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SECRETORY EFFECTS OF PSYCHIC STIMULATION AND INSULIN HYPOGLYCAEMIA ON HEIDENHAIN GASTRIC POUCHES IN DOGS

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The Heidenhain type of gastric pouch is generally regarded as vagally denervated, since, in its preparation, all layers of the gastric wall are divided. The sympathetic innervation, travelling along the blood vessels of the greater curvature, is believed to be intact. Several anatomists have shown, however, that the vagus trunks in the abdomen give off branches to the coeliac plexus (Brandt, 1920; McCrea, 1924; Mitchell, 1940), and from this point vagal fibres could enter the sympathetic distribution. It seems possible that, as suggested by Babkin (1950, p. 166), a few parasympathetic fibres may reach the Heidenhain pouch along the blood vessels.

Jemerin, Hollander & Weinstein (1943) investigated a number of procedures designed to test gastric pouches in dogs for the presence of vagal innervation. They found that insulin hypoglycaemia, provided that the blood sugar fell below 50 mg %, would reliably produce acid secretion in vagally innervated gastric pouches, but not in those of the Heidenhain type. They concluded that this was the most satisfactory method for the detection of vagal innervation, and out of this work developed the insulin test for completeness of vagotomy in man.

In 1949 one of us was investigating the pepsin secretion of Heidenhain pouches and was surprised at the variations in pepsin output in different animals. It seemed possible that one factor concerned might be the presence of small degrees of vagal innervation reaching some of the pouches, and it was decided to re-investigate this problem by a more sensitive technique for the detection of acid secretion than that employed by Jemerin *et al.* (1943).

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METHODS

The essential feature of the method is that the pouch is washed out every 15 min with a comparatively large volume of 0.005 N-HCl, and the washings titrated. This ensures complete recovery of even extremely small amounts of secretion and the weak acid prevents the inactivation of any pepsin in the secretion. So that the secreted juice should pass directly into the dilute acid as it was formed the following technique was adopted and used for almost all the experiments here reported.

Preparation and maintenance of animals

The animals used in this work were mongrel bitches of 9–14 kg. They were fed once daily on a standard diet of 3 parts minced boiled meat to 1 part bread, supplemented with cod-liver oil, dried milk and a salt mixture (3 parts NaCl to 1 part KCl). Between 300 and 400 g were given, the quantity being adjusted to the weight of the animal. Heidenhain pouches were prepared under thiopentone-ether anaesthesia from the usual region of the corpus. Measurements of the mucosal area of pouch and stomach remnant post-mortem suggest that about one-fifth of the total mucosa was included in the pouch. The right gastro-epiploic vessel was divided in the preparation, and a narrow bore silver cannula of the type described by Gregory (1950) inserted in the caudal end of the pouch closure. Drainage of the pouch has been found to be most efficient with the cannula in this position. The pouches secreted in most cases 100–200 ml. of juice daily on the above diet, and the animals remained in excellent health. The period of the present study has extended over 3½ yr; the longest period an animal has been under observation is 1½ yr.

Apparatus

Before an experiment the animals were fasted for 18 hr, access to water being allowed. During the experiment the dog stood in slings on a dog-stand of the usual type. A plastic tube of 4 mm bore connected the pouch cannula with the lower end of a 10 ml. graduated pipette mounted vertically. The pipette was enclosed in a condenser jacket through which water was circulated at 38° C. The upper end of the pipette could be connected through a 2-way tap with either a 20 ml. syringe or a float-type volume recorder, and its lower opening was approximately on a level with the neck of the pouch. The bore of the lower opening of the pipette was commensurate with that of the plastic tube so as to impose no restriction on the flow of fluid (Fig. 1).

At the start of an experiment, the plastic tube was disconnected from the vertical pipette and the pouch washed out first with water and then with 0.005 N-HCl, both at 38° C. Ten ml. of the 0.005 N-HCl were now run into the pouch from a bulb pipette and the plastic tube clipped. The tube was re-attached to the graduated pipette and the clip released. The fluid now flowed from the pouch into the pipette, and its level therein was set to the 5 ml. mark by means of the syringe which was at this stage connected with the upper end. The plastic tube was again clipped to retain the fluid at this level and the 2-way tap used to connect the volume recorder also with the pipette. By a further adjustment of the syringe the pointer of the recorder was brought to a reference line traced on the kymograph by a fixed marker. The tap was now turned so as to cut out the syringe but leave the volume recorder connected with the pipette, and the clip on the plastic tube again released. The contractions of the pouch now moved the fluid to and fro in the pipette, and ensured thorough mixing, while the volume recorder provided a tracing of the movements on a slow kymograph.

Towards the end of a 15 min period, the tap was turned to cut off the volume recorder and reconnect the syringe, which was used to drive all the fluid into the pouch. The clip was again applied to retain it there while the tube was disconnected from the pipette, after which the contents of the pouch were drained into a measuring cylinder. At 15 min a fresh supply of 0.005 N-HCl was run into the pouch and the whole procedure repeated. Thus each collection contained the secretion of the pouch during a period of 15 min. The manipulations described occupied less than 2 min so that an almost continuous record of motility was also obtained. The

setting of the volume recorder at each change, so that the reference line on the kymograph corresponded to a fixed level in the pipette, ensured a constant relationship between the tracing and the actual pouch volume throughout the experiment.

Estimation techniques

The volume of the washings was read and the fluid then filtered through washed glass wool to remove the visible mucus which was occasionally present. A 5 ml. portion was then titrated with 0.02 N-NaOH for free and total acid (either with *p*-dimethylaminoazobenzene and phenolphthalein as indicators or, in later experiments, by electrometric titration to pH 3.5 and 8). Results were expressed in ml. 0.1 N-HCl per 15 min period. The end-points of the titrations can be attained with

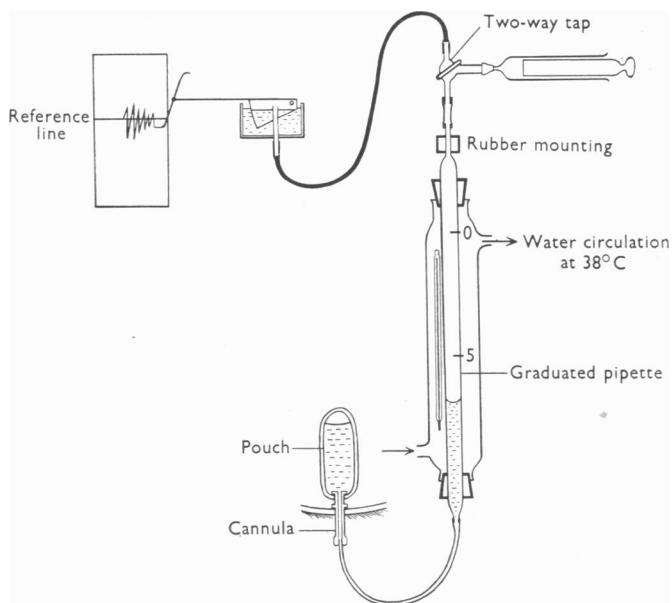


Fig. 1. Diagram of apparatus.

an accuracy of ± 0.05 ml. 0.02 N-NaOH, which is equivalent in the final result to ± 0.02 ml. 0.1 N-HCl. This does not take into account errors introduced in the measurement and recovery of the wash fluid, and this point will be considered later. Recovery of the wash fluid was normally good, the volume being 9.5–10.0 ml. and this was treated as 10.0 ml. in the calculation of the output. Increases in volume occurred only after the stronger forms of stimulation and were allowed for if above 1.0 ml.

Pepsin estimations were carried out on the remainder of the sample by the method of Hunt (1948). In view of the large number of samples from any one experiment only single estimations were made. An independent series of twenty-five duplicate estimations between pepsin values of 20 and 80 units/ml. showed a standard deviation of ± 1.1 units/ml. This s.d. showed no association with the pepsin concentration over this range. The washout sample was frequently more dilute than this, and in such cases digestion times were increased up to a maximum of 4 times in order to bring the final result within this range. No evidence was obtained of the presence of the pepsin inhibitor-pepsin complex described by Bucher & Beazell (1941) as dissociating on dilution. Hunt found the same degree of digestion when the substrate was digested by undiluted gastric juice

for 10 min or by the same juice diluted 1 in 10 for 100 min. These results have been confirmed by Linde (1950) and our findings support them also.

