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THE SECRETION OF GASTRIC ACID IN RESPONSE TO A LACK OF METABOLIZABLE GLUCOSE

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SUMMARY

1. The onset of the secretion of acid from the stomach of rats during insulin-induced hypoglycaemia has been found to correspond to a mean plasma glucose concentration of 72 mg/100 ml. (equivalent to a blood glucose concentration of 44 mg/100 ml.).

2. Infusions of the non-metabolizable sugar, 3-*O*-methylglucose, into rats with denervated adrenal glands caused a large and sustained rise in the gastric acid output. This gastric acid secretion could be prevented by prior vagotomy or stopped, when previously established, by cutting the vagi or by the administration of glucose.

3. A consistent relationship was demonstrated between the plasma concentration of glucose and the total concentration of glucoses (glucose plus 3-*O*-methylglucose) in the plasma at the onset of gastric acid secretion during the infusion of 3-*O*-methylglucose into these rats. The theoretical basis for this relationship is discussed.

4. 3-*O*-methylglucose did not cause the release of gastric acid when infused into rats with intact sympathetic nervous systems owing to the effects of the secretion of adrenaline provoked by this agent.

5. The qualitative and quantitative similarities between the factors governing both the secretion of gastric acid and the release of adrenaline in the absence of a sufficiency of metabolizable glucose are discussed. It is suggested that the reactions of both these systems under such circumstances are determined by chemoreceptors which possess identical characteristics.

INTRODUCTION

Two physiological responses to hypoglycaemia are directly dependent upon the autonomic nervous system, the release of adrenaline and the secretion of acid by the stomach. Adrenaline is secreted by the adrenal medulla when the oxidation of glucose by the brain is reduced either by insulin-induced hypoglycaemia (Kety, Woodford, Harmel, Freyhan, Appel & Schmidt, 1948), or by a non-metabolizable sugar which interferes with the process of glucose transfer into the brain (Himsworth, 1968*a*, *b*). During hypoglycaemia the secretion of adrenaline begins abruptly when the concentration of glucose in the blood falls below a critical level and ceases when that level is exceeded (Armin & Grant, 1959). It is believed that this response of the adrenal medulla is controlled by a receptor in the brain which may be stimulated by a lack of metabolizable glucose from any cause (Himsworth, 1968*a*).

The experiments described in this paper were undertaken to study the effect of a lack of metabolizable glucose upon the secretion of acid from the stomach and to test the hypothesis that the gastric and adrenal medullary responses to hypoglycaemia are determined by mechanisms with the same or similar characteristics.

METHODS

Animals. Male Norwegian hooded rats weighing between 280 and 340 g were used. They were starved for 24–48 hr before being used for an experiment in order to ensure that their stomachs were empty. The animals were anaesthetized by the intramuscular injection of urethane (150 mg/100 g body weight) or, in a few experiments, by subcutaneous pentobarbitone sodium (7·5 mg/100 g body weight). Urethane anaesthesia provokes a release of adrenaline from the adrenal medulla with resulting hyperglycaemia: this may be prevented by adrenal denervation (Kodama, 1930). The first stage of operative preparation, unless specifically omitted, was therefore to cut the spinal cord above the level of the sympathetic outflow. An airway was next inserted into the trachea and a catheter put into the right external jugular vein. A polyethylene tube was then passed down the oesophagus into the stomach and was tied in position by a ligature around the oesophagus in the neck. The abdominal cavity was opened in the epigastrium through the linea alba. A glass cannula was inserted in an incision in the duodenum and passed up into the stomach. This cannula, in the head of which were a number of openings, had a waist 2 cm from its end which was positioned at the pylorus. The cannula was tied into position by ligatures around the duodenum. All intra-abdominal manipulations were carried out gently with the least possible handling of the stomach. This experimental preparation was a modification of that described by Ghosh & Schild (1958). In some experiments the vagi were cut at this stage. In other experiments loose threads were placed around the vagi and both ends of each thread led through a short length of plastic tubing; the nerves could then be divided instantly and without disturbing the preparation by pulling the loop of thread through the plastic tube. Finally, any residual stomach contents were washed out with warm (37° C) physiological saline. Throughout the experiment the rat was laid upon a warm (37° C) surface.

