the heterologous Junin antigen are moderately high. However, the amount of antibody determined by the plaque neutralization test with Junin virus is negligible.

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Effects of Hydrogen Ion Concentration on Transport Characteristics of p-Aminohippurate by Rabbit Kidney Slices.* (30252)

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Earlier studies concerned with the mechanism by which p-aminohippuric acid (PAH) is actively transported by rabbit kidney slices have demonstrated that certain chemicals, when added to the medium, exert specific effects on the accumulation of PAH or on the displacement of intracellularly accumulated PAH(1-4). Although many parameters of this transport system have been studied, almost all of the data reported have been obtained from experiments conducted at pH 7.4.

In this paper are reported observations on the effects of certain hydrogen ion concentrations with or without various test agents on (a) the accumulation of PAH by slices and (b) displacement of preaccumulated PAH.

Materials and methods. Young adult rabbits weighing approximately 2 kg were sacrificed by a blow on the head, exsanguinated and the kidneys removed immediately. The kidneys were promptly placed in an ice cold saline bath of 0.13 *M* NaCl-0.02 *M* KCl. Thin slices were prepared by the method of Forster and Copenhaver(2). After brief blotting on filter paper, 280 to 320 mg of the slices were weighed and added to a 25 ml flask containing 2.7 ml of ice cold Cross-Taggart medium(1). It was necessary to replace 0.1 ml of the 0.1 *M* sodium phosphate with 0.1 ml 0.2 *M* 2-amino-2-methyl-1,3 propanediol (propanediol) in order to cope with the wide pH range (pH 5.0 to 12.0)

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and to maintain a balanced isotonic medium in each flask. Additional specific substances were brought to the desired pH with NaOH or HCl and added with the equimolar reduction of NaCl. In experiments designed to examine the effects of pH on intracellular accumulation of PAH, the basic medium included 6.7×10^{-5} *M* PAH, specific test agents, and the slices. The slices were incubated under constant oxygen tension in either Erlenmeyer or Warburg flasks. Upon completion of incubation, the flasks were chilled immediately in crushed ice after which the slices were rapidly blotted and weighed. Slices and media were analysed for PAH according to the method of Cross and Taggart(1). All experiments were conducted for 60 minutes at 30°C unless otherwise specified.

The effects of pH and various inhibitors and activators on the displacement of preaccumulated intracellular PAH were examined by methods similar to Copenhaver and Forster (4). Slices in isotonic medium containing PAH were incubated for one-half hour, chilled, then immediately transferred to respective isotonic media of specific pHs containing test agents, 0.01 *M* acetate, 0.01 *M* succinate, or 6.7×10^{-5} *M* probenecid without PAH. The slices were then incubated for one-half hour after which the flasks were chilled and the slices blotted, weighed, and analysed for PAH.

An investigation of pH effect on oxygen consumption with PAH was made with Warburg apparatus. Warburg flasks contained the basic medium including 6.7×10^{-5} *M* PAH and slices in the main compartment, 0.2 ml 20% KOH in the center well and oxygen in the gas space.

All pH measurements were made with a Leeds-Northrup pH meter. The pH of the medium was recorded before and after the slices were incubated. All pH values referred to in accompanying Figures and Tables are final values unless indicated otherwise.

Results and discussion. Accumulation of PAH. The accumulation of PAH by rabbit renal cortex slices was found to be dependent on the hydrogen ion concentration of the media. PAH accumulated intracellularly at all pH values tested with an optimum at pH