Blood-sugar estimations were performed, mostly in triplicate, by the Hagedorn-Jensen method as described by Harrison (1944), the deduction of 4 mg % from the 'enhanced glucose' value being applied. Venous blood was obtained usually from the cephalic vein and occasionally from the external jugular. From a series of 100 triplicate estimations the standard error of the mean of a batch of three replicates was ± 1.9 mg % and that of a batch of duplicates ± 2.4 mg %.

Methods of stimulation

Insulin. Boots's standard insulin in strength 20 units/ml. was administered intravenously in doses adequate to produce a reduction of blood-sugar level to at least 50 mg %. Like Jemerin *et al.* (1943) we did not regard a test as negative unless the blood sugar fell to this level or lower. For most experiments this required a dose of 1.5 units/kg.

Psychic stimulation. The form of psychic stimulation normally employed in these experiments was similar to the teasing procedure employed by Jemerin *et al.* (1943). The animals were shown a bowl of warm meat stew and allowed to smell but not taste it for a period of 30 min. Great care was taken to see that they did not lick up even the slightest trace of gravy and every effort was made to sustain the animals' interest in the procedure. The interest of some dogs was enhanced if the experimenter ate food during the stimulation period. After every few experiments one was interposed in which the animal was allowed to eat the food immediately after a period of teasing as above.

Sham feeding. To provide a more intense form of psychic stimulation and to exclude possible effects of swallowed saliva, two animals were prepared with oesophagostomies. The first was of the classic type in which the oesophagus was divided in the neck and the two ends were brought to the surface separately on the left side. The second was of the longitudinal slit type described by Gregory (1950). The slit was made 3-4 in. long. Both animals made a rapid recovery from the operation and remained in good health. The first was fed entirely on a liquid diet of homogenized meat, dried breadcrumbs, arachis oil and dried milk with vitamin and salt supplements. This was forced by air pressure through a plastic tube into the lower oesophageal opening. The second could eat solid food after a tight bandage had been applied to its neck in such a way as to keep the two sides of the opening in apposition. It was given a daily ration of meat in this way in addition to a supply of the liquid diet. Both animals were given a liberal water intake twice daily by stomach tube.

Sham feeding was continued for 30 min at a time. In the animal with the slit type of oesophagostomy the food bolus showed no tendency to go down the oesophagus when the neck was unbandaged. As a further precaution in this animal, to prevent the possible passage of small amounts of saliva containing shreds of food down the posterior wall of the oesophagus, this was swabbed repeatedly during the sham feeding. The stomach was washed out after several sham meals and no trace of meat was found in it.

Urethane of β -methylcholine hydrochloride. 'Mechothane' (Savory and Moore) (urethane of β -methylcholine hydrochloride) in sterile ampoules containing 5 mg/ml., was diluted immediately before use with sterile saline to a strength of 0.25 mg/ml. One ml. of this solution was injected intravenously.

Ethyl-3:3-dimethylallyl barbituric acid (compound 16A). A sample of the sodium salt was obtained from the Ely Lilly Co. The quantity required for a single dose was dissolved in sterile saline immediately before use and injected subcutaneously.

Experimental procedure

At the start of the experiments, following 18 hr fasting, the pouches were normally secreting no acid. No stimulus was applied unless during two or more 15 min periods the acid output remained at or very close to the control level, represented by the titration value of the 10 ml. 0.005 N-HCl placed into the pouch. The crucial feature of this method of following the pouch

secretion is that very steady base-lines can be obtained over long periods, against which even very small increases in acid output can be reliably detected (Fig. 6*b*). A resting blood-sugar sample was taken in the 15 min period before the insulin was given. After insulin, blood samples were taken at 15 min, 23 min, 30 min, 1 hr and thereafter at hourly intervals for the further duration of the experiment. In individual experiments there were variations from this scheme, the usual one being omission of the 23 min sample.

TREATMENT OF RESULTS

Acid secretion

This work is concerned with secretory responses which are close to the minimum detectable level, and the method of analysis must allow not only for the error of the titration, which has been mentioned and is comparatively small, but also for errors in measurement and recovery of the wash fluid and for spontaneous variations in basal acid secretion.

In each experiment, the basal level expressed in ml. 0.1N-HCl/15 min was calculated as the mean of the total acid figures for the three 15 min periods immediately preceding the application of the stimulus. Normally its value was very close to the titration value of the 10 ml. of 0.005 N-HCl which constituted the wash-out fluid. In some cases where 'Mechothane' had been given beforehand, only two periods intervened between the end of the secretory response to this drug and the application of the new stimulus. In such cases the mean of these two periods and the one immediately preceding the 'Mechothane' was taken. The output of total acid above this basal level was determined for each successive 15 min period following the stimulus.

With a few exceptions at the beginning of the work, the results on each dog with each form of stimulus were analysed as follows. For each experiment the difference was determined between the basal level defined as above and the mean total acid level over the three successive 15 min periods which normally included the maximum acid response to the appropriate stimulus. In the insulin response these were the second, third and fourth 15 min periods following the injection. During the first period the acid secretion was usually depressed. With the psychic stimulus the first, second and third periods after the start of the stimulus were used. This figure is the mean output above basal level for the appropriate three periods. For each series of results an overall mean output based on at least three experiments was calculated and its significance determined by Student's *t* test (Fisher, 1946).

In addition, the minimum output acceptable as a positive response to a stimulus in a single experiment has been defined. The aim was to select a level higher than the maximum likely to be produced by random variations in the base-line. For this purpose the basal level was determined as above from three 15 min periods in 100 consecutive experiments on six dogs (sixteen or seventeen on each animal). The mean of the standard deviations within the groups of three for the whole series of 100 sets was ± 0.05 ml. 0.1 N-HCl. The

minimum positive response was defined from this figure as follows: *During the first hour following the insulin injection or the start of the psychic stimulus the response must attain a total acid output of at least three mean s.d. (0.15 ml. 0.1 N-HCl) above the basal level in each of three successive 15 min periods, or in each of two successive 15 min periods it must attain an output of at least four mean s.d. (0.20 ml. 0.1 N-HCl) above the basal level.*

Mean response curves were prepared for total acid secretion with both insulin and psychic stimulation. These included only the animals finally classed as positive to the appropriate stimulus, but they included all the results on these animals irrespective of the individual assessment of each result. Acid outputs were determined as above and a mean value determined for each 15 min period after the stimulus. In the calculation of these means, negative differences between the acid level and the basal level were included. This is important, since in the majority of insulin responses the total acid during the first 15 min period following the injection was depressed below the basal level.

Pepsin secretion

The problems connected with the analysis of the pepsin results were similar in kind to those concerned with the acid, but greater in degree, since the method of estimation is less accurate and the spontaneous variation in output much greater. The pepsin output of the Heidenhain pouch at 18 hr after feeding was usually high and showed a wide variation in level between different animals and a distinctly smaller variation between experiments on the same animal. Even in the same experiment spontaneous variations often occurred, and in some animals these tended to follow a regular cyclic pattern. Basal outputs were calculated as the mean of three or, where possible, four resting periods, and mean response curves prepared as for acid. Owing to the variation in the basal output which occurred between one animal and another the results are presented in these curves as + or - a percentage of this output rather than in absolute pepsin units.

Some of the mean response curves for both acid and pepsin incorporate what may be called 'minimum significance levels'. These have been calculated from the s.e. of the mean output over the appropriate period and the value of *t* corresponding to the number of results used in calculating the mean. They indicate the minimum change above or below the basal level which would be significant at a *P* value of 0.05 with the appropriate s.e. and the number of results.