Measurement of gastric acid secretion. A known volume of an appropriate buffer was placed

in a reservoir which contained a magnetic stirrer and which was surrounded by a water jacket through which water at 37° C was constantly flowing. The buffer was continuously recirculated at 3·5 ml./min by a roller flow inducer (Watson-Marlow Ltd.). The buffer passed through the oesophageal tube into the stomach from which it then siphoned through the cannula back into the reservoir. The pH of the buffer in the reservoir was measured with a glass electrode (Radiometer) connected to a pen recorder (Multipoint Cleertrend, Leeds & Northrup). The volume of buffer used in each experiment was 20 ml. In those experiments where the time of onset of acid secretion was determined a succinic-propionic buffer (M/600) in normal saline was used over the pH range 4·5–5·6. When the quantity of acid secreted over a period of time was being measured a buffer of greater concentration (M/200) was used over the same pH range. The recorded change in pH could be recalculated as change in hydrogen ion excretion knowing the characteristics and the volume of the buffer in the system.

Blood sampling. Blood (approximately 0·2 ml.) for the estimation of the plasma glucose concentration was taken from the tip of the tail directly into heparinized micro-haematocrit tubes. These tubes were sealed and centrifuged immediately in a Hawksley micro-haematocrit centrifuge for 2 min. The tubes were then cut at the interface between the buffy layer and the plasma. The plasma was blown out of the tubes on to a watch glass. For the simultaneous determination of the concentration of glucose in blood and plasma a catheter closed by a three-way tap was placed in the common carotid artery. The rat was heparinized. The following sampling procedure was adopted. Blood (0·5 ml.) was aspirated through the catheter, the tap was turned and a further 0·4 ml. blood withdrawn into a clean dry syringe, the tap was returned to its earlier position and the first 0·5 ml. blood was returned to the circulation. Some of the sample so obtained was transferred to haematocrit tubes for the separation of the plasma; one such tube was reserved for the subsequent determination of the haematocrit.

Chemical methods. 0·02 ml. samples of plasma and 0·1 ml. samples of blood were deproteinized with zinc sulphate and sodium hydroxide. Glucose and 3-*O*-methylglucose were measured in the manner previously described (Himsworth, 1968*a*). All determinations were carried out in duplicate.

Materials. 3-*O*-methyl-α-D-glucopyranose was purchased from Koch Light Laboratories. Commercially available soluble insulin (Wellcome, 20 units/ml.) was used.

RESULTS

The administration of insulin (0·6 units by subcutaneous injection) to rats is followed after a variable interval by an increase in the rate of acid secretion from the stomach. The plasma glucose concentration at the onset of the change in the rate of acid secretion was determined in nine animals. In each experiment the spinal cord was cut above the level of the sympathetic outflow to denervate the adrenal medulla: this prevented any possible inhibition of gastric secretion by the adrenaline which would otherwise have been released in response to hypoglycaemia. After the injection of insulin the developing hypoglycaemia was followed by the rough estimation of the blood glucose concentration by means of glucose oxidase impregnated strips ('Dextrostix', Ames & Co.). When the blood glucose appeared to have fallen to between 50 and 60 mg/100 ml. frequent samples of blood were taken for the subsequent accurate measurement of the plasma glucose concentration. This sampling was continued for a short

time after the rate of acid secretion had increased. Sufficient glucose was then given intravenously to stop the increased output of acid. In some animals hypoglycaemia was allowed to develop once again and another series of blood samples was taken. The onset of the increase in the rate of secretion of acid by the stomach occurred when the plasma glucose concentration was between 69 and 78 mg/100 ml. in eight of the rats and at

