MECHANISMS RESPONSIBLE FOR SEX DIFFERENCES IN TRANSPORT OF ORGANIC IONS BY RAT KIDNEY CORTEX

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ABSTRACT

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By

Henry Michael Bowman

The ability of renal cortical slices to accumulate the organic acid paraminohippurate (PAH) is greater in tissue from male than female rats. The object of this study was to determine the specificity of this effect and to elucidate the mechanisms responsible for this difference.

Accumulation of organic ions by renal cortical slices was studied using an *in vitro* slice technique. This method involves incubation of thin slices of renal cortex in an oxygenated, buffered medium containing a low concentration of the substance to be measured. Data are expressed as the concentration of the substance accumulated in the slice factored by the concentration of substance remaining in the medium (S/M ratio). An S/M ratio greater than one is indicative of active transport and can be related to active secretion *in vivo*. PAH was used as the prototype for studying organic anion transport, while N-methylnicotinamide (NMN) and tetraethylammonium (TEA) were used as the prototypes for studying base transport. Sexually mature male and female Sprague-Dawley rats (12 weeks old) were used for this study.

Renal cortical slices from male rats developed higher S/M ratios for PAH than did slices from females. This difference was independent of incubation times (30-180 minutes) used. NMN S/M ratios were the same for either sex. However, a slight arithmetic difference was noted at each incubation time used. Another organic base (TEA) was therefore used because it exhibits much higher S/M ratios. Preliminary results indicated that renal cortical slices from male rats exhibited higher TEA S/M ratios than did females. This difference increased as the medium concentration of TEA was decreased. Incubation of renal cortical slices from male and female rats under nitrogen reduced PAH and TEA S/M ratios to one or less. Abolishment of the sex difference seen in the accumulation of both substances also occurred. Thus, the data demonstrate a lack of specificity in the action of sex hormones upon the accumulating mechanism for organic acids. The difference in S/M ratios results from the activity of an oxygen dependent active transport mechanism and not to a sex related difference in non specific binding to protein.

Since the S/M ratio is based upon tissue wet weight, differences could occur if slice composition varied with sex. However, total, extracellular, and intracellular water content in cortical slices from male and female rats was not different, nor was protein content.

Since the S/M ratio measured in the steady state is the sum of the individual components of entry, accumulation into

the slice, and runout from the slice, a difference in any of these processes could account for the sex difference seen. The two measurable components, uptake and runout, were therefore investigated in tissue from male and female rats.

Accumulation of PAH or TEA by renal cortical slices was measured during short times (2-15 minutes) when uptake is linear and relatively uninfluenced by other processes. No difference in accumulation of these substances between male and female was found. However, these measurements were made at only one medium concentration of PAH or TEA. Measurement of PAH uptake at PAH concentrations of 1, 2, 4 and 8×10^{-4} M was therefore done. PAH uptake by renal cortical slices from male rats occurred at a faster rate. This was evidenced by a greater maximal velocity (V_{max}) and apparent affinity constant (Km) for the male than was seen for female. Analysis of PAH uptake by renal cortical slices from male rats under varying experimental conditions indicated a depressing effect of probenecid and nitrogen on PAH uptake. Sodium acetate enhanced PAH uptake.

Runout of TEA from renal cortical slices of female rats appeared greater than from males. Likewise, runout of PAH from renal cortical slices of female rats appeared greater than from males. This was seen as a greater rate constant of the slow component and a shorter half time for runout exhibited by females. Runout of PAH from cortical slices

of male and female rats at 1°C was depressed. Incubation under nitrogen decreased the rate constant and increased the half time for runout.

It was concluded from these observations that the greater PAH S/M ratios exhibited by renal cortical slices from male rats results from a greater rate of uptake. This appears to be complimented by a slower rate of runout of PAH from cortical tissue of males.

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Henry Michael Bowman

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CHAPTER I

INTRODUCTION

Because of the many complex functions of the kidney, clarification of the individual mechanisms was long in coming. Since the time of Bowman, glomerular filtration was thought to be the principle mechanism responsible for clearing the body of waste products (Pitts, 1968). The first significant study to elucidate another kidney function was that of Marshall and Vickers (1923) who determined the method of excretion of phenolsulfonphthalein (phenol red) by the dog kidney. Their findings supported a theory that certain substances can be transported from the peritubular fluid to the tubular lumen by means of a specific secretory process. Other experiments by Marshall and Crane (1924) supported these observations. It appeared that the cells of the proximal tubule were able to pick up, concentrate and secrete into the urine a substance (phenol red) to which cells are generally impermeable. Added proof that phenol red passes solely in the direction from peritubular fluid to tubular lumen was obtained in studies done on mesonephric tubules from 9 day old chicks. The dye was admitted into cells only from that surface which is presented to the outside of the renal tubule (Chambers and Kempton, 1933).

The handling of phenol red by the mesonephric tubules of the chick was the same both *in vivo* and *in vitro*. Other data from these same experiments suggested some type of energy-requiring mechanism was responsible for transporting phenol red. This entire process could be inhibited when the temperature was lowered from 30°C to 3°C or when the *in vitro* incubation was not oxygenated. Since these early studies, it has been shown by many investigators that other organic acids and also organic bases are actively secreted by the cells of the proximal convoluted tubules in the cortex of the kidney (Cross and Taggart, 1950; Kandel and Peters, 1957; Farah *et al.*, 1959).

Techniques used to measure these secretory processes have been designed and utilized in both *in vivo* and *in vitro* systems. Use of the *in vivo* system for measurement is limited to a predetermined environment and can be used only for substances which do not have toxic effects on the animal. However, the renal handling of many organic acids and bases has been investigated using *in vivo* techniques. Malvin and Sullivan (1958), using a stop flow analysis, clearly demonstrated that the organic acid p-aminohippurate (PAH) is secreted in the proximal portion of the adult dog nephron. Other investigators, using clearance techniques, were able to demonstrate proximal tubular secretion of such organic bases as N-methylnicotinamide (NMN), tetraethyl-ammonium (TEA) and darstine and of organic acids such as

PAH, penicillin and phenol red (Beyer *et al.*, 1950; Kandel and Peters, 1957).

Methods for *in vitro* studies offer several advantages over *in vivo* systems. In such preparations various physiological and hormonal influences can be excluded, the problem of systemic toxicity due to various test substances is not present, and the conditions under which the measurements are made can be varied. One method employs the use of isolated renal tubules suspended in a medium containing various salt compounds and a colored dye such as phenol red. By use of a light microscope it is possible to demonstrate that the dye is transported by the cells of the tubules into the lumen. Unfortunately, such studies depend upon frequent direct visualization, and such a method is limited to colored compounds (Forster, 1948; Taggart and Forster, 1950). This method is strictly qualitative, but it is possible to demonstrate that a substance can be actively transported by the cells and that various agents can inhibit this. Cross and Taggart (1950) devised a technique which enabled investigators to quantify accumulation of substances by kidney cortical slices. This method employed thinly cut renal cortical slices incubated in an oxygenated buffered salt solution containing the substance to be measured. The amount of substance accumulated by the slice is compared to the amount of substance remaining in the medium and this is expressed as a slice to medium (S/M) ratio. This technique demonstrates the direct effect of such factors as temperature, pH,

oxygen, and other substances on the accumulation of various test materials (such as PAH) in an environment which is relatively free of normal physiological and hormonal factors.

The maximal ability of kidney cortical slices to accumulate PAH *in vitro* is 2-3 μmoles/gm tissue. This value compares well with the figure of 4-5 μmoles/gm tissue which represents the maximal capacity of rabbit kidney cortex to concentrate PAH *in vivo*. This close relationship between the results obtained *in vivo* and *in vitro* lends justification to the use of the slice technique (Foulkes and Miller, 1959a). The results obtained using the slice technique also lend support to the conclusion that the accumulation of various substances by kidney slices is closely related to the tubular secretion of these compounds in the intact animal (Cross and Taggart, 1950).

Sites responsible for the active transport of organic acids and bases are located in the proximal convoluted tubules of the kidney (Chambers and Kempton, 1933; Malvin *et al.*, 1958; Farah *et al.*, 1959). They involve similar transport mechanisms which are functionally distinct. Most organic acids share a common transport site and can competitively inhibit the transport of other organic acids (Knoefel and Huang, 1959; Huang and Lin, 1963). The same is true for the sites involved in base transport. Farah *et al.*, (1957) showed that uptake of TEA by dog renal cortical slices was inhibited by addition to the medium of

such organic bases as priscoline, NMN and guanidine. Neither class of compounds (organic acids or organic bases) interferes with the transport of the other, thus indicating separate and distinct transport sites for each (Farah and Rennick, 1956).

Active transport implies movement of a substance against an electro-chemical gradient. Energy required for this process is derived from metabolic activity involved in the maintenance of cellular function. Tubular secretion of organic acids and bases from the peritubular fluid requires active transport as a part of this process. Taggart and Forster (1950) observed that the ability of isolated flounder tubules to concentrate phenol red in the tubular lumen could be inhibited by interruption of an adequate oxygen supply or by cooling to low temperatures. The transfer of phenol red was also completely blocked by dinitrophenol (DNP). This inhibition was thought to be closely related to the ability of DNP to interfere with the formation of energy rich phosphate bond compounds by the mammalian kidney. Taggart and Forster (1950) concluded that the cellular transport of phenol red is dependent in part on utilization of this phosphate bond energy. These observations support the findings of Mudge and Taggart (1950), who studied the role of phosphate bond energy in renal tubular excretory processes in the intact dog. DNP had no effect on glucose reabsorption or on the excretion of sodium and potassium. However, it

did cause a prompt depression in the tubular excretion of PAH, diodrast and phenol red. This depression was attributed to a direct inhibition by DNP of the phosphate bond energy of the cellular transport mechanism involved in transporting these substances. Other evidence supplied by Cross and Taggart (1950) demonstrated that the addition of certain metabolic intermediates such as pyruvate and lactate to the medium enhanced the S/M ratios for PAH. Cross and Taggart (1950) attributed this enhancement to the ability of these substrates to donate energy for tubular transport. Other intermediates such as succinate and α-ketoglutarate depressed the ability of kidney slices to actively accumulate PAH. This action was the direct result of competition by these substrates for the same transport sites utilized by PAH (Cross and Taggart, 1950). Similar experiments done recently by Maxild and Moller (1969) support these findings. Most of these findings were involved in defining the cellular mechanism for organic acid transport. The metabolic requirements for secretion of organic bases are less well defined. Observations by Farah and Rennick (1956) suggest that energy for transport of organic bases is also supplied by oxidative phosphorylation. DNP was shown to cause a reduction of the NMN S/M ratio (Farah *et al.*, 1959). However, a variety of Kreb's cycle intermediates neither enhanced nor depressed base transport. Excretion of these substrates solely by way of the organic acid transport

system could account for this effect. Another possibility is that the organic base transport mechanism obtains its energy supply from a source other than the Kreb's cycle.

The stepwise nature of active tubular secretion has been studied extensively and it has been possible to characterize the intermediate events in the transfer process. Forster and Copenhaver (1956) visualized the first step in the renal tubular secretory process as an energy requiring step which results in passage of the secreted substance from the peritubular fluid into the proximal cells. More elaborate studies done by Foulkes and Miller (1959b) suggested four steps in the transfer of PAH from the peritubular fluid to the lumen in rabbit renal cortical slices. They demonstrated the existence of two intracellular fractions of PAH. One of these fractions rapidly diffuses and equilibrates with extracellular PAH. The other fraction diffuses and equilibrates slowly, and is responsible for the high slice to medium ratio. From these results, a model was proposed to explain the transport system for PAH (Figure 1). Step 1 consists of diffusion of PAH from the medium to the extracellular space in the tissue. Step 2 is a facilitated diffusion step at the peritubular cell membrane. Step 3 consists of a build up of a high tissue concentration of PAH. Step 4 results in the transfer of PAH across the lumen border of the cell into the tubular lumen (Foulkes and Miller, 1959b). Unlike Forster and Copenhaver

(1956) who visualized the active step as occurring at the peritubular cell membrane, Foulkes (1963) defined the intracellular concentration step (Step 3), at least in the rabbit, as the active step. Studies done by Farah *et al.* (1963) suggest that accumulation of PAH within the cell is due to a mechanism which controls the concentration of free diffusible PAH inside the cell. Since the S/M ratio truly represents uptake into the slice, accumulation in the cells, and runout back into the medium, uptake may involve steps 1, 2, and 3 and the runout may involve step 4. Work done by Ross *et al.* (1968) suggests this possibility, implying that uptake of organic acids and bases occurs at the peritubular side and that runout occurs at the lumenal side of the proximal tubules.

