bit atria with a combination of catecholamines and low potassium in the perfusion fluid. Under these conditions, atrial activity was present at temperatures below 5°C. Heretofore, spontaneous cardiac activity at such temperatures has been observed only in hibernating species.

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Absorption Through Unstimulated and Secreting Canine Oxyntic Glandular Mucosa.* (32583)

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A substantial body of literature discusses rates of diffusion of substances across the oxyntic glandular mucosa. [Work through 1964 is summarized in(1); a more recent important paper is(2).] A major difficulty encountered when one tries to interpret data on diffusion through the gastric mucosa is that the area across which diffusion occurs is unknown. Do ions and compounds diffusing from lumen to blood cross only the surface epithelial cells, or, passing down the tubules, do they diffuse across the oxyntic and chief cells as well? Since the surface area of the glandular cells of the tubules (excluding the canaliculi) is 13 times the macroscopic area of the surface of the stomach(3), one's conclusion about the permeability characteristics of the membranes forming the barrier to diffusion are grossly different if one considers substances to diffuse only across the surface epithelial cells or to cross both the surface cells and those lining the tubules.

We have attempted to solve this problem by measuring rates of absorption of three compounds (ethanol, thiopental and salicylic acid) whose absorption is accomplished entirely by passive diffusion. These compounds are uncharged in the acid solution we used, and the electrical gradient across the mucosa has no influence on their absorption. We have measured their absorption by the unstimulated, non-secreting, and by the maximally stimulated, secreting mucosa.

The dimensions of the dog's oxyntic glandular mucosa have been measured by Canosa and Rehm(3), and Rehm, Schlesinger and Dennis(4) have calculated the rate of diffusion of a solute from the lumen into the pits and tubules. C_o is the concentration of solute in an essentially infinite reservoir of fluid on the surface of the mucosa, and C is the concentration of solute at any time and at any distance from the surface when the solute diffuses into an infinitely long column of solvent of uniform cross-section. If the velocity of flow of fluid in the column is zero, the ratio C/C_o for a solute (HCl) having a diffusion coefficient of 2.7×10^{-5} cm^2 sec^{-1} is 0.4 at a depth of 1.5 mm from the surface at the end of 600 sec [Fig. 4A, reference(4)]. The diffusion coefficient of ethanol is about 1×10^{-5} cm^2 sec^{-1}, and that

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of salicylic acid is 1.2×10^{-5} in the same units(5). The diffusion coefficient of thiopental is not recorded, but it should be of the same order of magnitude. If we make the reasonable assumption that absorption in the gastric pits is not so rapid that the concentration of the solute at the entrance to the tubules is far lower than C_0, each of these compounds, when present in the luminal fluid of the unstimulated stomach, should reach a relatively high concentration in the fluid contained within the tubules. If the macroscopic area of the pouch is 60 to 100 cm^2 (as it would be if it were a sphere containing 35 to 70 ml), if the area of the pit lumens is 7% of the macroscopic area of the mucosa(3), and if the secretory rate were 30 ml in 30 min (as it was in the experiments reported here), the velocity of flow of fluid up the pits is of the order of 2.4×10^{-3} cm sec^{-1}. At this velocity of flow C/C_0 at the bottom of the pits is 0.0025 [Fig. 4B, reference(4)]. Therefore, under steady state conditions the concentration of solute at the entrance to the tubules would be very much smaller when the mucosa is rapidly secreting than it is in the depths of the tubules when the mucosa is not secreting. Consequently, if the walls of the tubules are an important site of entry into the mucosa, the rate of absorption by the secreting mucosa should be very much less than that by the non-secreting mucosa.

Methods. The dogs and the method of irrigating their pouches have been described in detail(6). Briefly, 3 unanesthetized dogs in good health, each having a separated vagally denervated (Heidenhain) pouch of the oxyntic glandular area of the stomach were used. The dogs were deprived of food, but not of water for 18-20 hours before use. At the beginning of each experiment the pouch was thoroughly washed with a solution of 100 mM HCl plus 54 mM NaCl and then with the same solution containing the test solute. The pouch was emptied, leaving in it a known residual volume characteristic of the particular dog and ranging from 4.7 to 5.3 ml. 40 ml of the test solution were placed in the pouch, thoroughly mixed and sampled, leaving a known initial volume between 35 and 38 ml. The fluid was allowed to remain in the pouch for 30 min, and it was thoroughly mixed by frequent withdrawal and reinfusion. At the end of 30 min the fluid was removed for measurement and analysis, again leaving a known residual volume. The whole procedure was repeated for 2 more 30-min periods when ethanol or thiopental was the test solute. Because salicylic acid damages the gastric mucosa, it was used for only one 30-min period, and the dogs were given ample time for recovery before being studied again.

