from hemolysis by 1.0*M* methylurea and thiourea but does not affect the hemolysis produced by acetamide. Similarly, 0.3*M* hydroxyurea protects red cells from hemolysis in 1.0*M* methylurea and thiourea but not from hemolysis by acetamide.

Figure 1 shows typical results (selected from four separate studies) of the flux measurements made with the solutions containing high concentrations of urea and no urea. It is evident that by the time the first sample of supernatant solution is obtained for measurement (generally after 5 minutes) equilibration of urea between cells and medium is approximately 95 percent complete. Thus there is apparently rapid bidirectional exchange of urea across the red cell membrane. By the end of 60 minutes net influx and efflux are essentially zero.

The term "facilitated diffusion" has been applied to the process by which glucose penetrates the plasma membrane of primate erythrocytes. The evidence for this mode of transport includes (i) the failure of primate erythrocytes to hemolyze in isotonic glucose (*7*); (ii) the apparent specificity of the transport process so that a given sugar may readily penetrate the red cell (*d*-xylose and *l*-arabinose) while other structurally similar compounds (*l*-xylose and *d*-arabinose) are excluded (*8*); and (iii) the competition between various substrates for the carrier so that low concentrations of glucose inhibit the penetration of other sugars like xylose and arabinose (*9*).

These features are clearly similar to the results obtained for the transport of urea into elasmobranch red cells. The failure of these cells to hemolyze in isosmotic urea; the hemolysis of these cells by methylurea and thiourea; and the inhibition of this hemolysis by small concentrations of urea indicate a similar mechanism. Our data also suggest that hydroxyurea shares the same carrier mechanism, whereas acetamide does not.

It should be emphasized that although our results are consistent with facilitated diffusion of urea, we have not rigorously established this as the mode of transport. The techniques we employed were chiefly qualitative. For the rigorous demonstration of facilitated diffusion, quantitative studies of the kinetics of urea transport; accurate measurement of changes in red cell volume; flux measurements of labeled urea; exploration of carrier inhibitors; and analysis of concentration gradients under a variety of circumstances will be required.

In view of the important role of urea in the economy of the elasmobranch, it is not entirely surprising that the individual cells of this group of fish possess unusual mechanisms for dealing with the compound. This demonstration of transport by facilitated diffusion may serve as an important model for studying the nature of urea distribution in other systems.

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5. Elasmobranch Ringer solution was prepared as follows: NaCl, 250 m*M*; KCl, 5 m*M*; $MgCl_2$, 3 m*M*; $CaCl_2$, 5 m*M*; NaH_2PO_4, 0.5 m*M*; $NaHCO_3$, 5 m*M*; urea, 350 m*M*. Water added to make volume 1 liter. The high urea medium contained 700 m*M* of urea per liter and only 75 meq of NaCl. The zero urea medium contained no urea and the amount of NaCl was increased to 425 m*M*. The *p*H of the solution averaged between 7.5 and 7.6.
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Strontium and Calcium Reabsorption in Renal Tubules of the Newt, Triturus pyrrhogaster

Abstract. *Reabsorption of calcium in the proximal tubule cannulated in vivo exceeded reabsorption of strontium. The diuretics Diamox and Salyrgan depressed the reabsorption of calcium but not strontium.*

During intestinal absorption and renal excretion in man and animals, if both calcium and strontium are present, calcium is preferentially absorbed (*1*). The phenomenon of preferential reabsorption of calcium is useful for evaluating the accumulation of Sr^{90} in man. Much less work has been done on reabsorption during renal excretion than during intestinal absorption because of the technical difficulty of performing experiments on a kidney in vivo. Although the importance of the renal tubules in the preferential absorption of calcium has not yet been demonstrated, it has been a subject of speculation (*2*).

Calcium reabsorption is thought to occur mainly in the proximal tubules of the kidney (*3*). We have measured in vivo the rates of reabsorption of Sr^{85} and of Ca^{45} in the proximal tubule of the newt, *Triturus pyrrhogaster*. The large size of the nephrons in the newt make this animal very suitable for such an investigation. A technique similar to ours was used previously for measuring reabsorption in single nephrons of *Necturus* (*4*).

A newt (weighing 4 to 5 g) was immobilized by destruction of the cerebrospinal system and the kidney was exposed under a microscope. A polyethylene tube (50 to 100 μ in diameter) containing a 0.6 percent NaCl solution between two air bubbles was inserted into a proximal tubule via the Bowman's capsule. Another tube, containing Ringer solution, was inserted into the corresponding distal tubule from the opposite side, and the Ringer solution was injected into the tubule.

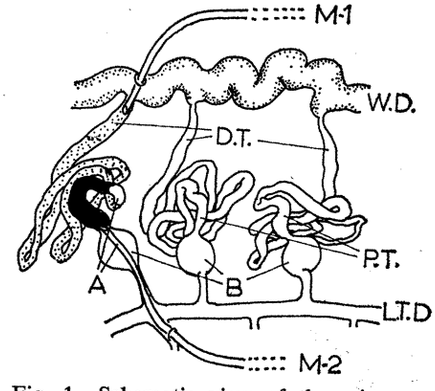

Fig. 1. Schematic view of the microperfusion method used for measuring the rates of reabsorption of Ca^{45} and Sr^{85}. *WD*, Wolffian duct; *M*, microinjector; *B*, Bowman's capsule; *PT*, proximal tubule; *A*, air bubble; the dark area represents NaCl solution containing either Ca^{45} or Sr^{85}; *LTD*, longitudinal duct.

Table 1. Percentage reabsorption of Ca^{45} and Sr^{85} in the renal proximal tubule of the newt. Each figure is the mean of six individuals (± standard deviation).