RESULTS

I. *Acid secretion*

Responses to insulin hypoglycaemia

In Table 1 are shown all the experiments in the present series, together with the proportion which attained the positive level defined above. In Table 2 are shown the individual mean results on each animal together with the statistical

TABLE 1. General classification of results

Dog	Psychic stimulation				Insulin stimulation	
	Without 'Mechothane' potentiation		With 'Mechothane' potentiation		Total	Positive
	Total	Positive	Total	Positive		
A2	1	1	.	.	1	1
S1		Tested only after insulin			2	2
T2	6	4	2	2	9	9
B4	8	6	5	4	3	3
M3	6	1	.	.	7	3
S2	4	1	6	5	8	0
J3	3	2	7	7	3	0
C1		Tested only after insulin			4	0
B2		Tested only after insulin			2	0
G3	2	1	6	6	3	0

TABLE 2. Acid response to insulin stimulation

Dog	No. of expts.	Mean acid output above basal level over three periods (ml. 0.1 N-HCl/15 min)	Statistical analysis		Conclusion
			<i>t</i>	<i>P</i>	
T2	9	0.33 ± 0.08	4.18	0.01 > <i>P</i>	Highly significant response
B4	3	0.27 ± 0.02	12.3	0.01 > <i>P</i>	Highly significant response
M3	7	0.26 ± 0.14	1.85	0.2 > <i>P</i> > 0.1	Response not significant
S2	8	0.04 ± 0.05	0.82	0.5 > <i>P</i> > 0.4	No response
J3	3	-0.01 ± 0.04	0.25	0.9 > <i>P</i> > 0.8	No response
C1	4	0.03 ± 0.04	0.76	0.6 > <i>P</i> > 0.5	No response
G3	3	0.07 ± 0.05	1.27	0.4 > <i>P</i> > 0.3	No response

analysis. Although there is a day-to-day variation in the response of individual animals, the positive level selected does in most cases differentiate them into those which attain it on the majority of occasions and those which do not. It was considered that an animal must attain the positive level on more than half the occasions tested to be classed finally as insulin positive. Dogs T2 and B4 were clearly insulin positive, both on the proportion of positive experiments and on the individual analysis of their results. Dogs A2 and S1 were the first animals tested. The responses they gave were very clearly defined and it was felt at the time that the response to psychic stimulation in dog A2 was adequate confirmation of the single result with insulin hypoglycaemia. It was not possible to carry out further experiments as the animals were being used in another investigation, and no individual analysis is possible on the number of results available. These animals were regarded as probably insulin positive and their experiments were included in the mean response curve.

The behaviour of dog M3 was abnormal. When first tested about 1 month after its pouch was prepared it gave a large positive response. It was not tested again for some weeks and subsequent testing with insulin showed a much reduced response which only just reached the positive level on two occasions out of six. The animal was therefore classed as 'insulin negative',

and the individual analysis also shows that the overall mean result on this animal is not significant. The remaining animals were clearly insulin negative.

Form of insulin response. A large and a small positive response to insulin hypoglycaemia are shown in Figs. 2 and 3. A negative response to this stimulus is shown in Fig. 4. The general form of the positive response is shown by the

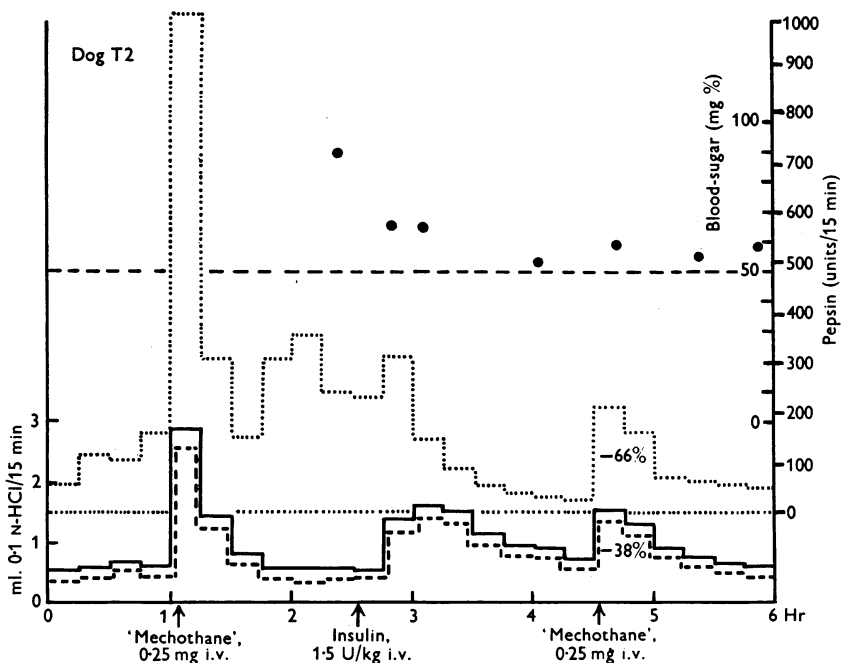


Fig. 2. Acid and pepsin responses to insulin hypoglycaemia in insulin-positive dog. Depression of 'Mechothane' response by hypoglycaemia. —, total acid; ----, free acid;, pepsin; ●, blood-sugar values. The horizontal heavy interrupted line indicates the critical blood-sugar level of 50 mg %, and the corresponding fine interrupted line the base-line of the pepsin scale. The figures on the second 'Mechothane' response indicate the changes in total output of acid and pepsin respectively expressed as percentages of the values of the first response. The same conventions and symbols are used in all the other diagrams illustrating results of single experiments.

mean curve based on the fifteen experiments on the four positive dogs (Fig. 5*b*). The mean total output is 1.34 ± 0.24 ml. 0.1 N-HCl. The acid response to the hypoglycaemia did not usually begin until the second 15 min period after the insulin. Usually in the first period there was a fall from the previous resting level and this can be seen in the mean response curve. The response normally reached its peak within 1 hr of the insulin being given, in the majority of cases in the third 15 min period after the injection. Thereafter it declined slowly.

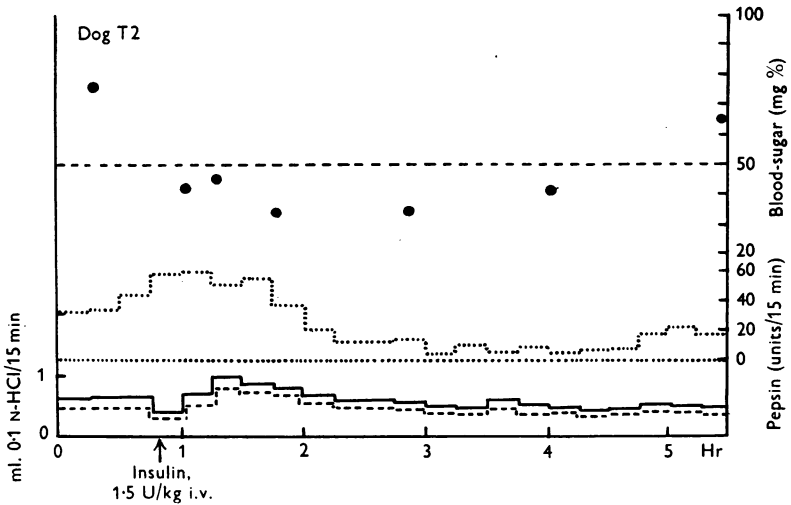


Fig. 3. Acid and pepsin responses to insulin hypoglycaemia in insulin-positive dog. Small acid response. Pepsin response shows only inhibitory effect with tendency for the level to rise again towards end of experiment.

