GASTRIC FREEZING *

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Gastric freezing in the treatment of chronic duodenal ulceration, pioneered by Wangensteen and his co-workers[16] in Minneapolis, has evoked widespread interest. The technique is currently being evaluated in many centres, and the early flush of enthusiasm for the postulate that this was a safe and easy way of achieving a 'physiological gastrectomy' is being tempered with the realization that many problems are still unsolved.[9,17]

In the Department of Surgery of the University of the Witwatersrand, the technique has been confined to the experimental laboratory. The effects of gastric and oesophageal freezing on the secretory function and histological appearance of the intact stomach have been studied in dogs. In addition, 2 patients who had been subjected to gastric freezing outside the Johannesburg General Hospital were available for investigation.

Materials and Methods

The intact stomach was frozen in 12 dogs and oesophageal freezing was performed in 3 dogs. The salient details of technique are given in Table I.

TABLE I. TECHNICAL DETAILS

Balloon volumes	600 - 800 ml.
Rate of perfusion	1,350 ml./min.
Perfusion temperatures (average)	
Inflow	−15·5°C
Outflow	−10·5°C
Duration of freezing:	60 mins.

1. *Weights of the dogs.* The dogs varied from quite small animals weighing 10 kg. to large animals weighing 26 kg.

2. *Balloons.* Standard human gastric and oesophageal balloons were used in all animals except one dog in which an ordinary condom with much thinner walls was employed. The volume of coolant (absolute alcohol) for gastric freezing varied from 600 ml. in the small animals to 800 ml. in the larger animals. For oesophageal freezing volumes between 150 ml. and 200 ml. were employed.

3. *Rate of perfusion* in the available apparatus (Swenko) was 1,350 ml. per minute.

4. *Perfusion temperatures.* Inflow temperatures averaged −15·5°C (range −13·5°C to −17°C). Outflow temperatures averaged −10·5°C. The lowest outflow temperature used was −12°C.

5. *Duration of freezing.* With the exception of two 75-minute freezes the duration of freezing was uniformly 60 minutes.

6. *Rectal temperatures* were monitored at 5-minute intervals and invariably showed a considerable and progressive downward drift despite the routine use of body warming in all except two animals. The average fall in rectal temperature at the end of an hour's freeze was 5·4°C with a range of 3°C to 7·5°C. One animal in which no body warming was employed died soon after the cessation of gastric hypothermia. Rectal temperature at the end of the gastric freezing was 27°C and the cause of death was almost certainly ventricular fibrillation. In 2 other animals the rectal temperatures fell to just under 30°C but, apart from some delay in the resumption of spontaneous respiration, recovery was untoward.

7. *Gastric secretory studies.* Maximal histamine tests, using 0·04 mg./kg. of bodyweight, were performed before and at intervals ranging from 20 minutes to 8 weeks after gastric and oesophageal freezing. Gastric secretions were collected through a large-bore stomach tube with multiple lateral perforations, using continuous manual suction with a Higginson's syringe for the basal and stimulated hours. The tip of this tube is easily palpable through the abdominal wall. Free and total acid were determined titrimetrically uing Töpfer's reagent and the results expressed in milliequivalents of hydrochloric acid per hour. Estimations of pH were performed on all specimens using a glass electrode.

8. *Histological changes in the stomach mucosa.* Detailed histological examinations were made of the entire stomachs of 2 dogs who came to autopsy. In the remaining animals gastric biopsies were performed before, and at frequent intervals after, freezing using a Wood's biopsy tube under general anaesthesia. Biopsies were taken from 2 or 3 areas of the stomach in each animal pre-operatively and 58 biopsies were taken at intervals ranging from 10 minutes to 8 weeks after gastric freezing. Sections were stained with haematoxylin and eosin and with periodic-acid Schiff.

