Accumulation of organic ions was expressed as the slice/medium (S/M) ratio. The PAH S/M ratio gradually increased from 2 days until 10 days and then declined to 14 days and began to rise until 20 days, but had not reached adult levels at 20 days. The NMN S/M ratio was also low at 2 days and increased until 14 days of age. After 14 days the NMN S/M ratio remained unchanged.

Increasing litter size markedly reduced body and kidney weight measured at 20 days, but no significant differences in accumulation of PAH and NMN by renal cortical slices were observed.

Removal of potassium and sodium from the medium markedly depressed the accumulation of PAH in renal cortical slices

from 10 day, 20 day and adult rats. The effect of altering potassium and sodium concentration on PAH accumulation was the same at all ages. NMN accumulation was not as sensitive to changes in these inorganic ion concentrations.

The rate of PAH uptake by tissue from 20 day was less than that from adult. The data suggest that the increase in accumulation of organic ions that occurs with age results from an increased number of functional transport sites.

Acetate markedly enhanced the rate of PAH uptake in adult tissue. But acetate had no effect on PAH accumulation by renal cortical slices from 2 to 12 days of age. Thereafter, the magnitude of acetate stimulation of PAH uptake increased with age. Acetate increased NMN accumulation by renal cortical slices at all ages. The magnitude of stimulation was lower than that of PAH and was the same at all ages. At high pH (8.2) acetate markedly increased the PAH S/M ratio in addition to the enhancement of the PAH (S/M) ratio at high pH.

The ability of renal cortical homogenates to synthesize PAH was used as an index of glycine acyltransferase activity in young and adult rats. This was done to test the hypothesis that acetate enhances PAH uptake by formation of acetylglycine, thereby decreasing available glycine in the tissue. PAH synthesis by renal cortical homogenates from 10 day old rats was lower than that from adults. The rate of PAH synthesis by renal cortical homogenates, measured by varying glycine concentration in the reaction mixture, also

was low in young rat tissue. Acetate had no effect on the ability of renal cortical homogenates to synthesize PAH under conditions studied (incubation time or varying glycine concentration) in either age group. In chicken kidney, acetate enhanced the PAH S/M ratio consistently but no PAH synthesis could be measured. These data suggest that the mechanism of acetate stimulation on PAH transport is not associated with the formation of acetylglycine.

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INTRODUCTION

Organic Ion Transport In The Kidney

Tubular secretion is a discrete renal process which is known to be involved in the elaboration of urine. Glomerular ultrafiltration and tubular reabsorption are also involved. The first positive demonstration of renal tubular secretion of a foreign substance was made in 1923 when Marshall and Vickers showed that the dye, phenolsulfonphthalein (phenol red, PSP) was excreted by the tubules of the dog kidney. Subsequent studies of Chambers and Kempton (1933) emphasized the active nature of this secretory mechanism. Using cultured cysts of chick embryo mesonephric tubules, they observed intraluminal accumulation of PSP in higher concentration than in the surrounding medium. Within the next few years similar studies on the excretion of PSP were performed in dog (Shannon, 1935) and man (Goldring et al., 1936; Smith et al., 1938). Since then a large number of other organic acids have been shown to be transported by the same secretory system. Some of these organic acids are Diodrast, hippuran, p-aminohippuric acid (PAH), penicillin, and probenecid. Similarly, organic bases such as N-methylnicotinamide (NMN), tetraethylammonium (TEA), quinidine, and guanidine are actively secreted by the kidney. Any substance secreted by the renal tubules may be filtered through the glomeruli. Hence, the rate of excretion

is the sum of the rate of filtration and the rate of tubular secretion. These substances compete with one another for what appears to be a common transport mechanism, but none of the substances of the organic acid group of compounds competes with or depresses the secretion of organic bases and vice versa. The site of secretion has been defined as the proximal tubules by the stop-flow technique devised by Malvin et al. (1958).

The overall process of transporting a substance from peritubular fluid into the tubular lumen involves at least two barriers or steps. Step 1 represents transport across the cell membrane on the peritubular side of the cell with subsequent intracellular accumulation. Step 2 is the transport across the luminal membrane into the tubular fluid (Taggart, 1958).

Cross and Taggart (1950) observed that thin slices of rabbit renal cortex are capable of accumulating PAH from a saline suspending medium against a considerable concentration gradient. They expressed the accumulation of PAH as the slice/medium (S/M) ratio. The S/M ratio was depressed by a variety of inhibitors such as 2,4-dinitrophenol. Cross and Taggart concluded that the accumulation of PAH by renal cortex slices in vitro and the tubular excretion of this compound in the intact animal are closely related phenomena. The most obvious difference between the two arises from the absence of a continuous filtration process in the slice and the consequent disturbance of the normal relationship between the cells and tubular urine. The S/M ratio, the concentration

gradient at any given time, is the result of two processes: active intracellular accumulation and diffusion of material back into the medium (runout). This is contrasted with 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, it is highly probable that the same biochemical processes operate in the two situations. Furthermore, the slice technique has certain advantages; for instance, the chemical composition of the ambient fluid can be fairly rigidly controlled and the influence of certain extrarenal factors which may alter tubular excretion in the intact animal are excluded.

Since Cross and Taggart introduced the use of the renal cortical slice technique, a great deal of work has been done in attempts to elucidate the nature and site of the PAH transport system. The intracellular accumulation of PAH is an active transport step (Forster and Copenhaver, 1956; Foulkes and Miller, 1959; Foulkes, 1963). Foulkes and Miller (1959) presented a four-step model of the PAH transport system in slices. Step I is diffusion of PAH from the medium into extracellular space in the tissue; step II is a facilitated diffusion step at the peritubular cell membrane; step III within the cell is a build-up of a high tissue concentration of PAH; finally, step IV transfers PAH across the luminal border of the cell into the tubular lumen from which it diffuses back into the medium. In this model, step III probably is the active transport system. Foulkes and Miller also proposed the existance of two intracellular fractions of PAH in renal cortical slices of the

rabbit. One of these fractions is rapidly diffusible and rapidly equilibrates with extracellular PAH. The other fraction, in contrast, diffuses and equilibrates slowly and is responsible for the high S/M ratio of PAH. Movement of PAH across the luminal membrane is by diffusion down a concentration gradient into the tubular lumen. Tune et al. (1969) also showed that PAH is actively transported into the tubule cell at the peritubular membrane and subsequently diffuses into the luminal fluid. Using a suspension of rabbit cortical tubules Burg and Orloff (1969) obtained similar results. Foulkes (1963) and Welch and Bush (1970) also suggested at least two intracellular pools, a bound or compartmentalized fraction and a freely diffusible pool. The subject is still unsettled, however, since Carrasquer and Wilczewski (1971) have presented evidence that energy is required for PAH to cross the peritubular membrane.

Cation Requirements for PAH Transport

In the early studies on the accumulation of PAH by thin slices of rabbit kidney cortex, it was noted that certain variations in the electrolyte composition of the medium could alter the rate of PAH transport (Cross and Taggart, 1950). In general, PAH transport operates maximally only when the potassium and sodium content of kidney cortex approximates normal values; potassium 40 mM/L, sodium 110 mM/L. It was demonstrated that the maintenance of normal cellular electrolyte composition is the most important single factor determing the rate of PAH transport (Taggart et al., 1953).

Foulkes and Miller (1960) observed severe depression of PAH accumulation in potassium-deficient kidney cortex slices of rabbits. They postulated that both the peritubular membrane step and the intracellular concentration reactions required potassium. A depression in rabbit cortical slice potassium by incubation with strophanthidin decreased the S/M ratio for PAH without significantly affecting oxygen consumption (Burg and Orloff, 1962).

Sodium also has an important role in PAH transport, although the specific effect on PAH transport has not yet been elucidated. Vogel et al. showed a proportional relationship between the transport of sodium and that of PAH in the doubly perfused frog kidney (Vogel et al., 1965; Vogel and Kröger, 1965 and 1966; Vogel and Stoeckert, 1966). Removal of sodium or calcium from the media resulted in a marked reduction of PAH and PSP transport (Chung et al., 1970).

Effect of Acetate on Organic Ion Transport

Many investigators have attempted to study the biochemical features of active transport in the kidney. Cross and Taggart (1950) demonstrated effects of various metabolic intermediates on PAH accumulation in rabbit renal cortex slices. Of all the substrates tested, acetate exhibited the most striking and uniform effect on PAH accumulation. Lactate and pyruvate also consistently increase the ratio, although never to the same degree as acetate. The members of the tricarboxylic acid cycle, α-ketoglutarate, succinate, fumarate,

and malate, all inhibited PAH accumulation. Oxalacetate, however, stimulated PAH accumulation, possibly as the result of its decarboxylation to yield pyruvate. Amino acids and fatty acids also inhibited PAH accumulation. Since there was no correlation between cellular respiratory stimulation and PAH accumulation, the workers suggested that a general acceleration of oxidative activity was not the underlying cause. These observations of acetate stimulation and fatty acid inhibition were to play a major role in subsequent investigations of the PAH transport mechanism. In the intact dog, Mudge and Taggart (1950) showed that acetate and lactate increased Tm_{PAH} whereas succinate and fumarate decreased Tm_{PAH}. The absence of any appreciable change in glucose Tm by acetate could be evidence that the number of active nephrons remained unaltered. Acetate also rapidly reversed the self-depressed Tm_{PAH} that occurs at high plasma PAH concentration (Schachter and Freinkel, 1951). Acetate increased PAH uptake (Ross and Farah, 1966) but decreased runout in slices of dog renal cortex (Farah et al., 1963; Farah and Frazer, 1964). Accumulation of the organic bases, NMN and TEA, was augmented by acetate also (Farah et al., 1959; Farah and Rennick, 1956). However, the stimulation of PAH accumulation was much greater. Schachter et al. (1955) investigated the possible role of acylglycines in the stimulation of PAH transport by acetate. These workers studied the synthesis, degradation, and active accumulation of a number of aliphatic acylglycines by kidney tissue of several species. It had been shown that synthetic reactions, like that for hippurate, involve the formation of the acyl thioesters of

coenzyme A which are the energy rich intermediates in acylglycine synthesis. The reactions are as follows:

1) $R\text{-}COOH+ATP+CoA\text{-}SH \rightleftharpoons R\text{-}CO\text{-}S\text{-}CoA+AMP+PPi$

2) $R\text{-}CO\text{-}S\text{-}CoA+NH_2\text{-}CH_2\text{-}COOH \rightleftharpoons R\text{-}CO\text{-}NH\text{-}CH_2\text{-}COOH+CoA\text{-}SH$

Further studies showed that a series of these acylglycines were themselves actively accumulated by kidney cortex slices (Schachter et al., 1955). However, since they are simultaneously hydrolyzed by enzymes in the kidney at rates decreasing with lengthening carbon chain of the acyl group, significant S/M ratios could only be observed with long chain acylglycines such as octanoglycine and caproylglycine. These series of aliphatic acylglycines and the aromatic acylglycine, hippurate, inhibited the active uptake of PAH. But acetylglycine did not inhibit at all. This observation provided a reasonable explanation of the inhibition of PAH accumulation in vitro and in vivo by fatty acids which undergo conjugation with glycine. The acylglycine thus formed could compete with PAH for transport. They also observed that acetate stimulation of PAH transport occurred only in those species (dog, rabbit, pigeon, rat and guinea pig) whose kidneys are capable of forming acylglycines. In those species unable to form acylglycines (chicken, turkey, duck, goose, and dogfish), PAH transport was not stimulated by acetate. The rat appeared to be an exception, for little stimulation of transport was reported to occur with acetate yet enzyme activity was relatively high (Despopoulos, 1956; Schachter et al., 1955). According to this hypothesis the administration of acetate decreases the

available glycine for the production of the long-chain acyl-glycines by forming acetyl glycine which does not compete with PAH for transport. Therefore, acetate, enhanced the PAH uptake by preventing the formation of inhibitors. However, this hypothesis has not been subjected to extensive experimental inquiries. Murdaugh and Elliot (1969) demonstrated that the presence of excess glycine did not inhibit the acetate enhancement of PAH transport. Another interesting point concerning the effect of acetate on PAH accumulation deals with alterations in pH of the medium. It has been reported that maximal PAH incorporation into renal cortical slices in rats and rabbits occurs above pH 8 (Copenhaver and Davis, 1965) and they observed that at this high pH acetate did not further enhance the accumulation of PAH. Thus, the exact mechanism of enhanced PAH transport by acetate is not yet elucidated.

The Maturing Kidney

Newborn mammals undergo dramatic physiological adjustments in their regulatory functions such as respiration, nutrition, circulation and excretion correlated with the achievement of independence of the maternal circulation.

Glomerular filtration rate in young infants is well below that observed in adults, even when corrected for body size (Barnett, 1940). This observation has been confirmed both in humans (Dean and McCane, 1947; West et al., 1948) and various animal species; rat (Horster and Lewy, 1970; Potter et al., 1969), piglets (Gruskin et al., 1970) and

guinea pigs (Spitzer and Edelmann, 1971). The ability of the kidneys of newborn rats to excrete a concentrated or dilute urine (Falk, 1955) as well as ammonia and titratable acid (Goldstein, 1970) is also significantly less than that of adults.

Morphological development of the rat kidney is dominated by nephrogenesis at the first week after birth. At the end of the fourth week nephrogenesis is maximal in all layers of nephrons (Baxter and Yoffey, 1948). Bogomolova (1961) also showed that the tubular system is not fully formed and the brush border of proximal tubule is very poorly developed in newborn rats.

The development of organic ion transport has been investigated in both man and animals. Calgano and Rubin (1963) reported that E_{PAH} in infants is low. PSP excretion in newborn rabbits increased rapidly and regularly in the first 10 days after birth but did not exist to an important degree before birth (Williamson and Hiatt, 1947). Rennick et al. (1961) demonstrated that PAH and TEA S/M ratios are low at birth. They suggested that the transport systems developed independently in fetal and newborn puppy and piglet. The ability of rabbit renal cortical slices to accumulate PAH at various ages was reported by Hirsch and Hook (1970). These authors further demonstrated that the newborn transport system was capable of responding to substrate stimulation. Treatment of young rats and rabbits with organic acid (penicillin, PAH), substrates of the organic anion transport system, resulted in increased accumulation

of PAH by renal cortical slices from these animals without altering uptake of the organic base, NMN (Hirsch and Hook, 1969, 1970).

Gluconeogenic renal enzymes, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase are present in late rat fetal tissue but the activity is very low and increases rapidly postnatally (Zorzoli et al., 1969). Alkaline phosphatase, glutaminase and carbonic anhydrase (Wacker et al., 1961) and several enzymes of carbohydrate metabolism (Burch et al., 1971) are lower than adult level in newborn rat kidney. Dicker and Shirley (1970) also reported that the rate of oxygen uptake and of glycolysis is low in the cortex of newborn rats.

Development of the kidney is altered by maternal protein restriction. Zeman (1968) reported that kidneys from protein restricted animals have fewer and less well-differentiated glomeruli, a greater proportion of connective tissue, and relatively fewer collecting tubules. Dams fed a protein-deficient diet during pregnancy produce offspring that have altered kidney function, with marked reduction in glomerular filtration rate and depressed urine excretion during both water diuresis and osmotic diuresis (Hall and Zeman, 1968). They suggested that these effects may also be influenced by postnatal diet.

Purpose

The overall purpose of these studies was to quantitatively describe the development of the organic ion transport systems in the newborn rat kidney.

These investigations were divided into two segments: Determination of the development of renal organic ion transport systems; Determinations of factors involved in acetate stimulation of PAH transport.