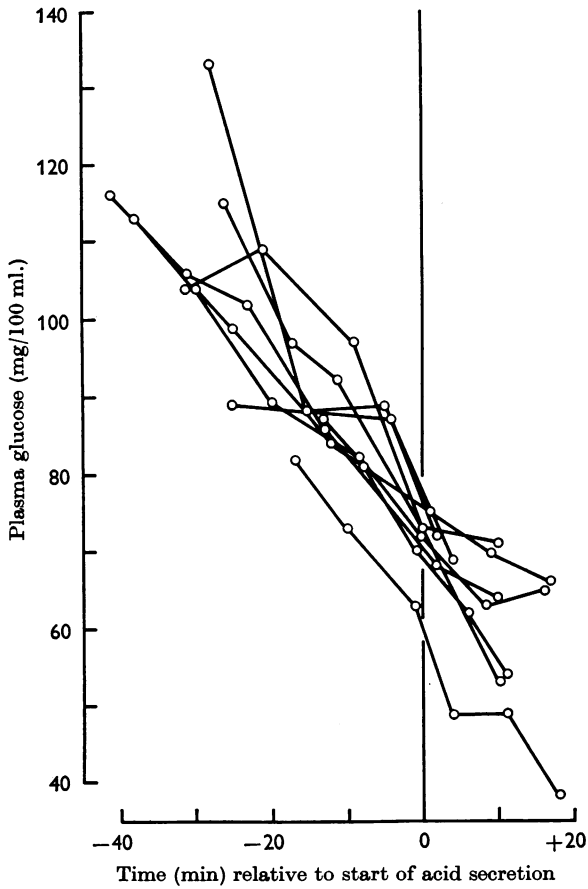

Fig. 1. The plasma glucose concentrations during developing hypoglycaemia in nine rats immediately before and after the onset of acid secretion.

60 mg/100 ml. in the ninth (Fig. 1) with a mean value of 72 ± 5·5 mg/100 ml. (± S.D.). Where the critical plasma glucose concentration was determined twice it appeared to be constant for each animal.

Simultaneous measurements of the concentration of glucose in the blood and plasma were made on five rats at intervals after the administration of insulin. No more than six measurements were made on any

animal. Blood samples were taken through a catheter in the carotid artery. The haematocrit was measured on each sample. Because of the small amount of glucose present in the red blood cells during hypoglycaemia it was found that minor variations in the haematocrit were accompanied by comparatively large changes in the blood glucose concentration relative to the plasma glucose concentration. However a plasma glucose concentration of 72 mg/100 ml. corresponded approximately to a blood glucose concentration of 44 mg/100 ml. (at a mean haematocrit of 48 % plasma glucose = 1·67 blood glucose + 1; $r = +0{\cdot}994$).

The effect of 3-O-methylglucose upon the secretion of acid by the stomach. The infusion of the non-metabolizable sugar 3-*O*-methylglucose into normal anaesthetized rats causes the release of adrenaline with a consequent rise in the plasma glucose concentration (Himsworth, 1968*a*). In the initial studies of the effect of 3-*O*-methylglucose upon the secretion of acid by the stomach this release of adrenaline, and any consequent rise of plasma glucose, was prevented by dividing the spinal cord above the level of the sympathetic outflow. Sectioning the cord in itself does not appear to influence the secretion of acid. During the control periods of the experiments the low and relatively constant basal rate of acid secretion was shown by the fact that the pH of the buffer recirculated through the stomachs of the rats varied only slightly. In general there was a small increase in acidity but this never exceeded 1 μ-equiv in any 5 min period.