Uptake of PAH and NMN was shown to be pH dependent, temperature sensitive, and dependent on the presence of potassium and calcium. Runout, however, was not affected by changes in any of these variables (Ross *et al.*, 1968). Uptake and runout, therefore, must be independent processes with separate mechanisms and transport sites. Ross and Farah (1966) and Ross *et al.* (1968) provide evidence for this. Runout of NMN was not affected by inhibitors that depress the rate of runout of PAH from kidney slices, thus suggesting that mechanisms for runout of organic acids and organic bases are different from each other (Ross and Farah, 1966).

Recently, identification of the actual carrier site for organic acids and bases has received much attention. Most of the work has been done on sites for base transport because compounds are available which can irreversibly inhibit the renal transport system of organic bases. Using C^{14} labeled dibenamine in substrate-protection experiments Magour *et al.* (1969) were able to label a protein in the dog kidney which seems to bind NMN specifically. This protective effect of dibenamine only occurred in cortical tissue slices and could be reduced by TEA and other quaternary ammonium compounds. This provides evidence for a possible carrier-mediated mechanism in the transport of organic bases. It has not been possible to do such studies with organic acids because no agent has been found which can bind specifically and irreversibly to proposed acid transport sites. Evidence that these transport sites exist has been provided from studies concerning PAH uptake by renal cortical slices. The effects of metabolic inhibitors in decreasing accumulation of PAH, the apparent saturation of the system with high PAH concentrations and the effects of organic acid competitors such as probenecid support the existence of such transport sites (Farah *et al.*, 1959; Huang and Lin, 1963; Berndt and Grote, 1968).

Other studies have shown that different stimuli, both physical and chemical, can affect renal organic ion transport. Hirsch and Hook (1970) have shown that treatment of young

animals with certain organic acids can increase the accumulation of PAH by slices of renal cortex. This effect was specific for organic anion transport and was thought to involve substrate induction of the transport mechanism. Direct stimulation of fully mature kidneys is somewhat different. The enzyme systems for transport are fully developed and appear refractory to substrate stimulation. Certain hormones, however, have noticeable effects on these transport sites. Farah (1952) reported that hypophysectomized rats have a decreased PAH S/M ratio. Administration of growth hormone returned the depressed PAH S/M ratio to normal. However, the PAH S/M ratio for normal adult rats was unchanged after treatment with growth hormone (Farah, 1952). These findings suggest that physiological levels of certain endogenous hormones may be necessary to maintain normal transport function.

While many substances appear to be specific stimuli for either organic acid or base transport systems; no substance has been shown to exert a direct, nonspecific effect on renal ion transport (Domer, 1960; Hirsch and Hook, 1970). Evidence available on the effect of sex hormones on renal transport of PAH in rats provide some interesting observations (Huang and McIntosh, 1955; Ferguson, 1963). Renal cortical slices from mature male rats had greater PAH uptake than did female kidneys (Huang and McIntosh, 1955; Ferguson, 1963). This difference was attributed to the continued

presence of testosterone in the male which in some manner was constantly stimulating transport of PAH. Injections of testosterone to females caused an increase in the PAH S/M ratio to the level of the male. Estrogen treatment of male rats reduced the normal PAH S/M ratio, but had no effect on PAH uptake by kidney slices of normal female and castrated male rats. These results demonstrated that the difference in the PAH S/M ratio between the sexes is due to the stimulatory effect of testosterone and not to the depressing effect of estrogen. More recently, Harvey and Malvin (1965) demonstrated higher creatinine clearances in male than female rats. Injections of testosterone into females increased creatinine clearance to male levels. Estrogen, however, did not affect creatinine clearance in either sex. No detectable sex difference in creatinine clearance has been observed in man (Harvey *et al.*, 1966). Dogs, however, exhibit a slight sex difference which is sensitive to testosterone (O'Connell *et al.*, 1962). These results suggest that renal tubular handling of creatinine is species dependent.

It was the purpose of this study to determine the specificity of testosterone induced renal transport changes and to elucidate the mechanisms responsible for the observed sex difference in rats.

CHAPTER II

METHODS

Accumulation of Organic Ions by Renal Cortical Slices

The ability of kidney cortical slices to actively accumulate organic ions was studied using the *in vitro* slice technique of Cross and Taggart (1950). PAH was used as the prototype for studying organic anion transport, while NMN and TEA were used as the prototypes for studying base transport. Sexually mature male and female Sprague-Dawley rats (12 weeks old) were used for this study.

Animals were killed by cervical dislocation and the kidneys removed immediately, weighed, and placed in ice-cold normal saline. Renal cortical slices weighing between 80 and 100 mg were prepared free-hand and kept briefly in cold normal saline until incubated. Slices were then incubated in 2.7 ml of the phosphate buffer devised 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} (1.15 mc/mmol). All 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 initial rate of uptake was determined. For those experiments, incubation times varied from 2 to 16 minutes.

After incubation the slices were quickly removed from the beakers, blotted and weighed. The weighed slices were macerated in 3 ml of cold trichloroacetic acid (10%) and the final volume of the homogenates was brought to 10 ml with distilled water and centrifuged. A 2 ml aliquot of media was similarly treated. PAH in the supernatant was estimated by the method of Smith *et al.* (1945). 1.0 ml aliquots of slice and media acid supernatant were added to scintillation vials containing 10 ml of modified Bray's solution (6 g of 2,5-diphenyloxazole and 100 g of naphthalene per liter of dioxane). The amount of NMN-C^{14} or TEA-C^{14} was determined in a Beckman LS-100 liquid scintillation counter employing external standardization. The transport that occurred was expressed as the slice/medium (S/M) ratio where S equals milligrams per gram of tissue (wet weight) or disintegrations per minute per gram of tissue and M equals milligrams per milliliter of medium or disintegrations per minute per milliliter of medium.

Analysis of Renal Cortical Tissue Composition

Extracellular space of renal cortical slices was determined by the method of Webber and Cairns (1958) using inulin-C^{14} (2 mc/g). Between 50 and 100 mg of sliced cortical tissue was placed in beakers containing .075 μc inulin-C^{14} in 3 ml of incubation media and incubated for 90 minutes. The procedure for analysis of tissue and medium homogenates was described previously. The samples were analyzed for

inulin-C^{14} using a Beckman LS-100 liquid scintillation counter. The inulin space as a fraction of wet weight was calculated by dividing tissue concentration by medium concentration. Total water content of cortical slices was determined by weighing the blotted wet tissue at the end of incubation and again after drying for 24 hours at 100°C. Tissue water was expressed as a fraction of wet weight. Intracellular space as a fraction of wet weight was calculated as the difference between total water content and inulin space.

Renal cortical slices were analyzed for protein content by the method of Lowry *et al.* (1951). Tissue samples weighing 100-150 mg were homogenized in 10 ml of 2 N NaOH and the contents were allowed to stand for 1 hour at room temperature. 1 ml aliquots were then diluted with water and analyzed for protein, and the results expressed as mg protein per 100 mg of wet tissue weight.

Analysis of Runout of PAH and TEA from Male and Female Rat Renal Cortical Slices

Runout of p-aminohippuric-(glycyl-2-H^3) acid (106.8 mc/mmol) and TEA-C^{14} was determined by the method of Farah *et al.* (1963). Preliminary loading of the slices with PAH-H^3 or TEA-C^{14} was accomplished by incubating at 25°C for 2 hrs in the normal media containing 6.0×10^{-4} M PAH-H^3 or 1.0×10^{-4} M TEA-C^{14}. Accumulation of PAH-H^3 or TEA-C^{14} was determined in representative samples of these slices and the

medium and the S/M ratios were calculated (Cross and Taggart, 1950). The remaining slices were removed from the PAH-H^3 or TEA-C^{14} containing medium, blotted on gauze and transferred at 1 min intervals through a series of 12 beakers containing the normal media, free of PAH or TEA. All runout beakers were equilibrated for 30 minutes at 25°C under a gas phase of 100% oxygen prior to runout. Runout experiments were performed in the Dubnoff metabolic shaker at 25°C under a gas phase of 100% oxygen. At the end of the runout experiment the beakers were removed from the incubator and aliquots from the beakers were analyzed as before. The amount of PAH-H^3 found in the samples was determined using a Beckman LS-100 liquid scintillation counter. The amount of substance in the slice at any one time is determined by subtracting the amount of substance appearing in the runout beakers up to that time from the amount of substance accumulated by the slice prior to runout.

Kinetic Analysis of PAH Uptake

To estimate rate of PAH transport, slices were incubated for 2 and 12 min at varying PAH concentrations (1, 2, 4 and 8×10^{-4} M). PAH was assayed by the methods described previously. The amount of PAH accumulated per gram of slice per min was calculated. The results were plotted using a Lineweaver-Burke plot (Clark, 1964), where the reciprocal of the rate of PAH uptake per minute was plotted against the reciprocal of the PAH concentrations.

The effect of sodium acetate (1.0×10^{-2} M), probenecid (5.0×10^{-6} M), and incubation under nitrogen atmosphere on the rate of PAH uptake in male rat kidney cortical slices was also determined. In those cases where the effect of sodium acetate and probenecid was being tested, addition of these agents was directly to the incubation beakers. The effect of nitrogen was determined by discontinuing incubation under oxygen and substituting nitrogen.

Statistical Methods

Data was analyzed using Student's "t" test for unpaired observations. The level of significance was chosen as $p<.05$. Initial rate of uptake or runout was determined by linear regression analysis (method of least squares, Goldstein, 1964). For those studies involving the effect of different PAH concentrations on the velocity of uptake, a kinetic analysis employing a Lineweaver-Burke plot was used (Clark, 1964).

CHAPTER III

RESULTS

Accumulation of Organic Ions by Renal Cortical Slices

PAH S/M Ratio

Adult male rats are heavier than adult females. Similarly, kidneys from males are heavier than kidneys from females. When kidney weight was factored by body weight the ratio for six males (.0067±.0001) was no different than that of six females (.0066±.0002). Nevertheless, renal cortical slices from male rats accumulated more PAH than slices from females (Figure 2). After ninety minutes of incubation, slices from males developed a PAH S/M ratio of 12.7±1.0, while an S/M ratio of 7.8±0.3 was seen with tissue from females. This difference was independent of incubation time as evidenced by the difference in S/M ratio at all incubation times used (Figure 2).

PAH Uptake

PAH uptake (µg PAH/100 mg tissue) by renal cortical slices was determined in a series of beakers over increasing incubation times. Uptake was found to be linear between two and fifteen minutes (Figure 3). Points on the graph represent the amount of PAH (µg/100 mg tissue) taken up by tissue slices during the short incubation times and when

factored by time, the rate of entry of PAH into the slices could be determined. The slopes (calculated regression lines) for the male and female tissue were 1.5 and 1.7 respectively. Thus the initial uptake rate of PAH for male and female was not different when measured in this manner.

NMN S/M Ratio

NMN S/M ratios for renal cortical slices from male and female rats were not significantly different (Figure 4). After ninety minutes of incubation values were 6.4±0.6 for males and 5.3±0.5 for females. However, a slight arithmetic difference between the ratio for male and female was noticed at all incubation times used (Figure 4). These studies were repeated at ninety minutes of incubation measuring PAH and NMN simultaneously. The data are summarized in Table 1. When measured in the presence of NMN, the PAH S/M ratio for renal cortical slices from male rat kidney (13.2±0.9) was again significantly greater than from females (7.9±0.3). The NMN S/M ratio in the same slices from males (6.4±0.6) was not significantly greater than from the females (5.3± 0.5).