Specific experiments were made to: 1) quantify the ability of rat renal cortical slices to accumulate PAH and NMN; 2) determine the effect of acetate upon the uptake of these organic ions; 3) determine the effect of nutritional changes on renal function by altering litter size at birth; 4) determine the potassium and sodium requirements of PAH and NMN transport system in tissue at varying ages; 5) determine, using a kinetic analysis, characteristics of the PAH transport system in tissue from newborn rats; and 6) determine the effect of acetate on PAH synthesis, which represents the activity of glycine acyltransferase (glycine N-acylase), in renal cortical tissue from young and adult rats.

METHODS

Development of Organic Acid and Base Transport in Renal Cortical Slices

Determination of Organic Ion S/M Ratio

Female Sprague-Dawley rats were bred in the departmental animal quarters so that young animals of known age could readily be obtained. Litter size was routinely reduced to 8 pups within 2 days of birth and young rats were left with their mothers until the time of experimentation.

Rats were sacrificed by a blow on the head and the kidneys removed immediately, weighed, and placed in ice-cold saline. Renal cortical slices were prepared free hand and briefly kept in cold saline until incubation. All incubations were conducted in duplicate. To obtain sufficient tissue the kidneys of several animals were pooled (i.e., at 2 days of age 8 pups were required for 1 duplicated incubation). Slices weighing approximately 100 mg were placed in 2.7 ml of the medium devised by Cross and Taggart (1950), and adjusted to pH 7.4. The concentration of PAH was 7.4×10^{-5}M and C^{14}-NMN was 6.0×10^{-6}M (2.5×10^{-2} µc/ml).

The slices were incubated for 90 min. under flowing 100% oxygen at 25° C in a Dubnoff metabolic shaker. After incubation the slices were quickly removed from the beakers, blotted on gauze and weighed. A 2 ml aliquot of medium was

taken from each beaker. To both tissue and media 3 ml of 10% TCA was added, the tissue was macerated, and the samples diluted to 10 ml with water. One ml aliquots of slice and media acid homogenates were added to scintillation vials containing 10 ml of modified Bray's solution (6 gm of 2,5-diphenyloxazole and 100 gm of naphthalene per liter of dioxane). Radioactivity was counted in a Beckman LS-100 liquid scintillation counter employing either internal or external standardization. PAH was measured by the method of Smith et al. (1945). Results were expressed as the final ratio of concentrations in slice and medium (S/M) where S equals mg/gm of tissue or disintegrations/min/gm of tissue and M equals mg/ml of medium or disintegrations/min/ml of medium.

Effect of Acetate

To determine the effect of acetate, 1×10^{-1}M sodium acetate was added to the incubation medium (final concentration, 1×10^{-2}M). To determine the effect of acetate in various concentrations in adult rat and adult New Zealand female rabbit cortical slices, the final concentrations of acetate in the medium were 0, 1×10^{-3}, 2×10^{-3}, 4×10^{-3} and 1×10^{-2}M.

Effect of Changing Litter Size

To determine the effect of litter size on development of organic ion transport, litter size was adjusted to 4, 8 or 12 pups within 2 days of birth. At 20 days of age, PAH and NMN S/M ratios were determined after 90 min incubation.

Effect of Potassium and Sodium

Tissue requirements for potassium and sodium were determined in slices from adult male, 10 and 20 day old rats. With sodium concentration constant at 100 mM the concentration of potassium in the medium was adjusted to 0, 5, 10, 20 or 40 mM. Similarly, with potassium concentration constant at 40 mM the concentration of sodium was adjusted to 0, 10, 50 or 100 mM. As potassium or sodium was removed, a constant medium osmolality was maintained by adding sucrose.

PAH Uptake

The rate of PAH uptake was determined in tissue from 20 day and adult rats. Slices from pooled kidneys were equally divided into 16 beakers (each experiment required kidneys from 3-4 adults or 16, 20 day old rats). Duplicate incubations were conducted at 4 PAH concentrations (1, 2, 4 and 8 x 10^{-4}M) and 2 times (2 and 12 min). The uptake of PAH/min at each concentration was determined and the data were plotted on Hofstee and Lineweaver-Burk plots (Christensen and Palmer, 1969).

Effect of Acetate and pH

The effect of acetate and high pH on PAH accumulation in renal cortical slices of 10 day and adult rats was also determined. To increase pH of the medium to 8.2, 0.1 ml of the 0.1 M sodium phosphate from Cross and Taggart medium was replaced with 0.1 ml of 0.2 M 2-amino-2-methyl-1,3 propanediol.

PAH Synthesis in Kidney Tissue

PAH Synthesis in Renal Cortical Homogenates

The rate of PAH synthesis which represents the activity of glycine acyltransferase was determined in renal cortex from adult and 10 day old rats and White Leghorn chickens.

Preparation of the enzyme: Sprague-Dawley rats were sacrificed by a blow on the head and the kidneys were removed immediately, and placed in ice-cold 0.25 M sucrose. Renal cortex was homogenized with cold 0.25 M sucrose in a glass homogenizer with a glass pestle.

Incubation procedure: The incubation mixture contained potassium phosphate buffer (pH 7.56), 25 µmoles; magnesium chloride, 5 µmoles; glycine, 45 µmoles; fumarate, 2.5 µmoles; ATP, 2.5 µmoles; p-amino benzoic acid, 3 µmoles; and 0.3 ml of kidney homogenate. The final volume was 1 ml. All the experiments were determined with and without 1×10^{-2} M sodium acetate in the reaction mixture. After incubation for 30 min (unless otherwise indicated) at 37° C in a Dubnoff metabolic shaker, the tubes were placed on cracked ice and 4 ml of 0.2 N trichloroacetic acid was added to each tube to stop the enzymatic reaction. The precipitated proteins were removed by centrifugation, and 2 ml of the supernatant was added to 3.1 ml of 0.1 N NaOH.

Extraction of p-amino benzoic acid: 1 ml of a phosphate buffer (pH 3.85) was added to 2 ml of the diluted and neutralized sample. Ether (10 ml) was added and the tubes

shaken for 5 min on a mechanical shaker. Extraction with ether was repeated one more time followed by extraction with 15 ml of benzene for 2 min. Ether and benzene fractions were discarded. Two ml of the aqueous phase was taken from each beaker for PAH analysis by the method of Smith et al. (1945).

Protein assays were performed by the method of Lowry et al. (1951) with bovine albumine fraction V as the standard.

Effect of Varying Concentrations of Glycine

To determine the rate PAH synthesis at various glycine concentrations, the final concentration of glycine in the reaction mixture was 0, 5, 25, and 50 µmoles or 0, 1, 2, 4, and 5 µmoles. The data were plotted on a Hofstee plot.

PAH Recovery

PAH recovery was determined with or without renal cortical homogenate in the reaction mixture in ten separate experiments. PAH (0.2 µmoles) was added to the reaction mixture instead of p-amino benzoic acid and the normal extraction procedure was followed. When tissue was in the reaction mixture the PAH recovery was 99.80±2.28%. With no tissue in the reaction mixture, recovery was 97.65±2.37%.

Statistical Analyses

All data are reported as means ± S.E. Differences between means were analyzed statistically using Student's "t" test, group comparison (Lewis, 1966) or Duncan's test following analysis of variance (Steel and Torrie, 1960).

RESULTS

Organic Ion Accumulation in Renal Cortical Slices during Development

Organic Acid and Base Accumulation

The accumulation of PAH and NMN by renal cortical slices was determined in young rats from 2 days to 20 days and in adults (Figures 1, 2). The PAH S/M ratio at 2 days (3.23±0.27) increased gradually until 10 days (6.93±0.33) and then declined at 12 and 14 days. The PAH S/M ratio at 14 days of age (4.94±0.28) was significantly less than at 10 days ($P<.05$). After 14 days the PAH S/M ratio increased but the value at 20 days (6.81±0.41) was still less than the value of adult tissue (11.66±0.23) (Figure 1).

The NMN S/M ratio was also low at 2 days (3.38±0.04), and increased until 14 days of age (6.92±0.21). From 14 days to 20 days (6.51±0.38), the NMN S/M ratio remained unchanged (Figure 2).

Effect of Acetate on Organic Acid and Base Accumulation

Addition of acetate to the medium (1×10^{-2}M) had no effect on PAH accumulation by renal cortical slices from 2 to 12 days of age. At 14 days, acetate increased the PAH S/M ratio from 4.94±0.28 to 7.33±0.84 (Figure 1). Thereafter, the magnitude of acetate stimulation of PAH uptake increased with

age. At 20 days, the PAH S/M ratio was increased from 6.81± 0.41 to 13.18±0.65 by acetate.

Acetate increased NMN accumulation by renal cortical slices at all ages. The magnitude of stimulation was lower than that of PAH and was the same at all ages (Figure 2).

Effect of Changing Litter Size

PAH and NMN S/M ratios were determined at 20 days after altering litter size from 4 to 12 at birth (Figure 3). Increasing litter size markedly reduced body and kidney weight measured at 20 days. Although the PAH S/M ratio appeared depressed in animals from the largest litters no significant differences in uptake of PAH or NMN were observed.

Effect of Potassium

The effect of varying potassium concentrations (0, 5, 10, 20 or 40 mM in the medium) on PAH and NMN was determined in renal cortical slices from 10 day, 20 day and adult rats. The effect of altering potassium concentration on PAH accumulation was the same at all ages (Figure 4). Removal of potassium from the medium markedly decreased the PAH S/M ratio (10, 20 day and adult rats were 2.16±0.17, 2.10±0.18 and 2.72±0.30 respectively). The PAH S/M ratio was low at 5 and 10 mM potassium in the medium but reached nearly normal value at a concentration of 20 mM. (The concentration of potassium in the normal medium was 40 mM).

NMN accumulation, measured in the same slices, was not as sensitive to changes in potassium concentration (Figure 5). The NMN S/M ratio at 20 and 40 mM potassium in the medium was essentially the same at all ages. In adult tissue, the NMN S/M ratio was slightly increased at the lower potassium concentrations.

Effect of Sodium

Tissue requirements for sodium were determined in renal cortical slices from 10 day, 20 day and adult rats. The concentration of sodium in the medium was 0, 10, 50 or 100 mM. The effect of altering sodium concentration on PAH accumulation was the same at all ages (Figure 6). Removal of sodium from the medium also markedly depressed PAH accumulation. The PAH S/M ratio appeared to be directly related to sodium concentration in the medium.

NMN accumulation, measured in the same slices, was not as sensitive to changes in sodium concentration (Figure 7). At all ages the NMN S/M ratio was essentially the same at any sodium concentration.

PAH Uptake

PAH uptake by tissue from 20 day and adult rats was linear between 2 and 12 minutes incubation. The rate of PAH uptake was determined as the difference in accumulation between 2 and 12 minutes incubation in four different PAH concentrations (1, 2, 4 and 8 x 10^{-4}M). The data were plotted on a double reciprocal plot analogous to a Lineweaver-Burk

plot (Figure 8) and a Hofstee plot (Figure 9). The maximal rate of uptake, estimated as the Y-intercept^{-1} in the Lineweaver-Burk plot and the Y-intercept in the Hofstee plot, was less in 20 day tissue (17.74 µg/gm tissue/min) than that from adult (27.02 µg/gm tissue/min). In contrast, the Km, estimated as X-intercept^{-1} in the Lineweaver-Burk plot and negative slope in the Hofstee plot, was not different in the two tissues (adult, 5.96; 20 day, 5.74 x 10^{-4}M).

Effect of Acetate on PAH Uptake

In the presence of 1 x 10^{-2} M acetate in the medium, the rate of PAH uptake by renal cortical slices from adult rat was markedly enhanced. Uptake was determined in four different PAH concentrations, 1, 2, 4 and 8 x 10^{-4} M and the data were plotted on a Hofstee plot (Figure 10). Acetate nearly doubled the maximal rate of uptake (Vmax) in the adult tissue (control, 14.71; acetate, 25.29 µg/gm tissue/min). But the Km (-slope) was not changed by acetate (control, 6.01; acetate, 7.21 x 10^{-4} M).

Effect of Acetate and pH

The effect of high pH (8.2) and the effect of acetate at high pH on PAH accumulation was determined in renal cortical slices from 10 day old and adult rats (Figure 11). The PAH S/M ratio was increased from 10.30±0.67 at pH 7.4 to 14.13 ± 0.37 at pH 8.2 in adult, from 4.87±0.25 to 6.96±0.45 in 10 days of age.

Acetate not only increased the PAH S/M ratio at physiological pH (7.4) but also at high pH. In adult tissue, the PAH S/M ratio was significantly enhanced in the presence of 1 x 10^{-2} M acetate at high pH (from 14.13±0.37 to 20.79± 0.20). Acetate slightly, but consistantly, increased the PAH S/M ratio at high pH in slices from 10 day animals (from 6.96±0.45 to 7.74±0.35).

Effect of Varying Concentrations of Acetate

PAH accumulation in renal cortical slices from adult rat and rabbit was determined at varying concentrations of acetate in the medium (0, 1, 2, 4 x 10^{-3} and 1 x 10^{-2}M) (Figure 12). Acetate increased the PAH S/M ratio more in renal cortical slices from rabbit (98% increase at 1 x 10^{-2}M) than from rat (44% increase at 1 x 10^{-2}M). In tissue from both species the PAH S/M ratio was maximal at 2 x 10^{-3} M of acetate.

PAH Synthesizing Enzyme System in Young and Adult Rats

PAH Synthesis in Renal Cortical Homogenates

PAH synthesis in renal cortical homogenates from 10 day and adult rats was determined in the presence and absence of acetate (1 x 10^{-2}M). Incubation time was varied from 10-90 minutes. Maximal PAH synthesis in tissue from 10 day old rats (2.39±0.47 µmoles/mg protein x 10^{-2}) was lower than that from adult (3.55±0.51 µmoles/mg protein x 10^{-2}) (Figures 13, 14). PAH synthesis in adult tissue was maximal at 30 minutes

incubation (Figure 13). Synthesis in tissue from 10 day old rats reached a maximal value at 60 minutes of incubation (Figure 14). Acetate in the reaction mixture did not significantly alter PAH synthesis in renal homogenates of either age animal.

Effect of Varying Concentrations of Glycine

The rate of PAH synthesis in renal cortical homogenates from adult rat was determined with varying concentrations of glycine in the reaction mixture in the presence of 1×10^{-2} M acetate (Figure 15). When the reaction mixtures containing 0, 5, 25 or 50 μmoles of glycine were incubated for 30 minutes, the rate of PAH synthesis was found to be maximal at 5 μmoles of glycine. Acetate produced no difference in PAH synthesis at any concentration of glycine.

Effect of Acetate on the Rate of PAH Synthesis

The rate of PAH synthesis was determined in renal cortical homogenates from 10 day and adult rat with varying concentrations of glycine (0, 1, 2, 4 or 5 μmoles) in the reaction mixture. Similar incubations were conducted in the presence of 10^{-2} M acetate. The data were plotted on a Hofstee plot (Figure 16). The maximal rate of PAH synthesis (Vmax) for the adult tissue (3.59 μmoles/mg protein/30 min $\times 10^{-2}$) was markedly higher than for the 10 day animals (1.55 μmoles/mg protein/60 min $\times 10^{-2}$). Acetate had no effect on the maximal rate of PAH synthesis or Km (-slope) in either tissue.

Effect of Acetate on PAH Accumulation and Synthesis in Chicken

The effect of acetate on PAH accumulation in renal cortical slices and on PAH synthesis in renal cortical homogenates from White Leghorn chicken was determined. Acetate increased the PAH S/M ratio consistantly but no PAH synthesis could be measured in this tissue (Table 1).

