Gastric freezing in the rat

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EDITORIAL SYNOPSIS This study reports the effects of gastric freezing on gastric secretion in the rat. The criteria necessary to induce achlorhydria are defined and secretory studies, performed at monthly intervals after freezing, recorded. The results show that gastric freezing can induce achlorhydria in the rat, so that freezing have may some therapeutic value if the technical problems in man can be overcome.

The treatment of chronic duodenal ulceration by gastric freezing, introduced by Wangenstein, Peter, Nicoloff, Walder, Sosin, and Bernstein (1962), was based on experimental studies which showed that achlorhydria, resistant to insulin, peptone, and submaximal histamine stimulation, could be induced in gastric pouches in the dog. Bernstein, Goodale, McFee, Madsen, Allcock, and Wangenstein (1964) reported enthusiastically on the therapeutic effects in man, but noted that a single freeze failed to induce prolonged secretory depression in the dog and is either slight or short-lived (Berg and Nanson, 1963; Clapp, Gilat, Tayao, Creemers, and Sherlock, 1963; Fores, Mitchell, and Weldon, 1964; Macleod, 1964). Similarly, in man the reduction in acid secretion after maximum histamine stimulation is frequently unsatisfactory (Nabseth, Seletz, Gentin, Gottlieb, and Zamcheck, 1964; Lawrie, Smith, Goodall, Pitman, and Forrest, 1964; Karacadag and Klotz, 1964) or recovery occurs within six to eight weeks (Hitchcock, Bitter, and Sutherland, 1964). In the only two reports of controlled clinical trials of gastric freezing for duodenal ulcer in man, one showed no benefit (Perry, Dunphy, Fruin, and Littman, 1964) while the other showed improvement in 75% of cases after six months, compared with 25% of controls (Rose, Fordtran, Harrell, and Friedman, 1964). In both cases, the numbers were small and the period of observations short.

Gastric freezing appears an attractive alternative to surgery, but several observations raised doubt about its therapeutic potential. Therefore it is essential to re-examine the experimental basis of this procedure and this paper reports our experience of gastric freezing in the rat. This animal was chosen partly because of the ease with which gastric freezing can be induced and observed, and partly because tests of gastric function are well established.

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MATERIALS AND METHODS

OPERATIVE PROCEDURE Male rats of the hooded Lister strain, weighing approximately 250 g., were used. Food was withdrawn for 18 hours before the procedure, but tap water was given to which tetracycline was added to provide an approximate oral dose of 50 mg./kg. Under ether anaesthesia, the stomach was delivered through an upper mid-line abdominal incision. A purse-string suture of 5/0 silk was inserted in the fundus of the stomach through which an opening was made; any debris was removed, but usually the stomach was empty. A small balloon, made from the end of a finger-cot and connected to the cooling apparatus, was inserted into the stomach, and a thermocouple, placed so that it lay between the balloon and the mucosa on the greater curve, recorded the temperature of the gastric mucosa. The purse-string suture fixed the position of the balloon which was filled with alcohol till it contained about 4 ml. Cold alcohol was circulated through the balloon, freezing occurred, and within one minute a fine frost appeared on the serosal surface. Throughout the procedure, the animal was placed on a heated table, and normal body temperature, recorded by a rectal thermometer, was maintained.

On completion of freezing the circulation was discontinued and the stomach allowed to defrost at room temperature, a process lasting about three minutes. The balloon was emptied and removed along with the thermocouple; the purse-string suture was tightened, the edges of the gastrotomy inverted, and the area oversewn. The abdomen was closed in layers with linen.

On the day after freezing, the animals received 5% dextrose in water with added tetracycline, and on the second day, they were allowed milk as they wished. By the third day, they had a soft food and water diet and thereafter received their normal diet of water and rat cake.

COOLING SYSTEM Ethanol was cooled and circulated as shown in Figure 1.

