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.

solution of known volume. The optimum mixture contains 60–80% acetone or dioxane, the exact proportion depending upon the relative amounts of fat and inorganic salts in the sample. If more than one dilution is made, it is economical to use acetone for the first dilution. However, because of its higher boiling point and density, dioxane[5] is to be preferred for the final dilution if the sample is to be counted as a liquid. In a few instances the mixing of acetone with concentrated nitric acid solutions was followed by evolution of heat and boiling of the acetone, which caused loss of sample. This difficulty was not encountered when dioxane was used for all the dilutions.

Using the above method we have been able to obtain good results with Au^{198} as well as with Hf^{181}. We were able to recover approximately 95% of radioactive colloidal gold added to a whole rat and to a dog liver before digestion. In another experiment the total activity found in a whole dog liver by this method differed less than 3% from the total activity found by counting the aqueous and fatty layers separately.

The use of acetone or dioxane to make a homogeneous nitric acid tissue digest is recommended as a more accurate and simple method for the determination of the isotope content of animal tissues.

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[5] Dioxane should be handled with due precautions in view of its inflammability, the toxicity of its vapors, and the possible presence of explosive peroxides.

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Effect of Ascorbic Acid on the Adrenal Weight of Normal and Hypophysectomized Rats[1]

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Ascorbic acid, in large doses, has been reported to increase the fall in adrenal cholesterol and to have other ACTH-like effects (*1–3*). It has also been shown that the same substance prevents the normal hypertrophy of the adrenals in animals exposed to cold (*4*), as well as the alarm reaction in the same conditions of exposure (*1*) or under epinephrine treatment (*5*). In the case of exposure to cold, ascorbic acid also increased the survival of the animals (*4*).

The purpose of the present investigation was to try to clarify and correlate the above findings. At least three working hypotheses might explain why ascorbic acid prevented the normal hypertrophy of the adrenals during stress: (1) ascorbic acid may inhibit directly the action of ACTH on the adrenal, although this seemed rather unlikely; (2) it has a corticotrophic effect; the hypersecretion of cortical hormones thus produced (an assertion substantiated by the fact that the fall in adrenal cholesterol is greater with, than without, ascorbic acid during exposure to cold [*1*]) would prevent, indirectly, the hypersecretion of ACTH, according to Sayer's theory (*6*); or (3) that ascorbic acid has a potentiating action on ACTH when the demand for cortical hormones increases. If the last possibility is correct, one would expect that in a case of stress the hypophysis would not be hyperstimulated by the lack of cortical hormones, which are produced in sufficient amount by the combined action of small doses (approximately corresponding to normal secretion) of ACTH and ascorbic acid.

Consequently, three series of experiments were performed. In the first, we wanted to learn whether ascorbic acid prevents the action of injected ACTH on the adrenals of normal rats. Sixty rats, averaging 200 g in weight, and fed Purina Fox-Chow *ad lib.*, were divided into 4 groups: One group (A) received by daily intraperitoneal injection, during 3 days, 150 mg of sodium ascorbate; a second group (B) received the same amount of sodium ascorbate plus 10 mg daily of ACTH by intramuscular injection; a third group (C) received sodium bicarbonate intraperitoneally, the amount of sodium injected daily being equivalent to that injected into the first group; and the last group (D) received sodium bicarbonate intraperitoneally plus 10 mg/day of ACTH by intramuscular injections. The results, presented in Table 1, show that ascorbic acid does not prevent the action of injected ACTH. The increases in adrenal weight in the ascorbate and bicarbonate groups are both statistically significant and do not differ from one another.

The second and third series of experiments were done on hypophysectomized rats, fed the special diet of Shaw and Greep (*7*). In each case, the left adrenal was removed 10 days after hypophysectomy and before any treatment. Comparisons were then made on each individual between the right adrenal after treatment, and the left one before treatment. Table 2 shows that ascorbic acid has no effect on the adrenal weight of hypophysectomized animals, at least for the 10-day period of treatment we have used.

In the third series of experiments, we tried the effect of a small dose of ACTH (0.5 mg/day) combined with ascorbic acid. Both were given by intraperitoneal injection. The dose of ACTH employed had no effect by itself on the adrenal weight, nor did the combination of ACTH (same dose) and bicarbonate (solution adjusted so as to inject the same amount of sodium daily into this group as into the sodium ascorbate group) have any effect; but the combination of the same dose of ACTH with ascorbic acid (150 mg/day) had a pronounced and significant effect. Whereas there is a small decrease in the first two groups (Table 3) between the weights of the right adrenal after treatment as indicated and the left adrenal before treatment, there is, on the contrary, an

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