significant action on the compound A spike of the desheathed sciatic-peroneal nerve of the bullfrog. Many experiments in this laboratory have shown that this concentration of NaCl is more than sufficient to maintain conduction in the A group of fibers in the bullfrog sciatic nerve. Curve *B* shows the rapid conduction block in the A fibers by a solution containing 0.0047 *M* amyl carbamate and 0.022 *M* NaCl. Addition of Ringer's solution at 16 min in place of the test solution caused a rapid return of the A activity. The crucial segment of the experiment is shown as Curve *C*. This illustrates the conduction block by a solution of 0.0047 *M* amyl carbamate and 0.022 *M* NaCl. At 26 min, when nearly complete block was achieved, the amyl carbamate solution was replaced by a second solution containing again 0.0047 *M* amyl carbamate but with the NaCl increased to 0.11 *M*. This caused a rapid recovery of conduction at a rate which was certainly no less than that of the previous run (Curve *B*). The experiment clearly demonstrates the reversal of block in the presence of amyl carbamate by increased sodium concentration.

Both types of experiments (Figs. 1 and 2) were simple, conclusive, and repeatable at will. The results suggest the existence of an intimate interaction between amyl carbamate and sodium. Present experiments, now in progress, suggest that other nerve-blocking drugs behave in a similar manner. The experiments, although subject to several interpretations, are most simply explained in the light of present knowledge on the assumption that amyl carbamate interferes, directly or indirectly, with a sodium mechanism. The experiments are of especial interest in relation to the sodium hypothesis of nerve conduction (*1*).

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The Action of Pectinol and Pectin Esterase on Sections of Rat and Guinea Pig Stomachs[1]

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McManus and Saunders (*1*) showed that periodic acid-Schiff (PAS) positive materials are removed with pectinase and less completely with pectinol. Pectin esterase did not remove these substances, but enhanced the PAS-coloration. The authors used human tissues (colon, bronchial mucins, and kidney) for their investigations. An attempt to compare the action of pectinol and pectin esterase on the stomach of the albino rat and of the guinea pig seemed of interest.

Ten 2–8-month-old nontreated rats of the Sprague-Dawley-Holtzman strain and 10 approximately 2-month-old nontreated guinea pigs (Rockland Farms) were killed by neckstroke. Their glandular stomachs were cut into 3 zones according to our standard procedure (*2*) and fixed in chilled ethanol, dehydrated, cleared in amyl acetate in the cold room at 4° C, and vacuum-embedded. Sections of 6μ thickness were mounted on slides with glycerine egg albumin, and left in a 50° C oven overnight. This step insured the adherence of the sections to the slides during incubation with the enzymes. The PAS-reaction (*3*) was carried out alone or after incubation of the sections with various enzymes.

The McManus reaction revealed PAS-positive fiber-like materials in the muscularis mucosae of the rat stomach. Those lying between the mucosa and the muscularis mucosae simulated a basement membrane. This was not observed in the guinea pig, in which PAS-positive materials were limited to the surface mucous cells.

Pectinol 100-D and Pectin Esterase No. 5 (Rohm and Haas) did not show any effect on the PAS-reaction at 1% strength and incubation for 30 min at 37° C. However, the same substances at 0.4% strength and incubation for 48 hr at 37° C, as recommended (*1*), showed the following results.

Pectinol 100-D incompletely removed the PAS-positive substances from the stomachs in accordance with previous findings (*1*). Furthermore, it brought out a positive PAS-reaction in parietal cells and erythrocytes that did not appear with the PAS-reaction alone or after saliva, ptyalin, or Taka-diastase incubations. Pectin esterase, also in accordance with McManus and Saunders (*1*), did not remove PAS-positive materials. It not only enhanced the coloration in the known PAS-positive constituents of the stomach, but caused a deep PAS-coloration in parietal and squamous cells, muscularis mucosae, submucosa, muscularis, and the walls of all blood vessels and erythrocytes. This was noted in the rat with greater intensity than in the guinea pig.

Incubation of rat stomach sections with Pectinol 100-D and Pectin esterase No. 5 at 37° C for 8 hr, followed by an accidental exposure to 60° C for 15 hr, gave the same results. Rat and guinea pig slides incubated with the same enzymes for only 8 hr at 37° C showed identical results. This demonstrated that 8 hr of incubation sufficed for the enzymatic action; furthermore, that a 15-hr incubation at 60° C did not affect the actions once they had taken place, although pectinases are rapidly inactivated at 60° C (*4, 5*).

