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THE EFFECT OF VAGAL STIMULATION ON PLASMA INSULIN AND GLUCOSE LEVELS IN THE BABOON*

BY P. M. DANIEL AND J. R. HENDERSON†

From the Department of Neuropathology, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E. 5

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SUMMARY

1. The concentration of insulin in the blood of fifteen fasting baboons, anaesthetized with pentobarbitone sodium, was measured by a specific radio-immunoassay method.

2. The mean resting concentration of insulin was: inferior vena cava (IVC) 22 μu./ml. and splenic vein (after splenectomy) 140 μu./ml.

3. Subdiaphragmatic stimulation of the cut right vagal trunk (at 10 impulses/sec, duration 1·5 msec, for 10 min) produced a mean increase of 50% over resting levels in IVC insulin concentration and an increase in splenic vein insulin of 30% over resting levels.

4. The mean resting level of blood glucose in splenic vein blood was 89·4 mg%, that in IVC blood was 79·4 mg%. The difference is significant ($P < 0{\cdot}005$).

5. Vagal stimulation did not alter these glucose levels, so that insulin release following vagal stimulation is not secondary to hyperglycaemia.

6. The increased insulin secretion following vagal stimulation might be expected to produce hypoglycaemia. Its failure to do so is discussed.

INTRODUCTION

It has been known for many years that the pancreatic islets are extensively innervated. In fact, Langerhans, in his original description of the rabbit's pancreas (1869), noticed the close relation between nerve fibres and islets, as well as the presence of scattered nerve cells in the substance of the islets. His findings were confirmed by Gentes (1902). Perhaps the most detailed description is that of de Castro (1923) who showed, using a Golgi double impregnation method on the mouse's pancreas, fine unmyelinated nerve fibres spreading over the blood vessels of the islets and

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ending on the endocrine cells. De Castro believed that different sets of nerve fibres innervated the endocrine and exocrine parts of the pancreas—but such a contention is very hard to support. Particular attention to nerve cells in islets was drawn by Van Campenhout (1927) and Simard (1942) who regarded the presence of nerve cells within some islets as suggesting a neurosecretory device for insulin release (though no attempt was made to prove this experimentally) and called these specialized organs 'les complexes neuro-insulaires'. Simard found the complexes in a varying proportion of the islets of every species of animal that he examined. The innervation of the islets of the cat was described by Richins (1945). Coupland (1958) using the cholinesterase technique of Koelle & Friedenwald (1949) showed the nerves to the islets and the nerve cells in the islets to be cholinergic. Some typical examples of islet innervation from our material are shown in Pl. 1. It is not known whether the islets are supplied with afferent nerves, or indeed whether the nerve cells in some islets are part of an afferent system.

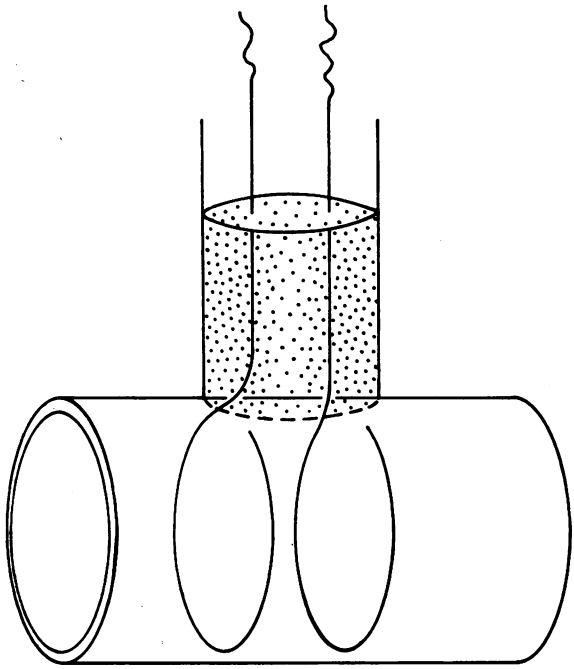
Vagal stimulation produces an exocrine secretion of the pancreas characterized by its low volume and high enzyme content (Brown, Harper & Scratcherd, 1967), and since the islets receive an innervation that is presumably vagal, many investigators have studied the influence of the vagus on endocrine function (Britton, 1925; Clark, 1931; Zunz & La Barre, 1927; Gellhorn, 1943). But the results have been inconclusive, since in all the experiments reported the changes measured have been in levels of blood glucose (or even reducing substances), and possible changes in insulin levels have had to be deduced from these values. Furthermore, the use of ether or chloroform (which causes a striking increase of 'resting' blood glucose) as anaesthetics, the stimulation of the vagi in the neck (causing widespread circulatory changes) and differences between species have all contributed to the confusion.

In 1948 Anderson & Long, using a bio-assay method to measure insulin, showed that glucose caused a striking release of insulin from the pancreas. Not surprisingly the 'neurogenic' hypothesis receded into the background, helped by the equivocal results obtained in earlier studies. However, the development of specific immunological methods for measuring insulin in blood made it seem worthwhile to re-investigate the possibility of neural control of insulin secretion. This paper reports an attempt to remove some of the earlier sources of confusion by measuring insulin levels in the blood directly, using pentobarbitone anaesthesia, and stimulating, with perhaps more physiological stimuli than those of earlier workers, the right cut vagal trunk below the diaphragm.