TEA S/M Ratio

Preliminary results using the organic base TEA (10^{-5} M) showed that the TEA S/M ratio for renal cortical slices from male rats (20.5±0.4) was significantly greater than those from females (17.4±0.4) (Table 1). Figure 5 illustrates that the difference in TEA S/M ratios (3.1±0.4 at 10^{-5} M TEA)

for renal cortical slices from male and female rats was magnified to 9.24±1.1 with a change from 10^{-5} to 10^{-7} M TEA in the medium.

When renal cortical slices from male and female rats were incubated under an atmosphere of nitrogen S/M ratios of approximately one were attained (Table 2). The difference in the S/M ratio for PAH and TEA between the sexes was also abolished.

TEA Uptake

Ability of renal cortical slices from male and female rats to accumulate TEA during short times was not different (Figure 6). The points on the graph represent the amount of TEA (x 10^{-2} µg/100 mg tissue) taken up by tissue slices during short incubation times. The slopes of the calculated regression lines for the male and female rate curves were 1.30 and 1.29 respectively.

Renal Cortical Slice Composition

Composition of slice components is illustrated in Figure 7. Total water content (expressed as a percent of tissue wet weight) of renal cortical slices from male (83.9±0.5%) and female (83.3±0.5%) rats was not different. Extracellular space (inulin space) was 25.4±0.9% for males and 26.6±0.6% for females. Consequently, intracellular space, the difference between these two measurements, was not different. Similarly, protein content of the cortex from

female rats (12.7±0.7%) was no different from that of the males (12.2±0.5).

Runout of Organic Ions From Renal Cortical Slices

Runout of PAH and TEA from preloaded renal cortical slices was exponential (Figures 8 and 9). The runout curves for PAH appear to be a composite of at least two separate components. The slow component of the curve could be described by the equation for a first order reaction. Thus the rate constant, k, for this part of the curve is calculated using the equation for a first order reaction:

$$k = \frac{2.303}{t} \log \frac{\text{concentration of PAH in the slice at zero time}}{\text{concentration of PAH in the slice at time t}}$$

The rate constant describing disappearance of PAH via the slow component from male tissue (.046±.004 min^{-1}) was less than that from female tissue (.070±.020 min^{-1}). This difference in rate constant for one portion of the curve was accompanied by a longer half-time of runout from the entire curve. The half-time for males was (8.0±0.81 min), compared to (6.0±1.4 min) for females (Table 3).

The rate constant for runout of TEA from renal cortical slices of female (.045±.002 min^{-1}) was greater than that of male (.036±.006 min^{-1}) (Table 3). These values were based upon two experiments. Comparison of the runout curves indicates that the half time for runout of TEA from male and female cortical slices is greater than twelve minutes (Table 3).

One PAH runout experiment was performed in an incubator chilled to 1°C. Runout of PAH decreased in both male and female tissues. The k value decreased to .019 while the half time for disappearance of PAH from the tissue slices increased to 12 minutes (Table 3).

Runout of PAH from male rat renal cortical slices was slightly decreased by incubating the runout beakers under nitrogen (Table 3). In these two experiments, half time for runout under nitrogen (9 minutes) was longer than half time for runout performed under oxygen (8 minutes) (Table 3).

Kinetic Analysis of PAH Uptake by Renal Cortical Slices

Male Versus Female

Rate of PAH uptake was determined at concentrations of 1, 2, 4 and 8 x 10^{-4} M PAH. The rate of PAH uptake for renal cortical slices from males was significantly greater than for slices from females. The experimental results were plotted on a double reciprocal plot and are expressed in Figure 10. The slopes of lines for male and female were 0.217 and 0.373 respectively. The plot for the male exhibited a V_{max} (in units of µg/g/min) of 27.02 while the V_{max} for the female was 7.75. Km values (in units of 10^{-4} moles/L) for male and females were 5.86 and 2.89 respectively.

Varying Experimental Conditions

The rate of PAH uptake by renal cortical slices from male rats was determined in the presence of sodium acetate,

probenecid, or nitrogen atmosphere (Figure 11). V_{max} (in units of μg/g/min) and Km values (in units of 10^{-4} moles/L) for PAH uptake were 27.02 and 5.86. In the presence of 0.2 M sodium acetate V_{max} was 50. and Km was 11.20 (Table 4). Addition of probenecid to the medium resulted in V_{max} and Km of 16.10 and 5.79 respectively (Table 4). Incubation of renal cortical slices from male rats under nitrogen resulted in a V_{max} of 9.61 and a Km of 7.10 (Table 4). Probenecid and nitrogen gas exhibited non-competitive type inhibition on PAH uptake. Sodium acetate, however, had an uncompetitive type effect on PAH uptake.

CHAPTER IV

DISCUSSION

Despite the fact that no sex difference was found in the kidney weight to body weight ratio, renal cortical slices from male rats accumulated more PAH than did slices from females (Figure 2). This difference was independent of incubation time from 30-180 minutes. The PAH S/M ratios developed at 90 minutes incubation for male (12.7±1.0) and female (7.8±0.3) are comparable to the values (15.4±1.0 and 8.4±0.50) obtained in the study of Huang and McIntosh (1955). These authors demonstrated that the difference in the transport of PAH between the sexes was due to the stimulating effect of testosterone and not to the depressing effect of estrogens (Huang and McIntosh, 1955). The kidneys of female rats have a lesser ability to transport PAH but they are susceptible to stimulation by testosterone. The kidneys of males appear to be refractory to any further stimulation by testosterone because of the endogenous presence of this hormone (Huang and McIntosh, 1955). Studies done by Harvey and Malvin (1965) support this observation. They reported that adult male rats have a higher creatinine clearance than do females. Injections of testosterone to females four hours prior to experimentation caused an increase in creatinine

clearance to the male value. Estrogen was without effect on creatinine clearance in males (Harvey and Malvin, 1965).

To determine the specificity of the effect exerted by testosterone, the uptake of the organic base NMN was determined in renal cortical slices from male and female rats. Since organic bases are transported by a system similar to, but distinct from, those for organic acids a sex difference in base transport would indicate a non specific effect of testosterone on renal transport systems. Results showed that over long incubation times (30 to 180 minutes) there was no statistical difference in the NMN S/M ratio between male and female rats (Figure 4). A slight arithmetic difference, however, was noticed at each incubation time used. To minimize variation these studies were repeated at ninety minutes of incubation measuring PAH and NMN simultaneously. When measured in the presence of NMN, the PAH S/M ratio in cortical slices from males was significantly greater than from females (Table 1). The NMN S/M ratio for cortical slices from males was not significantly greater than that of females (Table 1). Another organic base (TEA) was then used because it develops much higher S/M ratios. Development of high S/M ratios by TEA is attributed to its quaternary ammonium configuration. TEA is actively taken up by the tissue, but passage back into the medium is hindered by its structural configuration. The compound will stay in the tissue cells and this will result in high slice to medium

ratios. Since TEA is taken up by most species studied, it is a very useful substance to employ in studying organic base transport in kidney slices (McIsaac, 1969). Preliminary results showed that a statistically significant difference in the TEA S/M ratio for renal cortical slices from male and female rats did exist (Table 1). Experiments were then done to determine if this difference was physiologically real. It was reasoned, that if the effect were real we could magnify this difference by decreasing the concentration of TEA in the medium. Results illustrated in Figure 5 showed that with decreasing concentration of TEA in the medium the difference between male and female ratios was magnified. This indicates a real difference in accumulation of TEA between the sexes. Although it appears that the TEA S/M ratio for females is lower at a molar concentration of 10^{-7} than 10^{-6}, the S/M ratio at both concentrations is statistically the same. The magnification of the difference between the ratios is attributed more to the greater accumulation of TEA by male renal cortical slices at the lower concentrations. When using a TEA molar concentration of 10^{-4} the S/M ratio for renal cortical slices from male rats falls to 10, indicating saturation of the transport mechanism.

It was necessary in this study to demonstrate that the mechanism being studied is truly an active one. Since this type of mechanism is responsible for the S/M ratios that are

obtained for organic acids and bases, studies were carried out using nitrogen gas as substitute for oxygen in the incubation atmosphere. In a nitrogen atmosphere oxygen requiring processes are inhibited and certain cellular functions cannot be performed. Any effect on the S/M ratios for PAH and TEA under these conditions would indicate that an oxygen requiring transport mechanism is involved in the transport of these substances. This was shown to be the case (Table 2). An S/M ratio greater than one, which is indicative of active transport, was not obtained when incubation was carried out under nitrogen. The sex difference seen in the PAH and TEA S/M ratios was also abolished (Table 2). These data support previous studies which demonstrated that an oxygen requiring active transport mechanism is responsible for the accumulation of organic acids and bases by renal cortical slices (Cross and Taggart, 1950; Burg and Orloff, 1962). Furthermore, these results demonstrate that the sex difference seen in accumulation of PAH and TEA involves an active mechanism and is not due to a sex related difference in non specific binding.

Since the S/M ratio is based upon tissue wet weight and because differences could occur if slice composition varied with sex, it was necessary to measure total water content, extracellular space, intracellular space and protein content of the cortical slices. Results indicated

no difference between male and female when these components were measured (Figure 7). It was concluded from these observations that the difference in accumulation of PAH could not be attributed to a gross difference in slice composition.

It was of interest, therefore, to define the mechanisms involved in the sex difference seen in accumulation of PAH. Since the S/M ratio is determined in a steady state this value truly represents the algebraic sum of uptake into the tissue, retention in the tissue, and runout back into the bathing medium. Maximal rate of uptake occurs in the very early incubation times when intracellularly accumulated PAH is not yet concentrated enough to decrease the rate of uptake. Thus, if a difference exists in the maximal rate of uptake this should be seen during very short periods of incubation. A greater initial rate of uptake by renal slices from male rats would account for the higher S/M ratio for PAH seen in males. Measurement of initial uptake was made over the linear portion of the curve (2 through 15 minutes). Beyond 25 minutes the curve tends to plateau off because movement of PAH becomes multi-directional. In other words, runout and intracellular accumulation of PAH are affecting retention of PAH after 25 minutes.

No difference was seen, however, between the maximal rate of uptake of PAH by renal cortical slices from male and female rats (Figure 3). This was exemplified by similar

slopes for the male and female rate curves. Similarly, measurement of accumulation of TEA by renal cortical slices during short time periods showed no difference in the initial TEA uptake rate between male and female rats. This is exemplified by the parallelism of the slopes (Figure 6). These initial uptake rate measurements, however, were done at only one medium concentration of PAH or TEA. Since the sex difference in the TEA S/M ratio was magnified with varying concentrations of TEA in the medium, initial uptake rate could be affected by varying substrate concentration. This will be discussed later in the kinetic analysis of PAH uptake.

