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and in one dog in the fasting state there was a continuous secretion of acid from the pouch. These features are explained as the sequel to the daily administration of cortisone which we have shown in dogs to augment the gastric secretion from pouches, both in the fasting state and to a variety of stimuli.

The results reported here, together with the absence of any relationship between plasma cortisol levels and the second phase of insulin-induced gastric secretion, indicate that in dogs the second phase is not due to the stimulation of a pituitary-adrenal mechanism of gastric secretion as postulated by previous authors.

The results do not affect established observations on the influence of corticosteroids on the secretory activity of the parietal cells, in which they would appear to have both a permissive and an augmentation role. The mechanism of the biphasic response to hypoglycaemia requires another explanation than that suggested by French et al. (1953), and the results of our subsequent studies are reported in part 4.

4 The interrelationship of the phases of the response and the role of the antrum

It is possible that the phasic nature of the gastric secretory response to hypoglycaemia may be due either to the operation of different excitatory mechanisms after different intervals of time or to the temporary depression of a single excitatory mechanism by the operation of an inhibitory one. It was therefore considered prudent to examine both the influence of an anticholinergic compound upon the secretory responses to insulin and the role of the antrum in the pattern of the response to hypoglycaemia.

**FIG. 1b. The effect of bilateral adrenalectomy on the secretory response to insulin hypoglycaemia of an innervated gastric pouch in dog B. (Data are for the means of three experiments before and three after adrenalectomy.)**

*Continuous line = total acid  Broken line = volume*
three others the atropine was given intravenously 20 minutes after the insulin, and in the remaining three experiments the atropine was injected three and a half hours after the administration of insulin. The dose varied between 1 and 2 units of soluble insulin (B.P.) for each kilogram of body weight.

RESULTS

SIMULTANEOUS INJECTION OF ATROPINE AND INSULIN
In three of the four experiments on innervated pouches the first phase of the secretory response to insulin failed to appear (Fig. 1).

In all six experiments with both types of pouch the second phase appeared regardless of the presence or absence of the first phase of gastric secretion.

INJECTION OF ATROPINE 20 MINUTES AFTER INSULIN
Of the two experiments using innervated pouches, the first phase failed to appear in one but the response was unchanged in the other. In all three experiments using both types of pouch the second phase appeared unchanged in character.

INJECTION OF ATROPINE THREE AND A HALF HOURS AFTER INSULIN
In all three experiments the second phase of the response to insulin failed to appear throughout the nine hours of continued observation (Fig. 2).

DISCUSSION

It is shown by these experiments on gastric pouches that the appearance of the second phase of acid secretion in response to hypoglycaemia is not dependent upon the secretion by the same mucosa of the initial response.

The second phase of the response fails to appear if the timing of the injection of atropine is such that its anticholinergic effect operates during the period when this would normally be expected.

The secretion from the pouch can be inhibited by atropine in either phase of the response to insulin hypoglycaemia. Unfortunately, the full role of cholinergic mechanisms in the production of acid gastric secretion remains ill understood, but the ability of atropine, in our experience, to block to varying degrees the gastric secretory response to all modes of stimuli indicated that acetylcholine has at least an essential 'permissive role' to parietal cell activity as well as being an effective primary stimulus to secretion. In considering the significance of any changes in the secretory response to insulin after the injection of atropine the effect of the anticholinergic upon gastric emptying must also be taken into account.
account. Retention of secretion in the antrum of the main stomach could have important effects on mechanisms for both stimulating and inhibiting gastric secretion which may in turn influence the secretory behaviour of the gastric pouch. The two phases of the secretory response to hypoglycaemia may therefore represent the interruption by a period of inhibition of an otherwise continuous process of secretion initiated by a single mechanism of stimulation. Such a period of inhibition could be the result of a force operating either from the main stomach or centrally, and exerted in response to hypoglycaemia or to the secondary effects of vagal stimulation.

Gastric secretion induced by hypoglycaemia from mucosa with intact nervous and vascular supplies is subject to a number of interacting and opposing forces. On the one hand, direct stimulation of the parietal cells is accompanied by indirect stimulation from release of antral gastrin. On the other hand, the accumulating acid in the stomach will tend to inhibit the release of gastrin (Gregory and Tracy, 1960) and on passing through into the duodenum, will initiate acid-inhibitory mechanisms exerted through a neural pathway independent of the antrum (Sircus, 1958).

It is clear therefore that the role of the antrum in the secretory responses of gastric pouches to hypoglycaemia requires further consideration.