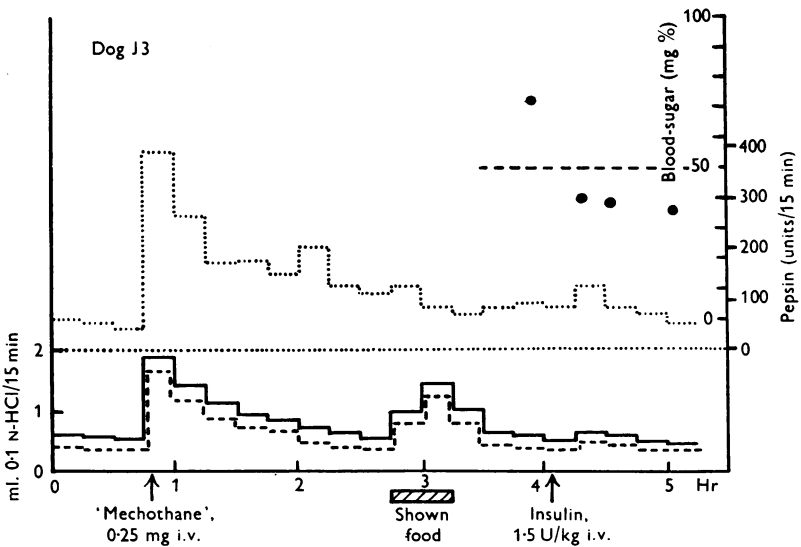


Fig. 4. Potentiated response to food showing. The acid secretion is well marked but the pepsin response is exceptionally small. The animal is insulin negative and shows no response to adequate hypoglycaemia applied immediately afterwards.

Correlation of secretory response with blood-sugar curve. Fig. 5a shows a composite blood-sugar curve derived from thirty-seven experiments on eight dogs in which a dose of 1.5 units of insulin/kg was given intravenously. Each point has been expressed as a percentage decrease from the resting level in order to eliminate the effects of wide variation in the absolute values. The response is of distinctive form. There is an initial rapid depression, followed in

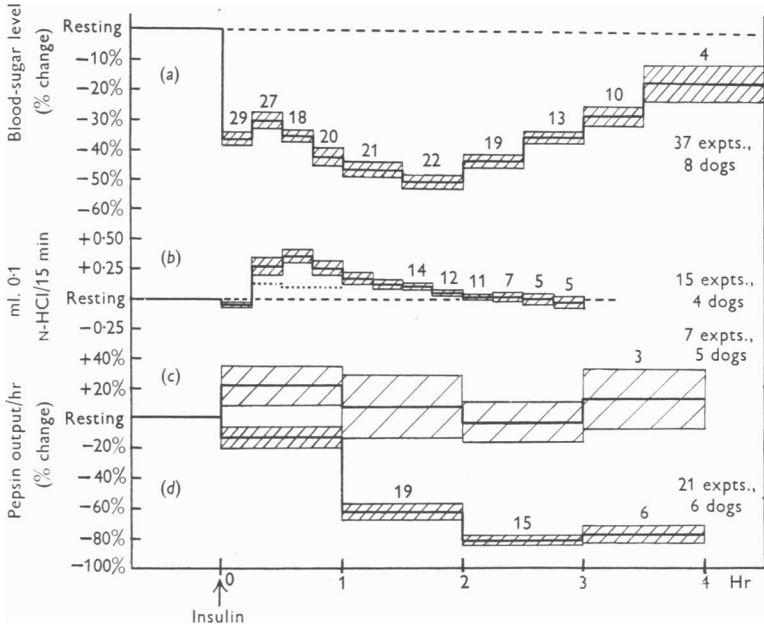


Fig. 5. Composite diagram to show the relationship between the mean blood-sugar curve (a), the mean acid-response curve (b), and the mean pepsin-response curve (d). (c) is a mean pepsin-control curve. In (a) each heavy horizontal line represents the mean of all blood-sugar values falling in the period which it covers. The number of such points in each case is indicated by the adjacent numeral. All values are expressed as % decrease from the resting level. In (b), (c) and (d) each heavy horizontal line represents the mean output over the period covered. Acid is expressed in absolute units, pepsin as percentage change from the resting level. The total number of experiments on which each curve is based is given at its end. Not all experiments continued for the same time and where the number of experiments in a given period is less than the total the actual number is indicated by an adjacent numeral. One s.e. on either side of every mean is indicated by cross hatching. The fine dotted lines on the second, third and fourth periods of the mean acid-response curve (b) are 'Minimum significance levels' (see text).

many experiments by a distinct rise. This feature was shown regularly by some of the dogs, but others showed it infrequently. To display it best it was necessary to take a blood sample half-way through the second 15 min period after the insulin injection and this was not done in all experiments. For these reasons the rise is not such a conspicuous feature of the composite graph as it is in many of the experiments on individual dogs. A well-marked example is

shown in Fig. 3. The most likely explanation of this rise seems to be that it is due to adrenaline release following the rapid fall in blood sugar in the first 15 min. A similar feature is seen in the blood-sugar figures of experiments in man published by Roholm (1930).

There was no correlation between the minimum blood-sugar level and the maximum acid secretion. The lowest blood-sugar levels were not attained until between 1 and 2 hr after the insulin, in most cases in the seventh and eighth 15-min period, while the peak of the acid response was normally passed in the first hour. The hypoglycaemia continued after the acid response was over, particularly with large doses of insulin. It seemed possible that this lack of correlation might be due to an inhibitory influence operating during the period of hypoglycaemia, and this possibility will be considered later.

Responses to psychic stimulation

All the experiments with psychic stimulation are recorded in Table 1, where they are divided into two groups, those with and those without 'Mechothane' potentiation. The individual mean results are given in Table 3. Unfortunately not all the animals were given a satisfactory psychic test. In the early stages of the work the opinion of Jemerin *et al.* (1943) that insulin stimulation is more reliable than psychic testing was accepted as correct. The psychic stimulus was only used occasionally, at the end of insulin experiments. It proved ineffective, although it was not applied until the animal had recovered from the central nervous depressant effects of the hypoglycaemia and was interested in the food. Later it was realized that these experiments were valueless since an inhibitory influence was operating at this stage after insulin (*see* Fig. 6 and section III which deals with inhibitory effects of insulin hypoglycaemia): by this time the earlier animals had been destroyed.

When applied without previous insulin hypoglycaemia, food showing proved more dependable than the latter stimulus. All the animals classed as insulin positive were positive to psychic stimulation when adequately tested, but not vice versa. Three dogs, S2, J3 and G3, responded to psychic stimulation but were insulin negative. The principal difficulty with the food-showing test is that if it is applied too often without reinforcement the conditioned response declines. Most of the recorded failures with this test are thought to be due to this effect, since the appearance of a negative response was the most reliable index of the need for reinforcement. So far no animal which has been adequately tested has been negative to psychic stimulation in the final assessment.

Potentiation by 'Mechothane'. It was found that in most dogs the psychic response could be potentiated by giving a previous dose of 0.25 mg 'Mechothane' intravenously, and applying the psychic stimulus as soon as the acid response to this drug had passed off, which was usually in 1½–2 hr. The potentiating influence of parasympathomimetic drugs and particularly of

the urethane of β -methyl choline hydrochloride on the secretion of the parietal cell has been previously described (Robertson & Grossman, 1948; Grossman, 1950; Langlois & Grossman, 1950), and the control experiments described later in section III suggest that some effect persists for over 3 hr following a single intravenous dose.

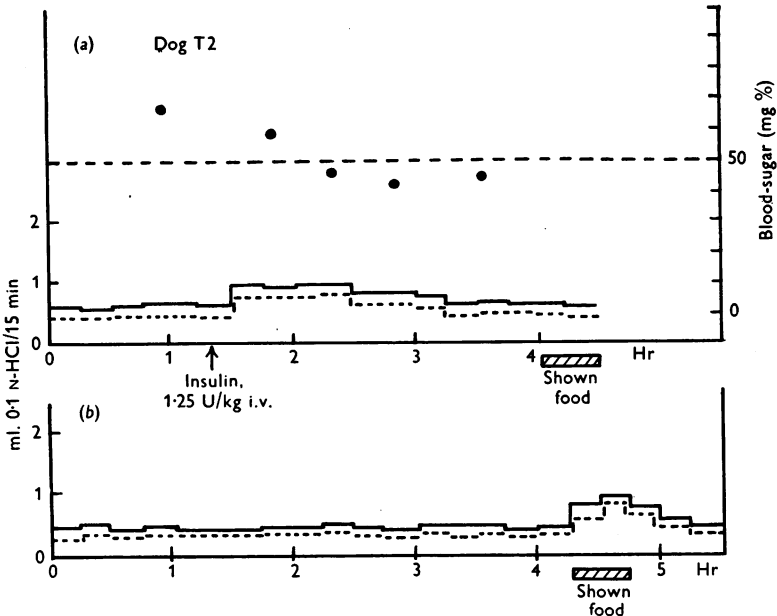


Fig. 6. Two experiments on the same dog to illustrate the failure of the psychic stimulus when applied after insulin. In (b) the animal has been kept on the stand for a corresponding period to that in the insulin experiment before the food was shown. This long period without stimulation shows the steady base-line which can be obtained by this method.

