of trimethyluric acid appears to be the oxidative ring opening of the pyrimidine ring to form 3, 6, 8-trimethylallantoin (V) which can then be further oxidized and hydrolized to more polar products such as 1, 6, 8-trimethylallantoic acid, glyoxylic acid, and mono-, and dimethylureas.

The hitherto known metabilites of caffeine viz, methylxanthines and methyluric acids, isolated from the urine of experimental animals represent only a fraction of the urinary metabolites of caffeine[11-13] Our results seem to explain the observed difficulty in recovering caffeine metabolites from the urine since methylated allantoins, allantoic acids and urea derivatives are highly polar and not easily extractable. Also these polar compounds may constitute the unidentified metabolites encountered by earlier investigators[14].

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Résumé. L'acide 1, 3, 7-triméthyldihydro-urique et la 3, 6, 8-triméthylallantoïne ont été identifiés comme métabolites nouveaux de la caféine dans l'urine de rat.

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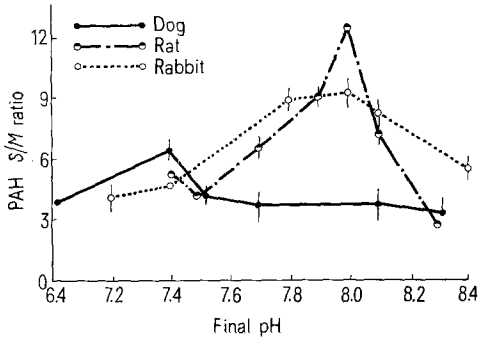
Effect of Medium pH on *p*-Aminohippurate Accumulation by Slices of Rat Renal Cortex[1]

Several techniques have been employed to study the transport of organic ions by the kidney, including clearance[2,3], micropuncture and microperfusion[4,5] and isolated tubules[6,7]. Probably the most widely used technique is the in vitro slice method of Cross and Taggart[8]. Even though the method is routine in many laboratories optimal conditions for incubation have not been completely elucidated. For instance, Cross and Taggart[8] observed that accumulation of the organic acid *p*-aminohippurate (PAH) by rabbit kidney cortical slices was dependent upon temperature and oxygen but was independent of pH over a relatively narrow range (pH 7.0–7.8). However, Copenhaver and Davis[9] found that over a wider pH range (5.0–12.0) the greatest accumulation of PAH by rabbit renal cortical slices was at pH 8.1 to 8.3. In contrast, Ross et al.[10] found the greatest accumulation of PAH by dog renal cortical slices to be at pH 7.4. To our knowledge comparable studies have not been conducted in tissue from the rat. Inasmuch as rat tissue is extensively employed for studies of renal transport in this laboratory, it was of interest to determine the effect of pH upon PAH accumulation by renal cortical slices from this species. For comparative purposes the effect of pH on PAH accumulation in rabbit and dog tissue was also determined.



Effect of medium pH on PAH accumulation (*S*/*M*). pH values shown are those at the end of 90 min incubation. Each point represents the mean (± SE) obtained in 5 duplicated experiments. When no vertical line is shown the variation is within the radius of the circle. In individual experiments a single dog or rabbit was used. For rat experiments, the kidneys of several animals were pooled and then treated as one.

Methods. Adult mongrel dogs, New Zealand rabbits and Sprague-Dawley rats were used in these studies. Dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.); rabbits and rats were stunned by a blow on the head. Kidneys were quickly removed, weighed and placed in ice-cold saline. Renal cortical slices were prepared free hand and briefly kept in cold saline until incubated. Slices (approximately 100 mg) were incubated for 90 min using either 0.1 *M* sodium phosphate buffer or 0.015 *M* 2-amino-2-methyl-1, 3 propanediol (propanediol) buffer, in medium containing 7.4×10^{-5} *M* PAH. All incubations were carried out in duplicate in a Dubnoff apparatus at 25 °C under a gas phase of 100% oxygen.

The pH of the incubation medium was initially adjusted to values ranging from 6.0 to 9.0. Renal cortical slices from individual dogs or rabbits were incubated in medium at several different initial pH values in each experiment. Kidney slices from 4 rats were pooled and treated as tissue from one animal. In the experiments with dog tissue phosphate buffer was used throughout the pH range, while propanediol was used in all rat and rabbit studies. Unless stated otherwise, the reported pH values are those measured after incubation. The final pH range was from 6.4 to 8.4. The effect of medium pH on the rate of PAH uptake was determined in phosphate buffer at initial pH values of 7.4, 8.0 and 8.5. Slices of rat renal cortex were incubated for periods of time ranging from 5 to 30 min.

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After incubation the slices were quickly removed from the beakers, blotted and weighed. Both the tissue and a 2 ml aliquot of medium were assayed for PAH[11]. Results were expressed as slice/medium (S/M) ratio, where S equals mg/g of tissue and M equals mg/ml of medium. Data were analyzed statistically using Student's t-test, group or paired comparison. All data are expressed as the mean $\pm$ standard error. The 0.05 level of probability was used as the criterion of significance.