RESULTS

A. *Effect of Freezing on Gastric Secretory Function*

In essence we have been unable to achieve any significant alteration in volume of gastric secretion, in basal or maximal free and total hydrochloric acid production, or in the pH of gastric secretions after gastric or oesophageal freezing. The results are shown diagrammatically in Figs. 1 - 8. Figs. 1 - 3 illustrate total acid content, free acid content, and pH of the gastric secretions in the maximally stimulated specimens. In Fig. 4 the maximal total acid output before and after gastric freezing is compared in individual animals. In 2 dogs, M.10 and M.8, there was in fact a slight but not significant increase in total acid production 1 and 3 weeks after gastric freezing.† In the other animals there was a small, but again not significant fall in total acid production after gastric freezing, except in animal M.12 where the reduction in acid output was slightly in excess of 50%. Note that in animal M.11 maximal total acid output estimated only 20 minutes after cessation of gastric freezing did not differ significantly from the pre-freezing value in the same animal. Fig. 5 compares the pH values of the maximally stimulated gastric secretion before and after gastric freezing in individual animals. In 3 animals, M.5, M.6 and M.10, there was a fall in pH after gastric freezing. In only 1 animal, M.12 again, was there a reasonable rise in the pH after gastric freezing. Fig. 6 illustrates the mean values for volume and pH of gastric secretion before and after freezing, both in the basal and maximally stimulated specimens. Fig. 7 compares mean values for basal and maximal free and total acid production before and after gastric freezing. The mean values reflect the findings in the individual animals.

Oesophageal freezing was performed in 3 animals, and the effects on gastric secretory function do not differ from those observed after gastric freezing (Fig. 8).

B. *Macroscopic and Microscopic Effects of Freezing*

Thoracotomies were performed in 2 animals during oesophageal freezing, and the temperatures along the

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† The figures above the shaded column in Fig. 4 indicate the number of weeks after the freezing was done.