The extent of this potentiation was very variable in different animals. In one dog (S2) it consistently had a large effect and made all the difference between a negative and a strong positive response (see Tables 1 and 3), and in another (J3) the mean output over the first, second and third periods after the start of the psychic stimulus was 0.26 ± 0.05 ml. 0.1 N-HCl in three experiments without 'Mechothane' potentiation, and 0.46 ± 0.04 ml. in seven experiments with it. The difference is significant, $t = 2.84$; $0.05 > P > 0.02$. The potentiating effect is illustrated in Figs. 7a and b, experiments on dog G3.

Table 1, in which the psychic stimulation experiments have been divided into those with and without potentiation, shows that in many cases the latter group gave a more reliable index of the response to this stimulus. It is not claimed that this has been demonstrated in all dogs, but the evidence for the potentiating effect is sufficiently strong for no animal to be classed as negative

to psychic stimulation unless it has failed to respond when tested in this way. 'Mechothane' potentiation was therefore incorporated in the routine procedure for an adequate psychic test. The observations on dog M3 were completed before this effect was established, and on the group of unpotentiated experiments performed on this animal it would seem to be negative to psychic stimulation.

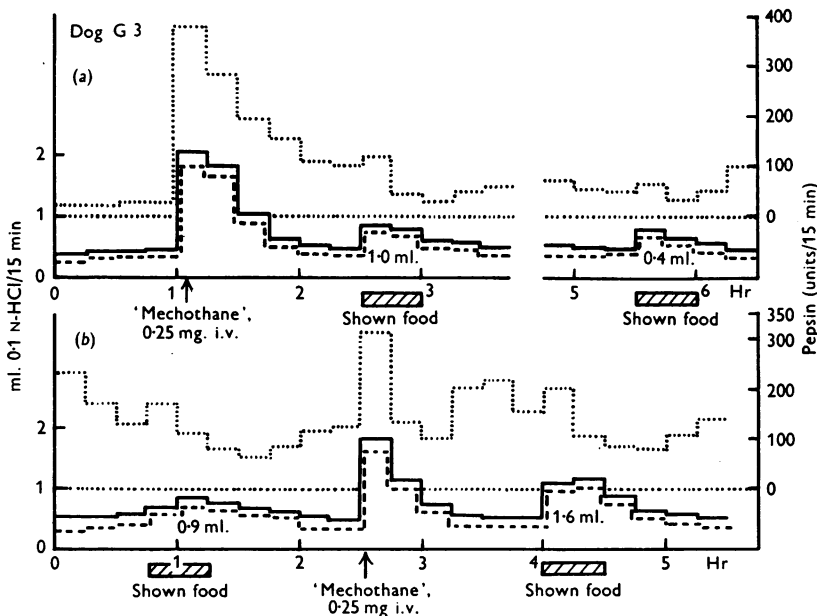


Fig. 7. These experiments on the same dog are intended to illustrate the potentiating effect of 'Mechothane' on the psychic response. It is shown best in (b) where the same stimulus has been applied before and after 'Mechothane'. (a) is designed to meet the objection which might be raised that the animal would be more hungry at the time of the second stimulation. The numerals adjacent to the responses indicate the total amounts of acid secreted above the basal level in ml. 0.1 N-HCl. The pepsin responses of these experiments are typical examples.

That the unpotentiated experiments were not an adequate test is suggested by the fact that several of the experiments just failed to reach the positive level, and the individual analysis of them in Table 3 shows that a statistically significant stimulation did occur.

The 'Mechothane' procedure did not reliably potentiate the insulin response. Occasionally there was a particularly large response after it (Fig. 2), but the effect was not at all consistent.

Experiments on oesophagostomized animals. The sham-meal procedure does not require periodic reinforcement and thus is undoubtedly the psychic stimulus of choice. By its aid it was possible to obtain larger and consistent

TABLE 3. Acid response to psychic stimulation

Dog	No. of expts.	Mean acid output above basal level over three periods (ml. 0.1 N-HCl/15 min)	Statistical analysis		Conclusion
			<i>t</i>	<i>P</i>	
T2	8	0.26 ± 0.07	3.69	0.01 > <i>P</i>	Response highly significant
B4	13	0.32 ± 0.06	5.71	0.01 > <i>P</i>	Response highly significant
J3	10	0.40 ± 0.04	9.49	0.01 > <i>P</i>	Response highly significant
G3	8	0.32 ± 0.05	6.60	0.01 > <i>P</i>	Response highly significant
S2(M)	6	0.33 ± 0.06	5.55	0.01 > <i>P</i>	Response highly significant
S2	4	0.03 ± 0.05	0.61	0.6 > <i>P</i> > 0.5	No response
M3	6	0.12 ± 0.04	3.14	0.05 > <i>P</i> > 0.02	Response just significant

In the case of dog S2, experiments with 'Mechothane' potentiation have been treated separately (under S2(M)) since this animal gave a significant response only when this technique was used. In other cases all experiments have been taken together. The experiments on dog M3 are all without 'Mechothane' potentiation.

TABLE 4. Comparison of acid responses with food-showing and sham-meal procedures

Dog	Food-showing		Sham-meal	
	Total	Positive	Total	Positive
B4	8	5	5	5
S2	4	3	3	3

psychic responses in the two dogs, B4 and S2, which were equipped with oesophagostomies. Table 4 shows the psychic stimulus experiments on these two dogs divided into food-showing and sham-meal experiments. For dog S2 only 'Mechothane'-potentiated experiments are included. The very small response of the pouch made it essential to show that it could not be merely secondary to stimulation of the main stomach either by swallowed saliva or by the accumulation of its own secretion within it. The rapid onset and decline of the psychic response are strongly against this interpretation. The positive response of the oesophagostomized animals to sham meals completely excludes an effect of swallowed saliva. The influence of acid in the main stomach is generally regarded as inhibitory to further secretion, and the infusion of a large volume of its own gastric juice into the stomach of an oesophagostomized dog via a tube passed through the fistula produced no stimulation of the pouch (Fig. 8).

Form of psychic response. Individual responses to psychic stimulation are shown in Figs. 4, 6*b*, 7*a*, *b*, 8 and 11. Fig. 9*a* shows a mean response curve prepared as for insulin stimulation. It is based on forty-five experiments on six dogs which have been adequately tested, and includes all the experiments performed on them. The psychic response rises rapidly during the first 15 min of application of the stimulus and reaches its peak in either the first or second 15 min period, usually in the latter. After the stimulus is withdrawn it normally falls off rapidly and is substantially complete within 1 hr. The mean total acid response is 1.11 ± 0.08 ml. 0.1 N-HCl. It will be noted that this is

smaller than the mean total acid response to insulin of 1.34 ± 0.24 ml., in spite of the fact that the psychic stimulus is regarded as more dependable. The extra size of the total insulin response is attained owing to its longer duration, and to the presence in the insulin series of occasional very large responses which elevate the mean result. The mean peak secretion rate in the psychic series is higher.

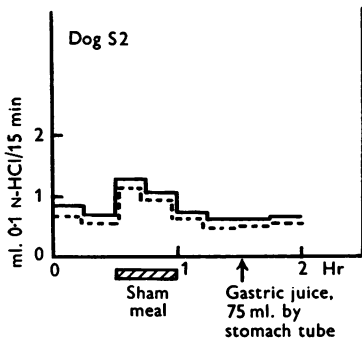


Fig. 8.

