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

Table 1. The *p*H and ethanol concentration of ruminal contents from sheep overfed with cracked wheat.

Time in relation to feeding (hr)	Sheep No. 6*		Sheep No. 7	
	*p*H	Ethanol (μmole/ml)	*p*H	Ethanol (μmole/ml)
−1	6.72	<1	6.60	<1
+6	5.42	2.6	5.55	3.1
14	4.80	3.6	4.65	14.0
22	4.45	13.0	4.88	9.0
28	4.47	18.0	4.90	5.9
30	4.55	19.0	4.85	11.0
37	4.55	33.0	5.25	9.0
46			5.35	6.0
54			5.40	4.1
70			5.80	0

* Sheep died by 46 hours after feeding.

as *n*-propanol, however, have been noted occasionally.

Table 1 gives data on ruminal *p*H and ethanol concentrations after intraruminal dosage of two sheep with 50 g cracked wheat per kilogram of body weight. Sheep No. 6 was a Hampshire cross wether weighing 82 kg and No. 7 was a Cheviot ewe that weighed 54.5 kg. Both sheep were prepared with ruminal fistulas (*5*) and with catheters in the carotid artery (*6*). Both sheep exhibited symptoms typical of ruminants overfed with large amounts of readily available carbohydrate (*2*). Microscopic studies of ruminal contents showed that in both animals, 14 hours after administration of the wheat, ruminal protozoa were no longer motile and there was a decided increase in the proportions of gram positive streptococci and rods. Presumably these were *Streptococcus bovis* and *Lactobacillus sp.* (*7*). Yeast-like cells were not numerous. Sheep No. 6 died 37 to 46 hours after the wheat was administered, while No. 7 recovered, regained its appetite, and had normal rumen motility 70 hours after dosing.

The only blood sample from sheep No. 6 that contained detectable ethanol was the sample collected 37 hours after the wheat was administered. This sample contained less than 1 μmole of ethanol per milliliter of blood. The blood from sheep No. 7 did not contain detectable ethanol during the experiment. The lower limit for detection was about 0.2 μmole/ml. These blood samples were taken from the carotid artery; perhaps ethanol would have been more readily detected in portal blood. Emery *et al.* (*8*) placed relatively large amounts of ethanol (approximately 110 μmole/ml daily) in the rumens of cattle during trials lasting 14 and 21 days. They found a mean concentration of 16 μmole of ethanol per milliliter of blood 2 to 4 hours after ethanol administration but did not report any signs of toxicity. Considering the acute nature of the overfeeding-indigestion syndrome and the absence or low level of ethanol in the blood, it seems likely that ethanol did not contribute significantly to the symptoms of overfeeding observed in our experiments.

Ethanol has been detected in ruminal contents of 12 out of 12 sheep fed cracked wheat. Another sheep dosed with 5.5 kg of crushed apples through a ruminal fistula had a ruminal ethanol concentration of 13 μmole/ml 26 hours after dosage. A 460-kg heifer dosed with 1 kg of glucose had 3 μmole/ml of ethanol in the rumen 8 hours later. Small but detectable concentrations of ethanol have been noted in the ruminal contents of four out of four steers that were on a high concentrate fattening ration (*9*).

Most samples of ruminal material from animals on hay rations contain very small quantities of ethanol or none at all. As part of another study, ruminal samples were obtained from eight lambs that had been maintained on a hay ration. Two of eight samples taken before feeding contained ethanol (1 μmole/ml and less than 0.5 μmole/ml) and one of the eight samples taken 5 hours after feeding hay contained 0.9 μmole/ml. All of these lambs had more than 1 μmole/ml of ethanol in their rumens after they had been fed wheat.

A number of important ruminal bacteria produce ethanol during growth in pure culture (*10*), but the near neutral *p*H and the low concentration of hydrogen in the rumen probably limit its formation (*11*). Perhaps the lowered *p*H after overfeeding inhibits methanogenesis and hydrogen then becomes available for ethanol production. The rate of metabolism of ethanol is slow and it is probably not an important intermediate in the rumen under normal conditions (*12*). However, it may be pertinent that a much greater rate of metabolism was noted when the concentration of ethanol was elevated.

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Parathion Activation by Livers of Aquatic and Terrestrial Vertebrates

Abstract. *Liver slices from seven terrestrial and six aquatic animals studied "activated" parathion, converting it to paraoxon, a potent anticholinesterase. Although there are similarities between the liver systems which activate parathion and which degrade drugs such as aminopyrine and phenacetin, liver slices of aquatic vertebrates can activate parathion but cannot degrade such drugs.*

The insecticide parathion is converted to paraoxon, a potent anticholinesterase, by liver slices (*1*) or by appropriately fortified liver homogenates (*2*). This activation process, in which the P=S group is converted to a P=O group, can also be accomplished by liver microsomes in the presence of magnesium and a reduced pyridine nucleotide (*3*). The same microsomal preparation can degrade a variety of drugs by such processes as dealkylation and hydroxylation (*4*).

Brodie and Maickel (*5*) showed that terrestrial vertebrates (including birds, mammals, and toads) could degrade such drugs as aminopyrine and phenacetin, but aquatic forms (including fish, turtles, frogs, and salamanders) could not. If the activation of parathion and the degradation of these drugs were carried out by the same enzyme system, one might expect to find that aquatic vertebrates could not activate