Time in proximal tubule (min)	Reabsorption (%)		Sr^{85}/Ca^{45} (%)
	Ca^{45}	Sr^{85}	
	No diuretic		
3	24 ± 4	10 ± 4	0.43
5	36 ± 6	15 ± 4	0.42
10	56 ± 6	30 ± 5	0.53
	Diamox		
5	15 ± 9	14 ± 5	0.91
	Salyrgan		
5	16 ± 9	13 ± 4	0.86
	Diuril		
5	25 ± 9	14 ± 6	0.58

The NaCl solution, which also contained either Ca^{45} (0.13 mg/ml; specific activity 50 μc/ml), or Sr^{85} (0.012 mg/ml; specific activity 50 μc/ml), was injected into the proximal tubule by means of a microinjector attached to the polyethylene tube. The microinjectors helped to maintain the polyethylene tube in a fixed position (Fig. 1). After 3, 5, or 10 minutes in the proximal tubule, the solution was forced back to the original tube and assayed for radioactivity. Separate measurements of Ca^{45} and Sr^{85} were made for several individual nephrons in a number of newts. The percentage reabsorption for each time period and the reabsorption ratio (percentage Sr^{85} reabsorption / percentage Ca^{45} reabsorption) were calculated (Table 1). The rate of Sr^{85} reabsorption was less than that of Ca^{45}. The reabsorption ratio was about 0.4 to 0.5.

Using the same microperfusion method, we also studied the influence of three diuretics which we believed might inhibit the active transport of ions in the membrane of the renal tubule. Newts were injected intraperitoneally with 5 mg of Diamox (acetazolamide), or 0.1 mg of Salyrgan (mersalyl and theophylline), or 0.2 mg of Diuril (chlorothiazide) 30 minutes before we tested for the reabsorption of Sr^{85} and Ca^{45} in the proximal tubule (Table 1). The rate of Sr^{85} reabsorption was unchanged, but the rate of Ca^{45} reabsorption was much less and the reabsorption ratio was close to unity with each of the diuretics except Diuril.

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Ethanol Accumulation in the Rumen after Overfeeding with Readily Fermentable Carbohydrate

Abstract. *A neutral volatile substance in ruminal contents from sheep suffering from acute indigestion due to overfeeding has been identified as ethanol. Ethanol was consistently observed in ruminal material from both cattle and sheep after they had been fed large amounts of readily fermentable carbohydrate.*

Information on the occurrence of ethanol in the rumen is limited. Cunningham and Brisson (*1*) found high concentrations of alcohol in the rumen, in urine, and in blood of lambs that were sick as a result of feeding a purified ration containing 8-percent glucose. They believed that the alcohol was produced by bacteria rather than by yeast. Krogh (*2*) noted an alcohol-like odor from ruminal contents of sheep fed sucrose and found that samples from the rumens of these animals contained large numbers of yeast cells.

Using gas-liquid chromatography, we detected a neutral volatile material in ruminal contents of animals overfed with cracked wheat and found that this substance increased in concentration as the animals became ill due to the overfeeding. This material has been identified as ethanol.

Gas-liquid chromatography was performed with a Beckman GC-2A gas chromatograph equipped with a hydrogen-flame ionization detector. The unknown material was eluted after methanol, before *n*-propanol, and coincident with both ethanol and isopropanol when chromatographed at 70°C in a 2-mm by 2-m coiled Teflon column packed with 7.5 percent (wt/wt) polyethylene glycol 400 monostearate (*3*) and 0.64 percent (wt/wt) phosphoric acid on acid-washed 60–80 mesh Chromosorb W (*4*). The flow rate of the carrier gas (helium) was 22 cm^3/min. The unknown substance did not appear to be an acid or a carbonyl compound since its elution was not affected by making the sample alkaline with sodium hydroxide or by treatment of the sample with 2,4-dinitrophenylhydrazine.

Ruminal fluid from a sheep that was sick after overfeeding was adjusted to *p*H 7.5 and distilled to one-half its original volume. A small sample of the distillate was analyzed for ethanol by gas-liquid chromatography and the remainder was oxidized with a potassium dichromate solution ($K_2Cr_2O_7$, 134 g/liter; 10*N* H_2SO_4, 675 ml/liter) in a boiling water bath for 10 minutes. The reaction mixture was steam-distilled, and the distillate was examined by gas-liquid chromatography, the polyethylene glycol monostearate column being used at 120°C. A peak with the same retention volume as acetic acid was the principal product of the oxidation, indicating that ethanol was the principal neutral volatile substance. Acetone was not detected.

Quantitative analysis for ethanol in blood and ruminal material was conducted by gas-liquid chromatography at 100°C, with a 4-mm by 2-m coiled copper column packed with 20-percent tetrahydroxyethyl-ethylenediamine (THEED) on acid-washed Chromosorb W (60–80 mesh). The inlet pressure of the carrier gas (helium) was 2.1 atm. The *p*H of ruminal contents was measured within 5 minutes of obtaining the samples. Two milliliters of 25-percent HPO_3 were added to 10 ml of strained ruminal fluid, the mixture was centrifuged, and 1 ml of the supernatant was added to a vial containing 200 μl of a 0.6-percent solution of *n*-butanol, which served as an internal standard during gas-liquid chromatography. Blood samples were deproteinized with phosphotungstic acid and the internal standard, *n*-butanol, was added prior to chromatography. Areas under the peaks were measured with a disc integrator.

On the THEED column the neutral volatile material in ruminal contents had a retention volume identical with that of ethanol and different from that of isopropanol. Trace quantities of a material with the same retention volume