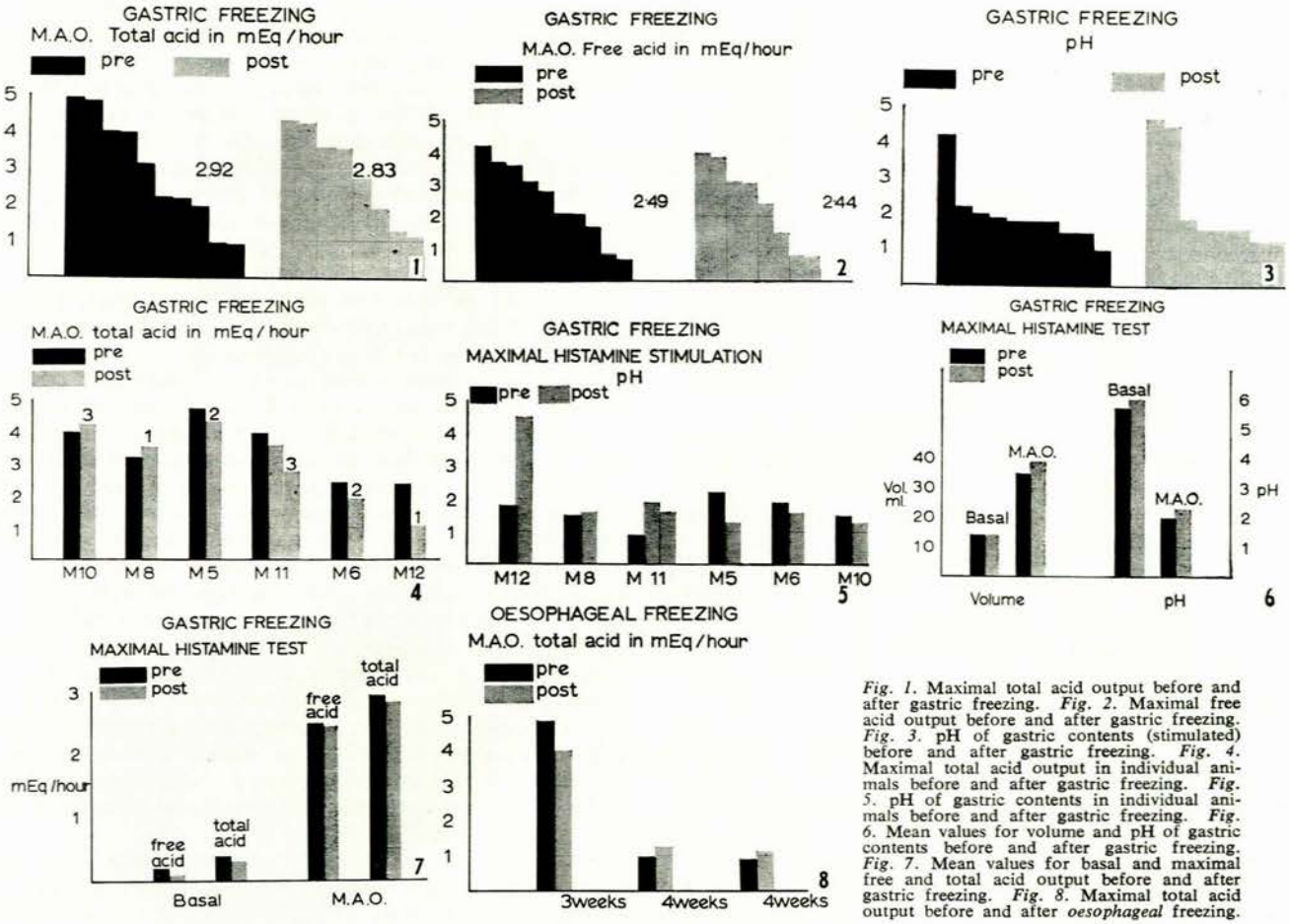

Fig. 1. Maximal total acid output before and after gastric freezing. *Fig. 2.* Maximal free acid output before and after gastric freezing. *Fig. 3.* pH of gastric contents (stimulated) before and after gastric freezing. *Fig. 4.* Maximal total acid output in individual animals before and after gastric freezing. *Fig. 5.* pH of gastric contents in individual animals before and after gastric freezing. *Fig. 6.* Mean values for volume and pH of gastric contents before and after gastric freezing. *Fig. 7.* Mean values for basal and maximal free and total acid output before and after gastric freezing. *Fig. 8.* Maximal total acid output before and after *oesophageal* freezing.

anterior and posterior vagal nerve trunks were monitored at 5-minute intervals with thermistor probes. In the first animal where a standard oesophageal balloon was used, the lowest temperature recorded along the anterior vagus was 21·8°C, and that along the posterior vagus was 24·6°C. No macroscopic freezing of the oesophageal wall was noted although the outflow temperature was maintained between −10°C and −11°C for 75 minutes. In the second animal where a condom was used, focal macroscopic freezing was observed in the oesophageal wall and also in the fundus of the stomach. Nevertheless the temperatures along the vagal nerve trunks were still disappointingly high. The lowest recorded temperature along the anterior trunk was 7·6°C, and that along the posterior vagal trunk was 8·8°C.

Laparotomies were performed in 2 dogs during gastric freezing. In the first animal in which an outflow temperature of −12°C was maintained for 60 minutes, fairly extensive but patchy solid freezing of the stomach wall occurred in the fundic portion of the stomach, particularly on the posterior wall, the greater curve and the adjoining anterior wall. This portion of the stomach was frozen

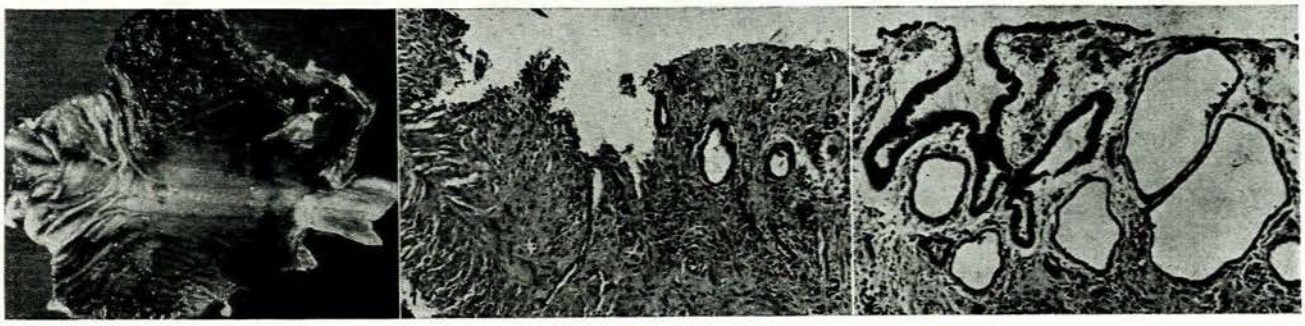

Fig. 9. Mucosal aspect of stomach showing large necrotic perforation in the posterior wall of the fundus.

