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)

HENRY D. JANOWITZ, FRANKLIN HOLLANDER, AND CLIFFORD JACKSON.
From The Mount Sinai Hospital, New York City.

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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ture area of the body of the stomach. These animals were invariably starved for 18-24 hours before each experiment. They were then suspended according to the technic usually employed in this laboratory for the collection of gastric juice from pouch dogs, and the spontaneous secretion was collected by soft rubber collecting tubes. The collection of unstimulated secretion was continued until its pH was at least 6.0, and only then was the stimulus applied. For such topical stimulation the animal was removed from its sling and the collecting device was replaced by a pezzer (mushroom) catheter—No. 18-22 Fr. The stimulating fluid was then slowly injected into the pouch, the volume being such as to fill the pouch completely without the application of any considerable amount of syringe pressure. This quantity ranged between 30 and 50 cc. The fluid was withdrawn at the end of 15 minutes, and a fresh supply injected for a second 15-minute period. At the completion of these instillations, the dog was returned to the sling, the excess stimulating fluid was drained off, and collection of mucus was begun. The volume secreted was noted at 30-minute intervals and collections were continued until the rate of secretion fell to insignificant values. Besides acetylcholine chloride (ACh) (1 mg/cc), mecholyl chloride, (0.5 mg/cc), and pilocarpine hydrochloride (1 mg/cc) were also used. The solutions were made up freshly each time, just prior to use, in normal saline or distilled water. These doses of the drugs were tolerated without any toxic manifestations. Viscosity measurements were done with the Fenske-Ostwald viscosimeter at 37°C. Measurements of pH were done either with the glass electrode or with indicators.

Results. Collection of mucus. Application of 30 to 50 cc of solution to the mucosa of the Heidenhain pouch for 30 minutes resulted in the secretion of an extremely viscous opalescent jelly-like mucus. This highly tenacious material was first noted in the collecting graduated tube during the second 30-minute interval following stimulation, and it continued to drain for 3 to 5 hours thereafter. Application of mecholyl or pilocarpine solutions for 30 minutes to these fundic pouches yielded similar material.

In 37 such experiments, the volume of mucus obtained during the entire collection period ranged from 1.7 to 12.0 cc (mean = 5.2 cc). The duration of these collections averaged 4.4 hours. The magnitude of the response appeared to be unrelated to this duration, probably because of the slow and irregular drainage of such viscous material. In the dosage employed, ACh gave a slightly greater response than did mecholyl or pilocarpine. Thus in 27 experiments with ACh, the volume ranged from 2.0 to 12.0 cc (mean = 5.8 cc); in 6 experiments with mecholyl it varied from 2.3 to 5.3 (mean = 4.5); and in 4 experiments with pilocarpine the range was 1.7 to 6.3 (mean = 4.0).

Characteristics of cell-free mucus. Grossly, the specimens were jelly-like, extremely viscous, translucent to opalescent, with occasional small particles of coagulated material. Microscopic examination of smears of this material, stained with toluidine blue or the periodic acid-Schiff technic revealed no individual intact columnar cells or groups of cells in the metachromatically staining background of mucin, and only a few scattered nuclei. The "relative" viscosity of this ACh-stimulated mucus freshly secreted ranged from 29 to 260 (mean = 93). Its pH was between 7.0 and 7.5. The material collected after stimulation with pilocarpine or mecholyl possessed essentially the same characteristics.

Discussion. These experiments indicate clearly that the topical application of acetylcholine or related parasympathomimetic drugs to the mucosa of Heidenhain canine pouches results in the secretion of highly viscid opalescent alkaline cell-free mucus. These findings are consistent with the reported results following the intra-arterial injection of ACh, and furnish a simple convenient method for collecting mucus secretion from fundic pouches for the study of its physical and chemical characteristics.

Of primary interest is the finding that this ACh-stimulated mucus is essentially free of cells. This is in marked contrast to the desquamating action of all the topical muci-

gogues previously studied, both in this laboratory and in others. It thus confirms the inference which we made on the basis of previous correlation studies: that visible mucus is secreted free of cells, and that the desquamation of surface columnar cells is not an integral part of the process of mucus secretion(3).

In contrast to the opacity of mucus secreted in response to the previously studied chemical mucigogues, which opacity has been found to be correlated in high degree with its columnar cell content, the mucus stimulated by ACh or related drugs is much clearer, being opalescent with a small number of more opaque fragments suspended in the mucoid mass. These fragments probably represent small particles of coagulated mucin.

In the concentrations used in the present work, ACh compares favorably with eugenol as far as rate of secretion is concerned. Thus in 65 experiments with 5% eugenol, the mean volume secreted was 6.4 cc(4), whereas in those with ACh it was 5.8 cc. But analysis of these rates is complicated by the extreme viscosity of the material secreted, with resulting slowness and irregularity of collection.

Because of its small cellular content and few gross particles, viscosimetry of native mucus proved feasible with the Ostwald technic. In this way, considerable variation in the viscosity of this ACh-stimulated mucus was observed from experiment to experiment. The meaning of such variations must await more detailed study of the chemical composition of specimens obtained by this procedure.

These studies do not furnish evidence on the mechanism of this ACh stimulation of mucus secretion, but experiments to this end are already under way.

Summary. The topical application of acetylcholine or related parasympathomimetic drugs to the mucosa of canine fundic pouches results in the secretion of alkaline opalescent viscid mucus which is free of columnar cells. These studies confirm our previous conclusion, that the exfoliation of gastric surface cells is not an essential part of the process of mucus secretion.

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Plasma Cholesterol Levels of Cholesterol Fed Control and Pyridoxine Deficient Monkeys.* (18565)

LOUIS D. GREENBERG AND JAMES F. RINEHART.

From the Division of Pathology, University of California School of Medicine, San Francisco.

There is little information on the effects of feeding cholesterol to the rhesus monkey. Mild increases of serum cholesterol have been observed in this species by Sperry, Jailer and Engle(1) following the feeding of whole egg or egg yolks as a source of cholesterol. The present paper describes the effect of cholesterol feeding on the plasma cholesterol of pyridoxine deficient and control monkeys and was carried out in connection with our studies on the development of arteriosclerotic lesions in vitamin B6 deficient monkeys(2,3).

Experimental. Studies on two groups of young rhesus monkeys are reported in the present paper. The first included 8 monkeys (7 males and 1 female) weighing 1.85-2.48 kilos which were housed in individual cages. They were fed *ad libitum* the modified M-3 basal diet and the complete supplements described in a previous publication(4) for a period of 39 days. Pyridoxine was then with-

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