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Relation of Nutrition to Gastric Function. I. An Experimental Method.

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We have developed an experimental procedure for studying the relation of nutrition to gastric function with the rat as the subject. Tentatively, we are determining the total acidity, the free HCl, and the mucin of gastric juice as a measure of gastric function. By "mucin" we mean that material which is precipitated by adding 4 volumes of alcohol to gastric juice and is determined as glucose by the orcinol method of Tilmans and Philippi.[1] Later we plan to study the enzyme and other constituents of the gastric juice.

As gastric stimulants we have used mecholyl (acetyl-β-methylcholine chloride) and histamine. The dosage used per kilo of body weight was 0.01 mg for mecholyl and 0.03 mg of ergamine phosphate for histamine. Each stimulant was dissolved in physiological saline and injected subcutaneously.

Our experimental procedure is as follows: Rats are placed in individual false-bottom cages and fasted for 48 or 72 hours. A meshed-wire jacket is placed upon the torso of each rat during the fasting period to prevent coprophagy and to keep the animal from licking its body, a process by which the rat ordinarily ingests considerable hair. This preparatory period of fasting in a jacket permits the rat's stomach to be cleared of solid contents and makes possible the collection of uncontaminated juice.

When a test is made the animal is anesthetized with nembutal and during the experiment nembutal is injected in small doses as needed to maintain anesthesia. A small incision is made in the abdomen and a tight ligature is tied around the duodenum near the pyloric sphincter. Such a ligature does not interfere with the nerve supply nor appreciably with the blood supply to the stomach and permits a secretory response of the whole stomach. The abdomen is closed with clamps. The gastric stimulant (mecholyl* or histamine) is injected subcutaneously at 15-minute intervals. One-half hour after beginning stimulation the abdomen is opened and the contents of

[1] Tilmans, J., and Philippi, K., *Biochem. Z.*, 1929, **215**, 36.

* We are indebted to Merck and Company for generous contributions of Mecholyl.

the stomach are removed by inserting a 20 gauge needle through a point in the stomach wall in the region of the rumen and aspirating with a syringe. In the same manner the stomach is emptied of gastric juice again in one-half hour and thereafter at one-hour intervals for 2 or 3 hours. The volume of the juice in each collection is recorded and the juice is analyzed.

With this experimental procedure regurgitation of intestinal contents into the stomach cannot occur; also saliva is not swallowed, a fact we demonstrated by placing a cotton plug in the esophagus. With the probable exception of the first collection, which might contain regurgitated material, the samples of gastric juice obtained are not contaminated by extra-gastric fluids. The only possible contamination that may occur is from bleeding at the sites of the needle punctures. This is very slight, and we have satisfied ourselves by control studies that it does not alter the analyses for the constituents reported in this paper.

For analysis a sample of juice (0.5 to 0.1 cc) is pipetted into a 50 cc conical bottomed centrifuge tube, a drop of Topfer's reagent is added, and the free HCl is titrated with 0.05 N NaOH from a microburette. A drop of phenolphthalein is added and the total acidity is titrated. The juice is made slightly acid again by adding acetic acid, and 4 volumes of 95% alcohol are added. The tubes are allowed to stand over night to permit maximum precipitation of the mucin, after which the content of the latter is determined.

Results obtained with the technic as outlined above upon 12 rats of weights ranging from 136 to 263 g, 6 receiving mecholyl stimulation and 6 histamine stimulation, are shown in Fig. 1. The rats used were young adults. These results are in agreement with the data obtained upon a larger number of normal rats used in developing this procedure. For total acidity the broken line curves represent individual values and the solid line is the average curve. The solid line curves for mucin and volume are composite curves showing the average values for the 6 rats in each case.