THE SECRETORY RESPONSE OF THE WHOLE STOMACH TO INSULIN

METHODS Three dogs were prepared with a gastric fistula by inserting a wide-bore stainless steel screw-capped cannula into the greater curvature at the junction of the antrum and the body of the stomach. Drainage was achieved by removing the screw-cap when, with the dog in the standing position, secretion was satisfactorily collected by gravity alone.

Seventeen experiments were made on the three dogs, and the response of the whole stomach to intravenous insulin determined with exactly the same conditions as previously described for pouch dogs.

RESULTS A first phase of acid secretory response to insulin was obtained in all 17 experiments.

A second phase appeared in eight of the 15 experiments but was absent from the remaining nine. The second phase was relatively smaller than that observed in the responses of innervated pouches in which, not infrequently, the output of acid in the second phase was even larger than in the first. From the whole stomach the output in the second phase, when it appeared, was approximately half that of the first phase. When the responses from whole stomachs are compared with those from innervated pouches the difference between the patterns is marked (Fig. 3).

FIG. 3. The secretory response to insulin compared in dogs with a gastric fistula and in dogs with innervated pouches. (The data represent the means from 16 and 18 experiments respectively.) Continuous line = output from whole stomachs Broken line = output from innervated pouches

THE SECRETORY RESPONSE OF THE WHOLE STOMACH TO TEASE FEEDING

Four experiments were made on three dogs using the technique previously described. The results were very variable. The response predominantly appeared to fall into three phases with a moderate output in the hour during and immediately after the teasing, a brisk larger outflow in the period two to three
hours after the teasing and a small third rise between five and six hours after the teasing (Fig. 4). The pattern of the response thus differed from that obtained from gastric pouches in response to tease feeding in which the phasing resembled that seen after insulin-induced hypoglycaemia.

THE EFFECT OF REMOVAL OF THE ANTRUM UPON THE SECRETORY RESPONSE TO INSULIN

After eight experiments had been made to establish the secretory pattern of the response to insulin in a dog with a gastric fistula, the antrum was excised. A complete strip of mucosa removed proximal to the line of excision was taken at operation and subsequent histology proved that the antrectomy was complete by demonstrating that the strip came from the parietal-cell bearing body area of the stomach. Subsequent to antrectomy, five further responses to insulin were studied.

RESULTS Before antrectomy a well-defined second phase appeared in four of the eight experiments. After antrectomy a second phase failed to appear in all five experiments (Fig. 5).

METHODS A dog was equipped with both an innervated gastric pouch and a wide-bore capped cannula draining the main stomach. Throughout the determination of the secretory responses of the pouch to insulin the contents of the antrum were sampled at 30-minute intervals and the pH measured electrometrically.

RESULTS Six experiments were made. In all six the pH in the antrum fell steadily during the time of the initial acid response to hypoglycaemia by the pouch, and when it reached 2.5 or below acid secretion from the pouch declined and was minimal one to one and a half hours after this pH level had been reached. The antrum pH remained between 2.5 and 3 for two and a half hours and then began to rise steadily until after two hours it was back to the pre-insulin level of 4 units, and during this period of rising pH acid secretion reappeared from the pouch (Fig. 6).

EFFECT OF SIMULTANEOUS DRAINAGE OF MAIN STOMACH UPON RESPONSE TO INSULIN OF A DENERVATED POUCH

A dog was equipped with a denervated pouch and a capped cannula draining the main stomach. Seven gastric secretory responses to hypoglycaemia were...
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Determined in three of which the cap was kept on the gastric fistula cannula and no drainage occurred from the main stomach, and in the other four the cap was removed and the main stomach drained throughout.

RESULTS In all three experiments in which the stomach was not drained a clear second phase response to insulin was obtained from the denervated pouch. In all four experiments in which the main stomach was drained simultaneously the second phase failed to appear (Fig. 7). It is of interest that the secretory pattern from the main stomach shows a small second phase in these four experiments in which antral drainage eliminated the second phase from the denervated pouch in the same dog (Fig. 8). This small second phase from the main stomach is, however, more prominent than that observed in the 16 experiments on dogs with gastric fistula (Fig. 3). It is probable that antral drainage is not as complete or as prompt where the simultaneous preparation of a pouch introduces the possibility of anatomical deformity of the main stomach.

DISCUSSION

These results indicate that the second phase of the acid secretory response to insulin hyoglycaemia only appears from either whole stomachs or gastric pouches when the secretion in the main stomach is allowed to make prolonged contact with the mucosa of the antrum.