The action of the peristaltic pump (A) on a piece of rubber pressure tubing forced the fluid round a circuit of 4 mm. internal diameter polythene tubing. A glass coil,

FIG. 1. A Peristaltic pump.
B Glass coil.
C Reservoir with alcohol, cooled by adding dry ice.

introduced into the circuit at B, was placed in a reservoir of alcohol C, cooled to between -30°C. and -35°C. by the addition of dry ice. The balloon was connected to the circuit using no. 2 polythene tubing (1.0 mm. internal diameter). A side arm D, in the apparatus, attached to a graduated burette allowed the balloon to be filled with a known volume of alcohol. This system permitted flow rates from 20 ml. to 300 ml. per minute, and the gastric mucosal temperature was accurately controlled by alteration of the pump speed during freezing. The mucosal temperature was recorded by a potentiometer connected to the thermocouple.

MEASUREMENT OF GASTRIC SECRETION Two procedures were adopted.

1 The Shay four-hour pylorus ligation technique (Shay, Sun, and Gruenstein, 1954) measured the gastric juice spontaneously secreted during the four-hour period following ligation.

2 The rat histamine test meal, described by Valberg and Witts (1961), measured the juice secreted during the 45-minute period following subcutaneous histamine which, in a dose of 50 mg./kg. body weight, provided maximum stimulation. An indicator dye, phenol red, incorporated in this meal, allowed gastric emptying time to be assessed.

DETERMINATION OF ACID AND PEPSIN IN GASTRIC CONTENTS Aliquots of gastric contents, each of 0.5 ml., were titrated in the inner compartment of a Conway unit against 0.1 N NaOH from a Conway microburette. The indicators used were phenol red for the juice obtained after pylorus ligation, and bromocresol green for the histamine-stimulated juice.

Pepsin was measured on 1.1 ml. samples by the method of Hunt (1948) and this was found by experiment not to be invalidated by the presence of phenol red.

RESULTS

Preliminary experiments were designed to establish the criteria of temperature and duration of freezing necessary to induce achlorhydria. A Latin square experiment was adopted using nine groups of six

rats in which the stomachs were frozen to temperatures of -5°C. , -10°C. or -20°C. for periods of one, five, or 10 minutes. The duration of freezing was measured from the time taken for the mucosal thermocouple to register the desired temperature until the circulation was discontinued. Freezing was usually induced within one minute but defrosting at room temperature varied from two minutes at -5°C. to about four minutes at -20°C. Three weeks after freezing, gastric secretion was measured using the Shay technique and the results are shown in Table I.

TABLE I

RESULTS OF SECRETORY STUDIES (SHAY TECHNIQUE $\mu\text{Eq. HCl/4 HOURS}$) THREE WEEKS AFTER FREEZING AT THREE SELECTED TEMPERATURES FOR DIFFERENT TIME INTERVALS

-5°C.	-10°C.	-20°C.
1 Minute		
450 $\mu\text{Eq.}$	360 $\mu\text{Eq.}$	561 $\mu\text{Eq.}$
364 $\mu\text{Eq.}$	48 $\mu\text{Eq.}$	Achlorhydria
260 $\mu\text{Eq.}$	Achlorhydria	Achlorhydria
180 $\mu\text{Eq.}$	Achlorhydria	Achlorhydria
Achlorhydria	Died	Died
Died	Died	Died
5 Minutes		
300 $\mu\text{Eq.}$	Achlorhydria	Died
95 $\mu\text{Eq.}$	Achlorhydria	Died
Achlorhydria	Achlorhydria	Died
Achlorhydria	Achlorhydria	Died
Died	Died	Died
Died	Died	Died
10 Minutes		
Achlorhydria	Died	Achlorhydria
Achlorhydria	Died	Achlorhydria
Achlorhydria	Died	Died
Achlorhydria	Died	Died
Died	Died	Died
Died	Died	Died