The highest average total acidities observed with mecholyl and histamine are 84 and 96 cc of 0.1 N acid per 100 cc of juice, respectively. The response is more rapid following histamine. With histamine the peak of the curve is observed most often in the half-hour collection, while with mecholyl the peak most often occurs between the first and second hour collections. The free HCl values obtained in these studies in general parallel the total acidity data, being 60 to 70% of the latter. The average values per 100 cc of gastric juice for mucin range from 23 to 40 mg as glucose following mecholyl stimulation, and 16 to 51 mg after histamine. The volume curve

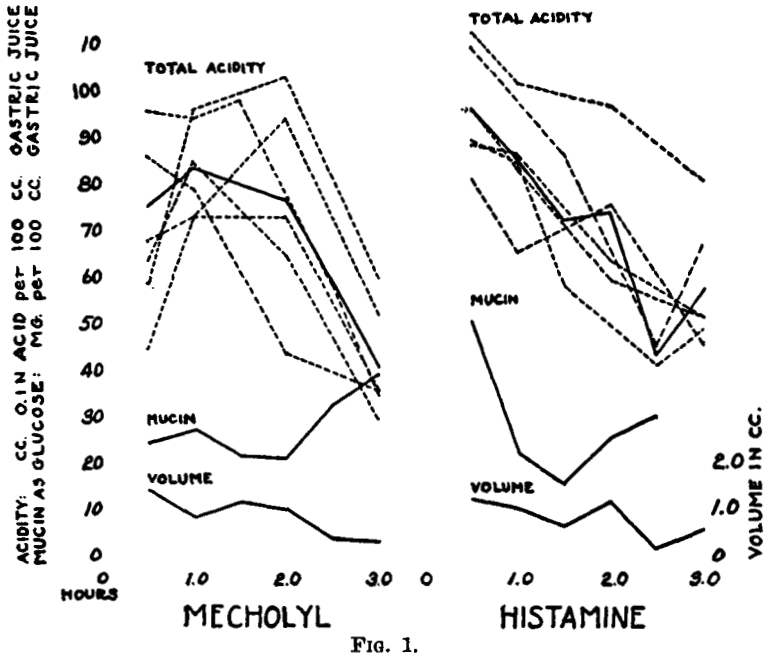

FIG. 1.

Effect of mecholyl and histamine upon gastric secretion in the rat.

shows a close parallelism between the concentration of acid and the amount of juice secreted.

The first sample of juice collected in each experiment may have been contaminated by residual material, hence the first point on all of the curves in Fig. 1 is probably atypical.

In general the degree of acidity, the amount of mucin, and the volume secreted are about the same with mecholyl stimulation as with histamine stimulation.

From the studies we have made thus far it appears that there is considerable similarity in the gastric secretions of the rat and man. With 10 human subjects, Helmer[2] found an average total acidity ranging from 82 to 111 cc of 0.1 N acid per 100 cc of gastric juice in a study by the fractional method following the administration of 0.5 mg of histamine hydrochloride. Our average curve with 6 rats receiving histamine ranges from 96 to 44 cc of 0.1 N acid per 100 cc of gastric juice. For mucin Helmer obtained values ranging from 12 to 18 mg per 100 cc as glucose. Our average curve for mucin with 6 rats after histamine stimulation shows values ranging from 16 to 51 mg per 100 cc as glucose. Our data with rats are

[2] Helmer, O. M., *Am. J. Physiol.*, 1934, **110**, 28.

thus comparable to the findings of Helmer for human subjects after the administration of a single dose of histamine.

The data of this report thus seem to justify the assumption that the rat is a subject with which studies of gastric function can be made that may have a bearing upon the mechanism of gastric secretion in man.

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Size of the Extracellular Compartment of Skeletal Muscle.

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The water of the animal body may be roughly divided into that contained within the cells, the intercellular fluid, and that contained in the fluid which bathes the cells but is not enclosed by the cell membrane, the extracellular fluid. There have been numerous attempts in recent years to determine the relative size of each of these compartments. In general, the methods employed consist of either (1) the determination of the amount of dilution of an injected, non-toxic foreign substance which does not enter the cell but is distributed uniformly throughout the extracellular water and (2) by the measurement of the amount of some normal constituent which is limited to and uniformly distributed throughout the extracellular water. Sodium thiocyanate has been suggested as a suitable substance for the first method[1-4] and chloride determination most convenient for the second method.[5-9]

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