Fig. 10. Superficial ulceration.

Fig. 11. Gross cystic change in gastric mucosa.

solidly to the diaphragm and the adjacent parieties. The body of the stomach was not frozen at all, and even direct manipulation at laparotomy was unsuccessful in keeping the tip of the balloon in the antrum. This animal died 1 week after freezing of a generalized peritonitis due to a large perforation on the posterior aspect of the fundus. Fig. 9 illustrates the site of the necrotic perforation and demonstrates the fact that the fundic portion of the stomach bears the brunt of the macroscopic changes following freezing. This stomach was examined in great detail microscopically and a variety of histological changes were seen. Superficial ulceration from the posterior aspect of the fundic portion of the stomach is shown in Fig. 10. Similar superficial ulceration occurred patchily in the anterior aspect of the fundic portion. Full-thickness necrosis of all layers of the stomach wall was observed in the region of the perforation. The oesophago-gastric junctional area showed fairly extensive cystic and atrophic changes, and more marked atrophic cystic change was noted focally in the anterior wall of the fundic portion of the stomach (Fig. 11). Numerous sections from the body of the stomach showed no essential microscopic abnormality, and detailed examination of the entire pyloric glandular area showed perfectly normal histological appearances.

Laparotomy in a second animal, where the outflow temperature was maintained at −10°C for 60 minutes, showed similar focal full-thickness gastric freezing. This animal died 1 hour after cessation of gastric freezing with a rectal temperature before death of 27°C. No specific measures had been taken to maintain body normo-thermia. Macroscopic examination of this stomach at autopsy (Figs. 12 and 13) showed gross mucosal oedema, congestion and haemorrhage, again largely confined to the posterior aspect of the fundic portion of the stomach. Detailed histological examination of the entire stomach showed appearances reflecting the macroscopical changes. The marked mucosal congestion and haemorrhage which were maximal in the fundic portion of the stomach are shown in Fig. 14. The rest of the body of the stomach and the pyloric glandular area were perfectly normal histologically.

In the remaining animals the histological effects of gastric freezing were studied by multiple serial gastric biopsies using a Wood's tube. In view of the considerable negative pressure employed during the performance of gastric biopsy, the histological appearances have to be interpreted with some caution. Pre-freezing control biopsies may show the typical microscopic architecture seen in the larger autopsy specimens, but more frequently there is some mucosal oedema separating the gastric tubules. Focal and even diffuse mucosal congestion can be produced by the negative pressure, and lymph follicles are not infrequently seen in normal biopsy specimens.

Bearing in mind the variation in the normal appearances obtained with this technique, the findings were that the vast majority of 58 post-freezing biopsies taken at intervals varying from a few minutes to 8 weeks after 1 or 2 freezing episodes were perfectly normal. Many gastric biopsies taken immediately and 2, 4 and 8 weeks after freezing show no significant histological abnormalities.

Pathological changes were, however, observed in certain biopsies. A clearly pathological degree of mucosal oedema in a biopsy taken immediately after freezing is shown in Fig. 15. One week later several gastric biopsies from this animal showed complete disappearance of the mucosal oedema. On occasions a degree of mucosal congestion which appeared to be in excess of that usually observed in the control biopsies was noted. More profound histological changes were also observed. Figs. 16 and 17 show superficial ulceration, atrophy of secreting tubules and chronic inflammatory-cell infiltration 2 days after a second freezing episode and 4 weeks after the first gastric freeze. Chronic granulation tissue was observed in a biopsy 5 days after gastric freezing. In another biopsy taken 1 week after freezing in another animal, merely a sheet of acute inflammatory cells was seen. These 3 biopsies present clear evidence of the occurrence of superficial ulceration in some animals after freezing.