Gastric freezing for one minute failed to abolish acid and pepsin secretion at all three selected temperatures; four of the six animals were capable of acid secretion after freezing at -5°C. , two at -10°C. , and one at -20°C. , though acid secretion was usually substantially less than normal. When freezing was prolonged to 10 minutes, or when the temperature was reduced to -20°C. , the mortality rate was high. As indicated in Table I, however, there were two groups of rats, frozen at -5°C. , for 10 minutes and -10°C. for five minutes respectively, where achlorhydria was induced without prohibitive mortality. The temperature of -10°C. for five minutes was selected for further studies. A control group of six rats was submitted to the same experimental procedure except that the intragastric temperature was maintained at $+37^{\circ}\text{C.}$ for five minutes. All animals recovered, and produced

normal amounts of acid and pepsin when tested three weeks later.

The cause of death in rats after freezing was usually peritonitis. Perigastric abscess formation was not uncommon though frank perforation was rare. Some of the deaths may have been due to poor operative technique and it was for this reason that tetracycline was given to all animals. Death occurred within the first week of freezing and the animals showed signs of ill health immediately after operation. The mortality was greatest at -20°C ., a temperature found to produce ice crystal formation within the gastric muscle cells, an event which results in necrosis (Buchan, Clark, and Love, 1965).

Having established a suitable technique of freezing to produce consistent effects, the duration of the alteration in gastric secretion was studied using the rat histamine test meal (Valberg and Witts, 1961). The reproducibility of this test in the normal animal is illustrated in Fig. 2 where adjacent columns represent the results of consecutive tests carried out at monthly intervals in five rats. There

was no significant alteration in secretion in any animal over seven months. In another group of 44 normal rats, all of which secreted acid and pepsin, the mean values were: volume 2.6 ml. (0.5-3.8 ml.), acid 275 μEq . (168-421 μEq .), pepsin 88 units (24-193 units), and the results compare with those of Valberg and Witts (1961).

Sixteen rats in which the stomach was frozen for five minutes at -10°C . have been studied for periods up to eight months (Table II). Each animal had has tests of gastric secretion before and at monthly intervals after freezing. Eleven animals have so far exhibited complete inhibition of acid and pepsin secretion for periods of three to eight months and of these, six have been achlorhydric for at least five months. In two rats which were initially achlorhydric a return of acid secretion has been demonstrated; in one, 35 μEq . was found at three months, no acid at four months but 50 μEq . at five months, and in the other 29 μEq . was secreted at the five-month test. In three other animals, gastric freezing appeared adequate but no significant

FIG. 2. Adjacent columns represent consecutive histamine tests at monthly intervals in the same (control) rats. (Pepsin is shown in Hunt units in juice collected 45 minutes after a maximum dose of histamine.)

TABLE II
RESULTS OF HISTAMINE TESTS ($\mu\text{Eq. HCl}$) AT MONTHLY INTERVALS IN RATS
FROZEN AT -10°C. FOR FIVE MINUTES

Rat No.	Pre-freeze	One Month After	Two Months After	Three Months After	Four Months After	Five Months After	Six Months After	Seven Months After	Eight Months After
4534	180	0	0	0	0	0	0	0	0
4536	350	0	0	—	0	0	0	0	0
5436	—	0	0	—	0	—	0	0	—
4533	168	0	0	0	0	0	0	—	—
5510	392	0	0	0	0	0	—	—	—
5534	98	0	0	0	0	0	—	—	—
5522	256	0	0	0	0	—	—	—	—
5526	276	0	0	0	0	—	—	—	—
4532	234	0	0	0	—	—	—	—	—
4537	292	0	0	0	—	—	—	—	—
5495	245	0	0	0	—	—	—	—	—
5535	187	0	0	0	0	29	—	—	—
5493	—	0	0	35	0	50	—	—	—
5478	215	81	148	237	—	—	—	—	—
5494	347	181	303	256	—	—	—	—	—
5528	261	186	344	161	83	—	—	—	—

