growth. The addition of yeast extract (Difco) at a concentration of 1 gram per liter resulted in good growth of these tips during the first week. Preliminary attempts were made to discover the active fraction of the yeast extract. None of the following substances were effective in eliciting tip growth at the concentrations tested: thiamin, nicotinic acid, pyridoxine, riboflavin, calcium pantothenate, biotin, and indoleacetic acid. Preliminary experiments testing the effectiveness of amino acid mixtures were inconclusive. It is anticipated that identification of the substances essential for root tip growth provided by the yeast extract will broaden materially our knowledge of the nutrition of the apical region of the root.

References

1. Bonner, J. and Addicott, F. Cultivation in vitro of excised pea roots. Bot. Gaz. **99**: 144, 1937.
2. Bonner, J, and Devirian, P. S. Growth factor requirements of four species of isolated roots. Amer. Jour. Bot. **26**: 661, 1939.
3. Brown, R. and Wightman, F. The influence of mature tissue on division in the meristem of the root. Jour. Exp. Bot. **3**: 253, 1952.

Tubular Transport of Phenol Red in the Flounder Kidney*

Karlman Wasserman, E. Lovell Becker,
and Alfred P. Fishman**
Tulane and New York Universities and
Mt. Sinai Hospital, New York City

The technique of Forster[1] as modified by Puck et al[2] was used to study 1) self-depression of phenol red as induced by high load/Tm ratios and 2) the influence of carbonic anhydrase inhibitor on acid secretion by the renal tubule.

Teased fragments of winter flounder, **Pseudopleuronectes americanus** were immersed in an oxygenated, temperature controlled (25°-28° C) electrolyte medium containing the following salts, (mM/L): NaCl 134, KCl 2.5, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.5, $NaHCO_3$ 40, phenol red 0.056 (2 mg%),

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with alterations as indicated in the text or tables. The tubules were observed under the microscope (125X) quantitation being made by the comparator block technique[2] or the 0—4+ semi-quantitative assessment.

Depression of Phenol Red Transport

In a previous publication[2], it was demonstrated that phenol red transport by the isolated flounder tubule was influenced by potassium and calcium concentrations in the medium. Two steps in the phenol red transport were postulated. The first dealt with the active uptake of the phenol red from the medium by the cell. Potassium was shown to be essential for this phase of transport to take place, its rate of reaction being proportional to the potassium concentration in the medium. The second step, the transport of the dye from the cell into the tubule lumen, required calcium in the medium since a calcium-free medium caused the dye to be trapped in the cells without significant concentration in the tubule lumen. An extension of these observations (Table I) demonstrates

Table I: Effect of Calcium on Phenol Red Transport

[Ca++]*	Lumen**	Cell**
0.0	0.0	0.9
0.19	0.9	0.5
0.38	3.8	0.5
0.75	5.0	0.0
1.50	5.0	0.0

*mM/L

**Concentration of phenol red (mg%) in matching filter.

that as calcium concentration is increased, without altering the concentration of any other solute in the basic medium, exit of dye from the cell into the lumen is potentiated. Higher calcium concentrations than 1.5 mM/L. could not be studied because calcium salts are quite insoluble at the high pH of the medium.

Very little dye is secreted into the lumen of the tubule when 1) no potassium is present, or 2) when high potassium concentrations (varies from fish to fish but may range from 10 mM/L on up) are present in the media (Table II). At high

Table II: Effect of Potassium on Phenol Red Transport

[K+]*	Lumen**	Cell**
0.0	0.0,0.5	0.0
1.2	10.0	0.0
2.5	5.0	0.0
10.0	3.8	0.5
20.0	5.0	2.5
30.0	1.9	5.0
40.0	0.5	7.5

*mM/L

**Concentration of phenol red (mg%) in matching filter.

concentrations of potassium when very little dye is present in the lumen of the tubule, the dye concentration in the cell is maximum. There is no blocking of secretion of phenol red into the lumen until high concentrations of phenol red appear in the cells.

Phenol red in the medium has a depressing effect on its own transport at high concentrations of phenol red (Table III). The depressing action appears to be on the transport of dye from cells into the lumen (Step 2). Step 1 (the active uptake of phenol red by the cell) appears to continue since dye is observed to accumulate in the cells.

Table III: Effect of Phenol Red Concentration in Medium on Phenol Red Transport.