In view of the focal nature of the macroscopic and microscopic changes observed when the entire stomach is examined, the essential normality of most of the biopsy specimens is not unexpected and certainly does not exclude the presence of such changes in many or perhaps all the stomachs. We can, however, conclude unequivocally that there has been no generalized atrophy of acid and pepsin secreting cells following gastric freezing. PAS stains likewise demonstrate that there has been no replacement of the enzyme-secreting tubular cells by the PAS-positive mucus-secreting cells. The histological appearances are therefore in complete accordance with the results of the secretory function studies in the same animals.

Histological observations were made on 2 patients who

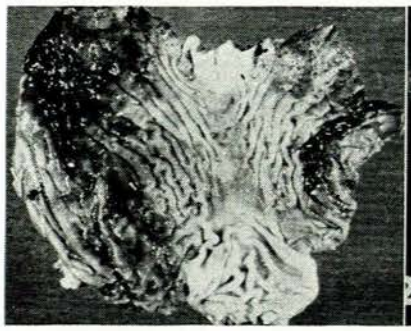

Fig. 12. Mucosal aspect of frozen stomach showing mucosal haemorrhage and oedema.

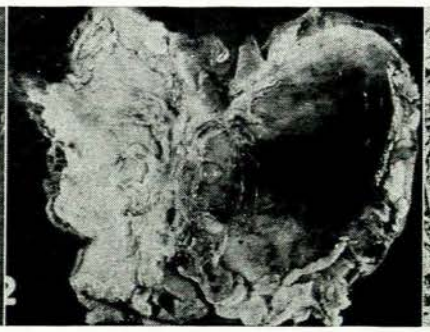

Fig. 13. Serosal aspect of frozen stomach showing maximal changes in fundic portion.

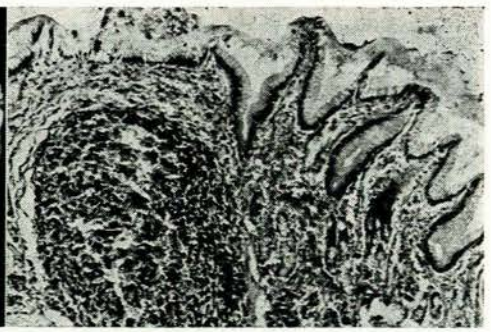

Fig. 14. Section from posterior aspect of fundus showing mucosal congestion and haemorrhage.

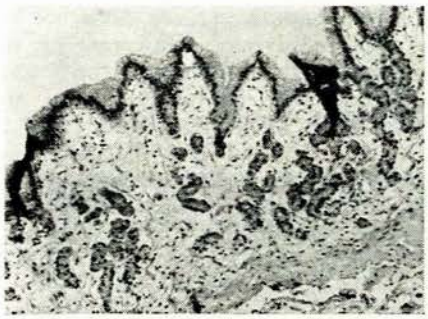

Fig. 15. Significant mucosal oedema immediately after freezing.

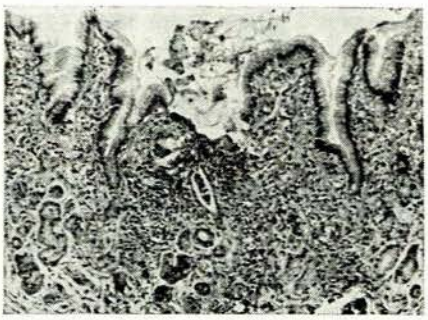

Fig. 16. Atrophic gastritis 2 days after 2nd freeze and 4 weeks after 1st freeze.

