guinea pigs on the scorbutogenic diet if antiscorbutic substances are withheld. Folic acid apparently did not affect the animals favorably or adversely. An adverse effect might have been expected if larger doses of folic acid were given, since the guinea pig is known to be more susceptible to toxic effects of folic acid than other animals(6). On a weight basis, the dosage given was greater than that used therapeutically in humans. The development of scurvy in monkeys receiving folic acid has been observed by May (4). The roentgen observations of amelioration were therefore puzzling. Later, a careful review of records revealed that a single dose of 200 mg of ascorbic acid, administered intravenously as part of a tolerance test performed to establish the diagnosis of scurvy, could have been responsible for the regression of the skeletal lesions noted roentgenographically.

Conclusions. Under conditions of vit. C deprivation, folic acid has no apparent antiscorbutic effect in guinea pigs.

6. Harned, B. K., Cunningham, R. W., Smith, H. D., and Clark, M. C., *Ann. New York Acad. Sc.*, 1946, v8, 289.

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Thioglycollate Inactivation of Posterior Pituitary Antidiuretic Principle as Determined in the Rat.* (18563)

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Recently, Ralli and her collaborators(1) suggested that "the antidiuretic activity of commercial Pitressin can be fractionated into at least two substances, one of which is active in the rat but not in the dog." This suggestion was based upon the observation that the antidiuretic action of Pitressin could be abolished by treatment with 0.01 M sodium thioglycollate(2) as determined by intravenous assay in dogs but not by similar treatment of Pitressin when assayed by intraperitoneal injection in rats. On the other hand, the chloruretic action of large doses of Pitressin treated with thioglycollate was reduced 45% in comparison with untreated Pitressin. (Whether the chloruretic action was abolished cannot be determined from the published experiments which lacked control groups receiving no Pitressin.) Ralli and her colleagues also concluded that in the dose range of 10-100 milli-units the chloruretic effect of Pitressin in rats was related to its antidiuretic action.

In their study of the antidiuretic action of thioglycollate-treated Pitressin, Ralli *et al.*(1) reported control experiments only with Pitressin containing a similar concentration of acetate. Sodium thioglycollate (1 ml of 0.01 or 0.05 M solution) has no antidiuretic effect after intravenous injection into the dog(2). However, its action on water diuresis in the rat after either intraperitoneal or intravenous injection has never been reported. In the experiments about to be described it was found that solutions of sodium thioglycollate have an "antidiuretic" action after intraperitoneal or subcutaneous injection into rats but not after intravenous injection.

Material and methods. Adult rats of either sex of the Long-Evans strain were used. In all the experiments a special lot of Pitressin†

* This investigation was in part supported by grants from the Playtex Park Research Institute and E. R. Squibb and Sons.

1. Ralli, E. P., Raisz, L. G., Leslie, L. H., Dumm, M. E., and Laken, B., *Am. J. Physiol.*, 1950, v163, 141.

2. Ames, R. G., Moore, D. H., and van Dyke, H. B., *Endocrinology*, 1950, v46, 215.

† The Pitressin was kindly furnished by Dr. A. C. Bratton of Parke, Davis and Co.

containing 80 units of antidiuretic (pressor) activity and 3.8 units of oxytocic activity per mg was used. When injections were made intraperitoneally or subcutaneously urine was collected from groups of 4 rats in metabolism cages. Each rat received by stomach tube at the start of the experiment 5 ml of water per 100 g weight. The volume of urine excreted was recorded every 15 minutes. The method of Jeffers, Livezy and Austin(3) was employed for the intravenous assays. The volume of each dose usually was 0.5 ml or less. The volume of urine excreted was determined every 5 minutes.

Experimental results. One of the 5 experiments performed in groups of rats receiving solutions either intraperitoneally or subcutaneously is summarized in Table I and is typical of the results obtained. The "antidiuretic" effect of sodium thioglycollate as a 0.01 M solution intraperitoneally was just as great as 25 milli-units of Pitressin with or without thioglycollate. The larger intraperitoneal dose of Pitressin, 100 milli-units, interfered with diuresis more than thioglycollate alone or thioglycollate and 100 milli-units of Pitressin. It must be emphasized that an effort to secure results of considerable accuracy by using a number of groups at each dose was not believed to be necessary. The

TABLE I. Action of Pitressin or Sodium Thioglycollate Solution or a Mixture of Both on Water Diuresis in the Rat.

Group*of 4 rats	Route of injection	Min. required for excretion of 50% of dose of water	
Control†	Intraper.	80	
.01 M NaTG‡	"	210	
.01 M NATG cont. 25 m.u. Pitressin	"	210	
25 m.u. Pitressin	"	195	
.01 M NaTG cont. 100 m.u. Pitressin	"	150	
100 m.u. Pitressin	"	255	
.01 M NaTG	Subcut.	>270	35%
.01 M NaTG cont. 100 m.u. Pitressin	"	>270	45%
100 m.u. Pitressin	"	>270	12%

* All doses 1 ml.
† Received isotonic saline sol.
‡ NaTG = sodium thioglycollate.