Fig. 8. Experiment in an oesophagostomized animal to show that neither swallowed saliva nor acid secreted into the main stomach is concerned in the response of the pouch to psychic stimulation. After the sham-meal response 75 ml. of the animal's own gastric juice were infused into the stomach through an oesophageal tube and no secretion occurred.

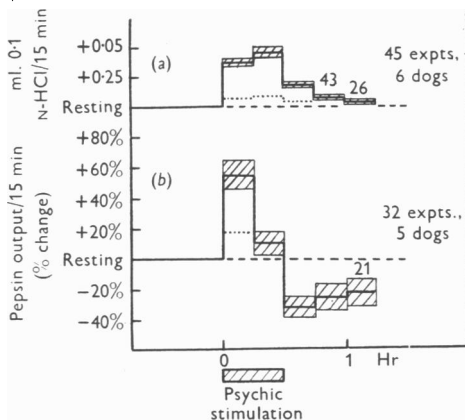


Fig. 9.

Fig. 9. Composite diagram showing the mean acid (a) and pepsin-response curves (b) to psychic stimulation. The mode of expression is the same as in Figs. 5 (a) and (d), save that the pepsin is here calculated as a 15 min output rather than as an hourly output as in the previous diagram. As before, the acid is expressed in absolute units, the pepsin as a % change from the resting level. 'Minimum significance levels' (see text) are shown by a fine dotted line for periods 1-3 of the acid response and period 1 of the pepsin response.

II. Pepsin secretion

Effect of insulin hypoglycaemia

The initial stages of the pepsin response to insulin hypoglycaemia were very variable, and complicated by the fact that not all the animals responded by acid secretion. Only one series of seven pepsin estimations on the insulin responses of a proved insulin-positive dog (T2) was obtained. Transient increases in pepsin output occurred at the beginning of many but not all of these (cf. Figs. 2 and 3). Owing to this variability mean 15 min outputs were not calculated. A comparison was made of the mean output of pepsin over the first hour following the insulin injection in this group of seven experiments and another of fourteen in five insulin-negative dogs. The results are expressed as \pm a percentage of the mean resting level. For the insulin-positive dog the

mean is $-2 \pm 13\%$ and for the insulin-negative group the mean is $-18 \pm 8\%$. The difference is not significant, $t=1.15$; $0.3 > P > 0.2$. Since the first hour covers the period of maximum acid secretion these figures show that any stimulant effect on pepsin output, even in the animal which gave the best acid response to insulin in the present series, is small and transient.

The most marked and striking effect on pepsin output was seen in the second and third hours following the insulin, and is common to both groups of animals. In the mean response diagram shown in Fig. 5*d* they have been combined. The mean output over each hour following the insulin is expressed as a percentage change from the basal level. During the second and third hours there was a marked depression of the resting pepsin output in all cases. Minimum levels were attained between 2 and 3 hr after the insulin, and for the whole group of twenty-two experiments the mean output over this period was reduced to $\frac{1}{2}$ ($-81 \pm 3\%$) of the basal level. The minimum blood-sugar level came shortly before the lowest pepsin output. There was a slight tendency for the pepsin output to rise in the fourth hour and more so in the fifth, but very few experiments extended so far and the mean figures are not reliable. Individual experiments showed a rise, as in Fig. 3, but this change seemed to lag behind the blood-sugar rise.

In view of the lack of experiments showing return to normal levels, it seemed important to show that the decline was a true inhibition and not merely a progressive washing out of pepsin from the pouch. A mean pepsin output diagram was therefore prepared from seven control experiments on five dogs (Fig. 5*c*). In these the pouch was washed out as usual but no stimuli were applied. It will be seen that although there was a good deal of hour to hour variation there was no tendency towards a sustained fall in output.

Response to psychic stimulation

In this case the stimulus was one which when applied with 'Mechothane' potentiation produced acid secretion in all the dogs tested. In these circumstances more uniformity in the pepsin results might be expected. Fig. 9 represents a mean response curve for thirty-two experiments on six adequately tested dogs in which a mean value has been calculated for each 15 min period after the start of the stimulus. The standard errors indicate the variation encountered. There is a highly significant increase in pepsin output during the first 15 min period after the start of the stimulus. Many of these responses occurred against a falling basal level, due to the tendency for the pepsin response to 'Mechothane' to be prolonged beyond that of the acid. Little weight can therefore be attached to the fall in pepsin output which occurs in the second and third 15 min periods, and no general conclusion as to the duration of the response can be reached. Pepsin responses to psychic stimulation are shown in Figs. 4, 7*a* and *b* and 11.

III. Inhibitory effects of insulin hypoglycaemia

The lack of correlation between the maximum acid secretion and the minimum blood-sugar level suggested that an inhibitory influence might be operating during the later stages of insulin hypoglycaemia. The experiments showed two other features which seemed consistent with this possibility. (1) As already mentioned above, the psychic response could not be obtained when tested in the later stages of hypoglycaemia, though the animal had recovered from the central nervous depressant effects and was obviously hungry and interested in the food. (2) In some experiments on insulin-negative Heidenhain-pouch animals where the basal level had been higher than usual the resting secretion showed a reduction during insulin hypoglycaemia.

The inhibitory influence of insulin hypoglycaemia on pepsin secretion has already been described in this paper (section II), since it is the most marked feature of this aspect of the response.

A further series of experiments was performed in order to confirm the inhibitory effects on acid and pepsin secretion induced by 'Mechothane'. The same method for assessment of secretion was used and two doses of 0.25 mg of the drug were given intravenously at intervals of 3-3½ hr. The total acid and pepsin outputs of each response were estimated, and any difference between the first and second expressed as + or - a percentage of the first. A control series showed for acid secretion a mean difference from nine experiments of $+16.4 \pm 7.8\%$ and for pepsin secretion a mean difference from eight experiments of $+0.1 \pm 8.2\%$. The agreement is quite good. The slightly higher acid of the second response seems to be due to the potentiating effect of the first dose of 'Mechothane'. Fig. 10 shows a single control experiment. In a further group of experiments insulin (1.5-2 units/kg) was given intravenously after the first 'Mechothane' response and the second dose of 'Mechothane' was given during the later stages of insulin hypoglycaemia when the acid secretion produced by the insulin was nearly or completely over (Fig. 2). The mean percentage difference for six experiments in each case was $-46.7 \pm 8.2\%$ for acid and $-64 \pm 2.4\%$ for pepsin. The reductions in each case are highly significant ($t=5.4$ $P < 0.01$ for acid, and $t=6.5$ $P < 0.01$ for pepsin).

IV. Ethyl-3:3-dimethylallyl barbituric acid

The report of Ballem, Noble & Webster (1948) suggested that this substance, compound 16A, which they have shown to produce gastric secretion in dogs by a central action on the parasympathetic nuclei, might be the ideal stimulant for an investigation of the present type. It should be free from any peripheral depressant influence, and Ballem *et al.* (1948) had reported it to be a more effective stimulant than insulin hypoglycaemia.