The intravenous administration of 3-*O*-methylglucose (a loading dose of 150 mg followed by an infusion at a rate of 20 mg per minute, given as a solution containing 30 g/100 ml. water) to prepared animals caused, after an interval, a marked outpouring of acid from the stomach. The interval before the onset of this response was prolonged if the plasma glucose concentration during the control period was high. The rate of secretion of acid rose rapidly to a peak, varying between 9 and 22 μ-equiv/5 min in eleven experiments, and then fell slightly to a rate which was maintained even after the end of an hour's infusion of 3-*O*-methylglucose (Fig. 2). The secretion of acid during the infusion of 3-*O*-methylglucose could be abruptly brought to an end by the intravenous administration of a sufficient quantity of glucose (Fig. 3).

Previous cutting of the vagi prevented the acid secretion that follows infusion of 3-*O*-methylglucose even though the plasma 3-*O*-methylglucose concentration was similar to that in the other experiments. Furthermore, division of the vagi during the infusion of 3-*O*-methylglucose in two experiments stopped the secretion of acid completely (Fig. 4). Cutting the vagi did not influence the plasma glucose concentration. At the end of those experiments where the secretion of acid in response to an infusion of 3-*O*-methylglucose had been either prevented by earlier section of the vagi or

stopped by the administration of glucose or cutting the vagi a dose of carbachol was given to show that the stomach was still capable of secreting acid.

In a series of ten experiments the onset of the gastric secretion of acid and its correlation with the plasma glucose and 3-*O*-methylglucose concentrations were investigated. In order that a wide range of plasma glucose

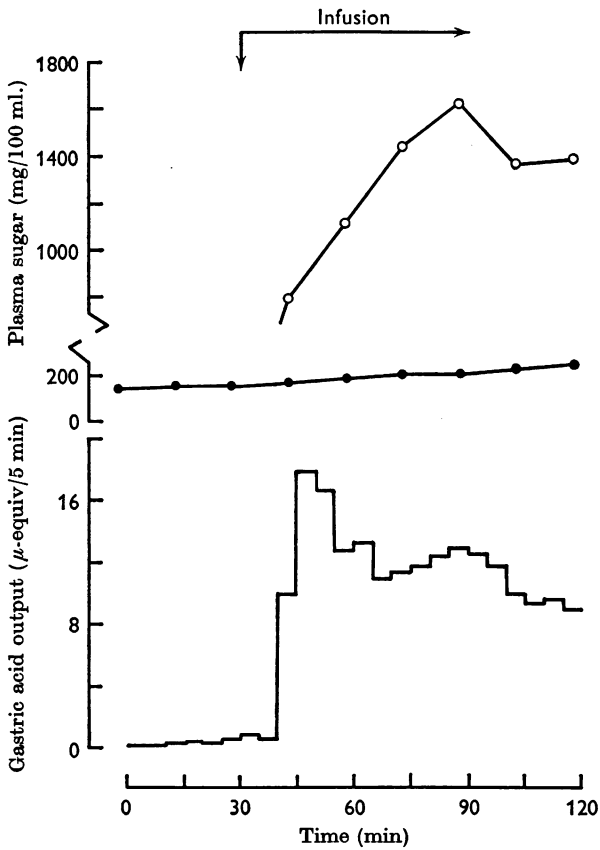

Fig. 2. A typical example of the effect of 3-*O*-methylglucose (loading dose 150 mg and infusion of 20 mg/min for 1 hr) upon the secretion of acid from the stomach of a rat with high level cord transection (urethane anaesthesia). The plasma concentrations of glucose (●) and 3-*O*-methylglucose (○) are also shown.

concentrations should be covered, the animals, prepared in the usual manner, were first given insulin (0·6 units by subcutaneous injection) to lower the plasma glucose. About 20 min later an infusion of 3-*O*-methylglucose (20 mg/min) was begun. This infusion was stopped at the first indication of a rise in acid secretion. After an interval of 3 min to allow equilibration of 3-*O*-methylglucose in the extracellular fluid a sample of blood was taken. Sufficient glucose was then given to terminate the acid

secretion. When this secretion had entirely ceased the infusion of 3-*O*-methylglucose was re-started and the cycle repeated two to four times. It was found that the plasma glucose concentration about the time of onset