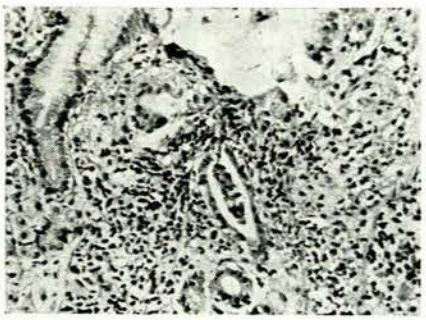

Fig. 17. High-power view of Fig. 16.

had been subjected to gastric freezing elsewhere than Johannesburg General Hospital. One patient had a gastrectomy performed 4 months after freezing for persistent duodenal ulceration. The stomach was macroscopically normal and numerous sections from the body of the stomach and from the pyloric glandular area showed no abnormality whatsoever. The second patient had had his stomach frozen for persistent minor dyspepsia following an antecedent vagotomy and pyloroplasty. Several suction biopsy specimens showed well-marked superficial and atrophic gastritis. This, however, is a frequent finding after gastric surgery,[4] and similarly we can draw no conclusions from the fact that he provided only 3·2 mEq. of hydrochloric acid per hour after maximal histamine stimulation.

DISCUSSION

Conflicting reports are appearing in the literature concerning the efficacy of gastric freezing in the reduction of acid output by the stomach. Wangensteen and his colleagues in Minneapolis, using isolated canine pouches of the Heidenhain, Pavlov and antral types,[1] and also intact stomachs in dogs, report a marked reduction in hydrochloric acid secretion following insulin and peptone stimulation in all cases. Furthermore, they report complete achlorhydria to peptone stimulation for periods up to 28 weeks after freezing. Similarly, in clinical studies[3] they report both achlorhydria and significant (over 50%) reduction in acid secretion soon after freezing. There is, however, a progressive rise in acid output as time passes, and after 6 months they found none of their patients to be achlorhydric and only 11% showed more than a 50% reduction in acid output.

Qualified support for these findings has come from Curtis Artz and his colleagues[2] who found a 70 - 100% reduction in basal acid output in 40% of their patients 48 hours after freezing. Their findings with regard to maximal acid output are less impressive. After 48 hours the average reduction in maximal acid output is 18·7% and 3 months later this figure had fallen to 5·7%.

The findings in the present investigation are supported by several reports which have recently appeared from other centres. Meredith and his colleagues[10] measured secretion from Heidenhain pouches prepared 1 week after freezing the entire canine stomach and they found the mean values of acid production to be actually higher than the values of control pouches from stomachs not frozen. As in the present study, they found individual examples where there was in fact a small rise in maximal acid output after freezing. Scott and his co-workers[15] found no consistent pattern of suppression of gastric acid secretion in 6-hour-fasting collections and after histamine stimulation. Similarly, in their experience, Hollander tests remained positive throughout a 4- to 11-month period after gastric freezing.

Uniformity of contact between the gastric balloon and the gastric mucosa is clearly an important technical consideration,[9] but it is doubtful whether this can explain all the reported discrepancies. Savage[14] and his colleagues in Seattle froze 20 isolated Heidenhain pouches in dogs and demonstrated a significant lowering of acid production in 8 out of 14. In the remaining 6 animals, there was no change in acid production in 4, and in 2 animals there was a rise in acid output after freezing. They report, however, that unless the pouch is distended virtually to the point of bursting, they were invariably unsuccessful in adequately freezing the pouches monitored by needle thermistors in the pouch walls. Morbidity and mortality in these animals was tremendous. Haemorrhage and oedema occurred in all animals. Three animals died of massive necrosis of the pouch within 24 hours after freezing, and 1 animal suffered a similar fate 3 weeks later. A further 3 animals developed enteric-pouch fistulae. These experiments demonstrate 'the uneasy transition zone in which freezing may significantly reduce acid secretion, or it may initiate massive necrosis, perforation and haemorrhage'. This was found to be the case even though variables of distension and depth of freezing are minimized by pouch exteriorization.

Wangensteen[3] acknowledges that this zone between a therapeutically effective freeze and one causing necrosis of the gastric wall may be narrow, but he feels that it can be defined. The Minnesota team feel that the answer lies in an improvement in techniques. Shortening the period of gastric freezing from 60 to 45 minutes, monitoring gastric mucosal temperatures with inlying thermistor electrodes, avoiding outflow temperatures below $-10°C$, better balloons, avoidance of general body hypothermia and the use of low molecular weight dextran to protect the gastric mucosa are some of the factors which are being evaluated in this laboratory. Wangensteen reports only one ulcer in 113 patients where the duration of freezing was limited to 45 minutes, and the outflow temperature was not allowed to fall below 10°C.