For stimulation experiments, the dogs were given subcutaneous injections of histamine and gastrin pentapeptide (I.C.I. 50,123) at 10-min intervals in doses of 50 μg histamine base and 8 μ gastrin pentapeptide kg^{-1} hr^{-1}. Juice was collected from the cannula draining the pouch, and after secretion was at least 8 ml in 10 min the procedure of filling the pouch was begun. Injections were continued, and secretory rate remained high.

All test solutes were dissolved in a solution of 100 mM HCl plus 50 mM NaCl. The initial concentrations are given in Table I. Ethanol was measured by the alcohol-dehydrogenase-DPN method of Bonnichsen and Theorell(7, Sigma Chemical Co.). Thiopental was estimated by measuring the optical density of alkalinized, diluted samples at 305 mμ (8). Appropriate blanks were used, and a standard curve was determined for each batch of thiopental used. Salicylic acid was measured by the method of Brodie *et al*(9).

Calculation of absorption constant. The rate ($\dot{Q}$) of disappearance of test substance from the luminal fluid was calculated by subtracting the product of initial volume and initial concentration from the product of final volume and final concentration. Values are reported in μM $(30\ min)^{-1}$ and are negative. In order to take into account the changing concentration of test substance within the luminal fluid, an absorption constant k was calculated from the equation

$$-\dot{Q}/30 = k([S]_0 + [S]_f)/2. \quad (1)$$

Here $\dot{Q}$ is the mass of test substance disappearing in the 30-min period, and $([S]_0 + [S]_f)/2$ is the arithmetical mean concentration of substance S in the lumen obtained by divid-

TABLE I. Absorption of Ethanol, Thiopental and Salicylic Acid by the Unstimulated and Stimulated Pouches of the Oxyntic Gland Area of Three Dogs.

Dog	Stim.	Vol_o, ml	ΔVol, ml(30′)$^{-1}$	$[S]_o$, mM	$\dot{Q}$, μM(30′)$^{-1}$	k, ml min^{-1}
			Ethanol			
1	—	35.7 ± 0.7	1.8 ± 0.9	15.9 ± 0.7	−183 ± 36	0.47 ± 0.10
1	+	36.0 ± 0.8	30.6 ±10.4	15.6 ± 0.6	−137 ± 32	0.41 ± 0.07
2	—	35.7 ± 0.3	1.4 ± 2.3	15.6 ± 0.7	−185 ± 54	0.49 ± 0.16
2	+	36.0 ± 0.1	24.3 ± 2.0	16.3 ± 0.5	−165 ± 49	0.48 ± 0.16
			Thiopental			
1	—	34.6 ± 0.1	1.0 ± 0.8	0.155 ± 0.006	−2.17 ± 0.26	0.59 ± 0.07
1	+	34.6 ± 0.1	33.0 ± 2.7	0.149 ± 0.009	−1.80 ± 0.63	0.61 ± 0.23
2	—	35.5 ± 0.1	1.8 ± 0.2	0.169 ± 0.007	−2.89 ± 0.44	0.76 ± 0.12
2	+	35.5 ± 0.1	38.4 ± 8.4	0.167 ± 0.007	−2.14 ± 0.32	0.65 ± 0.08
			Salicylic acid			
1	—	35.1 ± 0.1	4.8 ± 0.7	19.2 ± 1.7	−332 ± 28	0.80 ± 0.07
1	+	35.2 ± 0.4	37.3 ± 1.5	19.8 ± 0.6	−232 ± 52	0.59 ± 0.14
3	—	35.6 ± 0.1	0.9 ± 1.1	19.2 ± 0.6	−362 ± 64	0.86 ± 0.20
3	+	36.1 ± 0.9	31.4 ± 2.9	19.2 ± 1.0	−292 ± 42	0.79 ± 0.17

Means ± SD; n = 9 for ethanol and thiopental; n = 5 for salicylic acid.

ing the sum of initial and final concentrations by 2. The units of k are ml min^{-1}. Although evaluation of the constant by casting the equation into differential form and integrating might be more correct, this was not done for two reasons. There is very little theoretical and no practical difference between linear and exponential rates of change of concentration of the test substance in the unstimulated pouch where changes of concentration are small. (See, for example, the data in reference [10] where an exponential equation is fitted to similar data.) When the rate of change of concentration is high in the stimulated pouch, the rate of change imposed by dilution is actually linear.