The first phase of the secretory response of gastric pouches to insulin is probably due to a combination of three forces: the action of the vagus directly on the parietal cells of the pouch, the action of the vagus in releasing gastrin into the circulation from the antrum, and the effect of distension and muscular movement in the antrum upon the release of gastrin. The second phase, on the other hand, could either represent a delayed 'secretogenic' effect exerted by the acid stimulated in the first phase exciting the production of gastrin from the small intestine (Sircus, 1953) or the release of antral gastrin from the inhibiting effects of a highly acid juice bathing the antral mucosa. When the antrum is continuously drained it is probable that the pH of the juice on the mucosal surface of the antrum does not become low enough to inhibit gastrin release and the whole of the gastrin component of the hyoglycaemic secretogenic effect is discharged within the first two hours. The disappearance of the second phase after removal of the antrum indicates the dependency of this phase upon an intact antrum and presumably on the production of gastrin. Any contribution by the intestinal production of gastrin must be negligible as small quantities of acid passing through the pylorus to the duodenum during total stomach drainage would be more likely after antrectomy because of the removal of the pylorus sphincter; also

FIG. 7. The effect of simultaneous drainage of the main stomach upon the secretory response to hypoglycaemia of a denervated pouch. (Data represent the means of the seven experiments.)

Continuous line = the response with the main stomach undrained (means of three experiments)

Broken line = the response with the main stomach drained (means of four experiments)

FIG. 8. A comparison of the response to insulin obtained simultaneously from the main stomach and the denervated pouch. (Data represent the means of four experiments.)

Continuous line = main stomach output

Broken line = pouch output

FIG. 7

FIG. 8
it has been shown that acid stimulated by histamine did not promote a second phase of secretion.

The fact that the second response from the denervated pouches is also smaller when the antrum is draining simultaneously could be partly the result of the absence of normal pressure and motility, factors within the open stomach thus removing an additional force normally exerted on fundus and antrum which may contribute to the final total 'secretogenic' effect.

The inhibition of secretion of acid from body mucosa by acidification of the antrum has been shown for sham feeding using denervated pouches (Thein and Schofield, 1959a, b), for hypoglycaemic responses from innervated pouches (Harrison, Lakey, and Hyde, 1956; Andersson, 1960a; Chapman, Nyhus, and Harkins, 1960) and for meal responses by denervated pouches (Gouws and Harrison, 1958; Margolus and Harrison, 1956).

Two views exist concerning the mechanism of inhibition. The first, that the acidification releases a hormone suppressing acid secretion (Gouws and Harrison, 1958) is questioned by the observation that it fails to inhibit secretion stimulated by histamine (Andersson, 1960b). The second and more acceptable view is that acidification suppresses the release of gastrin from the antrum (Longhi, Greenlee, Bravo, Guerrero, and Dragstedt, 1958).

Andersson (1960a) states that inhibition is induced when the pH in the antrum falls to 2 and is maximal at a pH of 1-7. In our experiments in which the pH of the antral contents was determined the value dropped to 2.5 about two and a half hours subsequent to the injection of insulin and, within a further 30 minutes, a marked fall off in acid secretion appeared. It is possible that the pH of the gastric juice dispersed over and in close contact with the mucosa of the antrum may be lower than that of the main stream draining through the gastric fistula; the acid-inhibiting mechanism in the duodenum may be provoked by a fall in the pH of the duodenal contents to 2 units (Sircus, 1958).

It is conceivable that the overall pattern of the response of the parietal cells to hypoglycaemia is the product of the interaction of both excitatory and inhibitory stimuli. The stimulation of the vagus by hypoglycaemia directly promotes gastric secretion from the parietal cells and indirectly by the release of gastrin from the antrum. The resulting initial secretion of acid passing into the antrum inhibits the further release of gastrin. Consideration of the results of antrectomy indicate the possibility that whereas the direct effect of the vagus upon the parietal cells lasts for two to two and a half hours, the indirect effect secreted through the antrum may operate over five or six hours. The summation of these factors would tend to produce a biphasic response representing the intervention of a period of inhibition of antral gastrin formation in an otherwise continuous process of excitation of gastric secretion. This hypothesis we are submitting to further investigation by perfusion of antral pouchs with solutions of different pH while simultaneously stimulating innervated pouches by hypoglycaemia.

The frequent observation of a second phase larger than the first is in accord with previous experience that a period of inhibition of gastric secretion may be followed by a 'rebound' effect (Sircus, 1958).

We conclude, therefore, that it is the interaction of the acid-stimulating and acid-inhibiting mechanisms of gastric secretion mediated through the antrum which is responsible for the second phase and not the activation of a pituitary-adrenal pathway for gastric secretion.

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