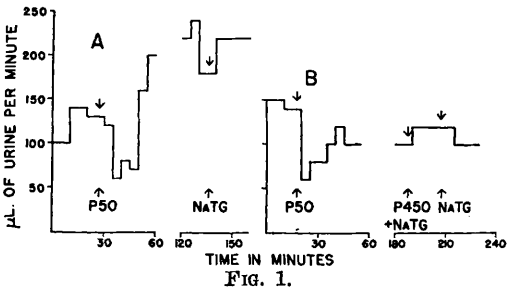

FIG. 1.

Effect of intravenous injections upon urinary excretion in adult rats. P50: 50 μu. of Pitressin in .5 ml of saline (both exp.). P450 + NaTG: 450 μu. in .5 ml of saline containing .01 M sodium thioglycollate (exp. B). NaTG: 1 ml of .01 M sodium thioglycollate (both exp.).

figures given in the experiment of Table I are consistent with the results of other experiments and explain the antidiuretic effect obtained in rats by Ralli and her co-workers when they injected mixtures of Pitressin and sodium thioglycollate intraperitoneally: the Pitressin could have been inactivated and the "antidiuretic" action of the mixture could have been attributed to the thioglycollate.

The delay in diuresis after the subcutaneous injection of the solutions as shown in Table I is, as would be expected, even greater than after intraperitoneal injection. The percentages in parentheses indicate the fractions of administered water excreted as urine after 270 minutes.

One ml of 0.01 M sodium thioglycollate administered to adult rats either subcutaneously or intraperitoneally is not innocuous. Deaths occurred (1 or 2 of the 4 in the group) 12 to 24 hours after injection in every group of Table I receiving thioglycollate except the one to which the Pitressin-thioglycollate mixture was administered subcutaneously.

In the intravenous assays in rats, several injections can be made into each animal. Typical results from 2 of the experiments are shown in Fig. 1. Although the volume of doses usually was 0.5 ml or less, even 1 ml of 0.01 M sodium thioglycollate intravenously caused no interference with diuresis during the period of observation. In experiment B, an inactivation of Pitressin by thioglycollate similar to that found by intravenous assays in the dog, was demonstrated.

Summary and conclusions. After intraperi-

3. Jeffers, W. A., Livezy, M. M., and Austin, J. H., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 184.

toneal or subcutaneous injection, an aqueous solution of sodium thioglycollate markedly reduces water diuresis in rats and cannot be used by either route to demonstrate unequivocally whether posterior pituitary antidiuretic principle has been inactivated. By the intravenous route in rats, thioglycollate does not interfere with diuresis and can be shown, as in the dog, to abolish the antidiuretic action of Pitressin. Therefore, there is no controlled experimental evidence that Pitressin contains more than one antidiuretic principle.

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Stimulation of Cell-Free Gastric Mucus by the Topical Application of Acetylcholine.* (18564)

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This laboratory has been concerned for several years with the systematic study of the characteristics of gastric mucus(1-4). In the course of these investigations the secretion of gastric mucus has been stimulated by the topical application of a wide variety of watery solutions and emulsions, of which eugenol (4-allyl,2-methoxyphenol), the chief constituent of clove oil, was found to be the most effective stimulus for mucus secretion. The mucus obtained by means of these topical stimuli usually, but not always, contained considerable numbers of intact columnar epithelial cells. Thus of 42 smears of specimens secreted in response to the topical application of 5% eugenol to canine fundic pouches, only 7 (17%) had few or no columnar cells [*i.e.*, at most a small number per low power microscopic field], while 35 (83%) had many columnar cells. These cells occurred not only in mucus stimulated by such irritating substances but also in that secreted "spontaneously" in very small amounts by the resting pouch. Thus, of 100 specimens secreted without topical stimulation, 70 (70%) had few columnar cells, while only 30 (30%) contained many. From these studies (3) it was concluded that pure mucus, as it is secreted, is of variable consistency but free of suspended desquamated columnar cells; although desquamation can occur concurrently with such secretion, the exfoliation of cells is not an integral part of the secretory process.

It therefore became necessary to develop a method for stimulating the output of cell-free gastric mucus in considerable quantity, in order both to confirm these inferences and to have a cell-free mucus suitable for further physical and chemical studies. Stavraky and Morton(5) had shown, in the dog, that intra-arterial injection of acetylcholine into the gastrosplenic artery (which supplies the corpus of the stomach along the greater curvature) results in the secretion of mucus. These experiments were performed with acute preparations, but they suggested that topical stimulation of the mucosa of canine fundic pouches by acetylcholine might be equally effective and more serviceable as a reproducible method for obtaining mucus in intact unanesthetized animals.

Methods. Four mongrel dogs with Heidenhain gastric pouches were employed in this study. The pouches were prepared with the conventional technic from the greater curva-

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1. Hollander, F., in *Postgraduate Gastroenterology,* (Editor-H. L. Bockus) W. B. Saunders Co., Phila., 1950, pp. 39-53.

2. Hollander, F., *Gastroenterology,* 1944, v3, 638.

3. Hollander, F., Stein, J., and Lauber, F. U., *Gastroenterology,* 1946, v6, 576.

4. Hollander, F., and Lauber, F. U., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 34.

5. Morton, G. M., and Stavraky, G. W., *Gastroenterology,* 1949, v12, 808.