DISCUSSION

Histologically the kidney of the newborn rat is embryonic in character; it contains nephrogenic tissue (Bogomolova, 1966). Postnatal nephrogenesis is marked in kidneys of newborn rats during the first and second weeks of life (Baxter and Yoffey, 1948). At 15 days nephrogenic tissue is no longer present and the major differences in structure between the convoluted and straight portions of the nephron are clearly evident (Bogomolova, 1966). Peripheral cortical tissue has been reported to be fully developed at 28 days (Baxter and Yoffey, 1948). Between one month and one year of age the kidney shows little change in structure other than the observation that the cortical layer becomes much thicker and the brush border reaches normal heights (Bogomolova, 1966).

The ability of renal cortical slices from newborn rats to accumulate PAH demonstrated a pattern of functional development similar to that seen in other species (Hook et al., 1970; Hirsch and Hook, 1970; Rennick et al., 1961). The PAH S/M ratio increased rapidly to a peak at 10 days and then declined to 14 days and began to rise, but had not reached adult levels at 20 days (Figure 1). Such a peak in PAH accumulation prior to adulthood is also seen in rabbits (Hirsch and Hook, 1970), piglets (Rennick et al., 1961) and

in puppies (Hook et al., 1970; Rennick et al., 1961). Rennick et al. (1961) observed a correlation between histological development of the renal cortex and the ability of the tissue to accumulate PAH. The data presented here are consistent with a similar correlation in very young rat tissue. Trimble (1970) suggested that the inability of the 10 day old rat to accumulate urea in the papilla may be caused by anatomical immaturities in the medulla. However, the decline in cortical activity after 10 days in rats and 4 weeks in rabbits and puppies (New et al., 1959; Rennick et al., 1961; Hook et al., 1970; Hirsch and Hook, 1970) demonstrates that physiological and histological maturation are not absolutely linked.

Rapid increases of gluconeogenic enzyme activity in mice and rat kidney cortex occur during the first 2 postnatal weeks (Zorzoli, 1968; Zorzoli et al., 1969). There could be a relationship between the patterns of biochemical and physiological development in the rat kidney. However, there did not appear to be a direct parallelism between gluconeogenic enzyme activities and organic acid accumulation in rat kidney cortex during development. Part of the explanation for the decline in PAH S/M ratio during development in rat tissue might be related to an active mechansim for exit of PAH from the tissue. Hirsch and Hook (1970) suggested that the mechanisms responsible for organic acid entry into renal tubular cells are fully developed at 4 weeks of age in rabbits but the mechanisms responsible for exit of organic acid might develop after this age.

Horster and Lewy (1970) measured a gradual increase in extraction of PAH with age in newborn rats. No peak in activity was seen prior to 20 days. The extraction of PAH in the dog also increased directly with age, reaching the mature value at approximately 10 weeks after birth (Horster and Valtin, 1971). A similar finding has been reported for maturation of the PAH transport system in the dog. Hook et al., (1970) observed a gradual increase in Tm_{PAH} in the maturing puppy and yet when measured in vitro PAH accumulation seemed to peak at 4 weeks of age. Thus the general pattern of development seen in vivo is different than that seen in vitro. The present data suggest that cellular function as measured by the S/M ratio develops rapidly in the first few days of life. The continual increase in functional maturity seen in vivo is, then, a reflection of both cellular maturation and total organ growth.

The pattern of development of organic base uptake in renal cortical slices was different from that of organic acid. There is a progressive increase in the ability to accumulate TEA by renal cortical slices of puppy (Rennick et al., 1961). Similarly, the pattern of development of NMN accumulation by rat tissue appears to be more closely associated with organ growth than that of PAH (Figure 2).

Altering litter size at birth markedly changed the normal rate of growth of kidney and body weight (Figure 3). Zeman (1968) and Hall and Zeman (1968) observed that maternal protein restriction resulted in definite morphological, histochemical and physiological changes in newborn rat kidneys.

Early malnutrition, from birth to weaning (21 days), impeded cell division in various rat organs including kidney and these animals did not recover normal growth when adequately refed (Winick and Noble, 1966). Edelman and Wolfish (1968) postulated that the magnitude of the working load imposed upon the kidney by diet might be a major factor in determining the rate of renal maturation after birth, when the investigators found that premature infants on a high protein intake for 4 weeks were shown to have approximately twice the tubular capacity to excrete PAH compared with infants fed low protein diets. Barnett et al. (1948), on the other hand, suggested that postnatal age may be a more important factor in determining the level of renal function than body size, and, as seen in this work, any changes in nutritional status produced by altering litter size from 4 to 12 pups was not adequate to markedly alter the development of the renal transport system (Figure 3). Thus, over the normal course of development factors other than nutrition must be the predominant controlling factors in the maturation of renal transport systems.

Altering the concentration of potassium or sodium in the medium produced the same effect on PAH transport in renal cortical slices from animals of all ages (Figures 4, 6). Foulkes and Miller (1960) suggested that there may be no correlation between PAH and potassium fluxes across the cell membrane. The effect of potassium on PAH transport probably is related to the role of potassium in the maintenance of a normal cellular environment. Bulger and Trump (1969) indicated that removal of potassium from isolated flounder tubules resulted

in changes in the basal cytoplasm region of the cell with disruption of the basal infoldings. They suggested that potassium is necessary to maintain the structural integrity of the basal architecture. The absence of potassium in the environment would change the cell membrane which, of course, is related to cell volume regulation and possibly to influx of organic anions. In the absence of sodium from the medium, removal of potassium or calcium or both ions induced no further depression in PAH or PSP S/M ratios, suggesting that sodium plays a dominant role in organic acid transport (Chung et al., 1970). The PAH S/M ratio seemed to be more directly related to sodium concentration in the medium than to potassium. Although the mechanism of the effect of sodium and potassium in organic ion transport is not known it is apparent that both ions are required for the transport of PAH. Accumulation of NMN, on the other hand, does not demonstrate the same sensitivity to inorganic ions in the medium (Figures 5, 7). Thus, while tissue requires an ionic environment similar to the in vivo situation to accumulate PAH, this does not appear to be true for NMN accumulation. This and the greater variation between litters seen in base accumulation suggest that a significant portion of this process may not be active transport but indiscriminate tissue binding as has been suggested previously (Ross et al., 1969).

The S/M ratio as used in this study was measured in a steady state system (after 90 minutes incubation). Thus, the data represent not only the transport capacity of the tissue but binding and runout capacity as well. Consequently, the

S/M ratio is truly a measure of the ability of the tissue to maintain a concentration gradient. During very short periods of incubation (such as between 2 and 12 minutes) the uptake of PAH into the renal cortical slices is linear, suggesting that intracellular accumulation of material has not reached a concentration high enough to alter the rate of influx of the material. Ross and Farah (1966) suggested that uptake over short periods of time adequately reflects the rate of transport of material into the tissue. The rate of PAH uptake was measured at a different PAH concentration and the data were plotted on a Lineweaver-Burk (Figure 8) and a Hofstee plot (Figure 9). In both types of plot the Km for PAH might reflect the relative affinity of a carrier substance for PAH and the Vmax might be an indication of the maximal velocity of the PAH accumulation process. Based on these assumptions the data suggest that the apparent affinity was not different in the tissue from two different age groups. However, the maximal velocity of transport appeared to be much greater in tissue from the adult animal. This suggests that the depressed ability of renal cortical slices to accumulate organic ions in the newborn results from a relatively small number of functional transport sites.

Acetate markedly increased the rate of PAH uptake into renal cortical slices from adult rats (Figure 10). However, the addition of acetate to the <u>in vitro</u> system did not enhance PAH transport in young rat tissue prior to 12 days of age (Figure 1). Cross and Taggart (1950) suggested that acetate

is actually one of the rate-limiting cellular components of the transport mechanism. Acetate could well play such a role since its concentration in body fluids and tissues is known to be extraordinarily low despite a relatively high rate of turnover. Acetate significantly increased the PAH S/M ratio in addition to the enhancement of the PAH S/M ratio at pH 8.2. But in renal cortical slices of rabbit, the stimulatory effect of acetate showed a gradual decrease between pH 7.4 and 8.2 (Copenhaver and Davis, 1965). There is no confirmed evidence that this difference is just from species differences or some other unknown mechanism. Schachter et al. (1955) presented hypothesis to explain the mechanism of acetate stimulation on PAH transport. This hypothesis though very attractive, has not yet been confirmed. As pointed out in the Introduction to this thesis, synthetic reactions, like that for hippurate, involve the formation of acylthioesters of coenzyme A which are the energy rich intermediates in acylglycine synthesis (Schachter et al., 1955). These workers suggested that the addition of exogenous acetate to the system should yield acetyl CoA as the most abundant acyl CoA available to the glycine acyltransferase system. The relative abundance of acetyl CoA may be expected to promote the synthesis of acetylglycine at the expense of the longer chain, inhibitory acylglycines. Since acetylglycine does not compete with PAH for transport (Schachter et al., 1955) the effect of the addition of acetate is a net increase in the transport of PAH. If this hypothesis were correct, the addition of acetate to the acylglycine synthesizing system should decrease acyl CoA thereby decreasing acylglycine compounds which are

supposed to compete with PAH for transport. No direct quantitative comparisons have been conducted to study this system. Lack of an effect of acetate on PAH uptake in the tissue from the very young animals could indicate that an acetate-requiring step is not rate limiting until 12 days of age. On the other hand, it could be that the system responsible for acetylation is not developed until 12 days of age. The tissue of the young rat prior to 12 days was used as a tool to gain further insight into the mechanism of acetate stimulation of PAH transport. The ability of rat renal cortical homogenates to synthesize PAH was used as an index of acylglycine synthesis (glycine acyltransferase activity).

Borsook and Dubnoff (1940) studied the biological synthesis of hippuric acid. They suggested that the synthesis of hippuric acid resembles in several respects the synthesis of the peptide bond. Cohen and McGilvery (1946, 1947) conducted a series of studies on the formation of PAH from p-aminobenzoic acid and glycine to explore peptide bond synthesis in rat liver slices and homogenates. They tested several kinds of tissues, liver, kidney cortex, testes, heart ventricle, thigh muscle, brain and spleen. But no tissue other than liver and kidney showed appreciable formation of PAH. This system involves three stages (Schachter and Taggart, 1954). Benzoyl adenylate is formed by pyrophosphate exchange with ATP. The adenylate moiety is then exchanged for coenzyme A to produce benzoyl-CoA. Finally, the latter reacts with glycine to give hippuric acid with the regeneration of free CoA. The first two reactions are catalyzed by a thiokinase, the last by glycine acyltransferase

(glycine N-acylase). PAH-synthesizing enzyme activity was found only in the mitochondrial fraction of liver and kidney homogenates from mouse (Kielley and Schneider, 1950).

Brandt (1960, 1964) showed that homogenates of liver taken from late fetal and early newborn rats were able to couple benzoic acid to glycine only to a slight extent. Hippuric acid synthesis increased slowly with age until shortly before weaning. Brandt (1966) also demonstrated that the activity of the glycine acyltransferase component of the system, measuring the activity of enzyme directly, varied with the age of the rat liver and in a similar pattern as hippuric acid synthesis. He suggested that glycine acyltransferase may be the rate-limiting step in the overall reaction. In mice liver homogenates, PAH synthesis was an age dependent reaction with minimal activity in the neonatal period and adult values attained at 1 month of age (Gorodischer et al., 1971).

Lower PAH synthesis in the 10 day old rat (Figures 13, 14) demonstrates that the activity of glycine acylase is low in young animals as Brandt (1966) reported previously. No effect of acetate on PAH synthesis by renal cortical homogenates was shown in 10 day old or adult rats (Figure 13, 14). Because of the different incubation time to obtain maximal PAH synthesis between 10 day old and adult rat (10 day old rat, 60 minutes; adult, 30 minutes), it would not be appropriate to discuss the maximal rate of PAH synthesis and Km. However, the rate of PAH synthesis for 60 minutes in 10 day old rat tissue was markedly lower than that of adult. Acetate had no effect on the maximal rate of PAH synthesis in either tissue (Figures

15, 16). Schachter et al. (1955) indicated that the enhancement of PAH transport by acetate appears to occur only in those species capable of glycine conjugation. The exception was the rat which has acyl glycinetransferase but reportedly little or no increase in PAH transport in response to acetate (Despopoulos, 1956). However, in the present work, acetate significantly and consistently increased the PAH S/M ratio in renal cortical slices of adult rat (Figure 12). Acetate has also enhanced the PAH S/M ratio in renal cortical slices of chickens despite the finding that no PAH synthesis occurred in the tissue. These data clearly show that there is no relationship between acetate and the acetylglycine synthesis system. Thus, the mechanism of acetate stimulation on PAH transport still remains unknown.

SUMMARY

The developmental pattern of organic ion transport was determined in renal cortical slices from newborn rats from 2 days to 20 days and in adults. PAH S/M ratios increased gradually from birth until 10 days and then declined to 14 days. After 14 days the PAH S/M ratio began to rise but had not reached adult levels at 20 days. It is suggested that cellular function as measured by the S/M ratio develops rapidly in the first few days of life. The NMN S/M ratio increased until 14 days of age and then remained unchanged, suggesting the pattern of development of NMN accumulation appears to be more closely associated with organ growth than that of PAH.

Nutritional changes produced by altering litter size from 4 to 12 at birth markedly depressed the normal rate of growth of kidney and body weight. No significant differences in accumulation of PAH or NMN by renal cortical slices from these rats were observed at 20 days of age. These data suggest that factors other than nutrition must be predominant in the maturation of renal transport systems.

The effect of altering the concentration of potassium and sodium in the medium on PAH accumulation was the same in renal cortical slices from 10 day, 20 day and adult rats. Removal of both ions from the medium resulted in marked depression of PAH S/M ratios, suggesting that sodium and potassium are

required for the transport of PAH. NMN accumulation did not show the same sensitivity to these ions in the medium.

The maximal rate of PAH uptake (Vmax), estimated from a Lineweaver-Burk and Hofstee plot, was markedly lower in renal cortical slices from 20 day old rats than that from adults. In contrast, the Km, which might reflect the relative affinity of a carrier substance for PAH, was not different in tissue from the two different age groups. These data suggest that the low ability of renal cortical slices to accumulate organic ions in the newborn rats results from a relatively small number of functional transport sites.

Acetate had no effect on accumulation of PAH by renal cortical slices from very young rats (2 to 12 days), whereas the PAH S/M ratio was markedly enhanced by acetate in tissue from adult. Acetate also increased the rate of PAH uptake in tissue from adults with no change in Km values. The effect of acetate on PAH accumulation by renal cortical slices from adult rats was smaller than that from rabbit, but the stimulatory effect was apparent. At high pH (8.2) the PAH S/M ratio was increased and acetate resulted in additional increases in the PAH S/M ratios in both age groups. Addition of acetate to the medium resulted in enhancement of NMN accumulation by renal cortical slices from all ages but the magnitude of stimulation was smaller than that of PAH.

The ability of renal cortical homogenates to synthesize PAH was used to estimate the activity of the enzyme acyl glycine-transferase (glycine N-acylase). Enzyme activity was low in renal cortical homogenates from 10 day old rats. The rate of

PAH synthesis observed by varying the concentration of glycine was also markedly low in the tissue from young rats (10 days). Acetate did not change PAH synthesis in the tissues from young or adult rats. These data discount the hypothesis that acetate enhancement of PAH transport is due to the formation of acetylglycine.