After an 8-hr pectin esterase incubation, followed by a 15-hr pectinol incubation, less PAS-positive materials were observed than after pectin esterase incubation alone. This proved that pectinol removes some of the PAS-positive materials, whereas pectin esterase broke down PAS-negative mucoproteins into simpler PAS-positive cleavage products. Differences were noted here between the rat and the guinea pig. In the rat, less PAS-positive substances were removed by

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pectinol. Furthermore, the PAS-positive basement membranelike structures were not affected. This showed that the rat stomach, in contrast to the guinea pig stomach, contained PAS-positive materials that were unaffected by pectinol following pectin esterase.

The results indicate a difference in the distribution and the chemical composition of mucoproteins and mucins in the stomach walls of the two species.

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The Preparation of Wet Ashed Tissues for Liquid Counting[1]

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The wet ashing of animal tissues with hot concentrated nitric acid usually leaves a small amount of fatty material undissolved. Comar (*1*) found in the course of extensive studies on the distribution of Cu^{64} and Mo^{99} that the amount of radioactive material contained in the fatty residue was negligible and that no significant error was produced by removing the fatty layer and discarding it. However, we found that the radioactivity of Hf^{181} and Au^{198} in the fatty material cannot be overlooked, since in some instances the counts per gram of undissolved fatty material were nearly ten times those of the aqueous solution (*cf.* 1 and 2, Table 1). The small amount of undissolved fat[3] in the digestion of the liver of a dog which had received Au^{198} in colloidal form was found to contain over 15% of the total activity of that organ.

The presence of relatively high activity in the fatty layer makes it difficult to obtain a representative aliquot portion for counting unless special precautions are observed. The fatty material tends to rise to the top of the mixture, and as a result the first sample poured off contains a higher proportion of this material than subsequent samples, whereas a sample of aqueous layer taken by pipette involves the opposite error (*cf.* 3 and 4, Table 1).

Since the fatty layer tends to float on top of the water layer, the radiation is not absorbed to the same extent as when the radioactive element is distributed uniformly. In cases where the thin floating layer has a higher specific activity than the water layer it causes too high a count. This effect has been noted when only a thin fatty film was visible on the surface of the liquid in the counting dish. The addition of a pinch of detergent (e.g., Tide) and agitation to break up the surface film have served to reduce the counting rate as much as 30–50% (*cf.* 5 and 6, Table 1).

TABLE 1

Example*	Specimen digested	Entire specimen diluted to	Sample counted	(Counts/min) $\times 10^{-3}$
1	Carcass†	1000 ml	10 ml aqueous phase	1.07
			10 g fat	10.3
2	Carcass†	1000 "	10 ml aqueous phase	0.12
			10 g fat	.96
3	Carcass†	1000 "	10 ml clear aqueous phase (not containing fat droplets)	.47
			10 ml after shaking (fat droplets included)	.76
4	Liver†	250 "	First 10 ml poured off (containing much fat)	8.47
			Second 10 ml poured off (containing less fat)	1.65
			Third 10 ml emulsified with Dreft	.35
5	Kidney‡	10 "	Entire 10 ml	5.78
			Same sample, after emulsification with Tide	2.41
6	Liver§ 0.59 g	10 "	Entire 10 ml	1.77
			Same sample, after emulsification with Tide	1.29

* Each example refers to a different animal.
† Of rat which had received injection of Hf^{181} sodium catechol disulfonate complex.
‡ Of rat which had received injection of Hf^{181} sodium gluconate complex.
§ Of rat which had received injection of Hf^{181} sodium mandelate complex.

In the past, investigators have sometimes dissolved the fatty layer in organic solvents such as amyl alcohol or ether-alcohol mixtures and counted aliquot portions of the aqueous and organic solutions separately (*2*). We have found it more convenient to add enough acetone or dioxane to bring both the fat and water into a single phase. The procedure is as follows: The entire organ or a representative sample of the minced organ was heated with a minimum amount of concentrated nitric acid until all particulate matter was dissolved and most of the excess acid had been boiled away;[4] the solution was cooled and diluted with acetone or dioxane and a little water to form a clear

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[3] The term "fat" is used loosely in this paper to refer to the fatlike material which remained undissolved after acid digestion. It is doubtful that the animal fat, before digestion, contained a high level of radioactive material.
[4] At this point a small amount of concentrated HCl is added to samples containing colloidal gold, to prevent the adsorption of radioactivity on the walls of the container. In the absence of HCl the loss of Au^{198} may amount to as much as 25% in 24 hr and more on longer standing.