The findings of Ross *et al.* (1968) provided evidence to indicate that the transport mechanism for runout is different from the uptake process. This being the case, a difference in the S/M ratio could occur if runout from male and female renal cortical slices was different. Experiments were performed to determine if this assumption was true. The runout of PAH and TEA from preloaded slices was exponential (Figures 8 and 9). Plotting the results on a semi-logarithmic scale gave non linear curves for runout of PAH (Figure 8). Both curves appear to be a composite of a fast and slow component. Similar curves have been observed with tissue from rabbit (Foulkes and Miller, 1959b) and dog (Farah *et al.*, 1963). Quite possibly the fast component represents runout of PAH which lies in the extracellular

space of the tissue slice, and that intracellular fraction which is in equilibration with it (Figure 1). The movement of this component would be primarily passive in nature. The slow component could be composed of the slowly equilibrating and slowly diffusible intracellular fraction of PAH. Movement of this fraction would be a combination of passive diffusion and active transport. Assuming the data for the slow component of the curve can be described using the equation for a first order rate reaction (Farah *et al.*, 1963), it is possible to calculate rate constants for the slow portion of the PAH runout curves for male and female. The rate constant of the female was found to be higher than the male value (Table 3). This is consistent with a faster runout of PAH from female renal cortical tissue. Realizing, however, that the entire runout curves are nonlinear, it may be a false assumption to state explicitly that the data fit exactly the equation for a first order rate reaction. It is highly unlikely that the release of PAH is a monomolecular reaction. Probably, runout is a composite of many different reactions and that one of the steps may behave as a first order reaction (Farah *et al.*, 1963). However, if one is unwilling to accept these assumptions, it can still be shown that half times for runout (measured over the entire curve) were different. In this case, the female value (6.0±1.4 minutes) was less than the male value (8.0±0.81 minutes) (Table 3). Both the greater rate constant and the shorter half time

for runout suggest that runout of PAH from female cortical slices is faster than from male slices. If runout from male kidney slices is slower, PAH will remain intracellularly longer than in female slices. Maximal S/M ratios are attained because the initial rate of uptake by necessity is much faster than runout from the slice (Ross *et al.*, 1968). More intracellular PAH as a result of slower runout from male slices will produce higher PAH S/M ratios.

Runout of TEA from renal cortical slices appears to be the same in both male and female (Figure 9) even though the calculated rate constants are different (Table 3). The half time for runout was longer than twelve minutes. This indicates that TEA remained in the slice, and that its rate of runout is normally slower than that of PAH. The structural configuration of TEA can account for this phenomenon (McIsaac, 1969). This also would explain the very high S/M ratio for TEA in comparison to other organic bases. Accumulation of TEA occurs because the substance is actively transported into the cells. Passage back out into the medium is severely limited because of the quaternary ammonium configuration. The molecule is always charged and cannot readily pass through the membrane. Although a sex difference may exist for runout of TEA between male and female, it may not be apparent because of the structural properties of TEA.

Runout of PAH is reduced when the temperature is decreased to 1°C. Farah *et al.* (1963) demonstrated a similar

reduction in runout of PAH from dog kidney slices. This was indicated by a decrease in the rate constant of the slow component of the PAH runout curve and an increase in the half time for runout. Similarly, incubation under nitrogen reduced the rate constant of the slow component and increased the half time for runout. It is possible that a change in temperature affects components in the cell which are involved in the intracellular concentration of PAH. The breakdown of this pool could be temperature sensitive, and at low temperatures it may not function at all. This would result in less PAH available for runout (Farah *et al.*, 1963). The effect exerted by nitrogen appears more complex. If runout is primarily a passive mechanism, nitrogen would have no effect on the rate constant for runout. But the breakdown of the intracellular accumulating mechanism for PAH could be energy dependent. Inhibition of breakdown of PAH to freely diffusible PAH for runout would result in a decrease in the total amount of PAH which could run out. This would affect the half time for runout of the total intracellular concentration of PAH.

Farah *et al.* (1963) have shown the effect of certain inhibitors on the runout of PAH. Their observations suggest that an outward transport mechanism may exist. Another hypothesis was proposed which suggested that runout is controlled mainly by an intracellular concentrating mechanism. Anything which decreased the activity of this concentrating

mechanism whether it was a metabolic or competitive inhibition should increase the rate of runout of PAH by increasing the concentration of intracellular free PAH. DNP, probenecid, octanoate were thought to be explained on this basis (Farah *et al.*, 1963). Similar agents (iodoacetic acid, DNP, and cyanide) had no significant effect on the runout of NMN (Ross *et al.*, 1968). This may suggest that runout of organic bases is strictly a passive phenomenon. The extent of passive diffusion that occurs during the runout of PAH is not known. High concentrations of inhibitors were thought to inactivate all of the postulated transport mechanisms in the cell. However, a secondary increase in the runout of PAH was observed when high concentrations of inhibitors were applied (Farah *et al.*, 1963).

In view of the possible difference seen for runout of PAH between male and female, the initial rate studies of PAH and TEA were in question. Preliminary results showed no difference in the initial uptake rate of PAH and TEA by male and female cortical slices. However, these studies were done at only one PAH or TEA concentration. It is therefore possible that differences in rate could exist at other concentrations. If the male is able to transport at a faster rate because of the existence of more transport sites than the females possess, a difference would not be apparent at a concentration which was not sufficient to involve all the transport sites. If this were the case, it

is very possible that the rate measured at one concentration is not truly indicative of the maximal transport rate by the male. A kinetic analysis was therefore done in order to determine the effect of varying PAH concentrations upon the rate of uptake of PAH. Results showed a large difference between the rate of uptake of PAH for male and female rats (Figure 10). This was evidenced by differences in Km and V_{max} respectively (Table 4). Figure 10 demonstrates that the PAH uptake steadily increases as the PAH concentration in the medium increases. Huang and Lin (1965) demonstrated comparable results using isolated renal tubules. These results indicate a faster rate of PAH uptake by male renal cortical slices. The differences in V_{max} suggest possible reserve transport sites which exist in the male. When the substrate load is increased these sites become functional, enabling the cell to transport more PAH. The Km value for male indicates that normally, the male has a greater load capacity. He is thus able to handle more PAH at any one time.

A greater initial uptake rate by males would account for a larger intracellular accumulation of PAH. This coupled with the slower runout of PAH from cortical slices of male kidneys would give male rats greater PAH S/M ratios. These results also support the existence of an active transport mechanism at the intracellular step. This would be step 3 proposed in the scheme of Foulkes and Miller (1959b)

(Figure 1). In order to support this, kinetic analysis of PAH uptake was determined under varying experimental conditions.

In the presence of acetate, uptake of PAH was significantly enhanced (Figure 11). Sodium acetate has been shown to increase the renal excretion of PAH, and also to enhance PAH S/M ratios *in vitro* (Cross and Taggart, 1950; Mudge and Taggart, 1950). The exact mechanism by which it exerts these effects is not known. Our results indicate that the effect exerted by acetate is evident during early rate of uptake.

Probenecid, however, caused a shift in the slope of the control curve (Figure 11). This appeared to be a non-competitive type inhibition as evidenced by the difference in V_{max} but the same Km. These results support similar findings done by Braun (1960). Findings from that study also demonstrated a non-competitive inhibition by probenecid on PAH uptake. However, Berndt (1966) has demonstrated tissue binding by probenecid in rabbit renal cortical slices. The theory proposed is that probenecid is taken up by tissue slices where it binds irreversible to the transport site. Consequently, PAH is unable to bind to the site. If this is true, then the term noncompetitive used to describe probenecid inhibition is not valid.

The effect of nitrogen atmosphere on PAH uptake merely demonstrates that some oxygen requiring system is involved in the uptake of PAH.

The data presented suggest that the uptake of PAH by cortical slices resembles the pattern of Michaelis-Menten kinetics. However, intracellular factors which are unaccounted for at the present time may be playing a role which is unexplained by the methods being used. This preparation was not intended to measure the velocity of an enzyme reaction. Its specific purpose was to quantitate the amount of a particular substance accumulated by renal cortical tissue slices. Kinetic measurements enabled us to detect a difference in rate of uptake of PAH by renal cortical slices from male and female rats. Any other similarity to enzyme kinetics would stop at that point.

The results of this study suggest that the effect presumably exerted by testosterone on active renal tubular secretory mechanisms is non-specific. The sex difference observed in the transport of PAH was shown to involve a difference in initial uptake rate between male and female. This appears to be complimented by a slower rate of runout of PAH from cortical tissue by males. As a result, the ability of renal cortical tissue slices from male rats to accumulate PAH will be greater than females. The male will therefore exhibit higher PAH S/M ratios.

CHAPTER V

SUMMARY

The sex-related difference in accumulation of PAH by rat renal cortical slices was studied using the *in vitro* slice technique of Cross and Taggart. Despite similar kidney weight to body weight ratios, tissue from adult male rats developed higher PAH S/M ratios than did tissue from females. This difference was independent of incubation time. No difference in accumulation of the organic base NMN by renal cortical slices from male and female rats was observed. However, a sex difference was observed in TEA S/M ratios. This effect was magnified by a decreasing concentration of TEA in the medium. The sex difference observed in PAH and TEA S/M ratios was abolished under nitrogen. It was concluded from these observations that the previously reported sex difference in PAH transport is not specific, but reflects a general stimulatory effect of testosterone on renal transport systems for organic acids and bases.

No differences were found in the total water content, extracellular space, intracellular space, or protein content of renal cortical slices from adult male and female rats. This indicated that a difference in slice composition

could not account for the sex difference seen in the PAH S/M ratio.

Measurement of the various steps responsible for the slice to medium ratio were made. Initial rate of PAH uptake by renal cortical slices from male and female rats during short incubation times was not different. The same was true for measurement of the initial uptake rate of TEA. These measurements, however, were done at only one medium concentration of PAH or TEA. Subsequent kinetic analysis of PAH uptake revealed significant differences. Measurement of PAH uptake at 1, 2, 4 and 8 x 10^{-4} M PAH showed a greater rate of PAH uptake by renal cortical slices from male rats. Sodium acetate had a stimulatory effect on early uptake, but probenecid and incubation under nitrogen depressed PAH uptake.

Runout of PAH and TEA from renal cortical slices was exponential. A difference in the rate constant for runout of TEA between male and female existed. Likewise, runout of PAH from renal cortical slices of female rats appeared greater than from males. This was seen as a greater rate constant of the slow component and shorter half time for runout of PAH from cortical slices of female rats. Runout of PAH from cortical slices of male and female rats at 1°C was depressed. Incubation under nitrogen affected both the rate constant and the half time for runout.

It is concluded from these observations that the greater PAH S/M ratios exhibited by renal cortical slices from male

rats results from a greater rate of uptake. This appears to be complimented by a slower rate of runout of PAH from cortical tissue of male rat kidney.

Table 1. Accumulation of organic ions by slices of rat renal cortex.[a]

	N	Female	Male
PAH S/M	5	7.9±.3	13.2±.9[b]
NMN S/M	5	5.3±.5	6.4±.6
TEA S/M	9	17.4±.4	20.5±.4[b]

[a]Simultaneous determinations of PAH and NMN S/M ratios were done on renal cortical slices from 5 males and 5 females. TEA S/M ratios were determined for 9 male and 9 female rats.

[b]Significantly greater than female ($p<.05$), group comparison.

Table 2. Effect of nitrogen atmosphere on accumulation of organic ions by slices of rat renal cortex.[a]

	Female	Male
PAH S/M	.93±.12	.99±.12
TEA S/M	.97±.08	1.13±.07

[a]Simultaneous determinations of PAH and TEA S/M ratios were done on renal cortical slices from 6 males and 6 females. Values are means ± S.E. from 6 such experiments.

Table 3. Runout of organic ions from rat renal cortical slices.[a]

	Runout	N	Rate constant for slow component min^{-1} ± S.E.	Half time for runout
Male	PAH	4	.046 ± .004	8±0.8 min
Female	PAH	4	.070 ± .020	6±0.4 min
Male	TEA	2	.036 ± .006	>12 min
Female	TEA	2	.045 ± .002	>12 min

Effect of different experimental conditions on runout of PAH.[a]

	N	Rate constant for slow component min^{-1} ± S.E.	Half time for runout
Male (incubation at 1°C)	1	.019	>12 min
Female (incubation at 1°C)	1	.019	>12 min
Male (incubation under N_2)	2	.030 ± .001	9 min

[a]For each experiment, renal cortical slices from 2 rats were pooled, equally divided among 3 beakers, and pre-loaded for two hours with PAH. At the end of this time, the amount of PAH accumulated by slices from two beakers was determined. Slices from the third beaker were transferred at one minute intervals through a series of 12 PAH-free beakers. From the results, the rate constants and half times for runout were determined as described in the text.