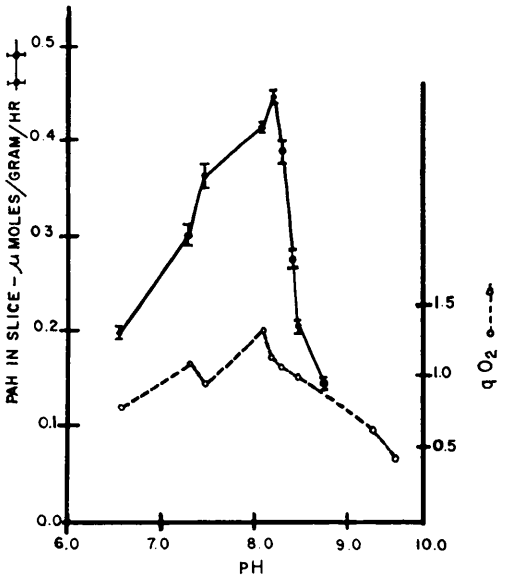

FIG. 1. Effect of hydrogen ion concentration on PAH accumulation and oxygen consumption in kidney cortex slices. PAH accumulation represents the average and S. E. of the mean from 5 experiments.

8.1 to 8.3 (Fig. 1). Values for intracellularly accumulated PAH obtained at pH 7.4 were in harmony with previous values(1,4). Oxygen consumption during PAH accumulation expressed as qO_2 (cmm of O_2 per mg initial wet weight of tissue per hour) shows no significant change between pH values 6.5 and 8.5 (Fig. 1).

The effect of pH is particularly noticeable when the accumulation of PAH is plotted against the final pH of the media, since the strong buffering capacity of the slices rapidly converted the pH of the medium towards the "physiological" pH. In example, medium with an initial pH of 9.7 dropped to 8.7 within 15 minutes after addition of slices and remained at that pH for the remainder of the incubation. Likewise, an initial pH of 5.8 was converted to 6.4 while other flasks in the same experiment with 17 different initial pH values all showed a change of pH toward 7.3 when slices were added.

Essig(6) reported values for PAH accumulation in rabbit renal cortex slices in media where the *initial* pH ranged from 6.3 to 8.5. His results show an increase in accumulation with increasing pH. However, in the light of

our experience and upon analysis of his methods it is very likely that Essig's studies extended only from pH 7.0 to 8.0 which probably explains why he did not observe the sharp pH optimum reported here.

The effects of several substances which influence the accumulation of PAH in renal cortex slices were studied at various hydrogen ion concentrations. A representative experiment comparing the results at pH 7.4 and 8.2 is summarized in Table I. Substances which are effective inhibitors of the PAH transport system at pH 7.4 are also effective at the pH optimum. However, acetate, which stimulates the uptake of PAH at pH 7.4, has no such effect at the pH optimum. Between pH 7.4 and 8.2 the stimulatory effect of acetate shows a gradual decrease.

TABLE I. Effect of Test Agents on Accumulation of PAH.

Test agent	μmoles/g/hr pH 7.4	μmoles/g/hr pH 8.2
Control	.344	.438
.01 *M* acetate	.524	.420
.01 *M* glycine	.308	.490
.01 *M* succinate	.175	.109
.01 *M* α-ketoglutarate	.120	.122
5×10^{-4} *M* 2,4-dinitrophenol		.123
6.7×10^{-5} *M* probenecid		.069
6.7×10^{-5} *M* probenecid and .01 *M* acetate		.045
.01 *M* diodrast		.087

Taggart(5) has proposed that acetate in the medium stimulates PAH accumulation because the acetate forms acetyl glycine intracellularly thereby preventing the formation of long chain acylglycines which inhibit the transport of PAH. In connection with this theory, experiments in this laboratory showed that glycine had no noticeable effect on PAH accumulation at pH 7.4 but at the optimum pH a small but consistent stimulatory effect was noticed (Table I). Measurements of PAH accumulation at the optimum pH and at various time intervals indicate that the glycine effect is consistent through the first hour of incubation. It is, however, independent of glycine concentration over the range of 2.5 to 15.0×10^{-3} M.

Schacter *et al*(3) have found that long chain fatty acids inhibit PAH uptake at the physiological pH, presumably by the formation of acylglycines(5). Addition of decanoate to the incubation medium depressed the uptake of PAH at the pH optimum (Table II). However, addition of glycine with decanoate did not enhance the decanoate inhibition. It would seem that addition of glycine, by promoting the formation of acylglycines, should inhibit the accumulation of PAH at the various hydrogen ion concentrations tested and would enhance the inhibition produced by decanoate. The lack of inhibition by glycine under the conditions reported here, and the stimulation of PAH uptake by glycine at the pH optimum raise some question as to the validity of the theory proposed by Taggart(5) concerning the stimulatory effect of endogenous fatty acids.