Results. Data on the absorption of the compounds by the pouches of 3 dogs are given in Table I. Although $\dot{Q}$ was always smaller for the stimulated pouch, the difference could be accounted for entirely by the effect of dilution resulting from copious secretion. There were no statistically significant differences between pairs of absorption constants.

Discussion. The results demonstrate that within the limits of experimental error very high rates of secretion have no effect upon the passive diffusion of our test substances into the oxyntic glandular mucosa. Therefore, the flow of water occurring during secretion does not oppose passive diffusion of substances into the mucosa. The major site of water flow during secretion is the lumen of the tubules, and at the rates of flow obtained in the present experiments the concentration of test substances within the lumen of the tubules must have been nearly zero. If absorption is not reduced under these circumstances, absorption from the lumen of the tubules cannot be any substantial fraction of total absorption when flow of water is low. We conclude that the major site of absorption in the non-secreting and secreting mucosa must be the surface epithelial cells. Although the data may be susceptible to alternative explanations, they must be taken into account when one attempts to frame a comprehensive theory of the permeability characteristics of the oxyntic glandular mucosa.

Summary. Rates of absorption of ethanol, thiopental and salicylic acid by pouches of the canine oxyntic glandular mucosa were measured when the pouches were unstimulated and not secreting and when they were strongly stimulated to secrete by injections of histamine and gastrin pentapeptide. No differences were found between the 2 situations when rates of absorption were referred to the mean concentrations of the compounds in the fluid contained in the pouches. It is concluded that the high rate of flow of water out of the mucosa when it is stimulated to secrete does not reduce the rate at which substances can diffuse passively into the mucosa, and it is

further concluded that the tubules of the gastric glands in which the rate of flow of water is greatest are not an important site of absorption.

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Inhibition of Rat Liver Microsomal N-Demethylase by α-Naphthylisothiocyanate: Studies with Puromycin Aminonucleoside.* (32584)

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Plaa *et al*(1) have shown that α-naphthylisothiocyanate (ANIT) inhibits the mouse liver enzyme(s) that catalyzes the side chain oxidation of hexobarbital and the ring hydroxylation of aniline, but does not inhibit the enzyme(s) responsible for the sulfoxidation of chlorpromazine. Inasmuch as ANIT was shown to be inhibitory over a longer time period than SKF 525A, it was of interest to study its effect on rat liver microsomal N-demethylase, *in vitro* and *in vivo*. The demethylation of puromycin aminonucleoside (PA) was used as the criterion of activity.

Materials and methods. α-Naphthylisothiocyanate, obtained from Eastman Organic Chemicals, New York, was recrystallized from hexane (decolorized with charcoal), m.p. 55.5-56.5° corr. Puromycin aminonucleoside was generously supplied by Lederle Laboratories Division, American Cyanamid Company. Other chemicals were purchased from commercial sources. Male albino rats were purchased from Simonsen Laboratories, White Bear Lake, Minn.

ANIT, in corn oil, was administered orally to rats at a dose of 80 mg/kg 2 hours before sacrifice or further treatment. The liver microsomes were isolated and analyzed for N-demethylase activity by measuring the formaldehyde formed according to Mazel *et al*(2). To study the effect of ANIT pretreatment on the metabolism of PA *in vivo*, PA (100 mg/kg, i.p.) was administered to rats 2 hours after ANIT, and urine was collected over the subsequent 72 hours. The urine was fractionated by ion-exchange chromatography and the aminonucleoside fraction separated by thin-layer chromatography into PA and its monodemethylated analog, MMPA,† as previously described(3). The ratio of PA/MMPA in the urine was used as the measure of microsomal N-demethylase activity *in vivo*.

Results and discussion. Pretreatment of rats with ANIT significantly inhibited the N-demethylation of PA (Table I). This was demonstrated by assay of the isolated micro-

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† 6-Methylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine.