

ciated with the metabolic processes of the blood and may modify or affect the structure of the hemoglobin molecule. Pauling, *et al.* (2) used tetrathionate in a buffer system to prevent formation of methemoglobin during electrophoretic analytical procedures and indicates that tetrathionate did not affect the structure of "sickle cell hemoglobin", during

these measurements. We propose to attempt to detect changes in the structure of the "sickle cell hemoglobin" in patients after treatment with tetrathionate. Clinical observation in regard to the use of this drug will be reported at a later date.

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### Confirmation of Presence of a Gastric Secretory Depressant in Gastric Juice of Humans. (17864)

CHARLES M. BLACKBURN, CHARLES F. CODE, DONALD P. CHANCE, AND  
EARL E. GAMBILL

*From the Divisions of Medicine, Physiology and Surgery, Mayo Clinic, Rochester, Minn.*

In a series of papers Brunshwig and his co-workers(1-5) have reported finding a gastric secretory depressant in gastric juice of human beings. The gastric juice tested was secreted in response to subcutaneous injections of histamine after an overnight fast. Samples of the juice or alcoholic precipitates of the juice were injected into dogs with subtotal, wedge, or Heidenhain types of gastric pouches while these were secreting in response to frequently repeated feedings of small amounts of meat. Although inhibition of secretion sometimes occurred during tests of acid gastric juice, it was observed more frequently after the injection of achlorhydric gastric juice and especially when the achlorhydria was associated with pernicious anemia or cancer of the stomach. Using a quantitative method for the determination of inhibition of gastric secretory activity(6), we have confirmed the finding of Brunshwig and co-

workers, that intravenous injections of alcoholic precipitates of achlorhydric gastric juice from human beings produce pronounced inhibition of gastric secretion in dogs.

*Methods and procedure.* Gastric juice was collected from human beings after an overnight fast by means of a Sawyer tube and intermittent suction. Histamine in a dose of 0.01 mg per kilo of body weight was used as the secretory stimulant. Achlorhydric gastric juice was collected from 2 groups of individuals: (1) 5 patients with pernicious anemia and (2) 8 persons who had no abnormality of the hemopoietic system and on whom roentgenographic studies of the stomach and duodenum were negative. Gastric juice containing acid was obtained from 9 individuals on whom tests of the stomach and upper part of the intestine revealed normal function. When the volume of juice obtained was small, samples from individuals in the same group were pooled. Alcoholic precipitates of the juice were prepared according to the method of Brunshwig and co-workers(3) by adding ethyl alcohol to the gastric juice until the concentration was 80%. After standing overnight the clear supernatant fluid was siphoned off, and the precipitate in the residue was collected by centrifugation. The

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1. Brunshwig, A., van Prohaska, J., Clarke, T. H., and Kandel, E. V., *J. Clin. Invest.*, 1939, v18, 415.

2. Brunshwig, A., Clarke, T. H., van Prohaska, J., and Schmitz, R. L., *Surg., Gynec., and Obst.*, 1940, v70, 25.

3. Brunshwig, A., Schmitz, R. L., and Rasmussen, R., *J. Nat. Canc. Inst.*, 1941, v1, 481.

4. Brunshwig, A., Clarke, T. H., van Prohaska, J., and Schmitz, R., *Ann. Surg.*, 1941, v113, 41.

5. Brunshwig, A., Rasmussen, R. A., Camp, E. J., and Moe, R., *Surgery*, 1942, v12, 887.

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6. Code, C. F., Livermore, G. R., Jr., Ratke, H. V., and Blackburn, C. M., (Abstr.), *Am. J. Physiol.*, 1948, v155, 430.

TABLE I.  
Gastric Secretory Inhibition Produced by Alcoholic Precipitates of Achlorhydric Gastric Juice from Patients with Pernicious Anemia.

| Precipitate tested* |             |     | Assay                                      |                 | Max. rise<br>of<br>R.T.† °F |
|---------------------|-------------|-----|--------------------------------------------|-----------------|-----------------------------|
| Batch               | Dose,<br>mg | Dog | Control rate<br>of secretion,<br>% of max. | %<br>inhibition |                             |
| A                   | 25          | D   | 41                                         | 60              | 1.4                         |
| A                   | 10          | C   | 45                                         | 86              | 1.9                         |
| B                   | 10          | A   | 56                                         | 64              | 0.7                         |
| B                   | 10          | D   | 54                                         | 63              | 1.1                         |

\* For explanation see text.

† R.T. = Rectal temperature.

precipitate was dried in air, ground to a fine powder and weighed samples were prepared for testing by grinding in a mortar with physiologic saline solution or mixing in a Waring blender for 5 minutes. The resulting suspension, in which some of the material was in solution, usually was administered intravenously to the test animals without further treatment. The suspensions obtained from the precipitates of unpooled acid gastric juice, however, were centrifuged and the clear supernatant solution only was given.

Assay of inhibitor activity was made according to a quantitative method developed by Code, Blackburn, Livermore and Ratke (7). For this procedure dogs with Heidenhain pouches were used, and the determination of inhibitor activity was made while these were secreting hydrochloric acid at fixed rates in response to repeated injections of histamine. In each assay the rate of gastric secretion employed was 41% or more of the maximal rate of output of the pouch. After a 2 hour control period during which secretion was maintained at this rate by injections of a fixed dose of histamine every 10 minutes, the material to be tested was given intravenously and the injections of histamine and collections of juice were continued for at least 3 more hours. Changes in output of hydrochloric acid during each of the subsequent 3 hours were expressed as per cent of the control rates, and the average of the changes in the second and third hours was

used as a single value to express the inhibitor activity of the sample. As a rule the body temperature of the animals was determined by rectum 2 or more times during the control period and every half to one hour after the injection of the precipitate. The temperatures recorded during the control period were averaged and this value used for comparison with subsequent readings.

*Results. Achlorhydric gastric juice from patients with pernicious anemia.* Gastric juice obtained from 3 patients who had pernicious anemia was pooled in batch A and juice from 2 other patients was pooled in batch B. In the determinations of inhibitor activity carried out on dogs doses of 10 to 25 mg of the alcoholic precipitate obtained from these batches produced 60 to 86% inhibition of gastric secretion (Table I).

*Achlorhydric gastric juice from persons without pernicious anemia or demonstrable disease of the gastro-intestinal tract.* Three batches of precipitate were tested. The first, batch A, was prepared from juice which had been collected from 3 individuals and then pooled; the second, batch B, was obtained from 3 persons and the last, batch C, from 1 subject. Seven determinations of secretory depressant action were made (Table II). A large dose of 200 mg produced achlorhydria in the dog. Six tests with doses of 25 mg resulted in inhibition of secretion ranging from 55 to 75%.

*Acid gastric juice from normal individuals.* Precipitates were prepared from separate samples of the gastric juice of 4 persons and from 2 batches of pooled juice. The first,

7. Code, C. F., Blackburn, C. M., Livermore, G. R., Jr., and Ratke, H. V., *Gastroenterology*, 1949, v13, 573.

TABLE II.  
Gastric Secretory Inhibition Produced by Alcoholic Precipitates of Achlorhydric Gastric Juice from Otherwise Normal Human Beings.

| Precipitate tested* |             |     | Assay                                      |                 | Max. rise<br>of<br>R.T.† °F |
|---------------------|-------------|-----|--------------------------------------------|-----------------|-----------------------------|
| Batch               | Dose,<br>mg | Dog | Control rate<br>of secretion,<br>% of max. | %<br>inhibition |                             |
| A                   | 200         | A   | 45                                         | 100             | 3.5§                        |
| A                   | 25          | E   | ‡                                          | 71              | 1.8                         |
| B                   | 25          | D   | 55                                         | 66              | 2.5                         |
| B                   | 25          | A   | 49                                         | 75              | .4                          |
| B                   | 25          | C   | 58                                         | 74              | .1                          |
| C                   | 25          | D   | 52                                         | 55              | .6                          |
| C                   | 25          | A   | 50                                         | 75              | .2                          |

\* For explanation see text.

† R.T.—Rectal temperature.

‡ Maximal rate of secretion not determined.

§ 4 episodes of emesis occurred.

TABLE III.  
Gastric Secretory Inhibition Produced by 100 mg Doses of Alcoholic Precipitates of Acid Gastric Juice from Normal Subjects.

| Precipitate tested* |   |     | Assay                                      |                 | Max. rise<br>of<br>R.T.† °F |
|---------------------|---|-----|--------------------------------------------|-----------------|-----------------------------|
| Batch               |   | Dog | Control rate<br>of secretion,<br>% of max. | %<br>inhibition |                             |
| A                   | A | E   | ‡                                          | 0               | 1.5                         |
| "                   | B | A   | 50                                         | 6               | 1.1                         |
| "                   | B | D   | 49                                         | 9               | 0.9                         |
| Subject             | 1 | H   | 66                                         | 43              | 2.2§                        |
| "                   | 2 | L   | 51                                         | 0               | 0.7                         |
| "                   | 3 | V   | 47                                         | 41              | 2.7                         |
| "                   | 4 | L   | 54                                         | 16              | 1.2                         |

\* For explanation see text.

† R.T.—Rectal temperature.

‡ Maximal rate of secretion not determined.

§ Animal vomited twice.

batch A, was composed of juice collected from 2 individuals and the second, batch B, of juice secreted by 3 individuals (Table III). All of the juice contained acid in amounts customarily found in the gastric juice of normal individuals. The precipitates were given in doses of 100 mg. Significant amounts of inhibition were noted on 2 of the 7 tests (Table III).

*Reactions.* Only 2 immediate reactions occurred in the 18 assays. In the first, 200 mg of precipitate was given. This was the largest dose used in the entire series. Immediately after the injection the pulse and respiratory rates increased, the animal showed signs of weakness without lying down, salivated and defecated. About 30 minutes

later the animal was alert and interested in its surroundings and within an hour had apparently fully recovered. Later the animal vomited several times. The other immediate reaction consisted of a brief period of pallor without apparent weakness or other symptom. A delayed rise in body temperature occurred in the course of all tests. In the course of half of the 10 tests in which the dose of the precipitates was 25 mg or less, the rise in body temperature was less than 1°F. The greatest increase was observed during the assay in which the largest dose of precipitate was used (Table II). When the maximal rise in body temperature observed on each test was plotted against the per cent inhibition of gastric secretion, no correlation

between the 2 was apparent. (Values for each are given in Tables I, II and III.)

*Comment.* In general, the results of this investigation confirm and extend the findings of Brunschwig and his co-workers. The pronounced secretory inhibition produced by 10 mg doses of precipitates of achlorhydric gastric juice from patients with pernicious anemia is in agreement with their earlier observations. We have not tested achlorhydric gastric juice from patients with cancer of the stomach which was the other juice in which they found a high degree of inhibitor activity. We did find, however, that precipitates prepared from achlorhydric gastric juice of a group of persons whose hemopoietic and gastro-intestinal systems were normal produced pronounced inhibition while precipitates of acid juice of normal persons were much less active. The implications that high degrees of depressant activity may be expected in achlorhydric juice and that concentration of inhibitor and acid may be inversely related, however, are not fully supported by the findings of Kirsner, Nutter and Palmer (8). Their study led them to the conclusion that there was no direct correlation between pH and presence or absence of inhibitor activity in gastric juice of human beings. All of the patients of Kirsner, Nutter and Palmer who had pernicious anemia, however, had achlorhydria and all of the samples of gastric juice tested from these patients showed inhibitor activity. All samples of achlorhydric gastric juice from patients with pernicious anemia which have been studied by Brunschwig and his co-workers and by us have, likewise, shown inhibitor activity. One definite conclusion, then, seems warranted. Achlorhydric gastric juice of patients

with pernicious anemia contains a substance or substances which are precipitated by alcohol and which when injected intravenously into dogs produce inhibition of gastric secretion.

The dogs used for tests of inhibitor activity by Brunschwig and his collaborators were secreting gastric juice in response to repeated feedings of small amounts of meat. With this stimulus inhibition of gastric secretion might conceivably be due to some inhibition of motility or digestion. The pronounced inhibition of histamine-provoked secretion produced by the injection of even small doses of active precipitates in our assays suggests rather more definitely than with secretion evoked by feeding that the precipitates have some direct action on the secretory mechanism in the gastric mucosa.

*Summary.* By use of a quantitative method for the determination of gastric secretory inhibitors the observation of Brunschwig and co-workers that the intravenous injection of alcoholic precipitates of achlorhydric gastric juice from patients with pernicious anemia depresses gastric secretion in dogs has been confirmed. The secretory depressant activity in such juice was shown by Brunschwig and his collaborators to reduce the secretion induced by feeding a meal. In this study it was found to be effective in inhibiting secretion provoked by histamine. The gastric secretory inhibitor was found in high concentration in achlorhydric gastric juice of persons without other demonstrable abnormalities in the gastro-intestinal tract or disease of the hemopoietic system. The gastric secretory inhibitor was absent or present in lower concentrations in gastric juice from normal individuals.

8. Kirsner, J. B., Nutter, P. B., and Palmer, W. L., *J. Clin. Invest.*, 1940, v19, 619.