Results and discussion. Accumulation of PAH by renal cortical slices from the three species studied was dependent on the hydrogen ion concentration of the medium. PAH S/M ratio in rat tissue appeared to increase from a low at pH 7.5 to a sharp peak at pH 8.0 (Figure). Rabbit tissue also developed maximal PAH S/M ratios around pH 8.0 but the peak was not as sharp as that with rat tissue. In contrast, the PAH S/M ratio from dog tissue had a slight peak at pH 7.4 and then remained rather flat over the rest of the pH range studied.

Phosphate buffer was used over the entire pH range with tissue from the dog. When similar experiments were attempted with rat and rabbit tissues, the pH was not well maintained but returned toward 7.4. COPENHAVER and DAVIS[9] also noted that phosphate buffer was ineffective at higher pH when using rabbit kidney slices and therefore used a phosphate-propanediol combination buffer in their study. Propanediol buffer was used exclusively for rat and rabbit tissue in this study, nevertheless the buffering capacity of the slices still tended to shift the medium.

Ross et al.[10] observed that the amount of PAH accumulated during short (2–10 min) incubations was linear with time and suggested that this early accumulation is a reflection of the maximal rate at which PAH enters the slice. To determine the effect of medium pH on initial rate of uptake, PAH accumulation by rat renal cortical slices was determined in phosphate buffer initially adjusted to pH 7.5, 8.0 and 8.5. In 5 experiments no significant effect of medium pH was demonstrable until 30 min. After 10 min uptake ranged from 2.2–2.7 µg/100 mg tissue. At 15 min tissue at pH 7.4 had accumulated 4 µg/100 mg compared to 5 µg/100 mg in tissue at high pH. After 30 min tissue incubated initially at pH 8.5 had accumulated 8.8 $\pm$ 0.8 (S.E.) µg/100 mg which was significantly more than that at pH 8.0 7.1 $\pm$ 0.5) and pH 7.4 (6.6 $\pm$ 0.3). If uptake of PAH in the first few min of incubation truly reflects the rate of PAH transport these data demonstrate that the difference in pH alters some other aspect of the accumulation process, possibly an intracellular 'trapping' mechanism or diffusion from the tissue back into the medium. After incubating 30 min the tissue initially buffered to pH 8.5 had accumulated significantly more PAH than that at 7.4. Although the initial pH had declined to 7.8 during incubation, the slices had been functioning at a pH higher than 7.4 for 30 min. This, then, demonstrates that the enhanced PAH S/M seen at pH above 7.4 with propanediol (Figure) was a function of pH and not the buffer used[12].

Zusammenfassung. Nierenschnitte von verschiedenen Arten akkumulieren PAH mit zunehmendem pH-Wert des Mediums bei einem deutlichen Maximum zwischen pH 7.4 und pH 8.0.

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A Difference in Creatine Uptake Between Pectoralis and Thigh Muscles of the Chicken[1]

We have previously described a specific, saturable process that serves to transport creatine into skeletal muscle[2,3]. We now describe a difference in creatine uptake between pectoralis and thigh muscles which suggests that membrane transport of creatine varies from one type of muscle to another.

Materials and methods. Newly hatched male chickens, hybrids of a cross between Hubbard hens and White Mountain roosters, were given a commercial diet[4] and water ad libitum until they were 15 days old, when their average weight was 186 g. Then 1-^{14}C-creatine (5.08 mC per mmole)[5] was injected rapidly into a brachial vein. In addition to receiving ^{14}C-creatine, some of the chickens also received 3 ml or less of 0.154 M NaCl i.v., either alone or as a vehicle for non-radioactive creatine. Neither the NaCl solution nor the nonradioactive creatine adversely affected the appearance of the chickens, and the NaCl solution alone had no effect on the distribution of ^{14}C-creatine to muscle. At selected time intervals after injection the chickens were decapitated, exsanguinated, and individual muscles were obtained for measurement of radioactivity. The muscles were homogenized in 10 to 20 volumes of distilled water using a glass homogenizer, protein was precipitated by adding enough trichloroacetic acid to achieve a final concentration of 5% (w/v); and an aliquot of the clear supernate was taken for measurement of radioactivity by liquid scintillation spectrometry. Using paper chromatography in complementary experi-

Table I. Concentrations of selected compounds in pectoralis and biceps femoris muscles

Compound	Pectoralis (µmoles/g wet weight)	Biceps femoris
Creatine, total	32 $\pm$ 3.0 (11)[a]	30 $\pm$ 5.8 (7)
Phosphocreatine	19.4 $\pm$ 4.5 (6)	10.4 $\pm$ 2.5 (7)
Inorganic phosphate	5.5 $\pm$ 2.7 (6)	5.9 $\pm$ 2.1 (7)
ATP	6.4 $\pm$ 1.0 (6)	4.0 $\pm$ 0.45 (7)
ADP	0.99 $\pm$ 0.32 (6)	0.69 $\pm$ 0.08 (7)

[a] $\pm$ Standard deviation. Number of animals is given in parentheses.

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