Table 4. Kinetic analysis of PAH uptake in rat renal cortical slices.[a]

	Slope[b]	Km (10^{-4} moles/L)[b]	V_{max} (µg/g/min)[b]
Control male	.217	5.86	27.02
Sodium Acetate	.224	11.20	50.00
Probenecid	.360	5.79	16.10
Nitrogen	.739	7.10	9.61
Female	.373	2.89	7.75

[a]The rate of PAH uptake (µg PAH/g/min) at PAH concentrations of 1, 2, 4 and 8 x 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes of incubation. Four such experiments were done for each different experimental condition.

[b]Values were obtained from the Lineweaver-Burke plot shown in Figures 10 and 11.

Figure 1. Steps in PAH transport by kidney slices as proposed by Foulkes and Miller (1959). The symbols and abbreviations are used as follows: ISF (interstitial fluid), ICF (intracellular fluid), M (medium), E (extracellular space), pah (intracellular fraction of rapidly diffusible PAH), and PAH (intracellular fraction of slowly diffusible PAH which is responsible for the high slice to medium ratio).

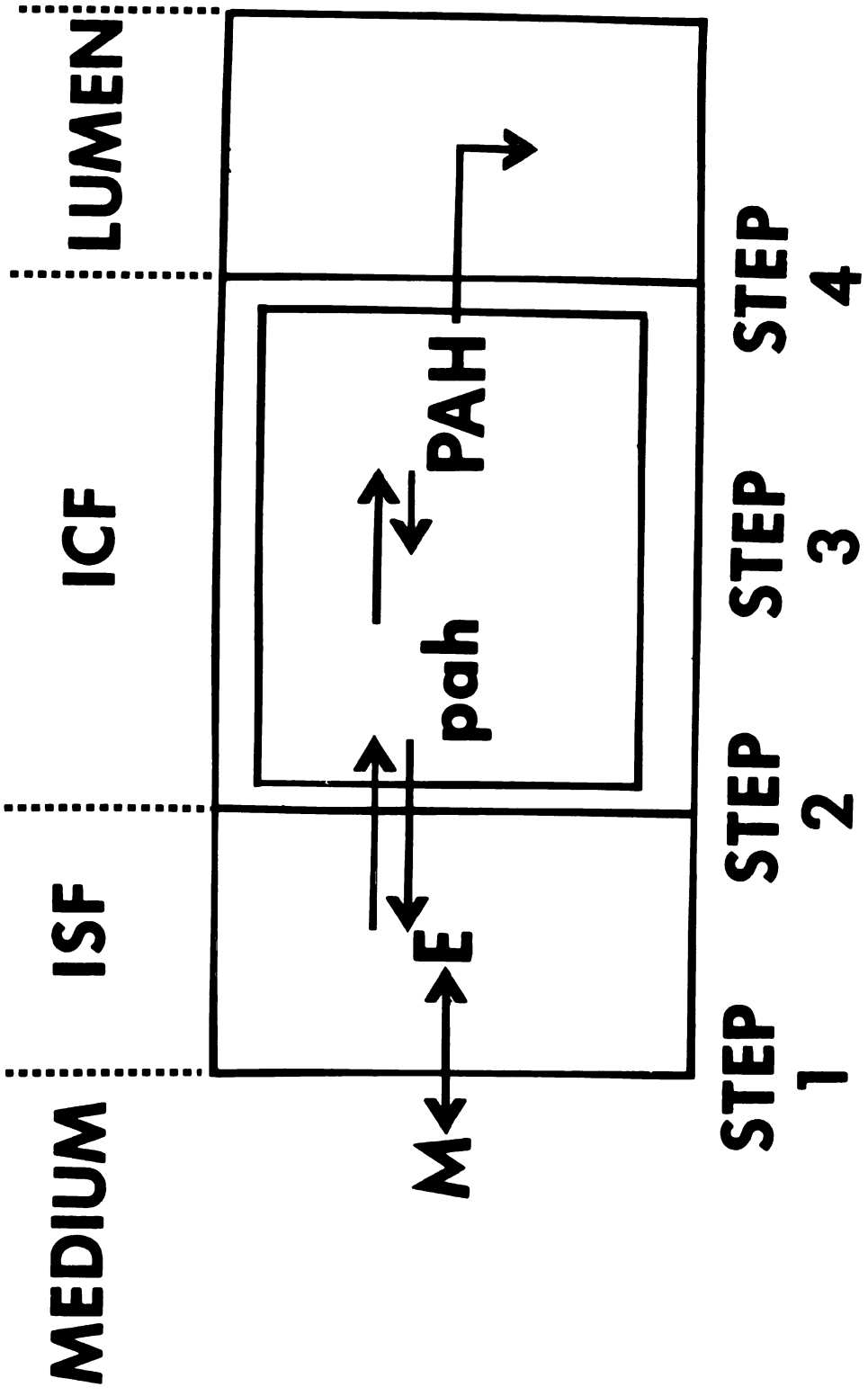

Figure 1

Figure 2. Accumulation of PAH by renal cortical slices of adult male and female rats. Kidney cortical slices from 3-4 animals were pooled and equally divided in a series of beakers, which were incubated for times ranging from 30 to 180 minutes. PAH slice to medium (S/M) ratios were measured. Points indicate means (± standard error) for six such experiments. Standard error was not shown in those cases where the vertical bars were less than the diameter of the points.

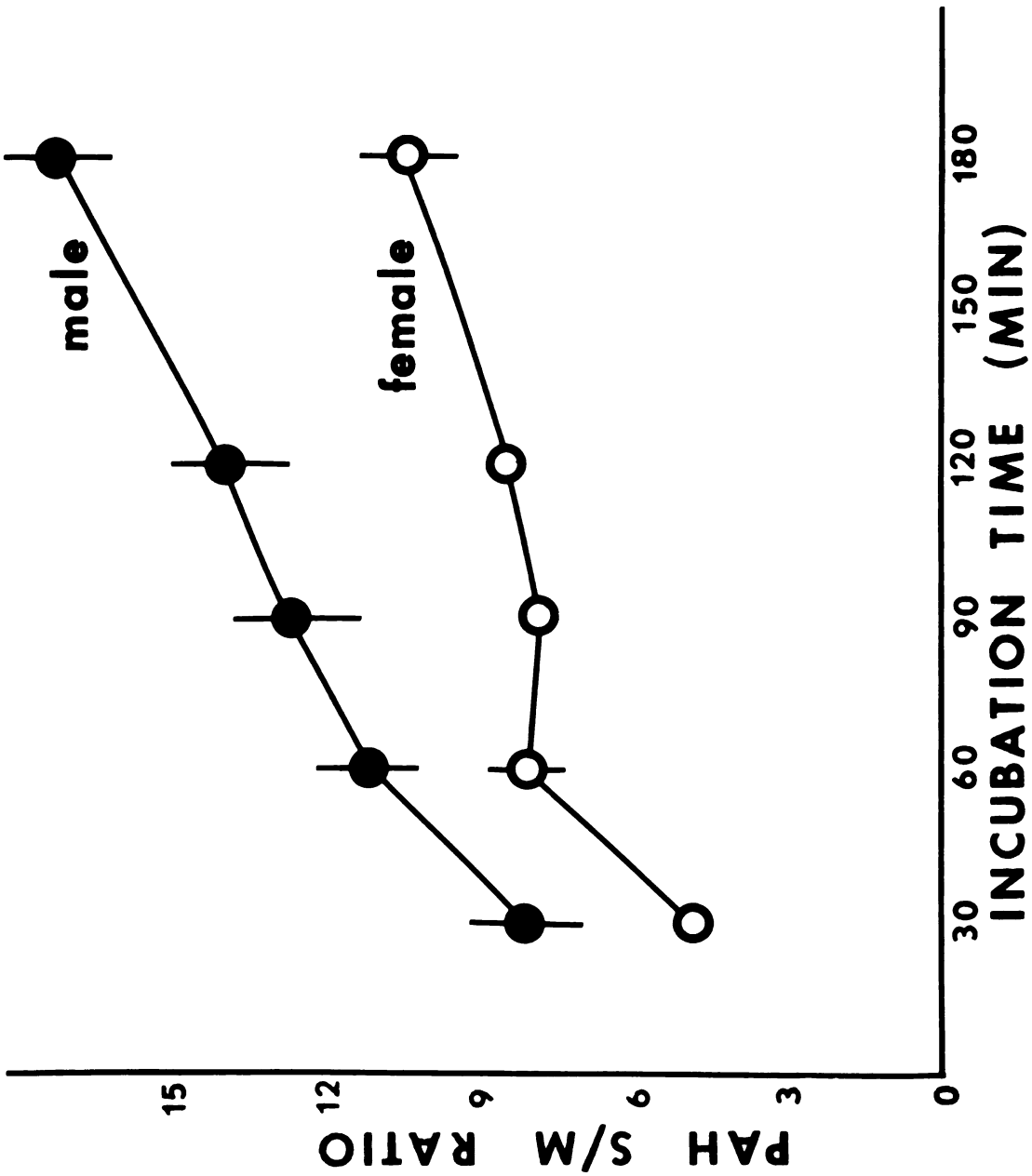
male
female
PAH S/M RATIO
0
3
6
9
12
15
30
60
90
120
150
180
INCUBATION TIME (MIN)

Figure 2

Figure 3. Comparison of PAH uptake by renal cortical slices from adult male and female rats after a series of short incubations. Points indicate means from four experiments, each representing pooled tissue from 3-4 animals. The lines are calculated regression lines. The slopes of the curves are 1.50 (male) and 1.70 (female).

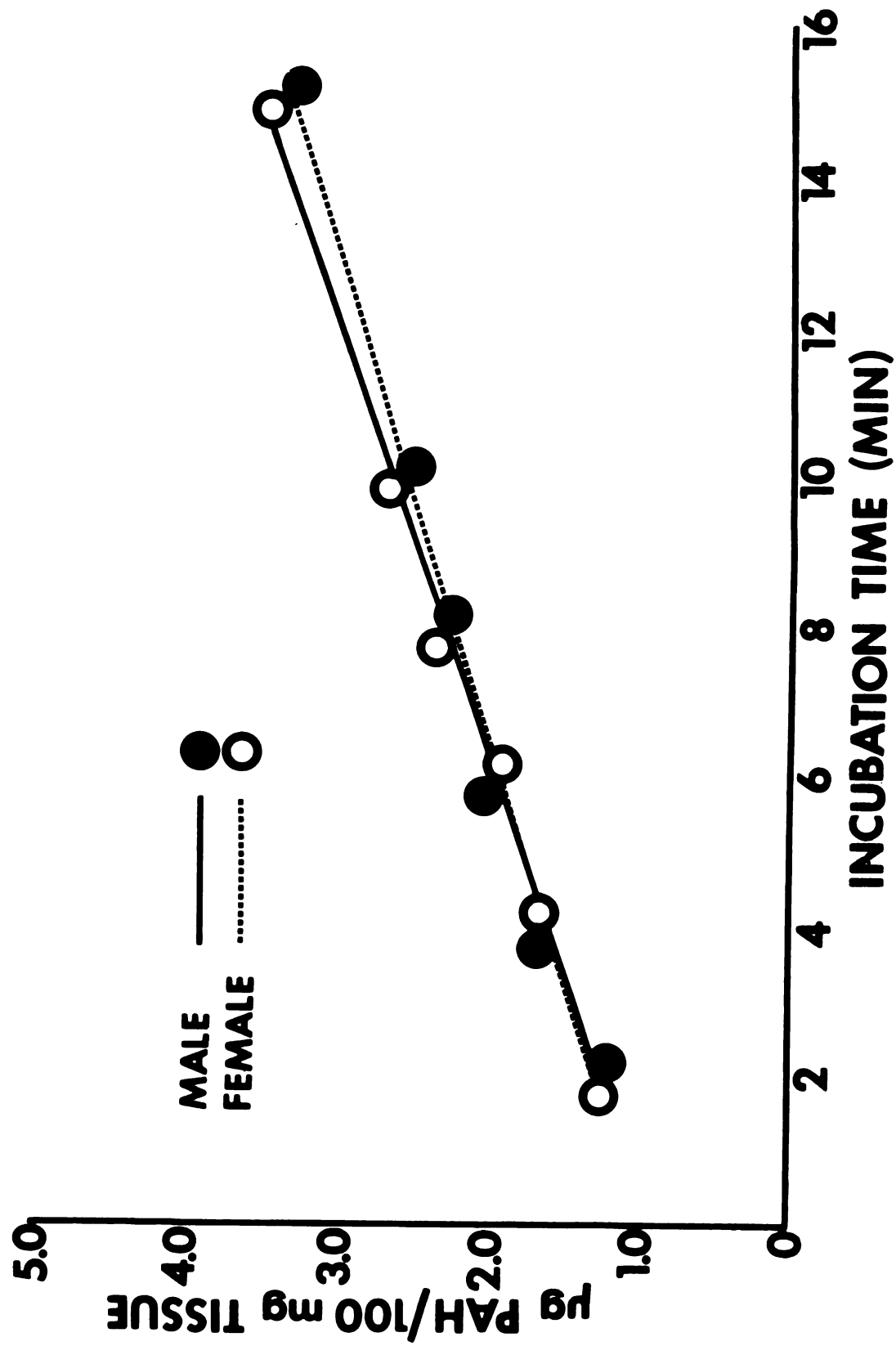
5.0
4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
14
16
INCUBATION TIME (MIN)
μg PAH/100 mg TISSUE
MALE
FEMALE

Figure 3

Figure 4. Accumulation of NMN by renal cortical slices of adult male and female rats. Kidney cortical slices from 3-4 animals were pooled and equally divided in a series of beakers, which were incubated for times ranging from 30 to 180 minutes. NMN slice to medium (S/M) ratios were determined. Points indicate means (± standard error) from six such experiments.

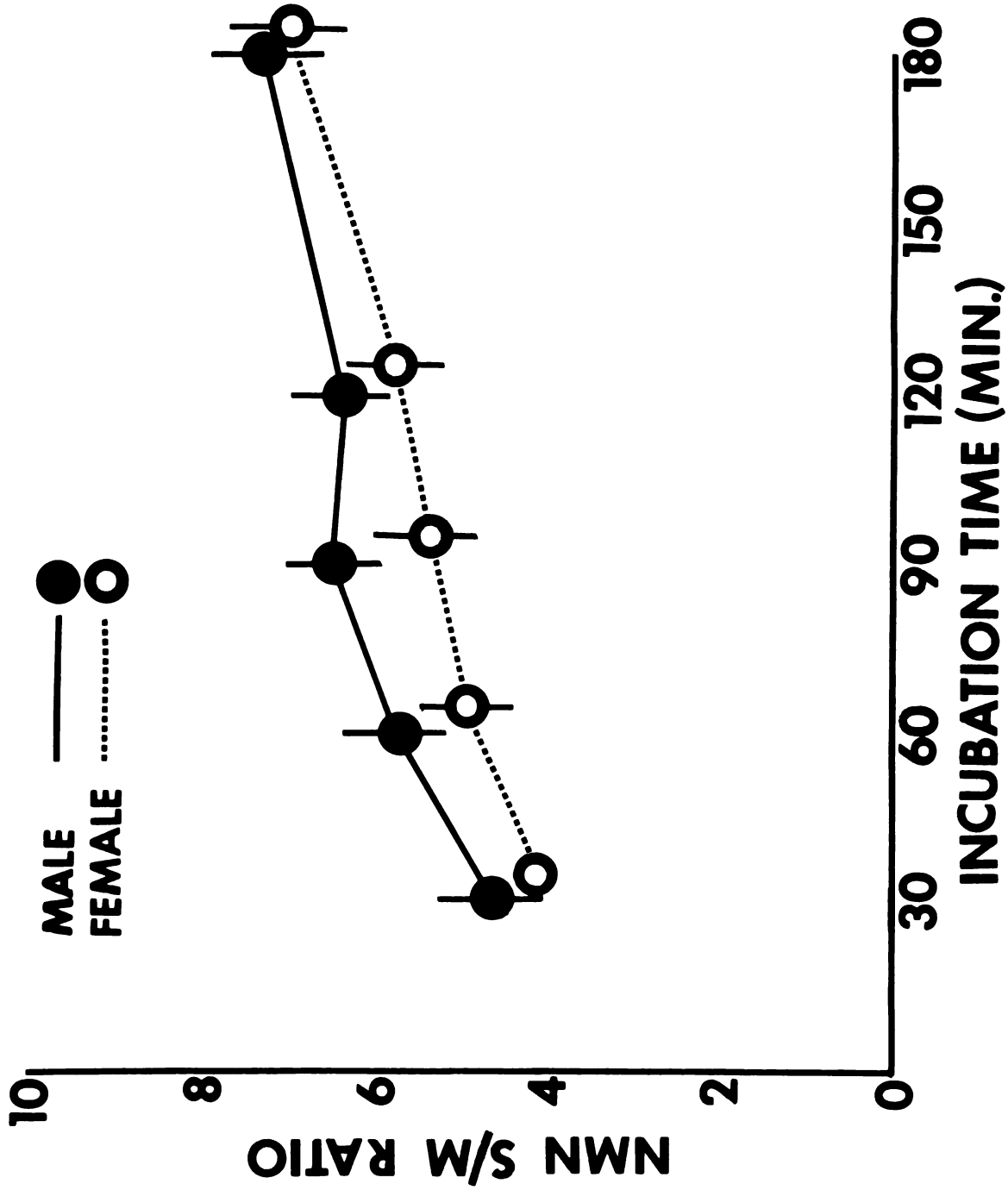
MALE
FEMALE
NMN S/M RATIO
INCUBATION TIME (MIN.)
0
2
4
6
8
10
30
60
90
120
150
180

Figure 4

Figure 5. Effect of increasing medium concentration upon the accumulation of TEA by male and female rat renal cortical slices. Kidney cortical slices from 3-4 animals were pooled, equally divided, and incubated at the different concentrations. Incubation lasted ninety minutes. Points indicate means (± standard error) from six experiments.

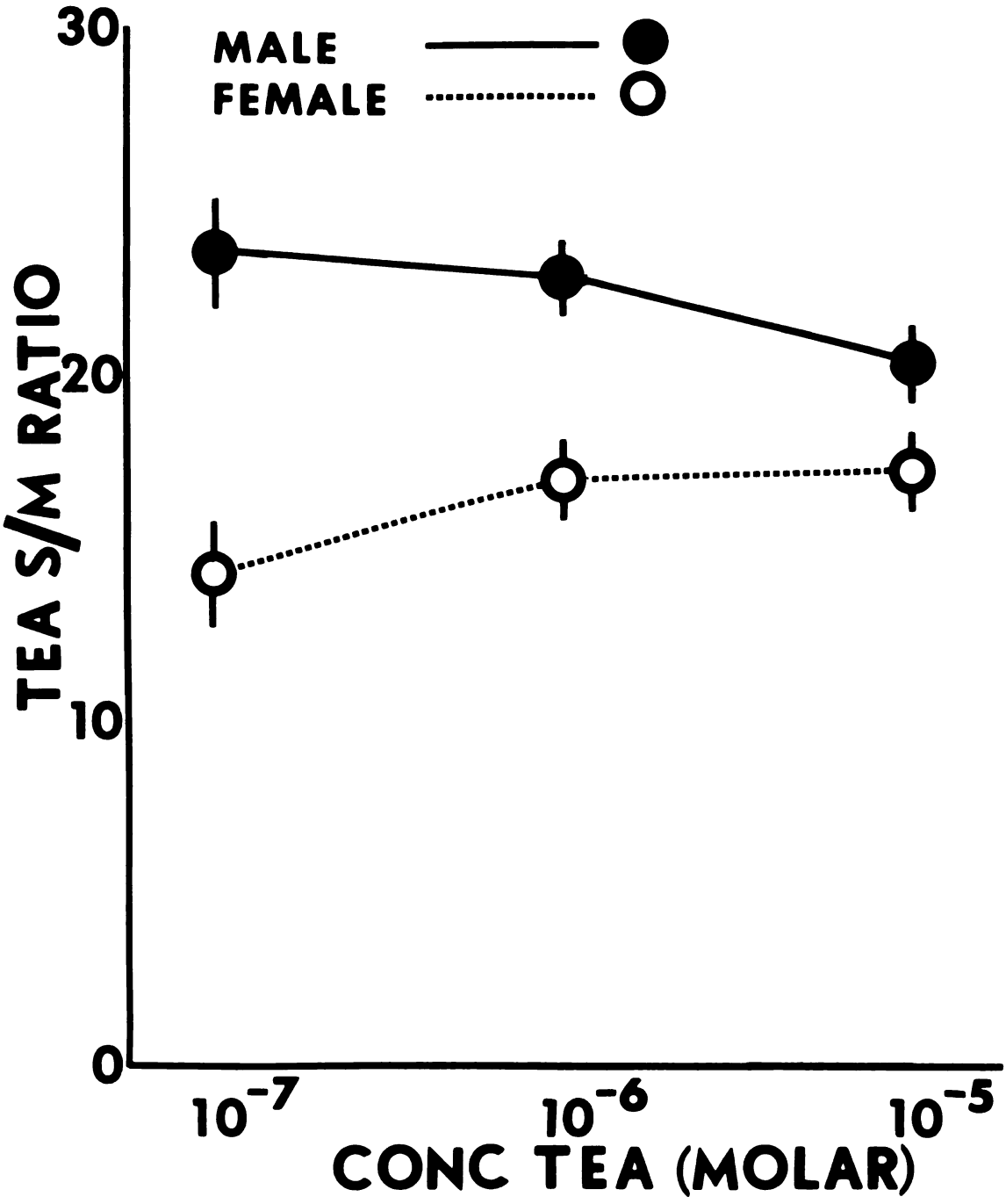

Figure 5

Figure 6. Comparison of TEA uptake by renal cortical slices from adult male and female rats after a series of short incubations. Points indicate means from four experiments, each represeting pooled tissue from 3-4 animals. The slopes of the curves are 1.30 (male) and 1.29 (female).