TABLE III
COMPARISON OF SHAY AND HISTAMINE TESTS IN NORMAL AND TREATED RATS
AFTER GASTRIC FREEZING TO -10°C. FOR FIVE MINUTES

Rat No.		Histamine Test			Shay Test		
		Acid ($\mu\text{Eq.}$)	Pepsin (units)	Volume (ml.)	Acid ($\mu\text{Eq.}$)	Pepsin (units)	Volume (ml.)
13 rats	Normal	326	124	2.9	626	487	6.2
5526	16 weeks post freeze	0	7	1.9	0	9	3.1
5522	18 weeks post freeze	0	—	0.9	0	0	3.2
5495	18 weeks post freeze	0	0	2.9	0	0	3.1
4156	20 weeks post freeze	0	—	0.4	19	—	1.8
4054	20 weeks post freeze	0	—	0.3	0	—	1.0
4165	24 weeks post freeze	0	0	1.7	0	—	1.8
4111	24 weeks post freeze	0	0	1.6	0	0	2.1
5115	24 weeks post freeze	0	0	0.1	0	6	2.8
4533	24 weeks post freeze	0	0	2.0	0	0	3.2

alteration of histamine-stimulated gastric secretion was demonstrated and no explanation can be offered for these anomalous results.

The possibility was considered that the results of the histamine test might be partly attributable to alteration in gastric motility. Fallacious results might occur if a small amount of acid was secreted in a rapidly emptying stomach. Since no such fallacy exists in the Shay tests we have compared this test with the histamine test in normal and treated groups. Table III shows the results in 22 rats which had both tests. In 13 normal rats, the mean values of acid and pepsin secretion are shown and it can be seen that the amount of acid in the four-hour Shay test is almost double that in the 45-minute histamine test. In nine rats in which stomachs were frozen (four from the group referred to in Table II and five which had not had pre-freeze or subsequent tests until the final one reported here), there was complete achlorhydria by the histamine test and only one rat secreted a small amount of acid (18 $\mu\text{Eq.}$) by the Shay test. These results suggest that the achlorhydria

demonstrated in treated animals is unlikely to result from experimental error.

DISCUSSION

Gastric freezing has aroused considerable controversy. A review of the clinical experience of eight investigators who treated 826 cases (A.M.A. Report, 1964) showed therapeutic success at six months in 50% of one series, but in only 13% of another. The relation between gastric secretion and relief of ulcer symptoms is not clear, though Bernstein, McFee, Goodale, Madsen, and Wangenstein (1963) reported that freezing depressed the secretory response to stimulation with insulin, peptone, and histamine, as well as the overnight secretion, by about 50%, and this effect persisted for some months. However, using the maximum histamine test, Nabseth *et al.* (1964) found that the depression of gastric secretion after freezing had recovered within one week. Artz, McFarland, and Barnett (1964), Karacadag and Klotz (1964), and Lawrie *et al.* (1964),

have shown similar recovery within six to eight weeks.

The fundamental approach to the treatment of chronic duodenal ulceration has been to induce, either by drugs or operation, a sustained depression of gastric acid secretion, and at present the clinical application of gastric freezing seems unpredictable and unsatisfactory. Wangenstein's observations in dog pouches (Wangensteen *et al.*, 1962) showed marked and prolonged alteration of gastric secretion, but Savage, Stavney, Stevenson, Harkins, and Nyhus (1963), who repeated the experiment using Heidenhain pouch dogs, achieved satisfactory results in only eight of 14 animals. Clapp *et al.* (1963) and Macleod (1964), using the standard technique of freezing, have both shown a return to normal values after histamine and insulin stimulation within a week. However, Fores *et al.* (1964) showed an immediate marked depression to about 10% of the pre-freeze value in 10 dogs, but all had returned to normal over a period of 10 weeks.