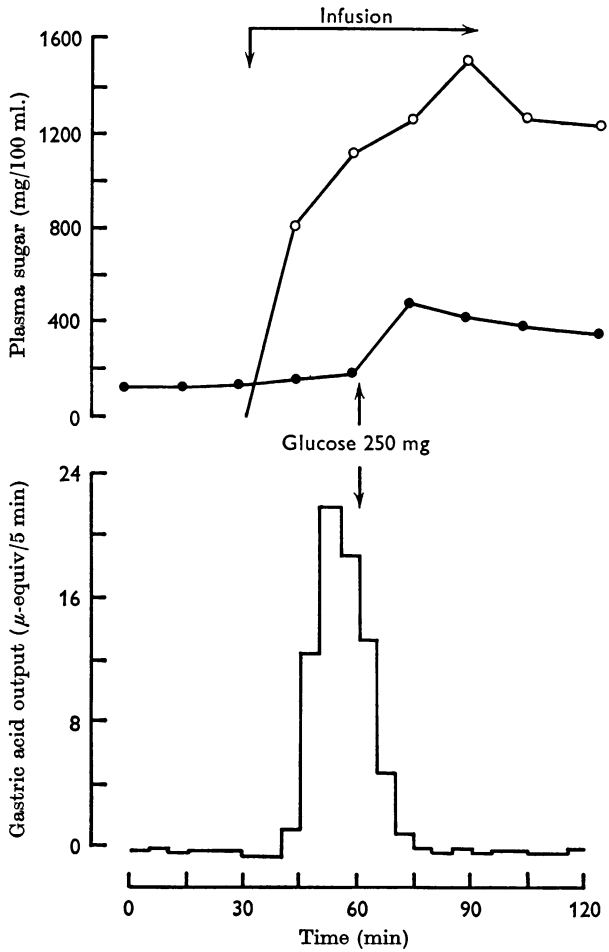

Fig. 3. The effect of the intravenous administration of glucose (250 mg) upon the gastric acid secretion caused by 3-*O*-methylglucose. Symbols as in Fig. 2.

of acid secretion by the stomach bore a consistent relation to the total sugar (glucose plus 3-*O*-methyl-glucose) concentration in the plasma (Fig. 5).

In apparent contrast to the preceding experiments no secretion of acid followed the administration of 3-*O*-methylglucose in five animals whose spinal cords were not cut and in which, in consequence, the innervation of the adrenal medulla was intact. In these experiments it was necessary

to anaesthetize the rats with pentobarbitone sodium (7·5 mg/100 g body weight by subcutaneous injection) as this, unlike urethane, does not cause a rise in the blood glucose concentration. Despite the fact that there was no acid secretion, the administration of 3-*O*-methylglucose (a loading dose of 150 mg followed by an infusion at a rate of 20 mg/min for 1 hr) still