Table 1. Effect of acetate on PAH transport and PAH synthesis in the chicken kidney.

Number of Determinations	PAH S/M Ratio		PAH Synthesis μmoles/mg protein x 10^{-2}	
	Control	+ Acetate	Control	+ Acetate
1	12.50	16.10	0.58	0.85
2	5.93	9.20	0.16	0
3	12.53	20.88	0.16	0
$\bar{x}$	10.32	15.39	0.30	0.28
(± S.E.)	(2.19)	(3.39)	(0.14)	(0.28)

Figure 1. PAH S/M ratio and the effect of acetate (10^{-2} M) with age in renal cortical slices from young and adult (12 weeks old) rats. Values are means ±S.E. of four experiments at each age. The absence of a vertical bar indicates S.E. is within the radius of the point.

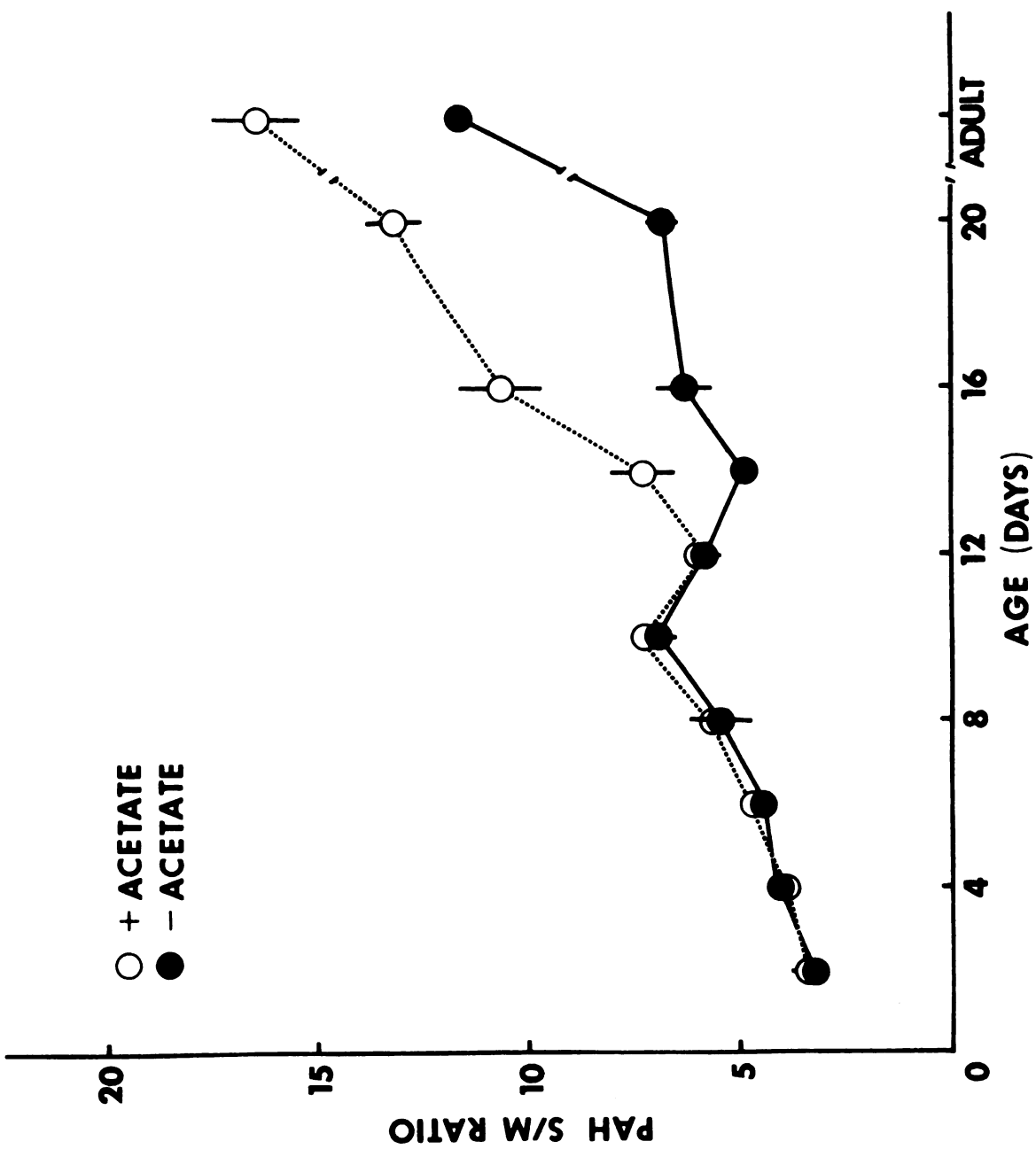
PAH S/M RATIO
20
15
10
5
0
4
8
12
16
20
ADULT
AGE (DAYS)
○ + ACETATE
● – ACETATE

Figure 1

Figure 2. NMN S/M ratio and the effect of acetate (10^{-2} M) with age in young and adult (12 weeks old) rats. Values are means ±S.E. of four experiments at each age. The absence of a vertical bar indicates S.E. is within the radius of the point.

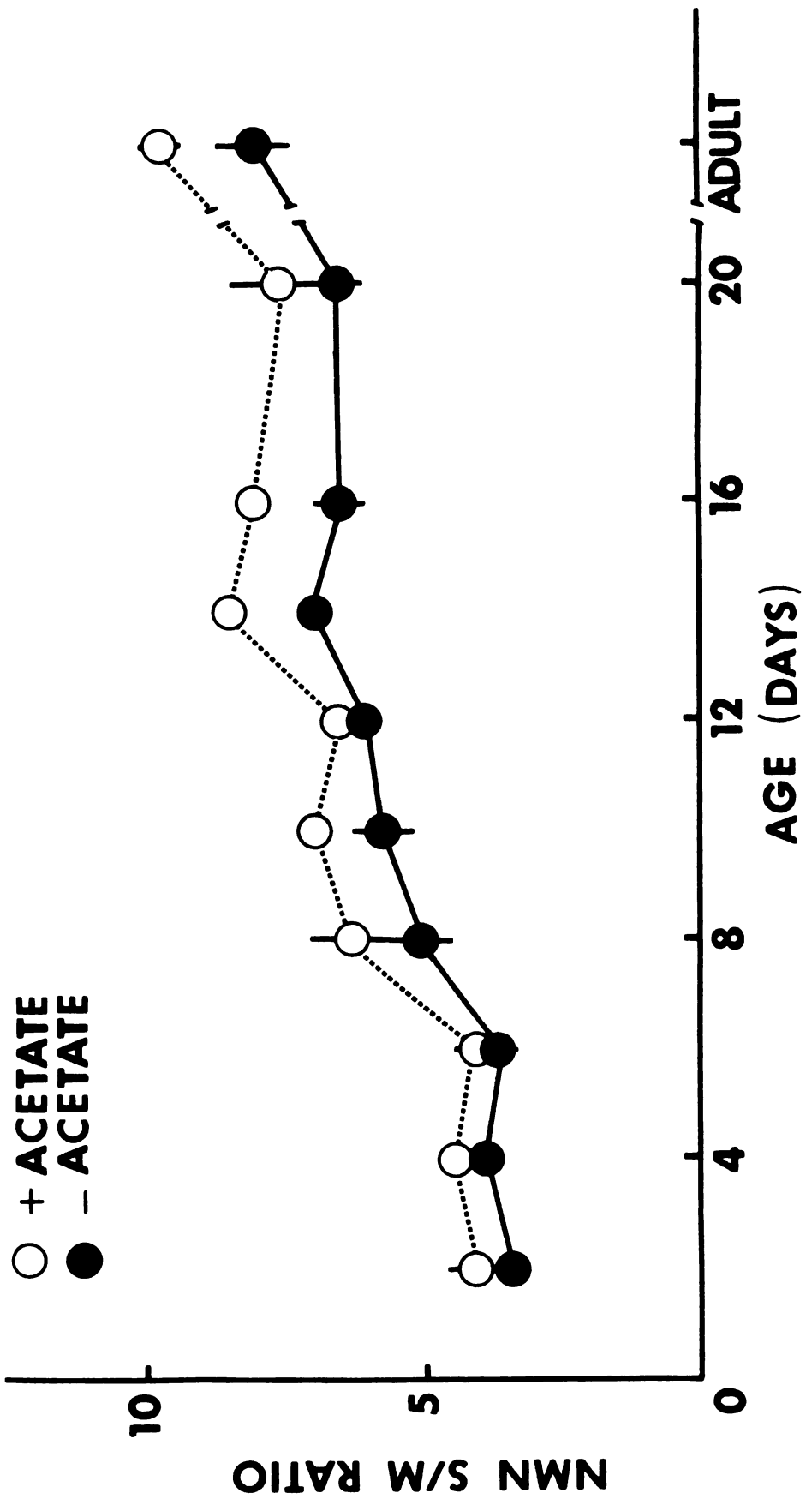

Figure 2

Figure 3. Effect of litter size on PAH and NMN transport and body and kidney weight in 20 day old rats. Litter size was adjusted within two days of birth. Values are means ±S.E. of four experiments at each litter size. Absence of a vertical bar indicates S.E. is within the radius of the point.

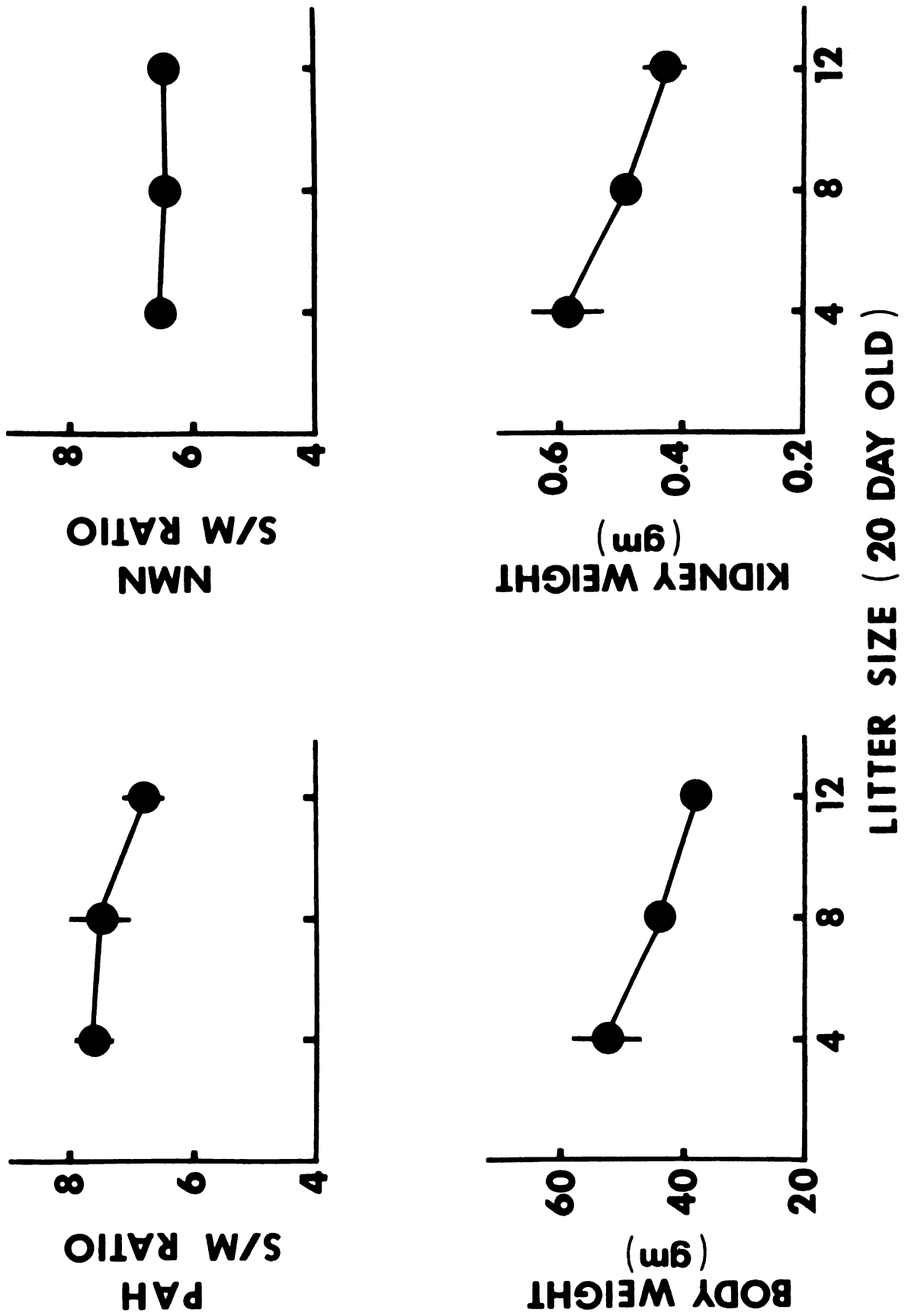
BODY WEIGHT
(gm)
60
40
20
4
8
12
KIDNEY WEIGHT
(gm)
0.6
0.4
0.2
4
8
12
LITTER SIZE (20 DAY OLD)
PAH
S/M RATIO
8
6
4
NMN
S/M RATIO
8
6
4

Figure 3

Figure 4. Effect of potassium on PAH accumulation in adult, 20 day and 10 day old rats. Values are means ±S.E. of four experiments. Absence of a vertical bar indicates that S.E. is within the radius of the point. The amount of potassium removed was replaced with sucrose to maintain a constant medium osmolality. Sodium concentration was 100 mM. (The potassium concentration in the normal medium was 40 mM.)