The best explanation of these results is found in the study by McIlrath, Hallenbeck, Allen, Mann, Baldes, Brown, and Rovestad (1963), who showed that the efficacy of freezing closely paralleled the size of the stomach; the larger the organ, the more unsatisfactory the result. Freezing was often focal, some areas were unfrozen, and others frozen so solidly that muscle necrosis subsequently occurred. Blumgart, Kay, Naylor, and Kugler (1964) have recorded widely varying temperatures in the gastric mucosa despite satisfactory inflow and outflow temperatures on the apparatus. The technical problems of achieving satisfactory freezing both in dog and man have yet to be overcome.

The potential of gastric freezing can only be assessed by a procedure giving consistent, reproducible effects and this has been achieved with the rat preparation. The present experiments are a re-evaluation of gastric freezing. The preliminary findings indicated that the temperature and duration of freezing are critical to achieve satisfactory results. A temperature of -10°C . for five minutes consistently resulted in achlorhydria without prohibitive mortality. Buchan *et al.* (1965) have shown that this effect depends on ice crystal formation in the gastric mucosa, while mortality is related to similar changes in muscle cells. Consistent effects were obtained in the rat probably because the small stomach obviated the difficulties encountered in achieving uniform freezing in larger animals. McFee, Stone, Goodale, Bernstein, and Wangenstein (1963) have used a similar preparation, but have experimented only with temperatures from 0°C . to -4°C . Stress-induced ulceration was used as a measure of the efficacy of freezing and it was found that 27% of the treated group showed ulcers

two weeks after freezing as against 92% of controls and this increased to 38% after five to six weeks. These results may be due to the fact that freezing for five minutes at 0°C . to -4°C . is almost certainly inadequate to produce achlorhydria, as shown by our own experiments in which two of the six animals frozen at -5°C . for five minutes secreted 300 μEq . and 95 μEq . respectively three weeks after operation (Table I).

The temperature of -10°C . for five minutes proved satisfactory to produce achlorhydria with remarkable consistency and the duration of the effect has been studied in 16 animals for periods up to eight months. In the majority, achlorhydria has persisted and, at present, no undesirable sequelae have resulted. Two animals have shown a return of a small secretion of acid; whether this represents the beginning of recovery of secretion rather than failure completely to abolish it, is uncertain. It is clear that in two animals secretion was unaffected, despite apparently normal experimental circumstances. This simply serves to emphasize that the technique must be closely controlled if these effects are to be kept to a minimum. In other experiments, it has been found that if a small portion of the mucosa is deliberately left unfrozen, the acid secretion will continue (Buchan and Clark, unpublished findings), and possibly this could have occurred in the two inconsistent experiments without being apparent on external observation.

The mortality rate of 15% in the animals may be related to operative technique and is identical to that reported by McFee *et al.* (1963), though recently it has fallen to 10%. The freezing procedure, however, is also in some way responsible for death, since there was no mortality in control animals in which the technique was identical, except that the mucosal temperature was $+37^{\circ}\text{C}$. This mortality therefore represents an unknown danger in the technique of freezing, the nature of which can only be conjectured, but the most likely cause would appear to be death of muscle cells due to intracellular ice crystal formation (Buchan *et al.*, 1965).

In larger animals a satisfactory technique of gastric freezing has still to be acquired and, until this can be done and the results assessed, there is little justification for gastric freezing in man. Assessment by an unsatisfactory technique is likely to lead to the premature abandonment of a procedure before its potential can be evaluated. Some indication of this potential is provided by the present study and further studies on the long-term effects of freezing on other alimentary functions are in progress.

SUMMARY

A simple effective method of gastric freezing in the

rat is described. A temperature of -10°C . lasting for five minutes is necessary to produce achlorhydria. Serial tests of gastric function using maximum histamine stimulation show that the achlorhydria after freezing persists for at least six months in 70% of rats.

We wish to acknowledge the invaluable technical assistance of Mr. A. Greig.

We wish to thank the Medical Research Council for a grant to one of us (R.B.).

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