strains produced pulmonary lesions in mice, whether inoculated intranasally or intraperitoneally. **Erwinia** strains at the same dilution rarely did so; if, however, larger numbers of organisms were employed in the inoculum, many strains of **Erwinia** were capable of inducing pneumonia in mice.

These data suggest that **Klebsiella** and **Erwinia,** both widely distributed in nature, may, depending on circumstances, shift from plant to animal hosts and possibly vice versa.

Studies on the Tubular Excretion of Creatinine and P-Aminohippurate in Thin Slices of Dogfish Kidney (Squalus acanthias)*

John V. Taggart, Roy P. Forster, David Schachter, Solomon A. Kaplan, and Edwin M. Trayner
Columbia University, Dartmouth College, and the University of Cincinnati

Studies on the metabolic processes associated with active cellular transport have been greatly facilitated by the recent development of a variety of experimental techniques utilizing either isolated renal tubules or thin slices of kidney cortex[1]. The transport and accumulation of colored compounds, such as phenol red, can be visualized directly with the microscope. The movement of other compounds, such as p-aminohippurate (PAH) or diodrast, can be followed quantitatively by chemical analyses of the tissue and ambient fluid. The **in vitro** techniques provide a convenient method for examining various metabolic intermediates and inhibitors for their effects on renal transport mechanisms.

In the present studies, the slice technique was used for observations on the tubular excretion of creatinine and PAH in the dogfish, Squalus acanthias. This species was selected for study because of the relatively high creatinine/inulin clearance ratios reported by Shannon[2]. The clearance data in Squalus indicate that, at low plasma levels of creatinine, tubular excretion accounts for 75 per cent or more of the urinary creatinine.

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Methods

Spiny dogfish weighing 1 to 2 kg were caught by line and stored in large live-cars for 4 days or less before use. An hematocrit of 20 per cent or more at the time of sacrifice was considered as indicating a satisfactory physiological state.

The slice technique was essentially as previously described[1]. The whole kidney was dissected free from the retroperitoneal space and cut into 5 to 10 mm blocks. Slices of 0.3 mm thickness were prepared in the Stadie-Riggs microtome. Slicing was performed in a chilled "elasmobranch saline"[3] of the following composition: NaCl 1.638% (280 mM/L), KCl 0.089% (11.9 mM), $CaCl_2$ 0.111% (10 mM), $NaHCO_3$ 0.038% (4.5 mM), NaH_2PO_4 0.006% (0.5 mM) and urea 2.16% (360 mM).

Preliminary studies with phenol red showed the excretory tubular tissue to be fairly uniformly distributed throughout the kidney and, therefore, the whole kidney was frequently used for slicing. Approximately 300 mg of slices were suspended in a Warburg vessel in 2.7 ml of elasmobranch saline which contained either 0.3 mM/L of creatinine or 0.067 mM/L of PAH. Other additions were dissolved in the saline and neutralized when necessary. The vessels were gassed with oxygen and shaken in a 25°C. water bath. After 20 to 80 minutes, the slices and an aliquot of medium were recovered for analysis. The accumulation of creatinine or PAH is reported as the final slice/medium concentration ratio (S/M).

The clearance studies were begun 9 to 20 hours after a single subcutaneous injection of inulin, creatinine or PAH. Serial urine collections were obtained with a rubber balloon attached to a catheter firmly tied into the male urinary papilla. Serial blood samples were obtained and the mid-point plasma level was estimated for each period. Clearance values have been expressed in ml/kg/24 hours.

Creatinine was estimated in tungstate filtrates by an apparently specific method using Lloyd's reagent[4]. Inulin was estimated in barium-zinc filtrates by the resorcinol method[5] and PAH and acetyl-PAH in trichloracetic acid filtrates by the method of Smith et al[6].