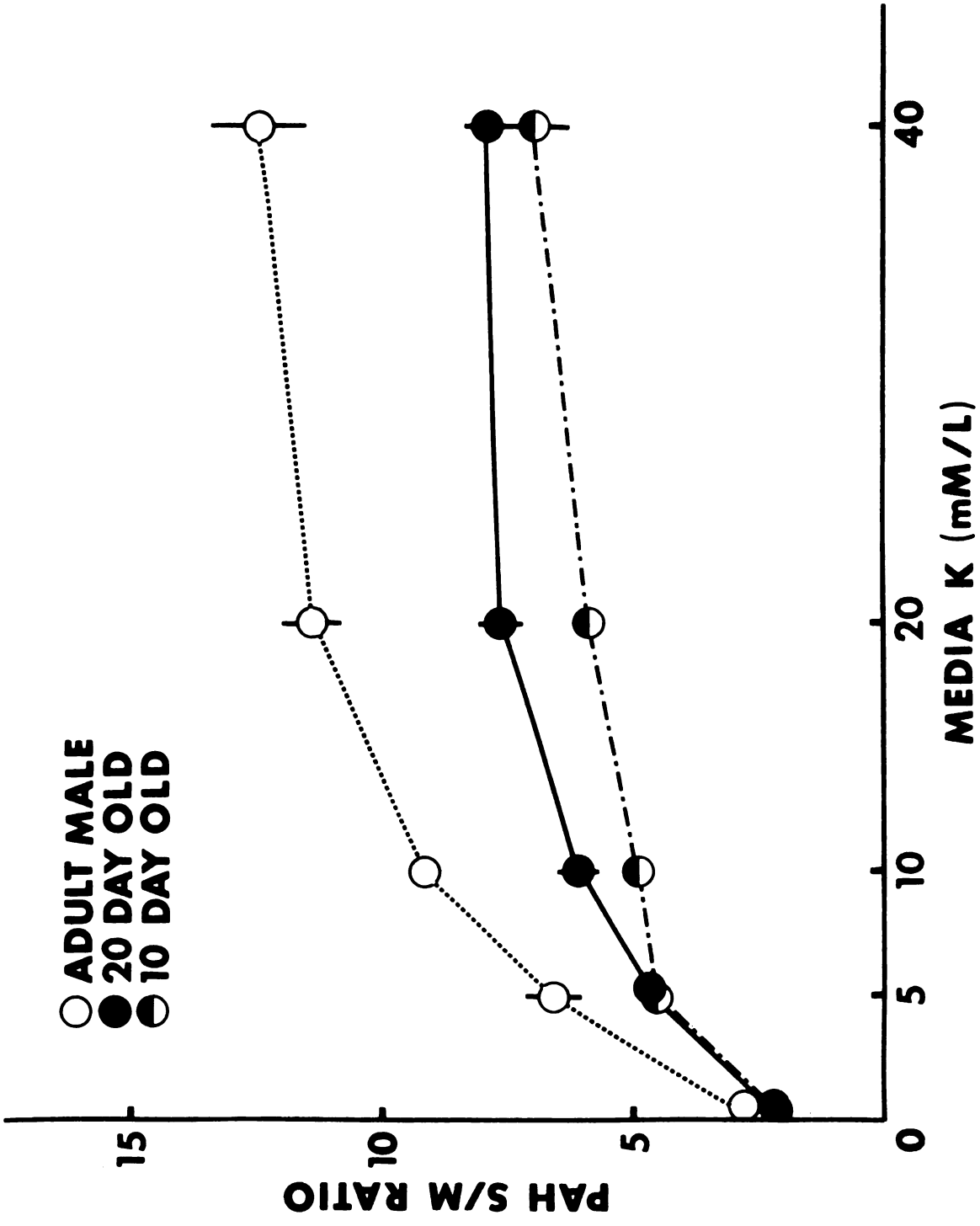
ADULT MALE
20 DAY OLD
10 DAY OLD
PAH S/M RATIO
15
10
5
0
5
10
20
40
MEDIA K (mM/L)

Figure 4

Figure 5. Effect of potassium on NMN accumulation in adult, 20 days and 10 day old rats. Values are means ±S.E. of four experiments. Absence of a vertical bar indicates S.E. is within the radius of the point. The amount of potassium removed was replaced with sucrose to maintain a constant medium osmolality. Sodium concentration was 100 mM.

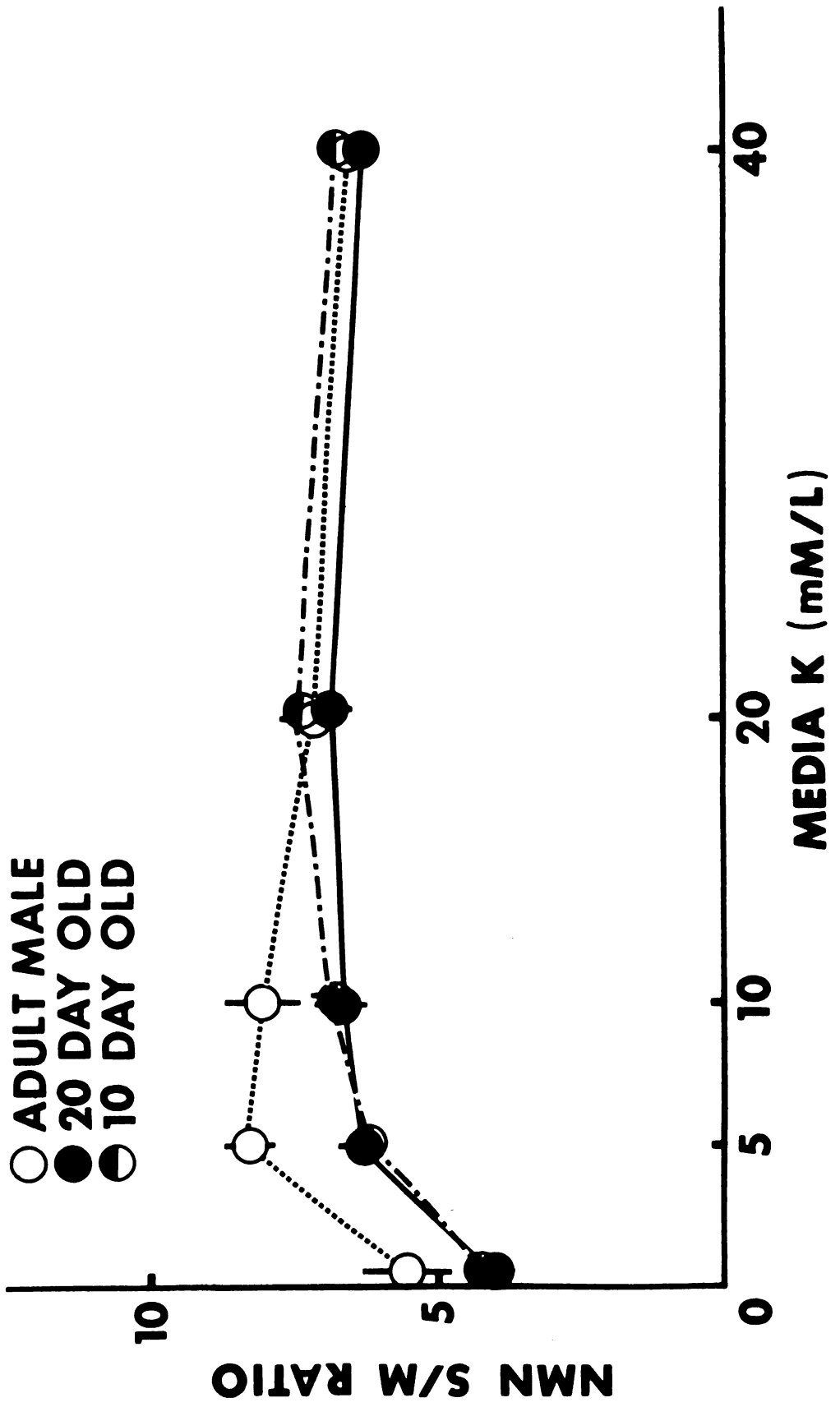
ADULT MALE
20 DAY OLD
10 DAY OLD
NMN S/M RATIO
10
5
0
5
10
20
40
MEDIA K (mM/L)

Figure 5

Figure 6. Effect of sodium on PAH accumulation in adult, 20 day and 10 day old rats. Values are means ±S.E. of four experiments. Absence of a vertical bar indicates that S.E. is within the radius of the point. The amount of sodium removed was replaced with sucrose to maintain a constant medium osmolality. Potassium concentration was 40 mM. (The sodium concentration in the normal medium was 100 mM.)

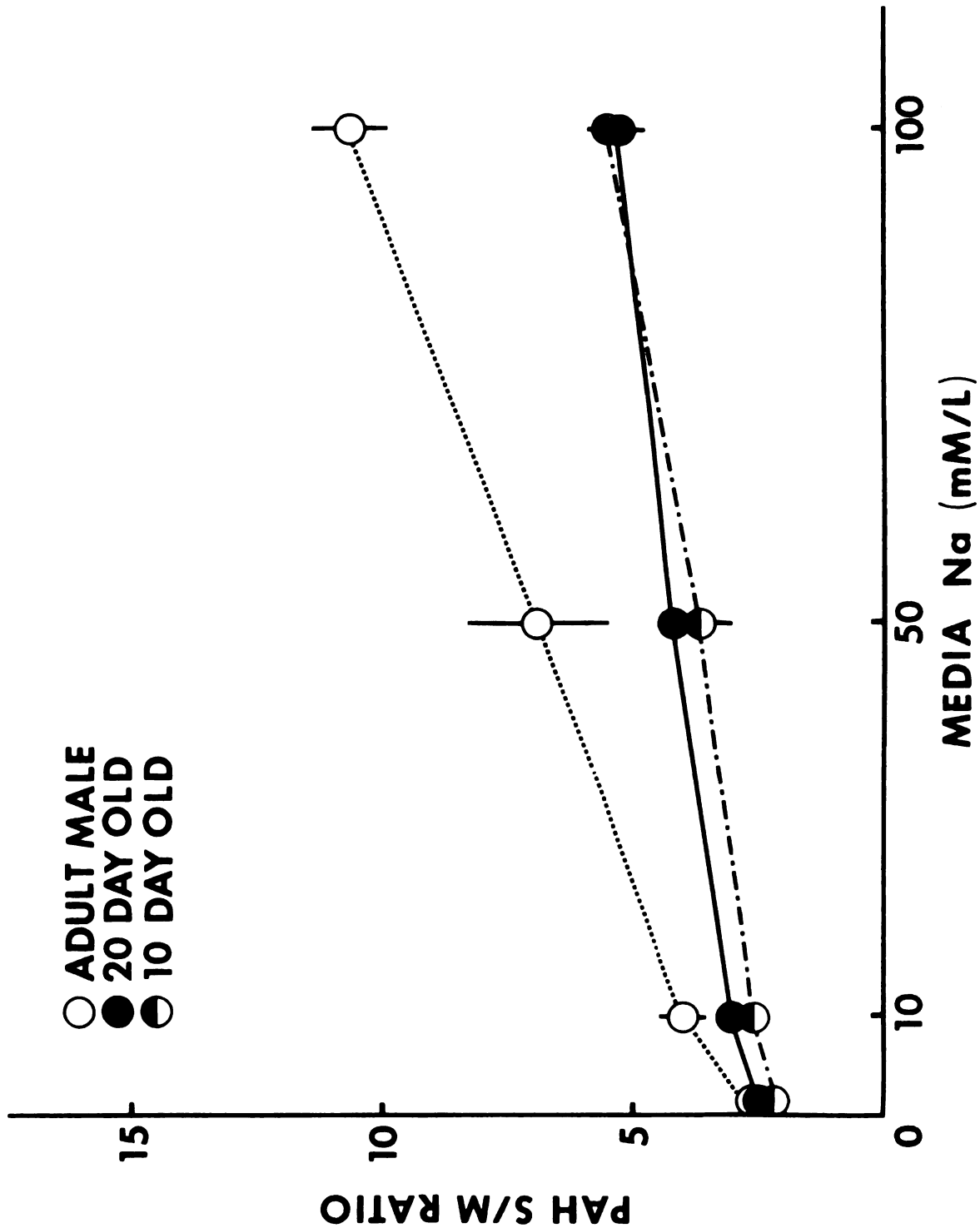
ADULT MALE
20 DAY OLD
10 DAY OLD
PAH S/M RATIO
15
10
5
0
10
50
100
MEDIA Na (mM/L)

Figure 6

Figure 7. Effect of sodium on NMN accumulation in adult, 20 day and 10 day old rats. Values are means ±S.E. of four experiments. Absence of a vertical bar indicates that S.E. is within the radius of the point. The amount of sodium removed was replaced with sucrose to maintain a constant medium osmolality. Potassium concentration was 40 mM.

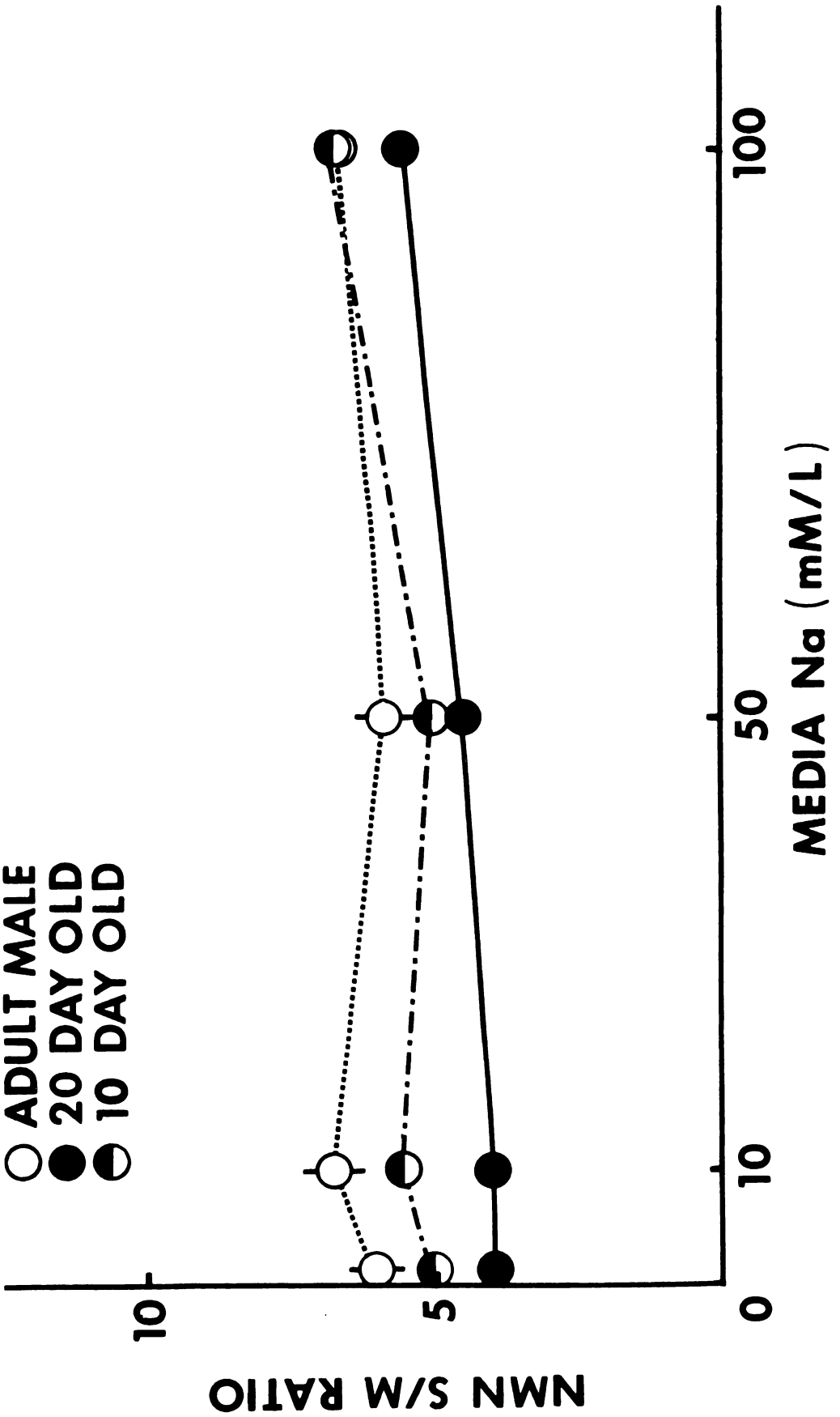
ADULT MALE
20 DAY OLD
10 DAY OLD
NMN S/M RATIO
10
5
0
10
50
100
MEDIA Na (mM/L)

Figure 7

Figure 8. Lineweaver-Burk plot of PAH uptake in renal cortical slices from adult and 20 day old rats. Values are average of four experiments at each concentration of PAH (1, 2, 4 and 8 x 10^{-4} M) and each incubation time (2 and 12 minutes). Uptake of PAH was determined as the minute rate of uptake between 2 and 12 minutes incubation.