Phenol Red. * conc. in medium	Lumen**	Cell**
2.0	7.5	0
10.0	5.0	0
20.0	10.0	0
40.0	0	3.8
60.0	0	3.8

*mg%

**Concentration of phenol red (mg%) in matching filter.

In order to determine the relationship between the depression of transport due to increasing the potassium concentration and that due to increasing the phenol red concentration, the effect of progressively increasing both potassium and phenol red concentrations in the medium were studied (Table IV). The combination of increasing the potassium concentration

Table IV: Effect of varying K^+ on self-depression of Phenol Red

Phenol Red conc. in Med.	2.5 mM/L K^+		10.0 mM/L K^+		20 mM/L K^+		30 mM/L K^+	
mg%	L*	C*	L*	C*	L*	C*	L*	C*
2.0	7.5	0.0	7.5	0	2.5	0.0	5.0	1.8
10.0	5.0	0.0	0	5.0	1.2	10.0	1.2	3.8
20.0	10.0	0.0	0	7.5	0.5	10.0	0.9	7.5
40.0	0	3.8	0	7.5	0.5	10.0	0.5	10.0
60.0	0	3.8	0	7.5	0.5	10.0	0.5	10.0

*Conc. (mg%) of phenol red in matching filter.

while simultaneously increasing the phenol red concentration in the medium, enhances the depression observed with just one variable, depression of Step 2 now occurring at lower phenol red and lower potassium concentrations.

Since calcium has an effect on Step 2 and the site of this depression is at Step 2, increasing concentrations of calcium in the medium were studied simultaneously with increasing concentrations of phenol red (Table V). The higher concentrations of calcium delay the appearance of depression despite progressively higher concentrations of phenol red. Thus, depression of step 2 in phenol red transport was found to occur in media containing high phenol red concentrations, high potassium concentrations, or low calcium concentrations, with all three depressing mechanisms being synergistic.

Table V: Effect of varying calcium on self-depression of phenol red.

Phenol Red conc. in Med.	0.4 mM/L Ca		0.8 mM/L Ca		1.5 mM/L Ca	
mg%	L*	C*	L*	C*	L*	C*
10	1.25	1.25	5.0	0	10.0	0
20	2.5	2.5	5.0	0	10.0	0
40	2.5	2.5	1.25	0	10.0	0
60	1.25	1.25	1.25	1.25	2.5	0
120	1.25	2.5	—	—	0	2.5

*Concentration (mg%) of phenol red in matching filter.

Action of Carbonic Anhydrase Inhibitor (#6063) On the Isolated Tubule

#6063 (2-acetylamino - 1,3,4 thiadiazole-5 sulfonamide) shown to be an **in vitro** carbonic anhydrase inhibitor[3], has been used by Berliner et al[4] to produce alkaline urine presumably by inhibiting carbonic anhydrase in the tubular epithelium of the kidney. The effect of #6063 in concentrations of 1 mg% on the uptake of phenol red and pH of the tubular urine was observed in the **in vitro** preparation previously discussed. The semi-quantitative 0 to 4+ technique was used to determine intensity of dye in the tubules, and the ability to maintain alkaline lumens (red or pink lumens) with and without #6063 was quantitated by observing the minimum bicarbonate concentration required in the medium in order to obtain alkaline lumens. When the urines became acid the endpoint (below pH 6.6) was an intensely yellow lumen. Preparations possessing orange or peach lumens or mixtures of yellow and pink lumens were plotted as having pH values between 6.6 and 8.2. The pH of the lumen was observed in a series of six petri dishes, with and without #6063, containing decreasing concentrations of $NaHCO_3$ 20, 10, 5, 2.5, 1.25, and 0.625 mM/L. The results indicated that in the presence of #6063 the lumen remained distinctly alkaline in as low bicarbonate concentrations at 1.25 mM/L, the turning point being at 0.625 mM/L of bicarbonate. Without #6063, the lumens were distinctly alkaline at levels

of 20 mM/L of bicarbonate. Mixtures of orange, red and yellow were observed at 10 and 5mM/L. At 2.5 mM/L and less the lumens were distinctly acid as indicated by intense yellow lumens. The media were always alkaline.

Where calcium was omitted from the basic medium, addition of #6063 to the medium caused the tubule to respond as though it were in a high potassium medium, i.e., there was a definite increase in phenol red uptake by the cells of 3+ intensity, while its control which lacked the #6063 showed a cellular uptake of only 1+ intensity.

In the normal basic medium, lumens gradually turn orange or yellow after 60 to 90 minutes. In the presence of #6063, the lumens remain pink with maximum intensity after 60 to 90 minutes.

References

1. Forster, R. P. Use of Thin Kidney Slices and Isolated Renal Tubules for Direct Study of Cellular Transport Kinetics. Science **108**:65, 1948.

2. Puck, T. T., K. Wasserman & A. P. Fishman, 1952. Some Effects of Inorganic Ions on the Active Transport of Phenol Red by Isolated Kidney Tubules of the Flounder, J. Cell. & Comp. Physiol. **37**:389, 1951.

3. Miller, W. H., Dessert, A. M. & Roblin, R. O., Jr., Heterocyclic sulfonamides as Carbonic Anhydrase Inhibitors J. Am. Chem. Soc. **72**:4893, 1950.

4. Berliner, R. W., Kennedy, T. J., Jr., & Orloff, J. Relationship Between Acidification of Urine and Potassium Metabolism. Am. J. Med. **11**: 274, 1951.