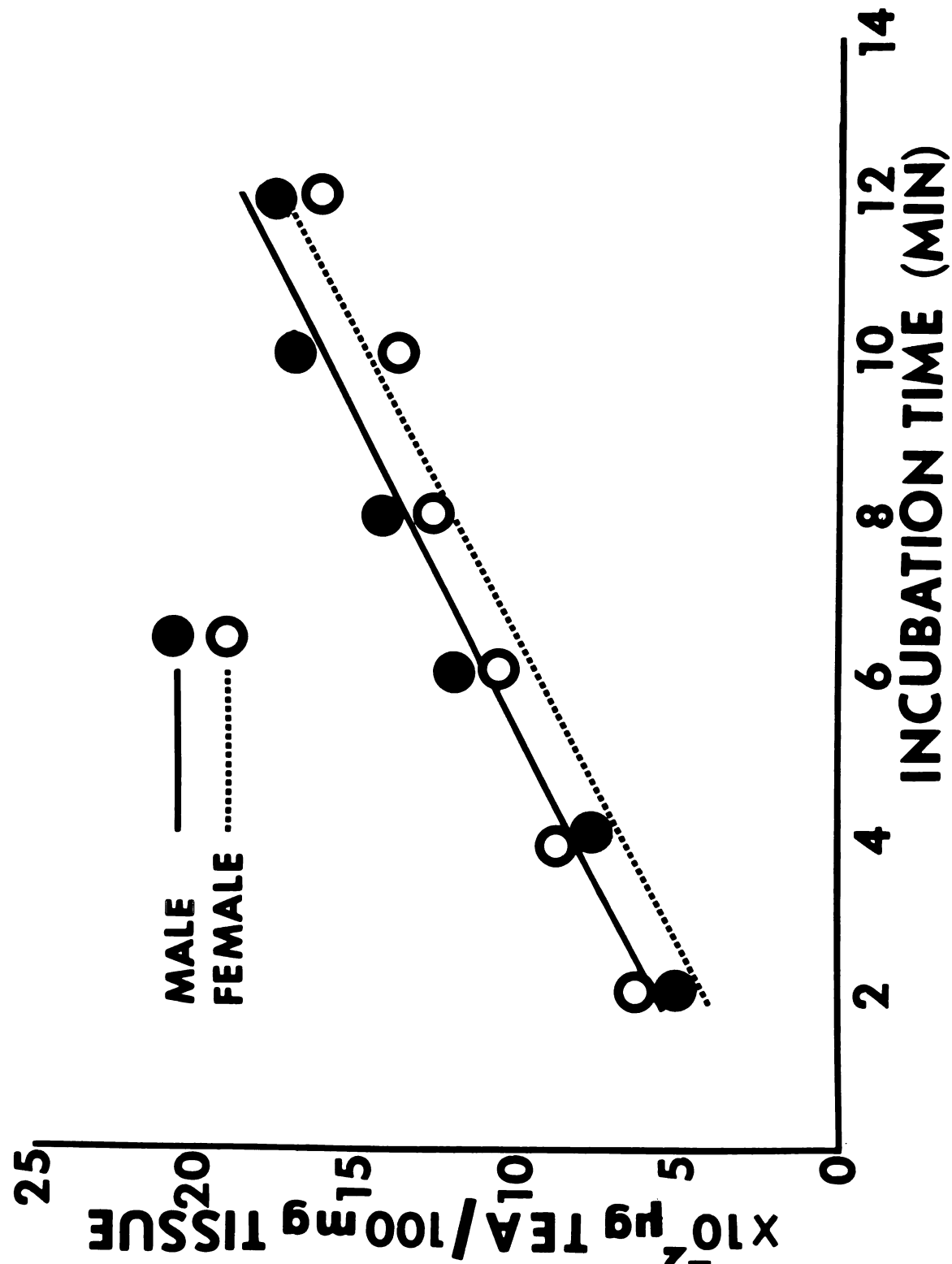

Figure 6

Figure 7. Comparison of cortical slice composition from male and female rat kidneys. Total water content, extracellular space (ECS), intracellular space (ICS) and protein content of male (M) and female (F) renal cortical slices were measured and expressed as a percent of tissue wet weight. Bars indicate means (± standard error) from six experiments.

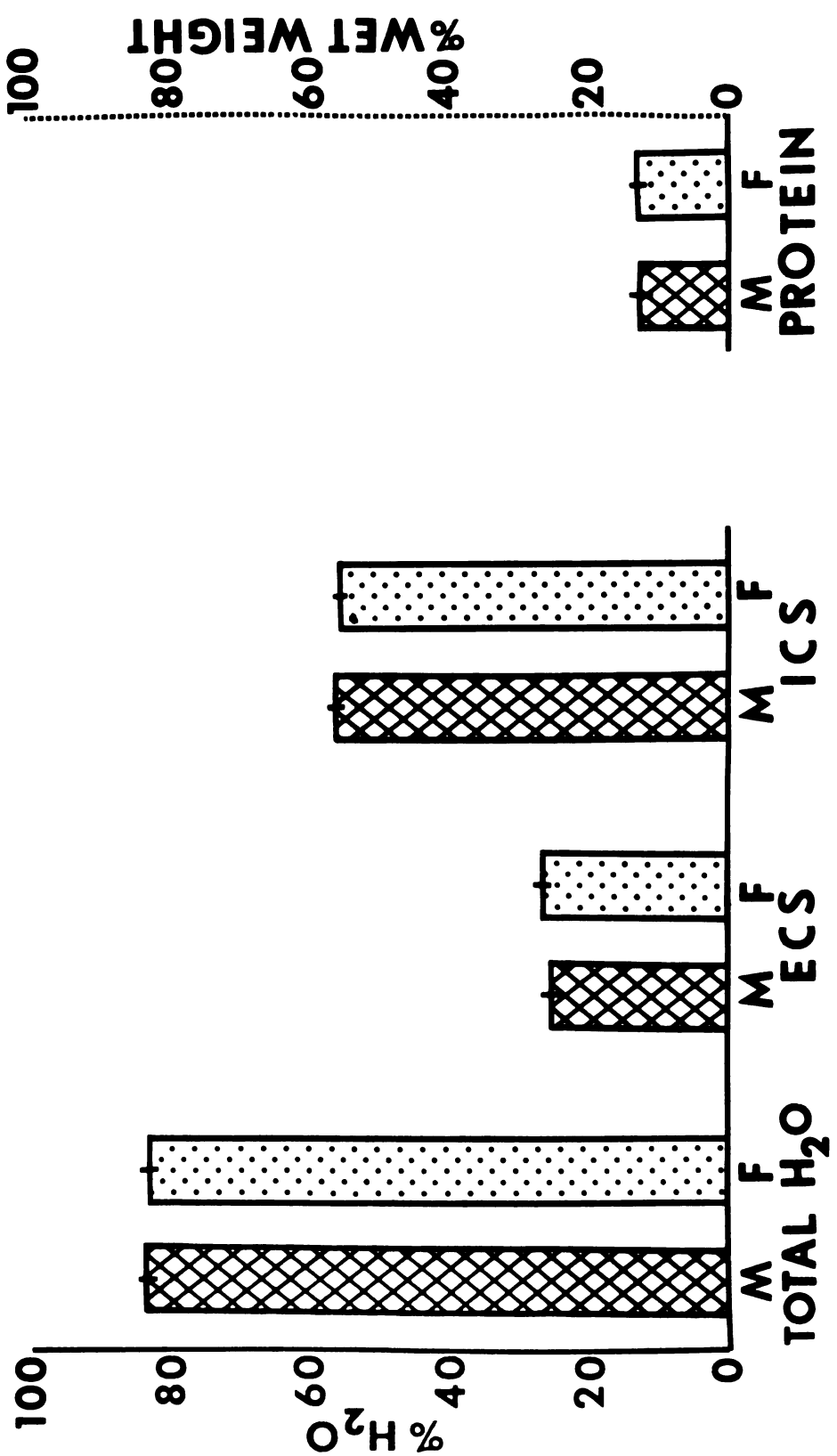

Figure 7

Figure 8. Determination of runout of PAH from renal cortical slices of adult male and female rats. Slices from 2 animals were pooled and pre-loaded for 2 hours with PAH and then transferred at one minute intervals into a series of 12 PAH-free beakers. Data represent concentration of PAH remaining in slices (initial concentration less amount appearing in beakers). Points indicate means for four such experiments.

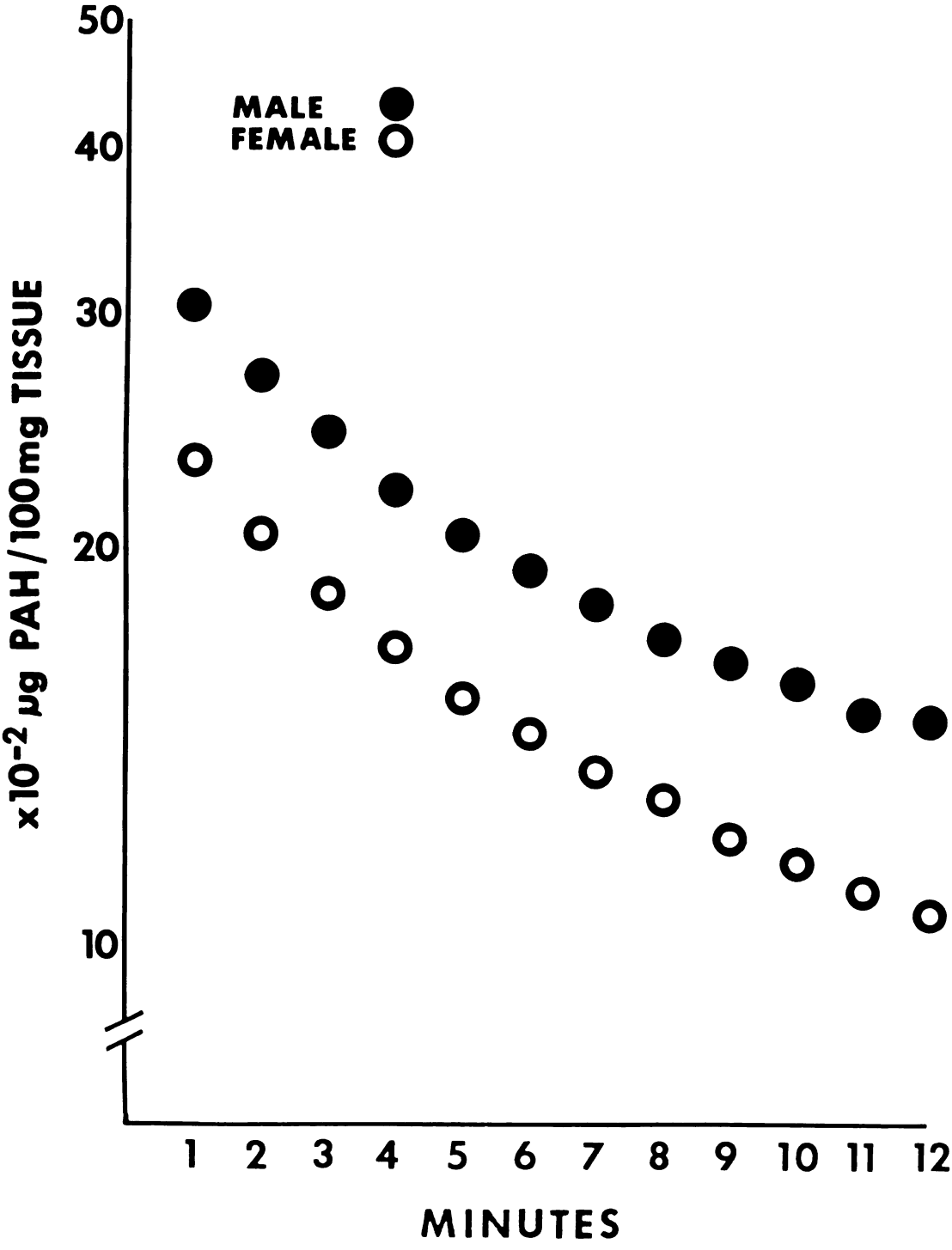
50
40
30
20
10
MALE
FEMALE
x10-2 µg PAH/100mg TISSUE
1 2 3 4 5 6 7 8 9 10 11 12
MINUTES

Figure 8

Figure 9. Determination of runout of TEA from renal cortical slices of adult male and female rats. Renal cortical slices from 2 animals were pooled and pre-loaded for 2 hours with TEA and then transferred at one minute intervals into a series of 12 TEA-free beakers. Data represent concentration of TEA remaining in slices (initial concentration less amount appearing in beakers). Points indicate means from two such experiments. The runout curves are calculated regression lines. The slopes of the curves are -1.04 (male and -1.01 (female).