A sample of the sodium salt of this compound was obtained from the Ely Lilly Co. who supplied it to the original workers, but trials of it proved most disappointing. In doses of 4-8 mg/kg subcutaneously it produced no stimulant effect in dogs whose Heidenhain pouches responded well to insulin hypoglycaemia and psychic stimulation. The results of three experiments are given in

Table 5. Fig. 11 shows an experiment in which the animal gave a large response to psychic stimulation immediately prior to the injection of the drug. The explanation of this failure is at present unknown, but may lie in the observations of Antia, Rosiere, Robertson & Grossman (1951)

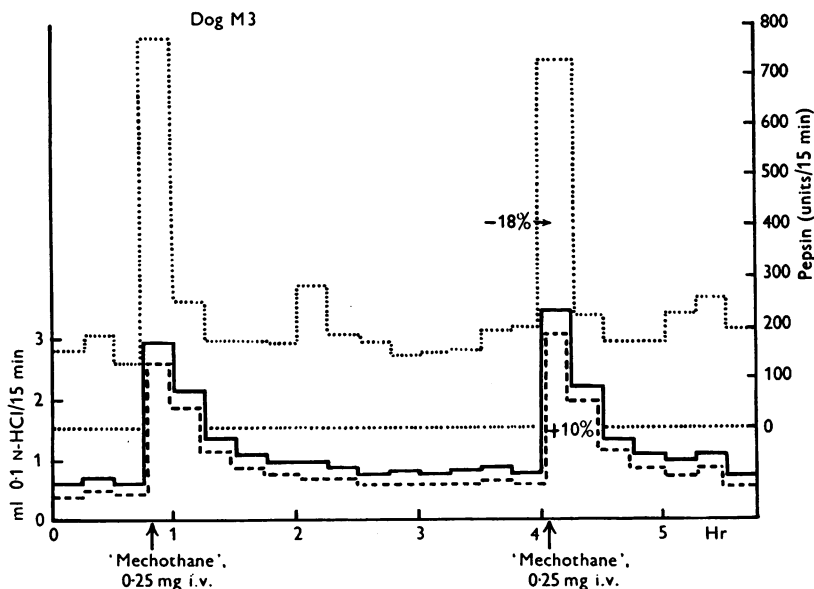


Fig. 10. Control experiment in which two doses of 0.25 mg 'Mechothane' have been administered intravenously at an interval of 3½ hr.

TABLE 5. Effect on acid secretion of compound 16A

Expt.	Dog	Subcutaneous dose (mg/kg)	Mean acid output above basal level over periods 1-3 (ml. 0.1 N-HCl/15 min)
137a	S2	4	0.08
152	B4	4	-0.03
154	B4	8	0.00

whose results on gastric fistula dogs show that compound 16A was a less effective stimulus than insulin hypoglycaemia. In one of the present experiments the dose was larger than that normally used by the earlier workers (3-4 mg/kg) but the animal did not show the pupillary paralysis or the marked signs of general central nervous excitation which they described as associated with larger quantities. In view of the failure of these initial experiments and development of a more reliable psychic stimulus no further investigations with this material were undertaken.

It has been learned subsequently from the Ely Lilly Co. that there is doubt as to the activity of the sample of compound 16A provided for testing. Its action is being reinvestigated.

V. Motility responses

In order to provide a complete description of the apparatus and methods used in the work an account has been included of the technique for simultaneous investigation of the secretion and motility of the pouch, but detailed results of motility studies are not presented here. The following is a brief account of the phenomena observed which are relevant to the present paper.

After insulin injections no convincing increase in motility was observed but a marked depression of tone and cessation of contractions commenced within the first half hour after the injection and continued for 3 or 4 hr. In experiments which were continued until the blood sugar returned towards the resting level, the motility also returned. This agrees with the previous observations of Templeton & Quigley (1930) on the Heidenhain pouch. No convincing increase in gastric motility following psychic stimulation was ever observed and in some experiments there was a reduction of previous contractions. Motility records during food showing were often confused by abdominal pressure changes caused by voluntary movements of the animal.

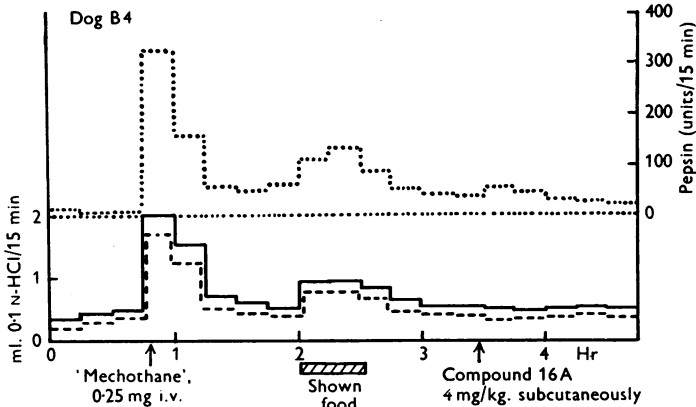


Fig. 11. Experiment in which a potentiated response to food showing is followed by a subcutaneous dose of 4 mg/kg of compound 16A. No response is produced by the latter, even though other experiments suggest that some potentiating influence should persist for this length of time. The pepsin response to the psychic stimulus is much larger than that usually observed.

DISCUSSION

The work reported here shows clearly that psychic stimulation, and in certain circumstances insulin hypoglycaemia, can produce a secretory response in Heidenhain pouches in dogs. This observation is in sharp contrast to the original findings of Jemerin *et al.* (1943) and to the more recent results of Janowitz & Hollander (1951) and of Lim & Mozer (1951). Jemerin *et al.* used only insulin and food showing, but both later groups of workers employed the sham-feeding technique which proved the most effective form of stimulus in the present work. The discrepancy is explicable on the grounds that none of these workers used a technique sufficiently sensitive to demonstrate the very small amounts of secretion produced. It is also possible that the method here described may slightly increase the sensitivity of the pouch itself by maintaining it in a state of mild distension. Lim, Ivy & McCarthy (1925) showed a small increase in the secretory activity of the Heidenhain pouch when it was distended by a balloon.

The interest of the present results lies in the explanation of the responses. With psychic stimulation there can be no reasonable doubt that the primary

excitation is in the central nervous system and its influence conveyed, at least so far as the main stomach, by a nervous pathway. With the insulin stimulus there is also the possibility that such a tiny response might be partly or even wholly due to a direct peripheral influence of the hormone or some impurity in the preparations used. Since previous workers have not observed any stimulant effect of insulin hypoglycaemia on the Heidenhain pouch, the possibility of a direct peripheral action on the pouch has not been considered by them. This point is being investigated, and for the present most stress is laid on the psychic responses, which in addition are more dependable and show larger peak secretion rates.

There are two possible mechanisms by which the central excitation may be conveyed to the Heidenhain pouch: (1) by a few vagal fibres reaching the pouch along the blood vessels of the greater curvature; and (2) by gastrin released from the main stomach following vagal stimulation.

The first of these is the one originally considered to be most likely, and the one which the experiments were designed to investigate. As already pointed out, it is possible that a few vagal fibres may reach the Heidenhain pouch along its blood vessels. In a recent discussion on gastric pouch techniques, Friedmann (1951) states that a Heidenhain pouch containing fundic mucosa adjacent to the cardia behaves like a vagally innervated pouch (Brestkin & Savitch, 1924) and that the degree of vagal innervation of a Heidenhain pouch varies with the amount of proximal fundus tissue incorporated. He quotes from a personal communication by Komarov (1950) the statement that even when an attempt is made to exclude this area it is not uncommon to find evidence of vagal innervation. The pouches used in this work did not include mucosa adjacent to the cardia. Volborth & Kudryavzeff (1927) postulated the existence of cholinergic gastric secretory fibres in the thoracolumbar outflow which reached the stomach via the sympathetic distribution. It is surprising that while Hollander (1948, 1951) is prepared to admit this mechanism as a possible cause of a positive insulin test following vagotomy, he continues to deny the possibility of a positive response to insulin by the Heidenhain pouch.

There were two main reasons why initially it was thought that the vagal fibre hypothesis was more likely to be correct. The first was that with the insulin test, on which originally most stress was laid, not all the animals gave a positive result. A response due to gastrin release should be given by all the animals, but there seemed scope for individual variation in the number of fibres reaching a pouch by a secondary route. Subsequently, the insulin hypoglycaemia stimulus has been shown to be less reliable than the psychic stimulus, and so far we have not found a single animal which is negative to the latter after adequate testing, i.e. 'Mechothane'-potentiated. This observation, while it does not exclude the vagal fibre hypothesis, is equally in keeping with the possibility of gastrin release.