Results

Creatinine Studies - No endogenous creatinine could be detected in control samples of plasma, urine or kidney tissue obtained from several specimens of Squalus. When kidney slices were incubated in a medium containing creatinine, the

creatinine distributed itself between the slices and medium and, subsequently, could be quantitatively recovered. However, the final S/M ratios for creatinine were very low, ranging from 0.8 to 2.3 with an average of about 1.4. In view of this finding, it seemed worthwhile to compare the accumulation of creatinine which occurs in kidney tissue **in vivo** with that which can be achieved with slices **in vitro.** Three dogfish were injected with 1.5 g of inulin and 100 mg of creatinine. Urine and blood samples were collected between the 9th and 21st hours after injection and the inulin and creatinine clearances were determined. At the termination of the clearance period, the fish were sacrificed and samples of kidney tissue taken immediately for creatinine estimations. The remainder of each kidney was sliced and used for the **in vitro** procedure.

The data from these experiments are summarized in table 1. The creatinine/inulin clearance ratios ranged from 1.8 to

Table 1.

	Experiment No.		
	1	2	3
Weight (kg)	1.99	1.82	1.48
Urine flow (ml/kg/24 hrs)	9.45	8.85	12.5
Inulin clearance (ml/kg/24 hrs)	49.0	32.4	46.0
Midpoint plasma creatinine (mg%)	2.99	4.73	4.65
Creatinine U/P ratio	15.7	12.1	6.50
Creatinine clearance (ml/kg/24 hrs)	148	107	81
Creatinine/Inulin clearance ratio	3.0	3.3	1.8
Accumulation **in vivo**			
Final kidney creatinine (mg%)	6.0	9.6	8.7
Final plasma creatinine (mg%)	2.0	4.1	3.1
Kidney/Plasma	3.0	2.3	2.8
Accumulation **in vitro**			
Final slice creatinine (mg%)	5.7	9.8	5.6
Final medium creatinine (mg%)	4.0	4.0	3.7
Slice/Medium	1.4	2.3	1.4

Table 1. The clearance of exogenous creatinine and the accumulation of creatinine in kidney tissue in Squalus acanthias.

3.3, all the values falling somewhat below the 4.0 to 7.0 range found at low plasma levels by Shannon[2]. Perhaps of greater importance was the finding that the creatinine U/P ratios were as low as 6.5 and never exceeded 15.7. The low U/P ratios are reflected in the limited accumulation of creatinine by kidney tissue both **in vivo** and **in vitro.** It was concluded from these experiments that the slice technique has a limited usefulness in studies on the transport of compounds which fail to establish high concentration gradients across the tubular epithelium. Further **in vitro** studies with creatinine, therefore, were abandoned.

PAH Transport - Thin slices of dogfish kidney rapidly accumulate PAH from low external concentrations. S/M ratios of 10 or more are achieved within 20 to 40 minutes after the Warburg vessels are introduced into the 25° bath. After the initial 40 minute period, the S/M ratio of PAH slowly declines as is shown in experiment 1, table 2.

Table 2.

Exper.	Time	Additions	qO_2	S/M
	Min.			
1	0	PAH		0.6
	20	PAH	0.77	10.0
	40	PAH	0.72	10.3
	60	PAH	0.77	9.6
	80	PAH	0.77	9.2
2	60	PAH	0.84	10.1
	60	PAH + acetate	1.01	5.8
	60	PAH + alpha-ketoglutarate	1.08	1.1
	60	PAH + succinate	1.15	1.5
	60	PAH + octanoate	1.11	0.9
3	30	PAH	0.62	10.1
	30	PAH + acetate	0.86	5.6
	30	PAAH	0.62	12.3
	30	PAAH + acetate	0.81	7.9

Table 2. Accumulation of PAH and PAAH in slices of dogfish kidney and the effects of various metabolic intermediates on transport. Respiration (qO_2) represents cmm of oxygen consumed per mg of tissue per hour. Metabolic intermediates were added in 0.01 M concentration. Temp. 25°, time indicated.

The addition of various metabolic intermediates to the suspending medium yielded the results summarized in experiment 2, table 2. In keeping with previous observations on mammalian kidney slices[7], the addition of 0.01M alpha - ketoglutarate, succinate or octanoate stimulated the respiration of slices, but blocked the accumulation of PAH. An unexpected finding was the apparent inhibition of PAH transport by 0.01 M acetate. With mammalian kidney, both **in vitro** and **in vivo,** acetate exhibits a striking stimulatory effect, usually increasing PAH transport by 50 to 85 per cent[7-8].