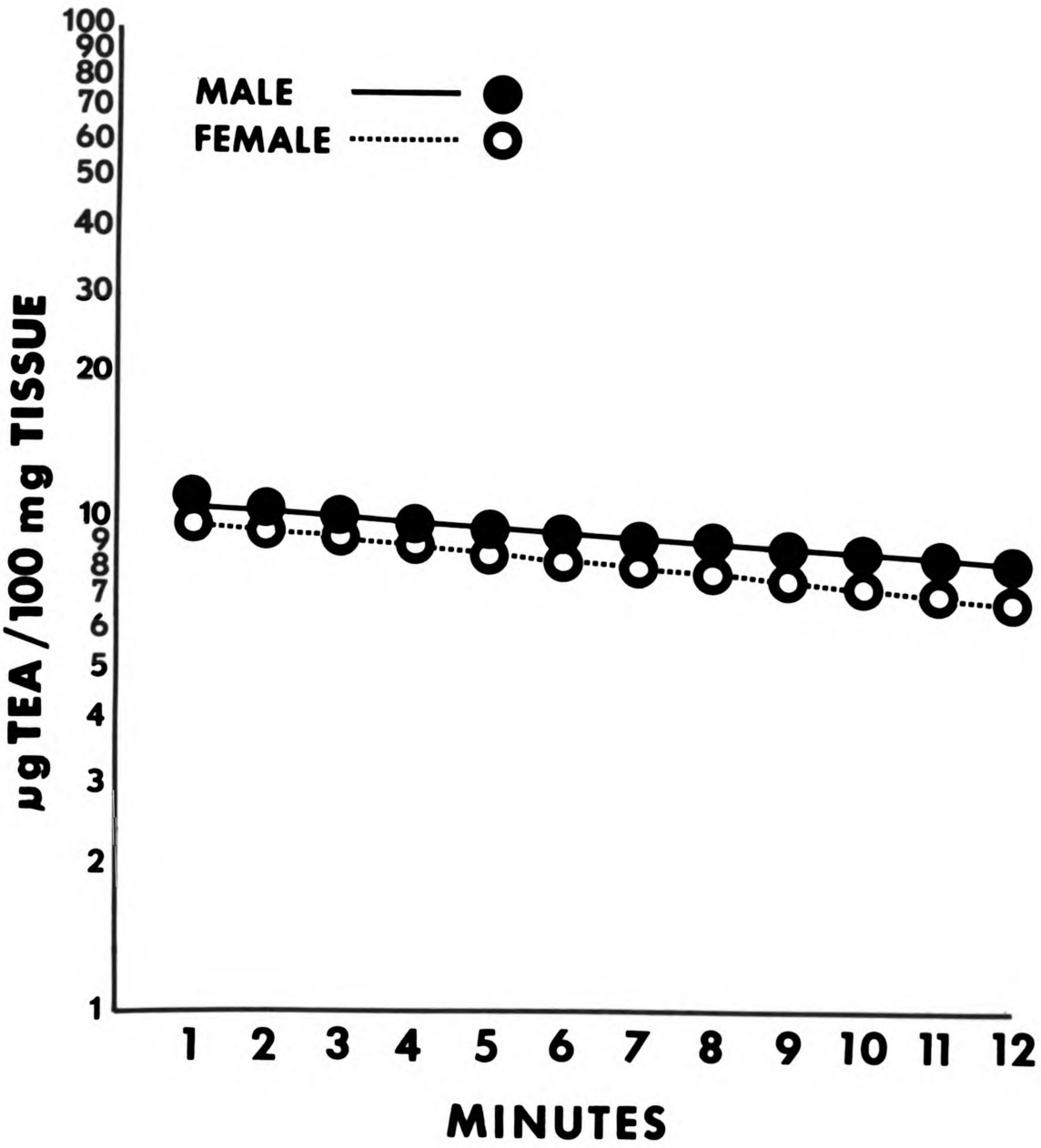
MALE
FEMALE
µg TEA /100 mg TISSUE
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12
MINUTES

Figure 9

Figure 10. Kinetic analysis of PAH uptake by renal cortical slices. The rate of PAH uptake (μg PAH/g/min) at PAH concentrations of 1, 2, 4 and 8×10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes of incubation. The points indicate the means from four experiments. The lines are calculated regression lines. The slopes of the curves are .217 (male) and .373 (female).

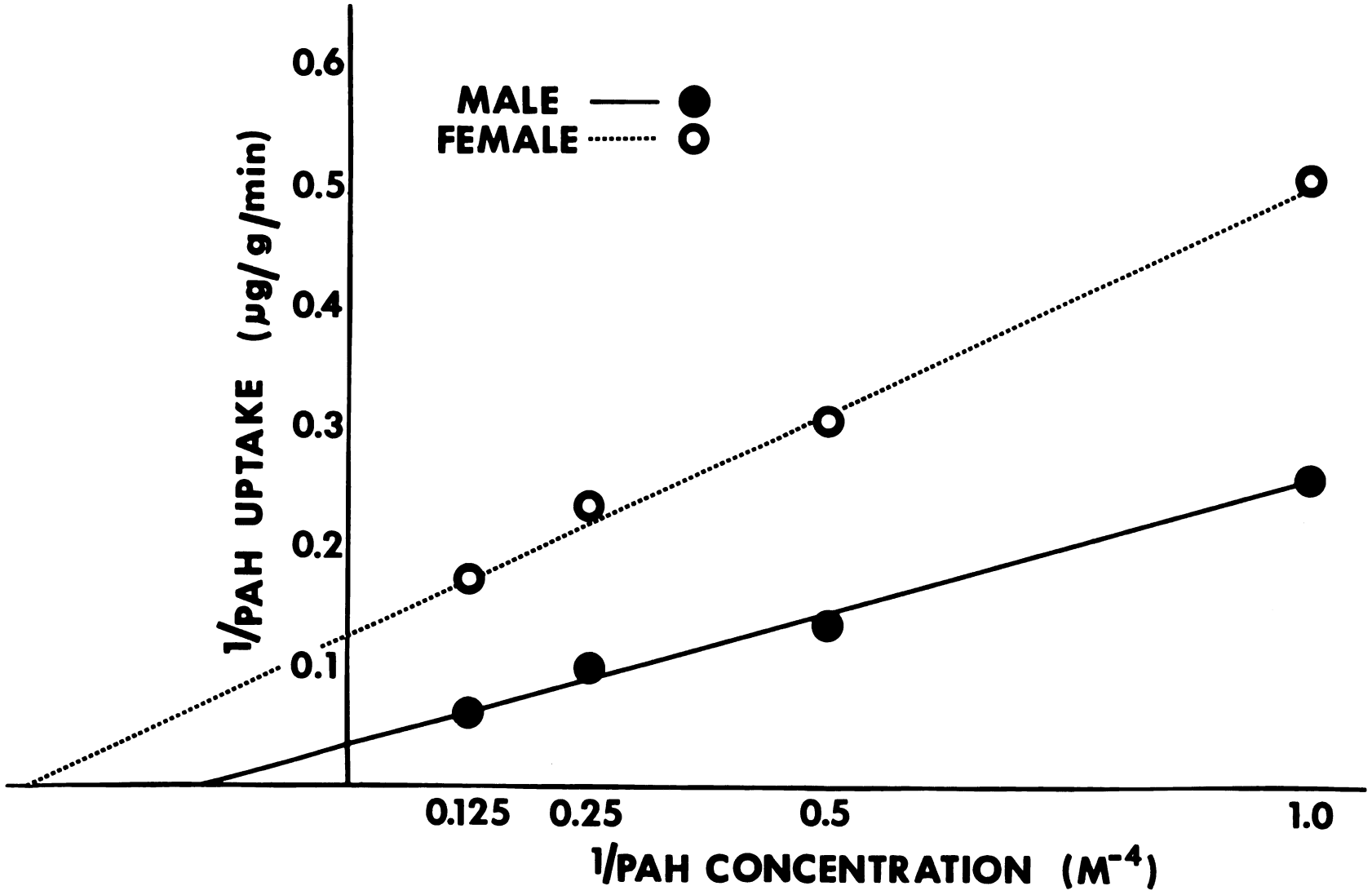
MALE
FEMALE
0.6
0.5
0.4
0.3
0.2
0.1
1/PAH UPTAKE (µg/g/min)
0.125
0.25
0.5
1.0
1/PAH CONCENTRATION (M⁻⁴)

Figure 10

Figure 11. Kinetic analysis of PAH uptake during varying experimental conditions. The rate of PAH uptake (μg/g/min) at PAH concentrations of 1, 2, 4 and 8 x 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes of incubation in the presence of sodium acetate, probenecid, or nitrogen gas. The various points for each curve represent the means from four experiments. The lines are calculated regression lines. The slopes of the curves are .217 (control), .224 (sodium acetate), .360 (probenecid) and .739 (nitrogen gas).

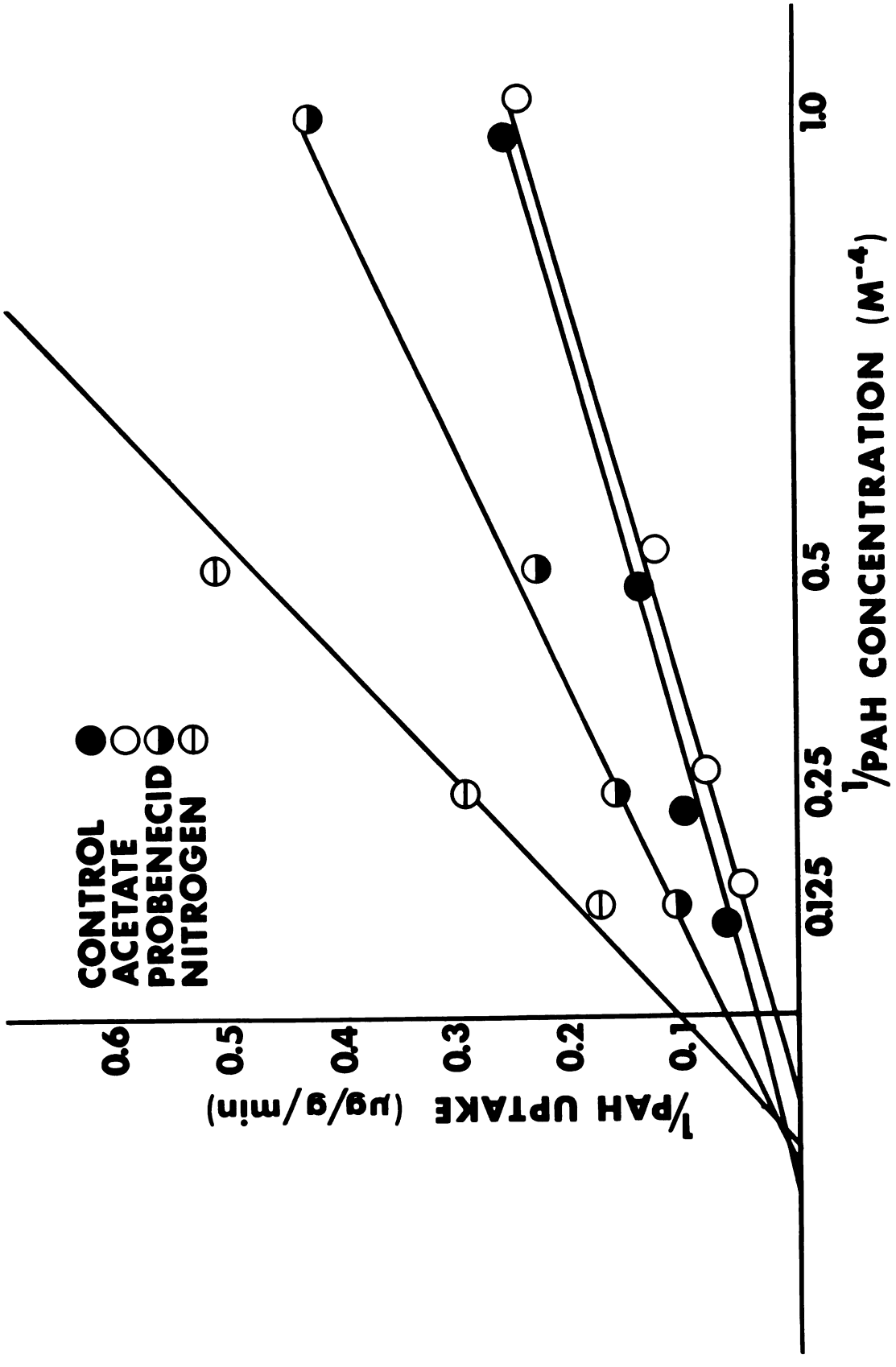
CONTROL
ACETATE
PROBENECID
NITROGEN
0.6
0.5
0.4
0.3
0.2
0.1
1/PAH UPTAKE (µg/g/min)
0.125
0.25
0.5
1.0
1/PAH CONCENTRATION (M⁻⁴)

Figure 11

BIBLIOGRAPHY

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