Scott and co-workers[15] sacrificed dogs daily for 7 days after gastric freezing for 60 minutes using balloon volumes of 600 ml. and outflow temperatures of −10°C, −12°C and −15°C. Gastric ulceration occurred in 62% of the 42 dogs used. With outflow temperatures of −15°C gastric ulceration occurred in 100% of the animals, but even when the outflow temperature was maintained at the currently recommended level of −10°C ulceration occurred in 43% of the animals.

At the present stage we are perturbed not only by the obvious discrepancies in reported results with apparently similar techniques, but by certain basic issues which have not yet been answered. If it is accepted that the rationale of gastric freezing in the treatment of chronic duodenal ulceration is the reduction of acid secretion, then freezing must achieve this either by reducing the parietal cell mass or by an effect on the mechanisms that stimulate the parietal cells.

The truly remarkable regenerative powers of gastric mucous membrane have been known for a long time.[5] Milton in Australia[11] stripped seven-eighths of the gastric mucous membrane in dogs and showed that the large denuded area is rapidly covered by epithelium which soon differentiates into perfectly normal glands with a full complement of parietal and peptic cells. This restoration to anatomical normality is accompanied by a return of maximal acid output which approaches pre-operative values in 3 - 6 months. The source of this new epithelium is of great importance. It is certainly derived in part from proliferation of the foveolar neck cells at the margins of the defect. There is also fairly convincing evidence that it is derived in part from residual groups of cells arising from the deeper portions of the gastric glands left behind on the muscularis mucosae during the stripping manoeuvre. Thus Longmire[8] has shown that a similar stripping manoeuvre in the cat occurs on a slightly deeper plane, and does not leave behind residual tubular cells. The denuded area in this animal heals in a completely different manner, namely, by wound contraction, granulation, scarification and relatively minor epithelial proliferation from the edges. In other words, a chronic gastric ulceration and gastric scarring are produced. Applying this knowledge to the demonstrable effects of gastric freezing, it is not surprising that acute superficial ulceration should heal rapidly and be difficult to detect clinically. Deeper mucosal sloughs, if they do not perforate, will heal more slowly by granulation. These ulcers will be more easy to detect clinically and it seems reasonable to assume that this is the main reason for the disparity in the incidence of gastric ulceration reported after freezing in man and the incidence demonstrated at autopsy in experimental animals.

In the light of this knowledge it is difficult to conceive how gastric freezing can inhibit permanently this phenomenal regenerative capacity of gastric mucous membrane without simultaneously producing permanent, profound, and potentially hazardous changes in the gastric mucous membrane. Marked atrophic and cystic changes do occur after gastric freezing. If these changes are irreversible, and if they are sufficiently extensive, one might expect a permanent reduction in acid and pepsin output. At the same time, however, it would be difficult to accept with equanimity such changes in view of the known incidence of chronic gastric ulceration and even gastric carcinoma on the basis of such atrophic changes. On the other hand, with available techniques, such atrophic changes are invariably patchy and focal, and furthermore, there is considerable doubt whether these atrophic changes are in fact irreversible. Milton[12] has elegantly demonstrated, by explanting gastric mucous membrane to the surface in dogs, that similar atrophic changes are completely reversible, both from the structural and from the functional point of view.

With regard to a possible effect of freezing on the acid stimulatory mechanisms, the antrum need not be considered at this stage because, with available techniques, the antrum is not frozen at all. A possible effect on the vagal innervation of the stomach has been postulated for a long time. Recently Karacadag and Klotz,[7] and also Hubel and his co-workers[6] have supported this concept. The former authors report a greater reduction in the so-called maximal insulin response after freezing than in the maximal histamine response. They interpret this as support for the view that gastric freezing produces a greater effect on the mucosal innervation than on the parietal cell itself.