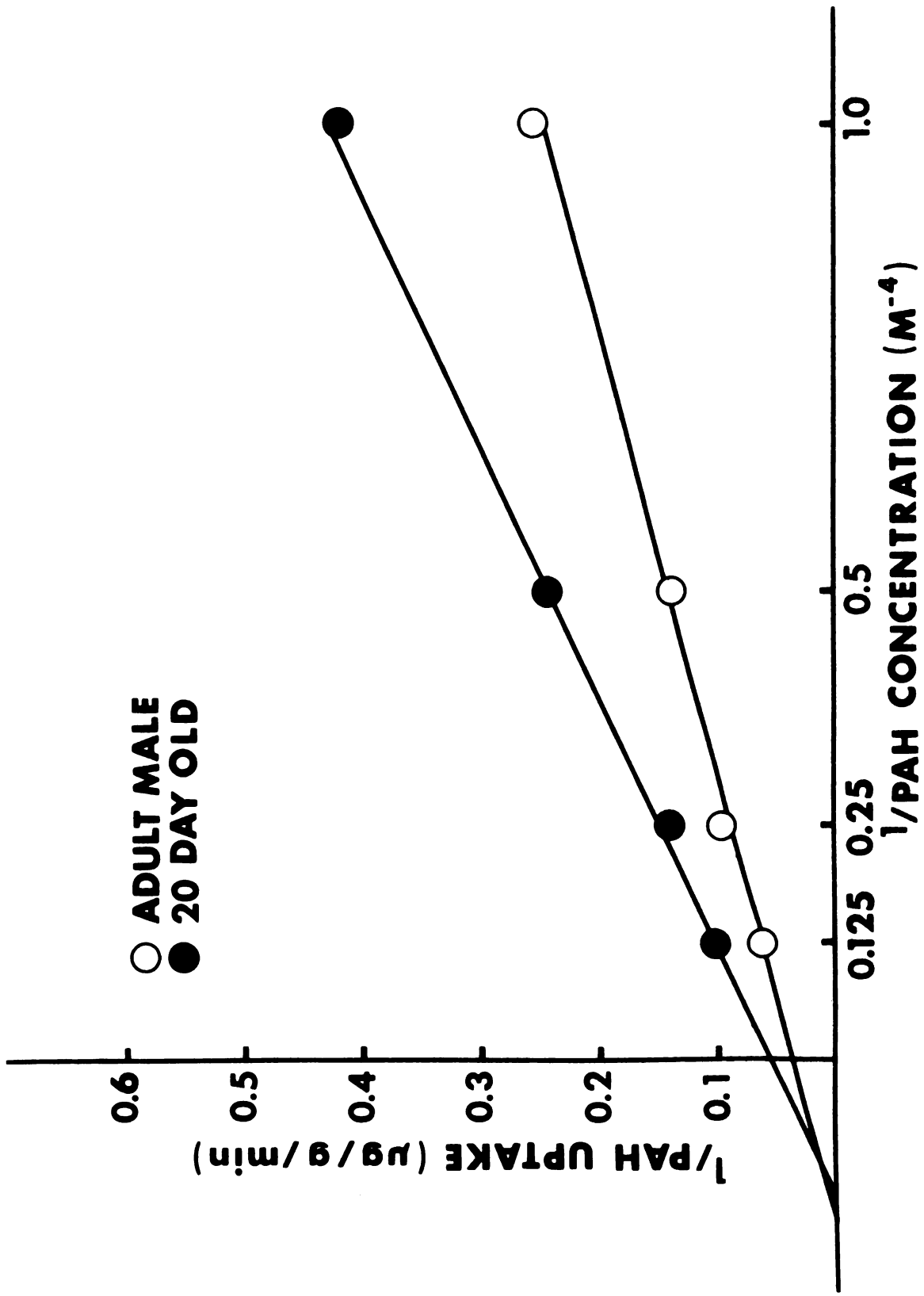
ADULT MALE
20 DAY OLD
1/PAH UPTAKE (µg/g/min)
0.6
0.5
0.4
0.3
0.2
0.1
0.125
0.25
0.5
1.0
1/PAH CONCENTRATION (M-4)

Figure 8

Figure 9. Hofstee plot of PAH uptake in renal cortical slices from adult and 20 day old rats. Values are averages of four experiments at each concentration of PAH (1, 2, 4 and 8 x 10^{-4} M) and each incubation time (2 and 12 minutes). Uptake of PAH was determined as the minute rate of uptake between 2 and 12 minutes incubation. Lines are calculated regression lines.

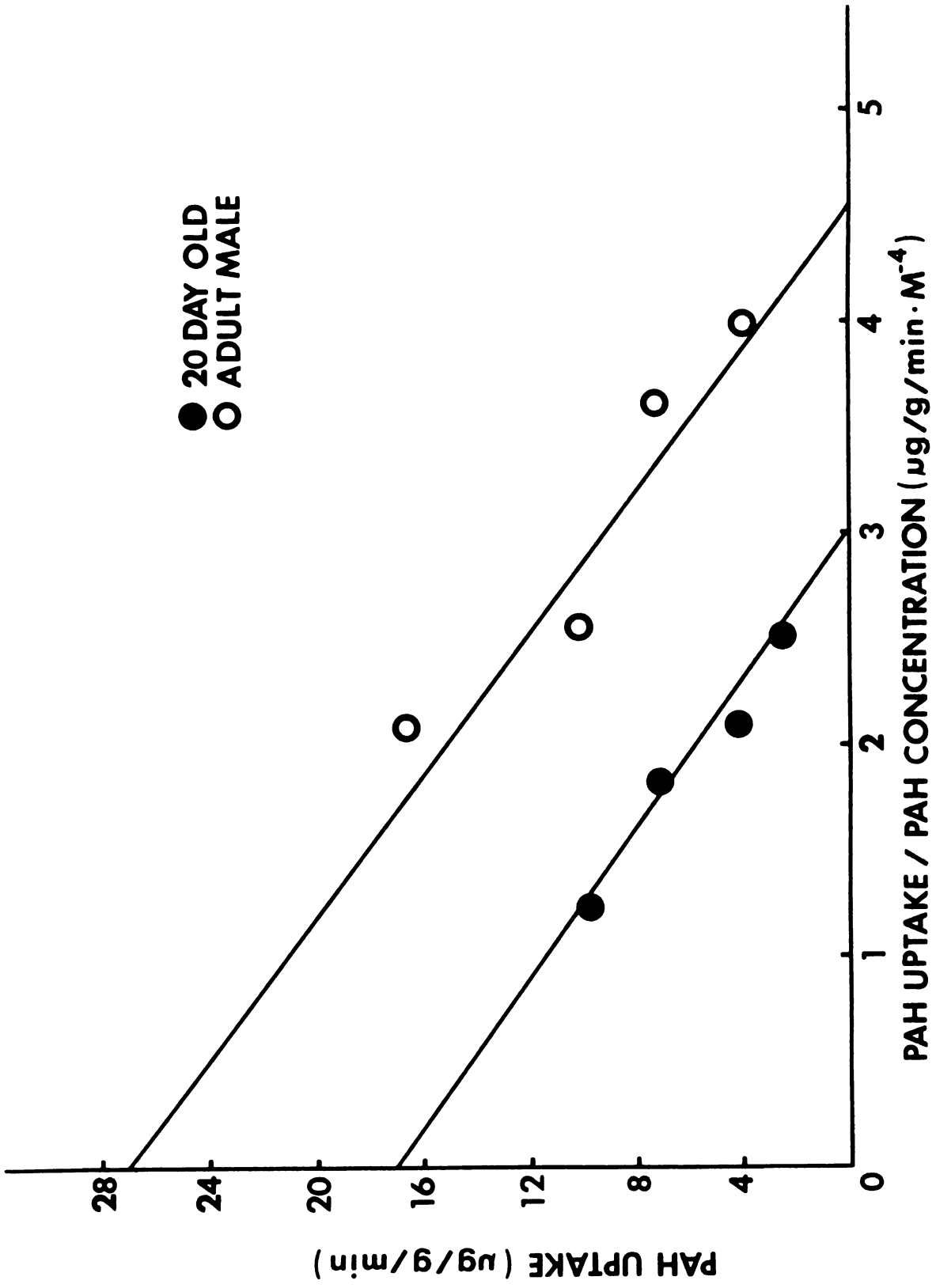
20 DAY OLD
ADULT MALE
PAH UPTAKE (µg/g/min)
28
24
20
16
12
8
4
0
1
2
3
4
5
PAH UPTAKE / PAH CONCENTRATION (µg/g/min·M⁻⁴)

Figure 9

Figure 10. Effect of acetate on PAH uptake in renal cortical slices from adult rat. Values are averages of four experiments at each concentration of PAH (1, 2, 4 and 8 x 10^{-4} M) and each incubation time (2 and 12 minutes). In this Hofstee plot, V represents PAH uptake (μg PAH/g tissue/min) and [S] represents PAH concentration in the medium. The two slopes (-Km) (control, 6.01; acetate, 7.21) were not different.

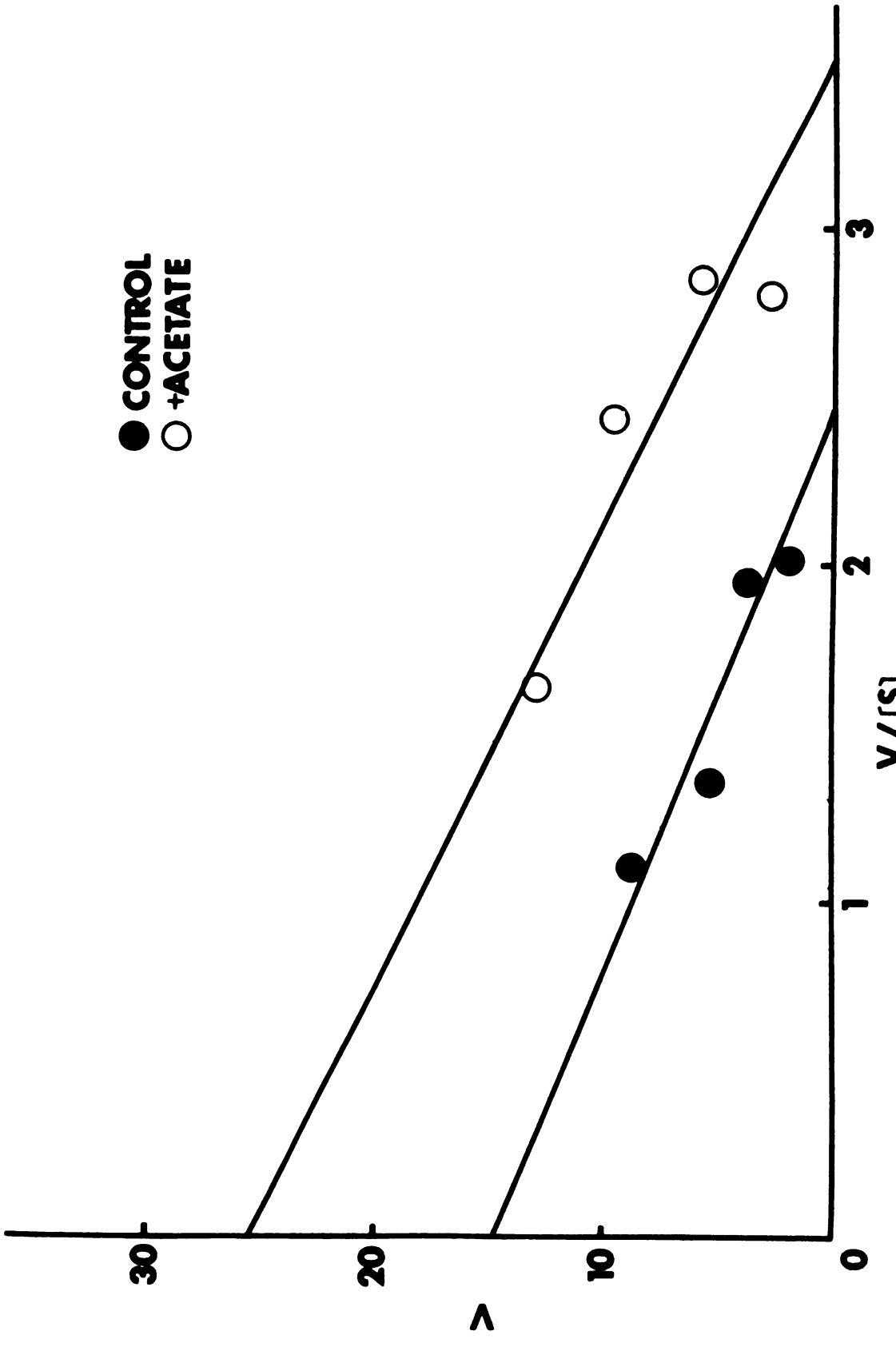

Figure 10

Figure 11. Effect of acetate (10^{-2} M) and high pH (8.2) on PAH accumulation in renal cortical slices from 10 day old, adult rats. Each bar represents means ±S.E. of four experiments. Acetate significantly enhanced the PAH S/M ratio at pH 8.2 in both tissues (Duncan's test).

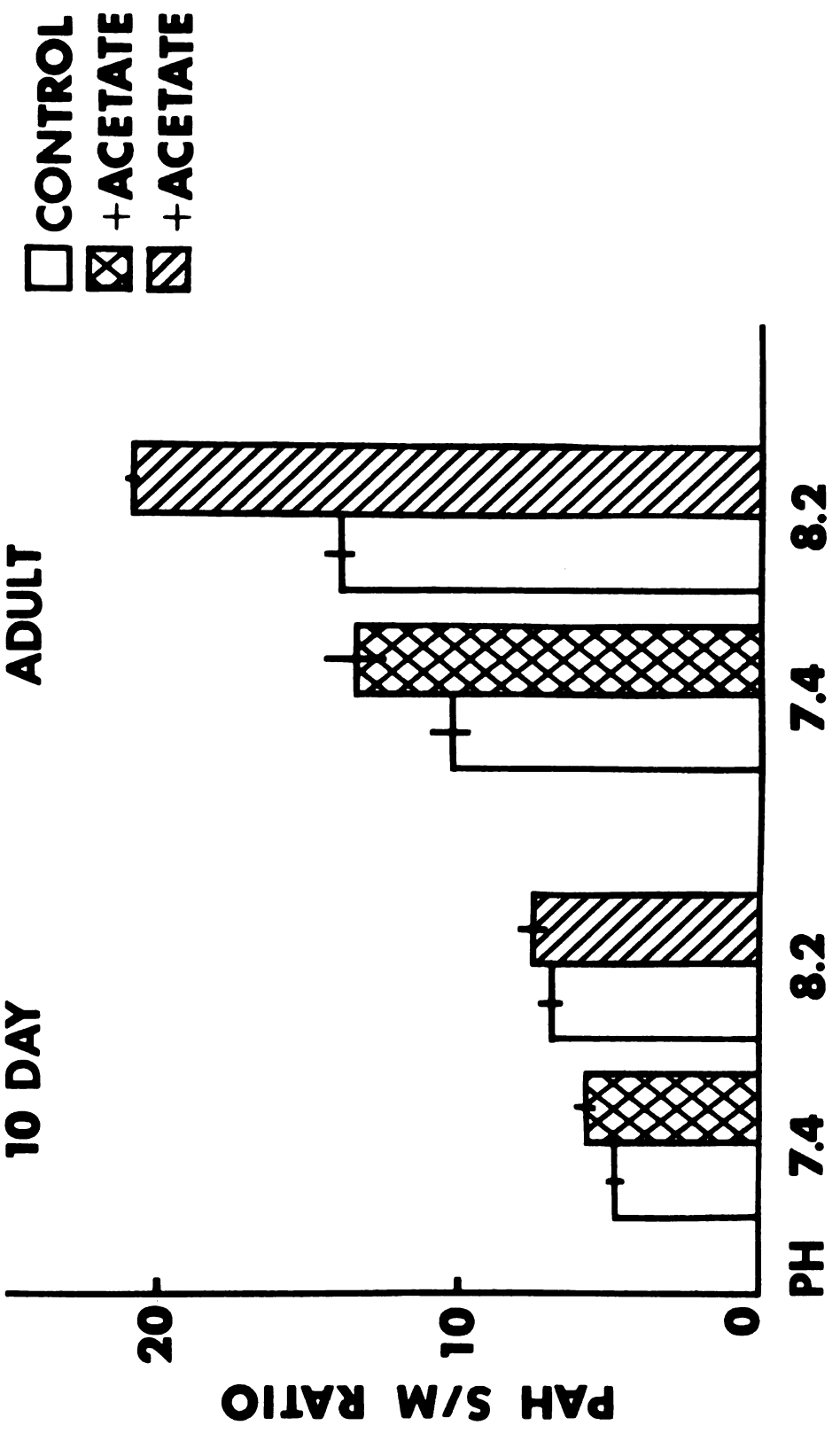
CONTROL
+ACETATE
+ACETATE
ADULT
10 DAY
PAH S/M RATIO
20
10
0
PH 7.4 8.2 7.4 8.2

Figure 11

Figure 12. Effect of acetate on PAH accumulation (S/M ratio) in renal cortical slices from adult rabbits and rats. The concentrations of acetate in the medium were, 1×10^{-3}, 2×10^{-3}, 4×10^{-3} and 1×10^{-2} M. Values are means ±S.E. of four experiments.