TABLE II. Effect of Glycine and Decanoate on PAH Accumulation.

	μmoles/g/hr pH 8.2
Control	.404
.01 *M* glycine	.490
.001 *M* decanoate	.222
.01 *M* glycine and .001 M decanoate	.288

Displacement of PAH. Previous studies(4) have shown that at pH 7.4 preaccumulated PAH tends to run out of kidney cortex slices after transfer to PAH-free medium. In a representative experiment reported here, slices were allowed to accumulate PAH at pH 8.2 for 30 minutes at which time the concentration of PAH was 0.327 μmole/g. Slices were then transferred to PAH-free media at various pH values and incubated for 30 minutes after which the amount of PAH remaining was determined (Table III). It is apparent that the displacement of PAH does not show a sharp pH optimum.

TABLE III. Effect of Hydrogen Ion Concentration on PAH Displacement.

Final pH	PAH remaining μmoles/g
6.4	.132
7.4	.184
8.0	.200
8.2	.193
8.3	.202
8.4	.183
8.5	.181
8.8	.158

The effects of acetate, succinate and probenecid on the displacement of intracellularly accumulated PAH were studied at various pH values ranging from 7.4 to 8.9. Acetate, which inhibits the loss of accumulated PAH at pH 7.4(4), has no such noticeable effect at lower hydrogen ion concentrations. Succinate and probenecid stimulate the displacement of PAH at all tested hydrogen ion concentrations without exhibiting a noteworthy pH dependence.

Summary. Studies indicate that PAH accumulation by thin slices of rabbit renal cortex shows a sharp pH optimum at 8.1 to 8.3. At the pH optimum, acetate does not show stimulation of PAH uptake that is characteristic of its effect at physiological pH. The displacement of previously accumulated PAH does not show a sharp pH dependence.

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Inhibition by Actinomycin D of Bone Resorption Induced by Parathyroid Hormone or Vitamin A.* (30253)

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Both parathyroid hormone (PTH) and vitamin A (vit. A) stimulate the slow development of bone resorption in tissue culture (1). PTH inhibits osteoblasts and stimulates fibroblastic and osteoclastic proliferation as well as inducing calcium release and resorption of bone matrix. Cell proliferation is less prominent with vit. A. In several recent studies(2-5), prior administration of Actinomycin D *in vivo* was found to inhibit the calcium mobilizing response to exogenous PTH but not its effect on phosphate transport. Since Actinomycin D inhibits DNA-dependent RNA synthesis, it was suggested that the calcemic effect of PTH depended upon new RNA synthesis. On the basis of similar experiments an RNA-dependent step has been postulated for a number of other hormones (6). The effect of Actinomycin D on the stimulation of bone resportion in tissue culture by both PTH and vit. A was examined in the present study, since these agents are thought to act by different mechanisms.

Methods. The methods employed have been described(1). Paired bone shafts of radius and ulna from 19-day rat embryos were cultured for 72 hours in 50% human serum in Eagle's medium in an atmosphere of 5% CO_2 in air. Embryonic bone rudiments were obtained from pregnant rats in which Ca^{45} was injected 36 hours prior to sacrifice. The ratio of the release of previously incorporated Ca^{45} from treated bones to that from paired controls served as a measure of bone resorption. In addition, each bone was examined histologically. Three groups of experiments were performed.

Group I. Bones were treated with submaximal doses of purified PTH (0.12 μg/ml of a purified preparation with a potency of 2500 U/mg, kindly provided by Dr. G. Aurbach) or with vit. A (3 μg/ml). Varying doses of Actinomycin D (kindly provided by Dr. N. G. Brink of Merck Sharp & Dohme Research Laboratories) were added to both the treated and paired control cultures. All agents were added to the medium prior to explantation of the bone rudiments. *Group*

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