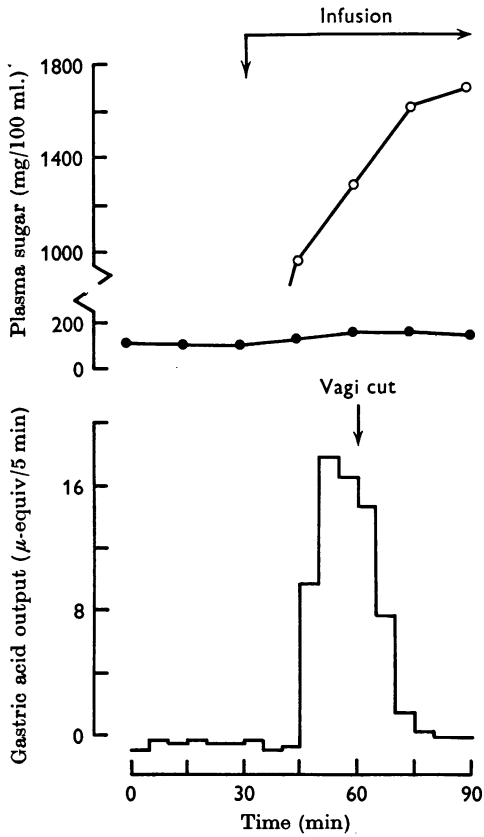

Fig. 4. The effect of cutting both vagi upon the secretion of gastric acid caused by 3-*O*-methylglucose. Symbols as before.

caused the expected rise in plasma glucose concentration. That pentobarbitone sodium in this dosage does not itself directly inhibit the secretion of acid by the stomach was however demonstrated in three animals where the spinal cord was cut before the infusion of 3-*O*-methylglucose and in which a rate of release of acid of comparable magnitude to that occurring in rats anaesthetized with urethane was observed. Acid secretion occurred after 3-*O*-methylglucose, therefore, when a rise in plasma glucose level was prevented but not when it was permitted.

DISCUSSION

The secretion of acid by the stomach during insulin-induced hypoglycaemia has been extensively studied. This response which is thought to be initiated from within the central nervous system (Jögi, Ström & Uvnäs, 1949) is mediated by the vagi and can be ended by the administration of glucose. The response may be modified, especially in its initial stage, by the activity of the sympathetic nervous system (Bachrach, 1963). In most of the experiments described in this paper any such effect of sympathetic activity was prevented by division of the spinal cord above the level of the sympathetic outflow. The onset of the gastric secretory response to hypoglycaemia has been shown to be independent of both the dose of insulin and the rate of fall of the blood glucose concentration (Davis, Brooks & Robert, 1965). Secretion of acid begins when the blood glucose has fallen below a critical concentration. This concentration is generally below 50 mg/100 ml. and has been found to vary with the individual animal, the species and the method used to measure the blood glucose. In the present experiments the onset of the secretion of acid by the stomach during insulin-induced hypoglycaemia in the rat was found to correspond to a mean plasma glucose concentration of 72 mg/100 ml. If the increase in the rate of secretion of acid is due to deprivation of the central nervous system of an adequate supply of glucose such a response will be more dependent upon the concentration of glucose in the plasma than the concentration in the blood as a whole. In the rat a plasma glucose concentration of 72 mg/100 ml. was equivalent to a blood glucose concentration of about 44 mg/100 ml. This value, obtained for the critical concentration of glucose at the onset of gastric acid secretion in the rat after the administration of insulin, is therefore in accord with those values obtained in other species when the blood glucose concentration was measured under similar conditions.

It was observed in these experiments that the start of the secretion of acid by the stomach occurred at the same plasma glucose concentration in any one individual rat and that, in the series as a whole, the critical plasma glucose values fell, with one exception, within a narrow range. The experimental preparation behaves, therefore, in a consistent and predictable fashion.

The intravenous infusion of 3-*O*-methylglucose into fasted rats, in which the sympathetic nervous system had been separated from its higher central connexions by cutting the spinal cord, resulted in a large, sustained secretion of acid from the stomach. This secretion was shown to be mediated by the vagi for it could be prevented by prior vagotomy, or a secretion previously established by infusion of 3-*O*-methylglucose could be abruptly

terminated by cutting these nerves. The acid secretory response of the stomach to 3-*O*-methylglucose is therefore determined by the same final pathway as the gastric response to insulin hypoglycaemia, namely the vagi. Furthermore, acid secretion in response to both hypoglycaemia and 3-*O*-methylglucose may be stopped by the administration of a sufficient quantity of glucose. The inference is, therefore, that both these stimuli to acid secretion achieve vagal excitation by acting upon the same mechanism or mechanisms within the central nervous system. That the response is not directly dependent upon a low concentration of glucose in the plasma is shown by the experiments with 3-*O*-methylglucose in which the release of acid was provoked without any depression of the plasma glucose level and those in which it was shown that at high initial plasma glucose concentrations a larger amount of 3-*O*-methylglucose was needed to start the secretion.