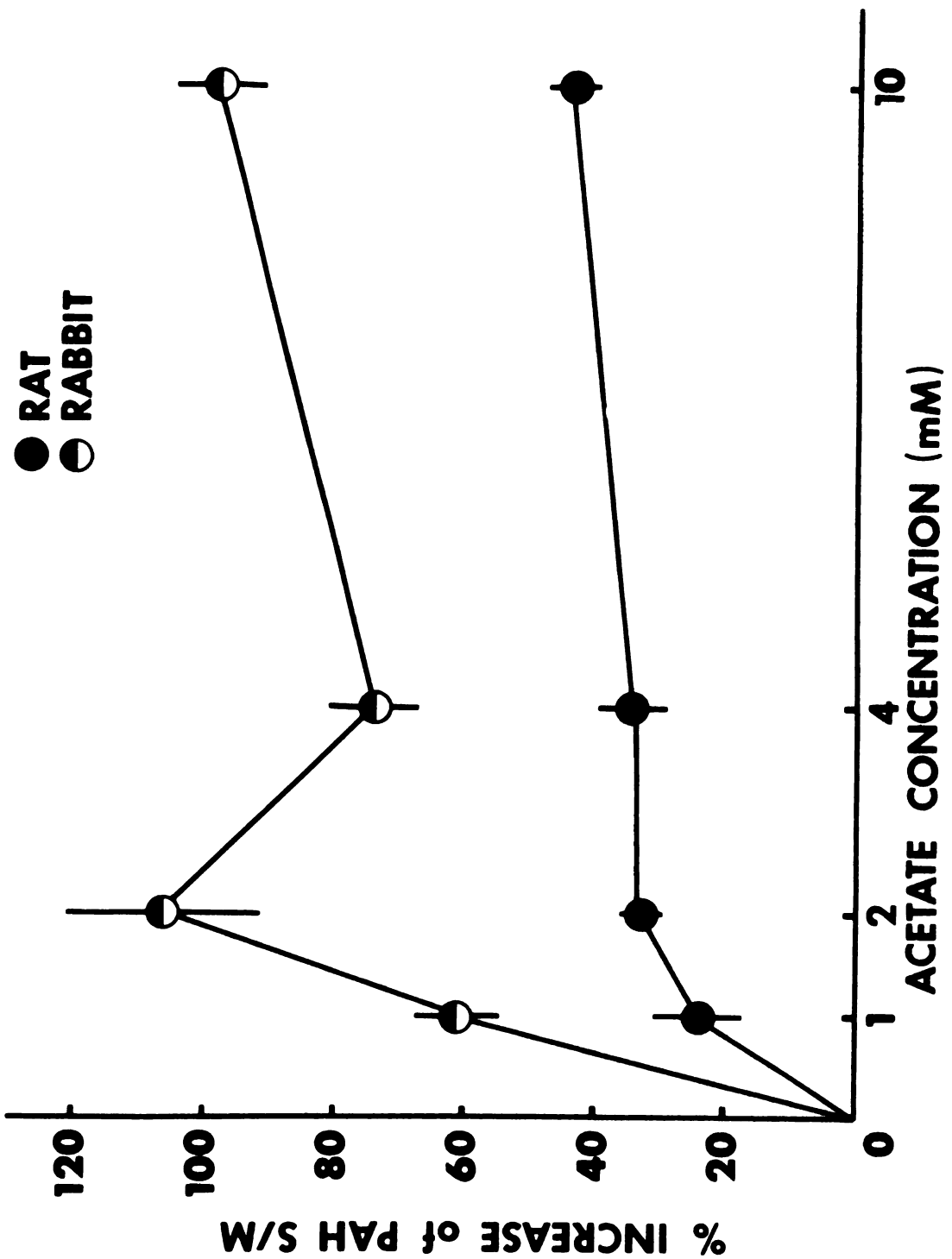
RAT
RABBIT
% INCREASE of PAH S/M
120
100
80
60
40
20
0
1
2
4
10
ACETATE CONCENTRATION (mM)

Figure 12

Figure 13. Effect of acetate on PAH synthesizing enzyme system in the renal cortical homogenates of adult rat. Values are means ±S.E. of four experiments, each experiment was the average of triplicate determinations. The absence of a vertical bar indicates S.E. is within the radius of the point.

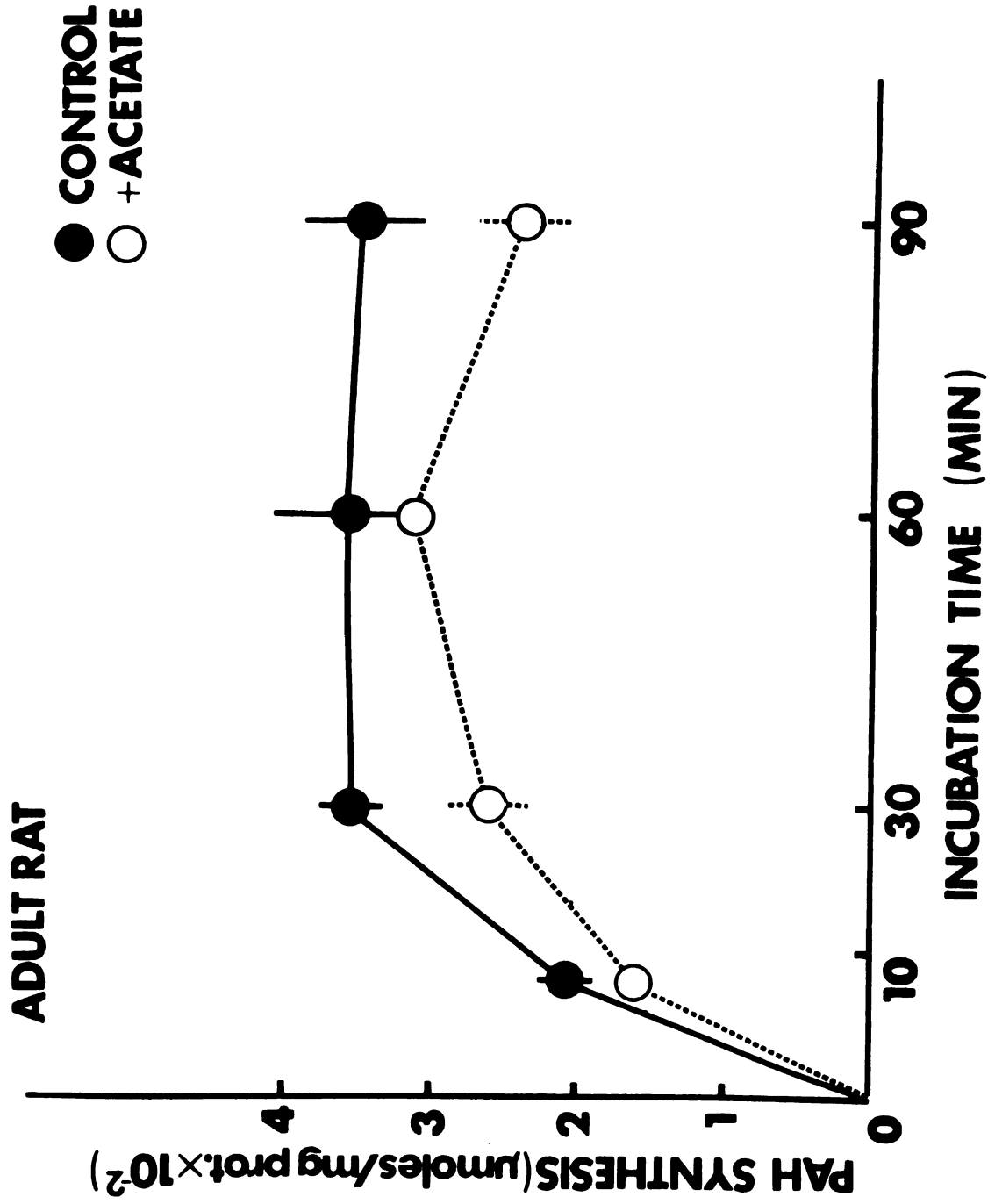
ADULT RAT
● CONTROL
○ +ACETATE
PAH SYNTHESIS (μmoles/mg prot.×10⁻²)
0
1
2
3
4
10
30
60
90
INCUBATION TIME (MIN)

Figure 13

Figure 14. Effect of acetate on PAH synthesizing enzyme syste in renal cortical homogenates of 10 day old rats. Values are means ±S.E. of four experiments, conducte in triplicate.

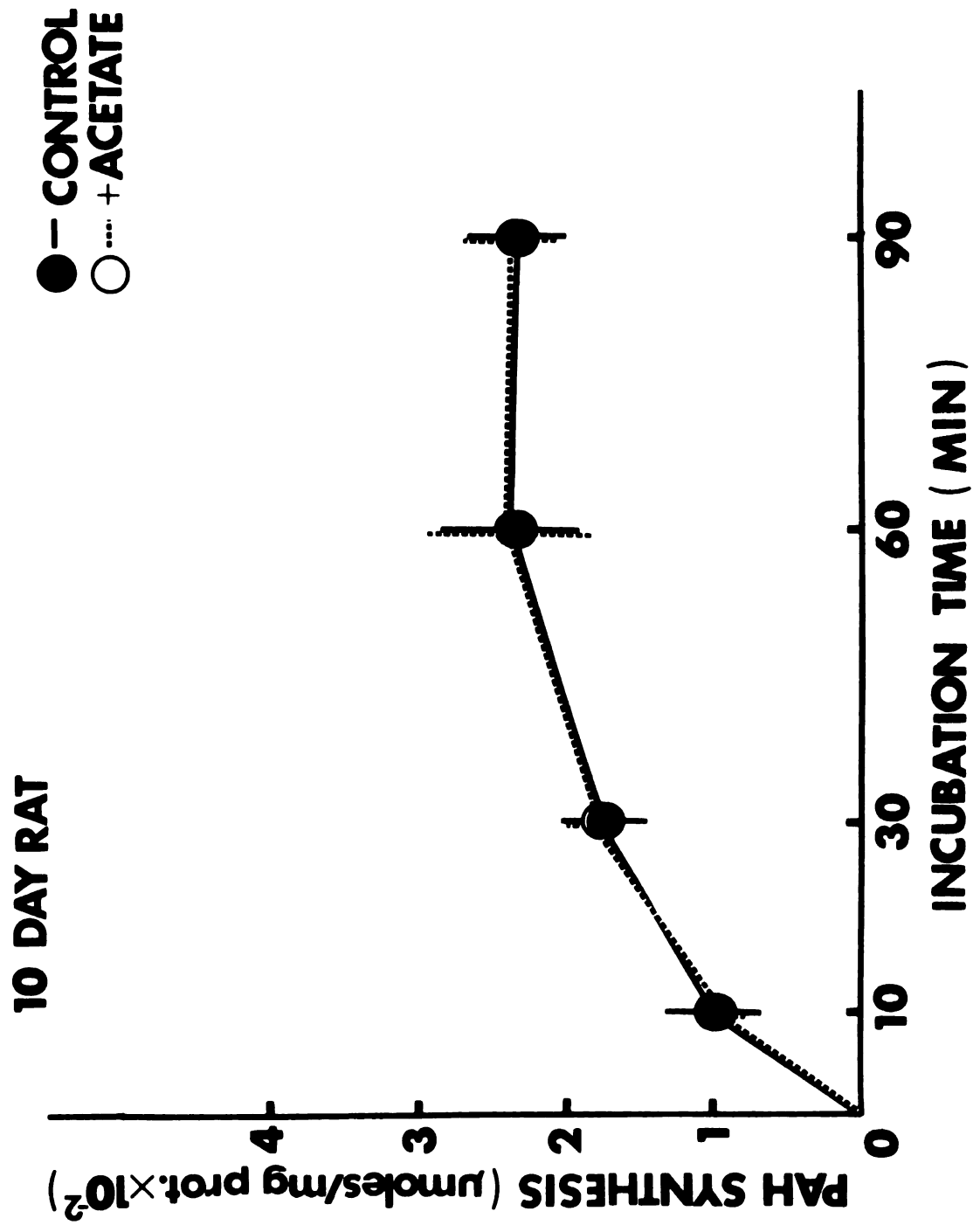
10 DAY RAT
● — CONTROL
○ ··· + ACETATE
PAH SYNTHESIS (μmoles/mg prot.×10⁻²)
0
1
2
3
4
10
30
60
90
INCUBATION TIME (MIN)

Figure 14

Figure 14. Effect of acetate on PAH synthesizing enzyme system in renal cortical homogenates of 10 day old rats. Values are means ±S.E. of four experiments, conducted in triplicate.