The second reason was the very small size of the responses observed. It seemed likely that gastrin, released as part of the physiological mechanism of vagally induced gastric secretion should produce a much larger response in the pouch. The possibility did remain, however, that gastrin is liberated in small amounts from the pyloric mucosa during vagal stimulation, and that this normally acts synergistically on the parietal cell with the direct vagal innervation. In the Heidenhain pouch, where the synergistic vagal effect is absent or minimal, the circulating gastrin from the antrum of the main stomach would produce only a very small response. The work of Lim & Mozer (1951) supports this point of view. They have shown that the cephalic phase of secretion in an innervated fundic pouch is reduced by cocainization of the pyloric mucosa. They also claim that the response to sham feeding is frequently abolished by this procedure. They failed to obtain a response to sham feeding in a denervated fundic pouch (Heidenhain) which they attribute to the amount of gastrin released being subthreshold for this preparation. This possibility has also been suggested by Linde (1950). The present experiments, in which by the use of a more sensitive method and by potentiation with 'Mechothane' it was possible to detect a very small acid secretion in the Heidenhain pouch on vagal stimulation, fit in very well with this concept.

Other evidence which suggests that the release of gastrin from the pyloric mucosa forms at least part of the mechanism of the gastric response to vagal stimulation has recently accumulated. The work of Robertson, Langlois, Martin, Slezak & Grossman (1950) has shown that acetylcholine will release gastrin from the pyloric mucosa in dogs. Linde (1950) has provided confirmation of much of the earlier work of Uvnäs (1942) on the cat. In man, Glass & Wolf (1950) have shown that resection of the distal $\frac{2}{3}$ to $\frac{3}{4}$ of the stomach abolished the rise in acidity and volume of juice produced by insulin hypoglycaemia though the rise in pepsin and mucoprotein persisted. Noring (1951) carried out sham-feeding tests on ulcer patients before and after partial gastrectomy of the Billroth II type and found a marked and sustained reduction in the volume and acidity of the secretion and a less marked reduction in the pepsin secretion.

The main reasons for preferring the vagal fibre hypothesis are thus no longer valid in the light of later evidence, and at this stage both it and the alternative possibility of gastrin release must be given equal consideration. The pepsin studies might have been expected to produce some evidence bearing on this point. Direct vagal stimulation is a strong pepsin stimulant, whereas gastrin preparations excite a pure acid secretion and there is as yet no definite evidence of the existence of a hormonal mechanism for pepsin. Unfortunately, the pepsin results available do not permit a definite answer on the crucial question as to whether the pepsin response is to be regarded as a primary stimulation or a secondary washing out effect. The strong inhibitory influence operating

in the experiments with insulin hypoglycaemia render these useless from this point of view, and in the case of psychic stimulation the general variability of the response and the falling background in most experiments make it impossible for the duration of the marked initial increase in output to be satisfactorily defined.

In the presence of direct vagal innervation, the pouch might be expected to show motility responses to central parasympathetic excitation, but a convincing increase in motility has not followed either method of stimulation. However, with insulin hypoglycaemia there is a strong inhibitory influence on motility, and with food showing the movements of the animal complicate the record. Lorber, Komarov & Shay (1950) recorded motility by balloons in gastric-fistula dogs and found that sham feeding was followed by cessation of peristaltic contractions, increase of fundic tone and decrease of antral tone. This work makes it difficult to predict what type of motility response to expect from a Heidenhain pouch with minimal vagal innervation, and the motility studies have been of no value in deciding whether the pouch responds to direct vagal innervation or to blood-borne gastrin. Experiments are in progress designed to differentiate finally between the two possibilities by investigating the effect on the secretory response of antrectomy, and of prolonged irrigation of the pouch with solutions of local anaesthetics. The preliminary results by both these methods support the gastrin hypothesis.

The inhibitory effects which we have found to be associated with insulin hypoglycaemia are of great importance in the assessment of the value of this form of stimulus. The existence of such an influence was suggested by the experiments of Necheles, Olsen & Scruggs (1942). These workers found that the stimulation of acid secretion in Pavlov pouches was less with the deeper levels of hypoglycaemia, and also that the secretion to a meat meal was reduced when the meal was given along with an injection of insulin. Karvinen & Karvonen (1951, personal communication) report that a high rate of histamine-induced secretion in Heidenhain pouches is depressed by approximately 50% during insulin hypoglycaemia.

The present results provide no definite information about the mechanism of the inhibitory influence. The effect does not seem fully established until the lower levels of hypoglycaemia have been reached. It seems possible that it may be due to a direct peripheral influence of the hypoglycaemia on the gastric secretory cells. Alternatively it may be a consequence of central sympathetic excitation, secondary to the hypoglycaemia. Both these possibilities have been put forward to account for depression of gastric motility by insulin hypoglycaemia (Lalich, Youmans & Meek, 1937; Necheles & Olsen, 1941). The inhibitory effect on pepsin secretion may also be a direct action of hypoglycaemia on the secreting cell. It is interesting that, in the few experiments followed so far, the return to normal of pepsin secretion seems to lag behind

that of blood-sugar level. The influence of insulin hypoglycaemia on the enzymes of the pancreatic secretion has been studied by several workers, and both inhibitory and stimulant effects have been reported. The position is confused and has been discussed by Babkin (1950, pp. 873-888). Kneller & Nasset (1949) have reported that a severe hypoglycaemia with blood-sugar levels of less than 25 mg % produced a marked depression of the output of sucrase and peptidase in the jejunal secretion of fasted dogs.

It would appear that the presence of these inhibitory influences affords a satisfactory explanation of the relative inefficiency of insulin hypoglycaemia as compared with psychic stimulation for the demonstration of the small acid responses to vagal stimulation found in the Heidenhain pouch. Evidently the response to insulin hypoglycaemia represents the resultant of stimulant and inhibitory influences.

Sharick & Campbell (1951) have recently shown in single experiments on nine patients that severe hypoglycaemia produced a well-marked gastric secretion in five cases, a slight to moderate response in three and no response in one. They suggest that in man there is no depression of the gastric response with the deeper levels of hypoglycaemia and that there may be a species difference between the dog and man in this respect. This claim seems unjustified since if the gastric response to insulin represents a balance between stimulant and inhibitory influences, both may be enhanced in severe hypoglycaemia and the effect observed in a given individual will depend on the relative strengths of the two mechanisms at the appropriate blood-sugar level. The position is likely to be very different in Heidenhain pouches or after partial vagotomy from that in intact individuals. The stimulant effect depends only on the vagus, and will thus be much reduced, whereas the depressant effect depends on non-vagal factors and should be unchanged. Consequently, the balance is likely to be heavily weighted towards inhibition. These are the conditions under which the insulin test for completeness of vagotomy is performed in man. A stimulus which involves an inhibitory influence seems an unsatisfactory way to test for minute degrees of residual innervation. Even if a positive response is obtained, the extent of the innervation may be much greater than the amount of acid secreted would imply. This may well be an important factor in the poor correlation between the insulin test and clinical results after vagotomy which has been the subject of recent discussions by Weinstein, Hollander, Lauber & Colp (1950) and Walters, Belding & Smith (1951). This criticism of the test will remain valid whatever is established as the actual mechanism of the response of the Heidenhain pouch to vagal stimulation.

SUMMARY

1. A method is described for the detection of minimal changes in acid and pepsin secretion in gastric pouches in dogs. It will also allow a simultaneous motility record.

2. Four out of ten Heidenhain-pouch dogs responded to insulin hypoglycaemia by a small but definite acid secretion. The form of the response and its relation to the blood-sugar curve are considered.

3. A method for the production of a potentiated response to psychic stimulation is described. All five animals so tested gave a definite increase of both acid and pepsin output.

4. Three animals gave a positive response to psychic stimulation but not to insulin hypoglycaemia.

5. Insulin hypoglycaemia has been shown to exert a depressant influence on both acid and pepsin secretion, particularly on the latter. This is considered to be the probable explanation of its lower efficiency compared with psychic stimulation. The bearing of these results on the insulin test is discussed.

6. The mechanism of these responses is considered. They could be due either to a small vagal innervation reaching the pouch or to the effect of gastrin released from the main stomach during vagal stimulation. Preliminary evidence favours the latter possibility.

7. A sample of ethyl-3:3-dimethylallyl barbituric acid (compound 16A) was found to be ineffective as a stimulant of the Heidenhain pouch.

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