These findings may be explained on the basis of the known properties of 3-*O*-methylglucose. This substance enters the rabbit brain by the same facilitated transfer system as glucose and has the same affinity as glucose for this system (Agnew & Crone, 1967). The facilitated transfer system for glucose in the dog brain is saturated at a blood glucose concentration of about 70 mg/100 ml. and at higher blood glucose levels the amount of glucose entering the brain increases only slightly by means of passive diffusion (Crone, 1965). 3-*O*-methylglucose when present in the plasma of fasted rats in a high concentration impedes the movement of glucose into the intracellular compartment and causes a marked reduction in the rate of oxidation of glucose (Himsworth, 1968*b*). In fasted animals the brain is the major site of glucose consumption and following 3-*O*-methylglucose the decline in glucose oxidation is comparable to that observed during hypoglycaemia. It seems likely therefore that insulin-induced hypoglycaemia and 3-*O*-methylglucose deliver equivalent stimuli to the brain. Both restrict glucose entry, the one by lowering the plasma concentration of glucose, the other by competing with glucose for the facilitated transfer system, and thus both reduce the availability of metabolizable glucose to the brain.

If the foregoing explanation is correct it follows that the amount of glucose entering the brain by means of the facilitated system in the presence of 3-*O*-methylglucose will depend upon the ratio of the concentration of glucose to the total concentration of sugar (glucose plus 3-*O*-methylglucose) in the plasma if this total is sufficient to saturate the system. As the onset of acid secretion by the stomach during insulin hypoglycaemia occurred consistently at the same plasma glucose concentration, a similar correlation was sought for in the ratio of glucose to total sugar in the plasma at the beginning of acid secretion. In order that these experi-

ments should cover the widest possible range of plasma glucose values insulin was given to the rats at the start of the experiment to lower the plasma glucose concentration, but not so far as to initiate gastric acid secretion. As insulin does not itself affect the rate of passage of glucose into the brain (Crone, 1965), this preliminary measure should not influence the results. It was found that there was a clear correlation ($P < 0{\cdot}001$) between the plasma glucose concentration and the total plasma sugar concentration (glucose plus 3-*O*-methylglucose) at the time at which acid