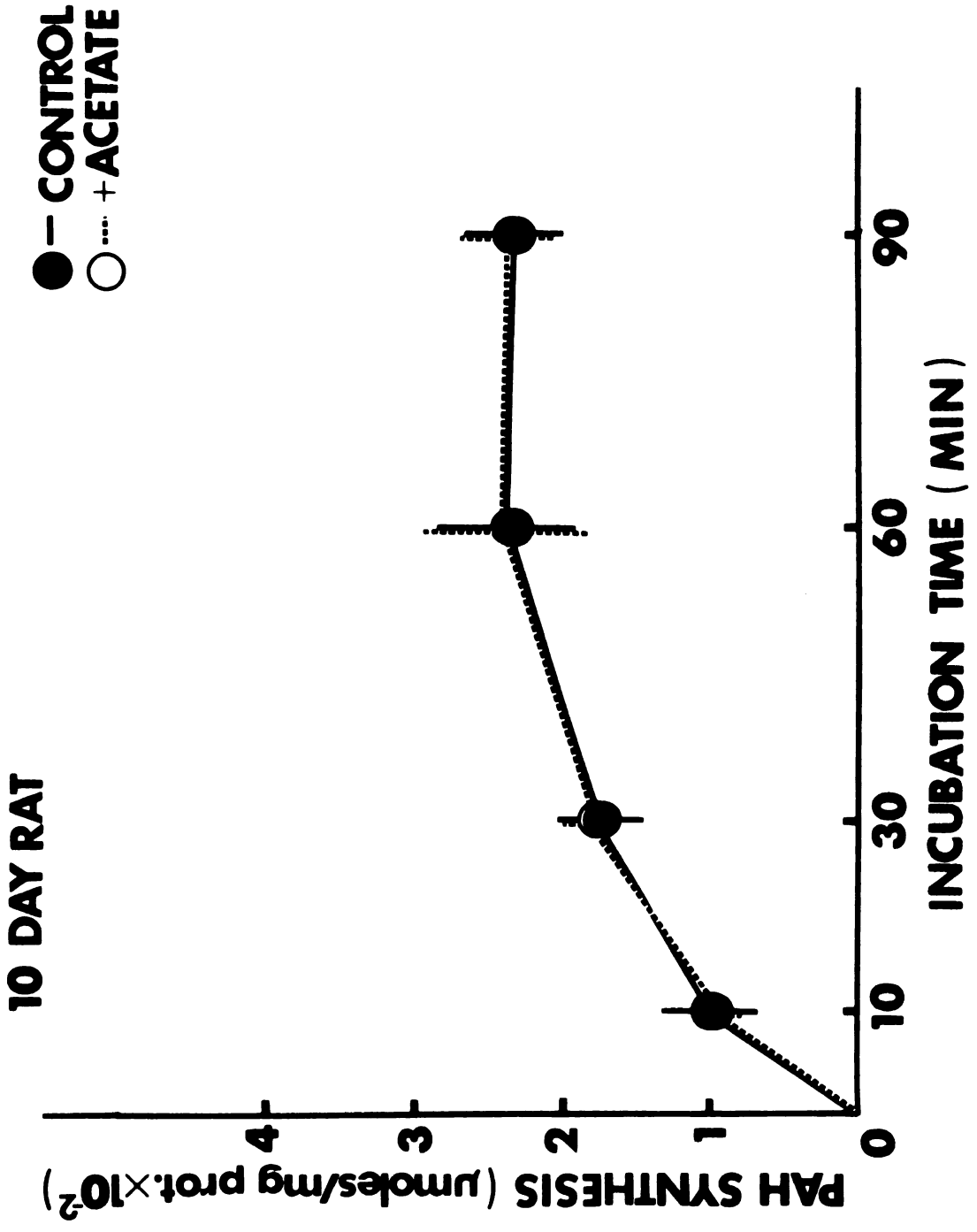
10 DAY RAT
● — CONTROL
○ ···· + ACETATE
PAH SYNTHESIS (μmoles/mg prot.×10⁻²)
0
1
2
3
4
10
30
60
90
INCUBATION TIME (MIN)

Figure 14

Figure 14. Effect of acetate on PAH synthesizing enzyme system in renal cortical homogenates of 10 day old rats. Values are means ±S.E. of four experiments, conducted in triplicate.

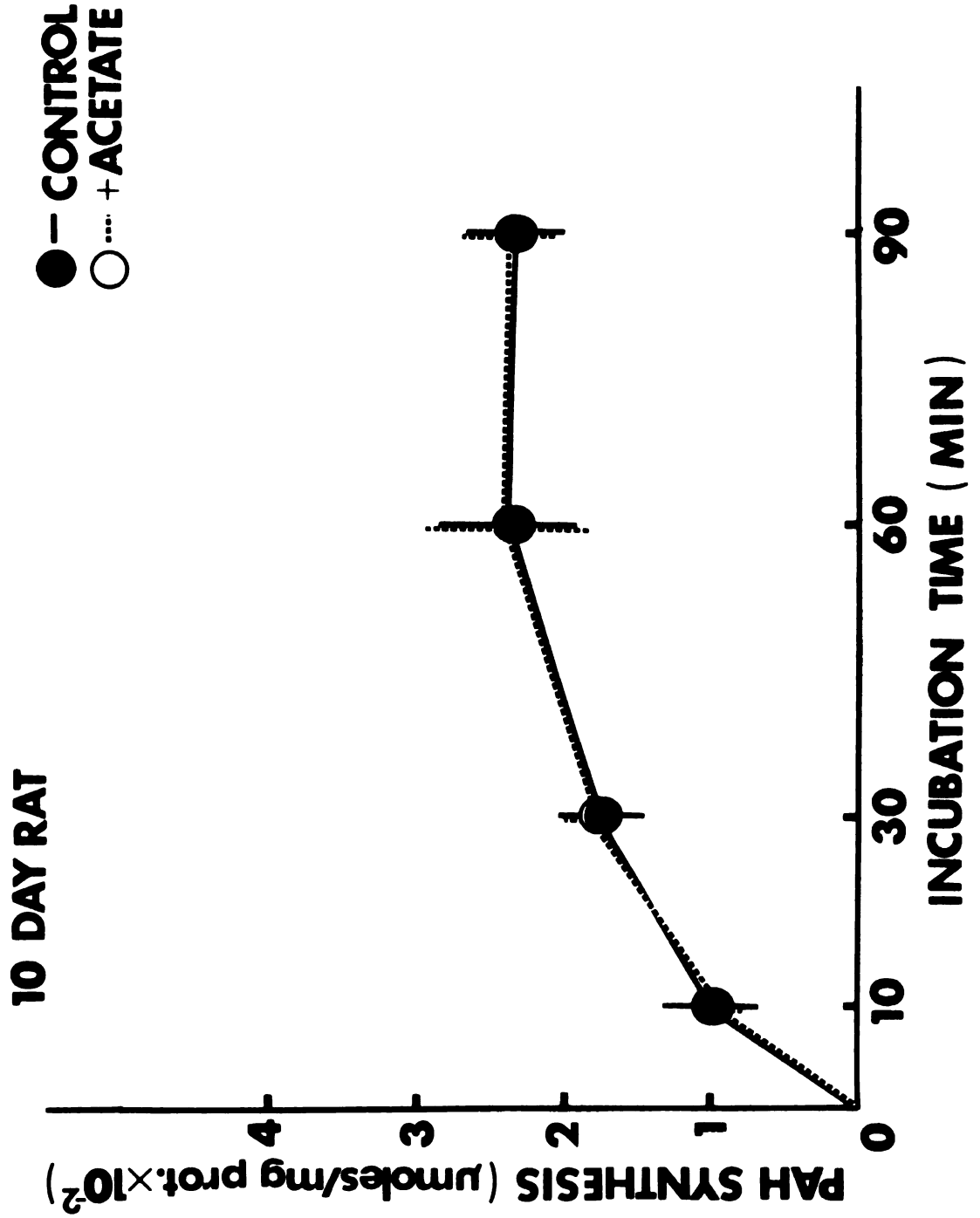
10 DAY RAT
● — CONTROL
○ ··· + ACETATE
PAH SYNTHESIS (μmoles/mg prot.×10²)
0
1
2
3
4
10
30
60
90
INCUBATION TIME (MIN)

Figure 14

Figure 15. Effect of varying glycine concentrations of the PAH synthesizing enzyme system in renal cortical homogenates of adult rats. Values are averages of three determinations. The glycine concentrations in the reaction mixture were 0, 5, 25 and 50 umoles (the glycine concentration in the normal reaction mixture was 45 umoles). PAH synthesis was expressed umoles/mg. protein/30 min x 10^{-2}.

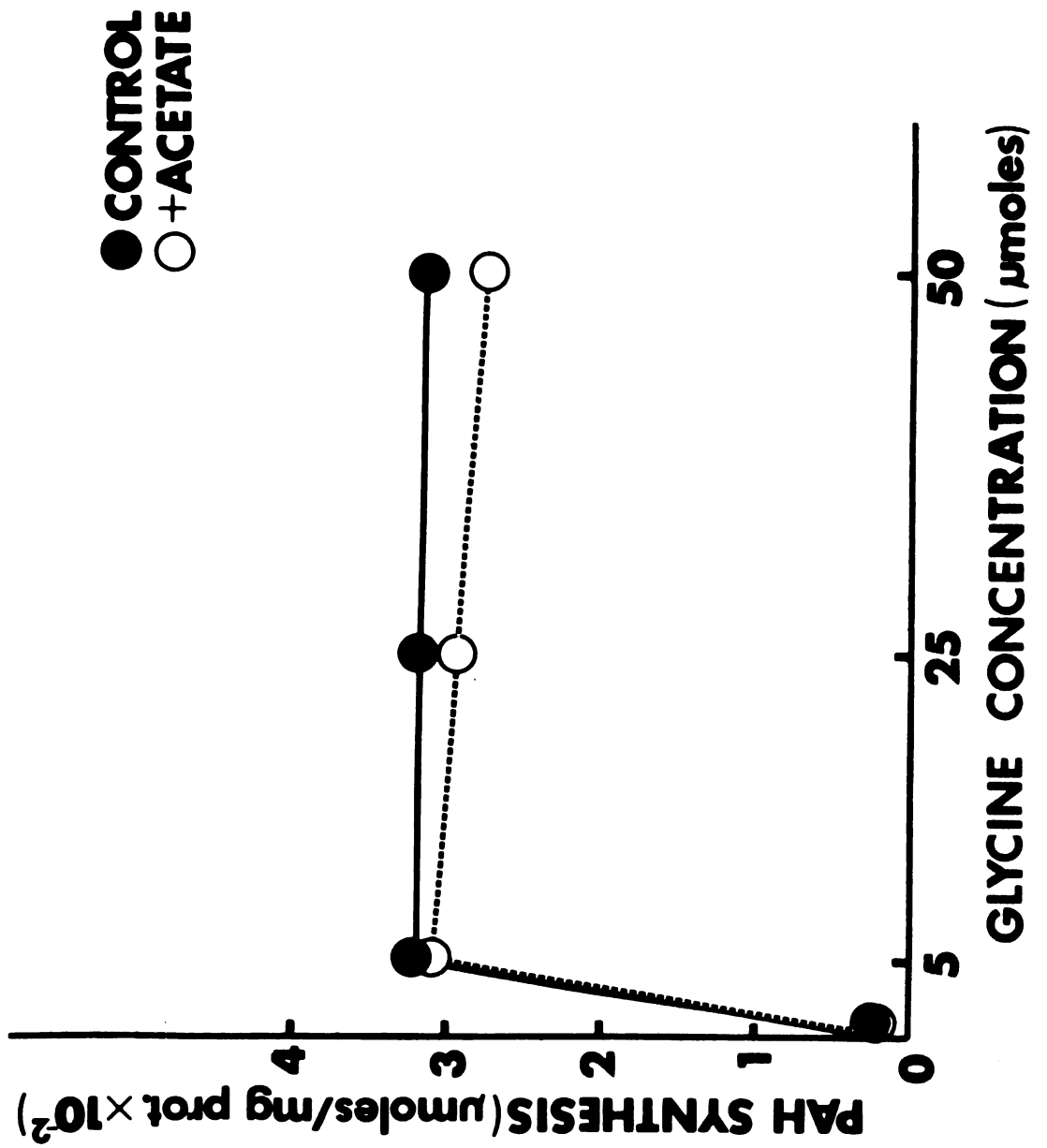
●CONTROL
○+ACETATE
PAH SYNTHESIS(μmoles/mg prot.×10⁻²)
0
1
2
3
4
5
25
50
GLYCINE CONCENTRATION (μmoles)

Figure 15

Figure 16. Effect of acetate on the rate of PAH synthesis in renal cortical homogenates of 10 day old and adult rats. The glycine concentrations in the reaction mixture were 0, 1, 2, 4, and 5 μmoles. In this Hofstee plot, V represents the rate of PAH synthesis, μmoles/mg protein/30 min x 10^{-2} in adult, μmoles/mg protein/60 min x 10^{-2} in 10 days, [S] represents the concentration of glycine in the reaction mixture. Lines are calculated regression lines. Two lines within an age group are not significantly different (Student's "t" test for differences between slopes).

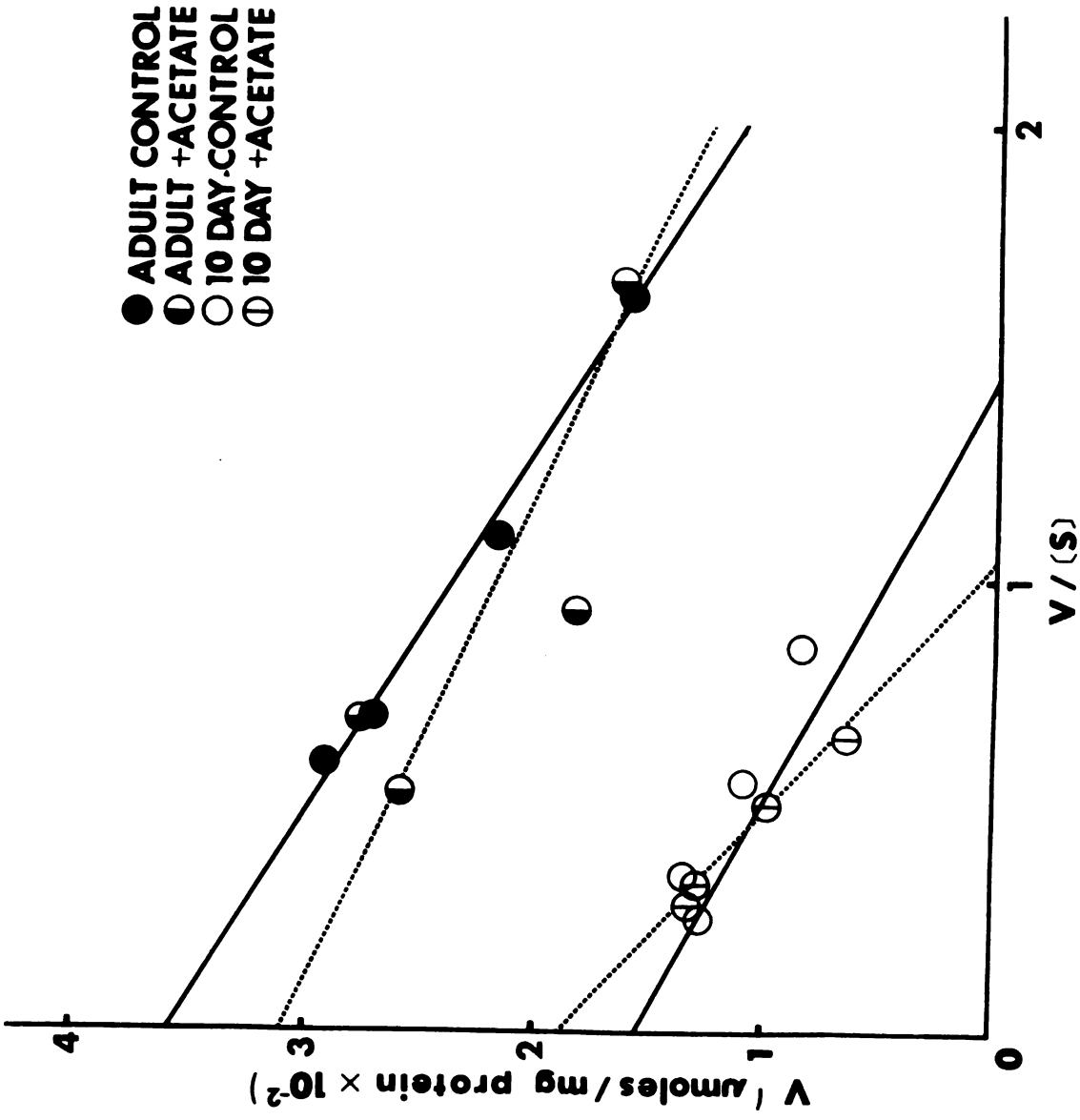
● ADULT CONTROL
◐ ADULT +ACETATE
○ 10 DAY CONTROL
⊖ 10 DAY +ACETATE
V (μmoles / mg protein × 10⁻²)
V / (S)
0
1
2
3
4

Figure 16

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