

that the return of bile to the upper gastrointestinal tract, either by mouth or by an indwelling tube, prevents the formation of duodenal ulcers.

B. *Incidence of ulcer following pancreatic exclusion.* With regard to the question of pancreatic exclusion, we have observed 19 dogs in which the pancreas was separated from the duodenum; in this preparation there is, of course, no entrance of pancreatic juice into the intestine.

The dogs survived from 21 to 720 days after the operation, with malnutrition varying from slight to severe (50% of body weight); in only one instance was a duodenal ulcer observed at autopsy. These findings agree with those of Ivy,¹¹ who reported one case of true ulceration in 24 dogs with ligated pancreatic ducts. The problem of ulcer formation following external drainage of pancreatic juice involves not only exclusion of the juice from the duodenum, but also the very serious matter of fluid and salt loss, and our present report is not concerned with this aspect of the problem. It may be accepted as an established fact that duodenal ulcer only rarely results from absence *per se* of pancreatic juice from the upper intestine.

Summary. Of 15 dogs with complete external drainage of bile, all being supplied with bile by mouth or by a tube entering the upper intestine, duodenal ulcer was observed in 2 cases; only one instance of ulcer was noted in 19 dogs with exclusion of the pancreatic juice by separation of the pancreas from the duodenum.

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Development of Refractoriness to Enterogastrone Preparations.*

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It has been established that after prolonged administration animals may become refractory to crude hormone preparations, particularly to those of hypophyseal origin, which are water-soluble and protein-like in nature. It has not been demonstrated that animals may become

¹¹ Ivy, *Arch. Int. Med.*, 1920, **25**, 6.

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refractory to preparations of the water-soluble duodenal hormones, although allergic types of reactions have been observed.¹

Preparations of enterogastrone, a duodenal chalone which inhibits gastric motility and secretion,^{2, 3} have been found to induce a refractory state in certain dogs. Of 8 dogs with pouches (vagotomized) of the entire stomach that have been repeatedly injected over long periods of time with enterogastrone preparations, 2 have very definitely become refractory within one month, whereas the remaining 6 dogs have given no indication of altered tolerance over periods varying from several months to several years. In the case of one of these animals it has been possible to demonstrate that the tolerance is directed toward the extracts, presumably impurities, and not toward the chalone elaborated by the animal itself.

During the months of April and May, 1936, 2 total pouch dogs, T-1 and T-2, were each injected 5 times with enterogastrone preparations of known potency. The resulting degree of inhibition of continuous histamine secretion was consistent and comparable in the 2 animals. Early in June attempts to assay chemically separated fractions of these preparations were unsatisfactory because of inconsistency in the responses of the 2 animals. Accordingly, a standard preparation to which both animals had previously responded was again administered to both dogs. Dog T-2 failed to respond, whereas dog T-1 exhibited its original degree of inhibition. This procedure has been frequently repeated and dog T-2 has never again exhibited an inhibitory response to the administration of enterogastrone preparations. Fortunately the degree of inhibition produced by the intraduodenal administration of olive oil had been determined in this animal before it had become refractory. A second determination performed after the dog had become refractory yielded practically the same degree of inhibition as had previously been obtained. These results demonstrated clearly that although the animal had become refractory to the enterogastrone preparations, it had not become refractory to the chalone which was elaborated by its own duodenal mucosa when exposed to the action of fat.

In September, 1937, dog T-4 received several injections of enterogastrone to which it gives responses comparable to those of the other animals in use at the time. Later when attempts were made to assay preparations which had been subjected to purification procedures,

¹ Voegtlin, W. L., Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1934, **110**, 198.

² Gray, J. S., Bradley, W. B., and Ivy, A. C., *Am. J. Physiol.*, 1937, **118**, 463.

³ Ivy, A. C., and Gray, J. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**, 405.

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TABLE I.
Development of Tolerance to Enterogastrone Preparations.

| Dog | Date | Substance Assayed | Quantity | % Inhibition |
|-----|----------|-------------------|----------|--------------|
| T-2 | 4/15/36 | Prep. T-5 | 1 unit | -60 |
| T-2 | 5/7 | Olive oil | 50 cc | -20 |
| T-2 | 6/16 | Prep. T-5 | 1 unit | -7 |
| T-2 | 6/17 | Olive oil | 50 cc | -23 |
| T-4 | 9/11/37 | Prep. T-43 | 1 unit | -54 |
| T-4 | 11/22/38 | " W-5 | 100 mg | +4 |
| T-4 | 11/23 | " W-5 | 100 " | +11 |
| T-4 | 12/7 | " W-5 | 100 " | -16 |

it was found that this animal yielded results quite inconsistent with other animals. This animal was then used for a considerable period for other studies. In November, 1938, a modified method of assaying enterogastrone against histamine secretion was studied. It was found that dog T-4 failed to respond to 100 mg doses of a standard preparation to which 3 other dogs gave an average (7 assays) of 74% inhibition. The pertinent data demonstrating that dogs T-2 and T-4 had become refractory to enterogastrone preparations are exhibited in Table I.

Although it has been more difficult to demonstrate conclusively, we have received the impression that unoperated dogs occasionally develop a tolerance after they have been repeatedly injected with small doses of enterogastrone for purposes of assaying their potency in inhibiting gastric motility.

Since the methods for assaying enterogastrone have always required chronic animals, the development of tolerance has been a hindrance to reliable assays. The purpose of this report is to call attention to this difficulty for the benefit of those who may be engaged in assaying duodenal extracts.

Conclusions. (1) After repeated injections dogs occasionally become refractory to preparations of the duodenal chalone, enterogastrone. (2) This refractoriness is probably due to impurities in the extracts, since continued response to the chalone elaborated by the animal's own duodenum has been observed.