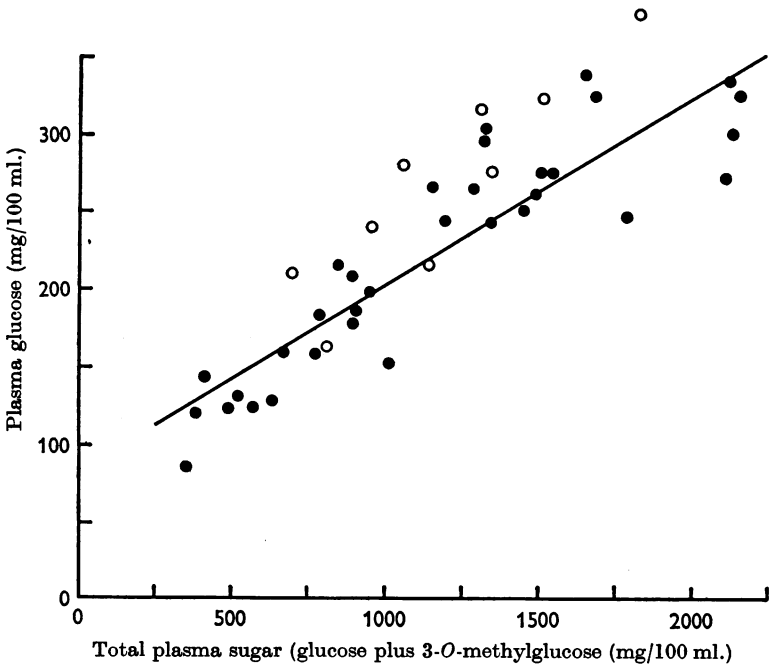

Fig. 5. The plasma glucose concentration plotted against the total plasma sugar (glucose plus 3-*O*-methylglucose) concentration at the onset of gastric acid secretion (●) in rats with high level cord transection during the infusion of 3-*O*-methylglucose (plasma glucose = 0·12 total plasma sugar + 81; $r = +0{\cdot}902$; $P < 0{\cdot}001$). Also shown are the results, plotted in the same manner, from experiments in which rats with intact spinal cords were given infusions of 3-*O*-methylglucose (○). For further details, see text.

secretion started (Fig. 5). This finding of a persistent proportionality between the two concentrations is consistent with the hypothesis that 3-*O*-methylglucose restricts the access of glucose to a mechanism which controls the vagally determined secretion of gastric acid, by competing with glucose for a carrier mediated transfer system of limited capacity.

The infusion of 3-*O*-methylglucose into rats anaesthetized with pentobarbitone sodium and with their spinal cords intact did not cause a secre-

tion of acid from the stomach. The infusion was, however, accompanied by a rise in the plasma glucose concentration. This increase in the plasma glucose has previously been shown to be due to the release of adrenaline by the action of 3-*O*-methylglucose (Himsworth, 1968*a*). The absence of acid secretion in these experiments is not attributable to the anaesthetic agent for the infusion of 3-*O*-methylglucose into rats anaesthetized with this substance, but in which prior denervation of the adrenal glands had been carried out, caused a massive release of acid. The explanation of these findings appears to be as follows. Throughout the infusion of 3-*O*-methylglucose into rats whose sympathetic nervous systems were intact the plasma glucose concentration remains slightly too high relative to the total concentration of sugar (glucose plus 3-*O*-methylglucose) in the plasma for the ratio between these to fall to the requisite level for the secretion of acid to be provoked (Fig. 5). The absence of any secretion of acid may therefore be largely attributed to the adrenaline mediated compensatory rise in the plasma glucose concentration that accompanies the infusion of 3-*O*-methylglucose into normal rats, reinforced by the inhibitory action of adrenaline upon the liberation of gastric acid.

These experiments show clearly that the stomach secretes acid in response to vagal activity consequent upon a lack of metabolizable glucose in the central nervous system. A similar lack provokes, by other pathways, the release of adrenaline from the adrenal medulla. Both these physiological reactions of the autonomic nervous system are thought to originate from within the central nervous system. Each may be prevented or stopped by cutting the appropriate nerves or remedying the lack of metabolizable glucose. The release of adrenaline occurs during insulin-induced hypoglycaemia in rats when the blood glucose falls below 40 mg/100 ml. (Himsworth, 1968*a*). The secretion of acid starts under similar conditions at a blood glucose concentration of 44 mg/100 ml. In neither case is the response graduated to the severity of the hypoglycaemia but rather is 'on-off' in character. Nor is either reaction directly dependent upon the concentration of glucose in the plasma for each may be provoked, in the presence of a normal or even somewhat raised plasma glucose concentration, if the availability of glucose to the brain is reduced by giving adequate amounts of 3-*O*-methylglucose. Furthermore, each response may be produced by intracellular inhibitors of glucose metabolism such as 2-deoxyglucose. The factors which govern the secretion of gastric acid and the release of adrenaline in the absence of a sufficiency of metabolizable glucose are therefore qualitatively and quantitatively identical. Each of these reactions by the autonomic nervous system is initiated before the lack of metabolizable glucose causes any general neurological disturbance. Both therefore originate in areas of the brain more than usually sensitive to such a lack.

The existence may thus be inferred of a class of chemoreceptors whose quiescence depends upon the maintenance of a relatively high rate of glucose metabolism.

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