It was noted in the course of studies with acetate that recoveries of PAH decreased with time to as low as 60%. Since all of the added PAH could be recovered with acid hydrolysis, it was concluded that acetylation of the p-amino group had occurred. The rate of PAH acetylation was found to be approximately 0.2 micromole per gram of tissue per hour*. This finding appeared to provide a possible explanation for the inhibition of PAH transport by acetate. It was assumed that the acetyl-PAH formed by the slices could compete with PAH for accumulation. That such an explanation is invalid is shown by experiment 3, table 2. While acetyl-PAH (PAAH) is actively accumulated by slices, its accumulation is inhibited by acetate in a manner very similar to that observed with PAH.

Although the anomolous behavior of acetate could not be explained, it was considered desirable to determine the extent of PAH acetylation in the intact animal and to note the effect of this process on the apparent clearances of PAH and PAAH. Three dogfish were injected with 91 mg of PAH and blood and urine collections were obtained between the 20th and 24th hours after injection. All samples were analyzed for both PAH and PAAH. The data summarizing these experiments are contained in table 3. It may be noted that significant quantities of PAAH were present in both urine and plasma samples. The wide discrepancy between the clearances of PAH and PAAH clearly reflects the synthesis in kidney of the latter compound. The clearance of PAH + PAAH probably provides the best approximation of the effective renal plasma flow. On the basis of reasonable calculations it may be concluded that 30 to 60 per cent of the urinary PAAH had been synthesized in the kidney. These observations clearly demonstrate the spurious clearance values which are obtained when renal synthesis of the measured compound occurs.

*Although the acetylation of foreign amines is generally confined to the liver, it has previously been noted that guinea pig renal cortex is also capable of acetylating PAH[7].

Table 3.

	Experiment No. 1	2	3
Weight (kg)	1.82	1.48	1.39
Urine flow (ml/kg/24 hours)	11.6	13.4	8.2
Plasma Concentration (mg%)			
PAH	2.19	2.95	1.94
PAAH	0.27	1.46	0.52
PAH + PAAH	2.46	4.41	2.46
Urine Concentration (mg%)			
PAH	139	112	162
PAAH	47	116	172
PAH + PAAH	186	228	334
Clearance (ml/kg/24 hrs)			
PAH	737	510	686
PAAH	2020	1062	2710
PAH + PAAH	878	692	1110

Table 3. The clearances of p-aminohippurate (PAH) and p-acetylaminohippurate (PAAH) in Squalus acanthias 20 to 40 hours after a single subcutaneous injection of PAH.

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The Nutrition of the Apical Region of the Pea Root

John G. Torrey
University of California

Isolated four millimeter root tips excised from germinating pea seeds show good growth on a relatively simple synthetic medium composed of inorganic salts, thiamin and nicotinic acid, and 4% sucrose[1-2]. Although the longer tips usually develop normally on this medium, tips one millimeter or less in length seldom develop on the simple medium. Recent work by R. Brown of England[3] has suggested that, in pea roots, the apical meristem itself is dependent upon metabolites provided by the more mature tissues of the root.

Attempts were made to culture 0.5 millimeter root tips of the pea, **Pisum sativum,** variety Alaska, using a complex nutrient medium. Four millimeter root tips were excised aseptically from pea seeds after 48 hours germination and were transferred to control medium containing 0.5% agar in Petri dishes. After one week of culture in the dark at 25 degrees C., the terminal 0.5 millimeter tip of each root (including approximately 300 microns root cap and 200 microns of the apical meristem) was excised with a sharpened needle under a dissecting microscope. Tips were transferred by needle to modified Syracuse dishes, 27 mm. diameter, containing 1 ml. of the medium to be tested. Eight such dishes were placed in each Petri dish and returned to the culture chamber.

On the control medium, the 0.5 millimeter tips did not grow, but each tip became a rounded mass of small undifferentiated cells. Increasing the concentration of sucrose (6%) or agar (0.5-2.0%) in the medium had no effect on tip