In our opinion the evidence for this concept is not very convincing for the following reasons:

1. The above-mentioned results do not appear to be particularly significant on careful analysis.

2. In the present study, temperatures attained along the vagal nerve trunks, even with direct oesophageal freezing, remain well above freezing point and do not support the concept of a thermal truncal vagotomy.

3. No consistent or significant changes have been demonstrated after gastric freezing in Meissner's or Auerbach's plexuses.

4. Vagotomy invariably produces a consistent and significant drop in maximal histamine response,[13] probably because vagal influences are essential in a synergistic or at least in a permissive capacity. Failure of gastric freezing to suppress significantly the maximal histamine response with available techniques in our hands and in the hands of many other workers does not support the postulate that freezing has a significant effect on parietal cell innervation.

CONCLUSIONS AND SUMMARY

1. Standardized and currently recommended techniques of gastric and oesophageal freezing have failed to produce significant alterations in the volume of gastric secretion, basal or maximal hydrochloric acid output, or the pH of gastric contents in the dog.

2. The pathological effects of gastric and oesophageal freezing are invariably focal in distribution and include patchy mucosal haemorrhage and oedema, superficial ulceration, atrophic gastritis and massive necrosis. The greater part of the body of the stomach and the entire pyloric glandular area are unaffected by freezing. There has been no generalized atrophy of parietal and chief cells.

3. Improved techniques may achieve a more uniform freeze of the stomach, but the problems involved are formidable and still unsolved. The ultimate objective of the technique is obscure in the light of the phenomenal regenerative capacity of gastric mucous membrane. There is no convincing evidence that freezing produces any significant or lasting effect on parietal cell innervation.

4. The routine use of gastric freezing in the treatment of chronic duodenal ulceration is not justifiable. In the words of

Wangensteen, general employment of this technique 'should remain limited to those university hospital centres where it may receive critical evaluation and realistic appraisal'.

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REFERENCES

1. Allcock, E. A., Carpenter, A. M., Bernstein, E. F., Peter, E. T. and Wangensteen, O. H. (1963): Surgery, **53**, 764.
2. Artz, C. P., McFarland, J. B. and Barnett, W. O. (1964): Ann. Surg., **159**, 758.
3. Bernstein, E. F., Goodale, R. L., McFee, A. S., Madsen, A. J., Delaney, I. P. and Wangensteen, O. H. (1964): J. Amer. Med. Assoc., **187**, 436.
4. Du Plessis, D. J. (1962): S. Afr. Med. J., **36**, 471.
5. Fergusson, A. N. (1928): Amer. J. Anat., **42**, 403.
6. Hubel, K. A., Faber, L. Kent, T. H., Clifton, J. A. and Mason, E. E. (1964): Amer. J. Dig. Dis., **9**, 328.
7. Karacadag, S. and Klotz, A. P. (1964): *Ibid.*, **9**, 319.
8. Longmire, W. P., Beal, J. M., Lippman, H. N. and Bishop, R. C. (1952): Surgery, **32**, 384.
9. McIlrath, D. C., Hallenbeck, G. A., Mann, C. V., Baldes, E. J., Brown, A. I. and Rovelstad, R. A. (1963): Gastroenterology, **45**, 374.
10. Meredith, J. H., Myers, R. T. and Bradshaw, H. H. (1963): Ann. Surg., **158**, 440.
11. Milton, G. W., Maxwell, G. A. and Finckh, E. S. (1960): Brit. J. Surg., **47**, 562.
12. Milton, G. W. and Finckh, E. S. (1960): Gastroenterology, **39**, 312.
13. Ross, B. and Kay, A. W. (1964): *Ibid.*, **46**, 387.
14. Savage, L. E., Stavney, L. S., Stevenson, J. K., Harkins, H. N. and Nyhus, L. M. (1963): Arch. Surg., **87**, 690.
15. Scott, H. W., Shull, H. J., O'Neill, J. A. and Richre, R. E. (1964): Ann. Surg., **159**, 769.
16. Wangensteen, O. H., Peter, E. T., Bernstein, E. F., Walder, A. I., Sosin, H. and Madsen, A. J. (1962): *Ibid.*, **156**, 579.