METHODS

Fourteen male baboons and one female baboon, weighing 3–7 kg, were used. Each animal was fasted for 18 hr before the experiment. Anaesthesia was induced with intramuscular 'Sernylan' (phencyclidine hydrochloride, Parke Davis) 2 mg/kg and maintained with pentobarbitone sodium (Martindale Samoore). Since the resting levels of blood glucose and blood insulin found in these baboons were similar to those in conscious man, we have assumed that the anaesthetics do not affect the levels of these substances. A polythene catheter was passed up the femoral vein, reaching 2–3 cm above the iliac bifurcation in the inferior vena cava (IVC), through which a slow drip of 0·9% saline (containing pentobarbitone) was run throughout the experiment. The catheter was also used for blood sampling.

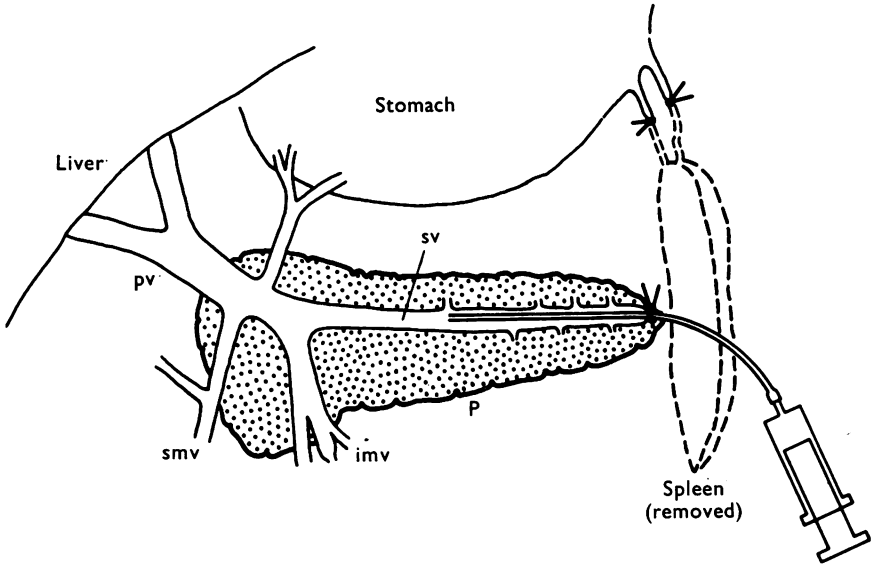
A laparotomy was then performed, the stomach gently retracted downwards and the right vagal trunk identified on the right and posterior surface of the oesophagus. The nerve was dissected off, care being taken not to damage the adjacent pleura, and cut as high up as possible. The right vagal trunk usually consisted of only one trunk at this level, though it often divided a short distance below. The phrase 'right vagus' is a bad one, since below the vagal plexuses in the thorax a given vagal trunk contains fibres from both right and left *cervical* vagi. In this paper 'right vagal trunk' is taken to mean that part of the nerve which leaves the thorax under the right crus of the diaphragm and runs along the right posterolateral surface of the oesophagus. The cut end of the nerve was then passed through an electrode holder, modified after a design suggested by Professor C. G. Phillips (Text-fig. 1). Care was taken not to stretch the nerve.



Text-fig. 1. Diagram of electrode holder for vagal stimulation. The silver electrodes are embedded in dental acrylic resin contained in the vertical polythene tube. They then run round the wall of the horizontal polythene tube, through which the vagus is threaded and secured.

In eight animals the splenic vein was catheterized. The short gastric veins running in the lieno-gastric ligament were tied off, as were the branches of the splenic artery and vein just as they entered the spleen. The spleen was then removed, the object of this being to exclude any possible changes in splenic flow produced by vagal stimulation. The catheter was inserted so that its tip lay about half way along the splenic vein (i.e. 2–3 cm from the distal end of the vein) and so collected blood solely from the tail of the pancreas (Text-fig. 2). In the remaining seven of the animals a catheter was passed high up into the portal vein via the inferior mesenteric vein in order to obtain samples to be used in another series of experiments.

The abdomen was then closed, and the animal left undisturbed for $1\frac{1}{2}$ hr.



Text-fig. 2. Diagram to show position of the catheter in the splenic vein. Note that the splenic catheter is sampling only pancreatic venous blood. P = pancreas; pv = portal vein; smv = superior mesenteric vein; sv = splenic vein; imv = inferior mesenteric vein.

Stimulation. The vagus was stimulated with a pulse frequency of 10 impulses/sec at a duration of 1·5 msec at 5 V for 10 min. Within a few seconds of beginning the stimulation the small bowel and stomach showed active peristalsis, and this continued throughout the period of stimulation. Each animal had its right vagal trunk stimulated on two occasions during the 8 hr experiments. In four experiments an arterial catheter in the aorta connected to a manometer showed no change in blood pressure on stimulating the nerve.

Sampling and assay of insulin and glucose. Two or three blood samples were taken before the abdomen was opened, and three more samples in the 30 min before stimulation. The next sample was taken 5 min after the end of stimulation, and others at 10 min intervals for 30 min and then at 15 min intervals for the following 2–3 hr. Samples were always taken simultaneously from the IVC and from the splenic vein. Blood samples (0·8 ml.) were collected in heparinized syringes and in some experiments 0·2 ml. of this blood was transferred to an auto-analyser cup containing dried sodium iodoacetate. The remainder of the blood was centrifuged, and the plasma deep frozen. Glucose was measured by a glucose oxidase method (Auto-analyser, Technicon). Insulin was measured in plasma by the radio-immunoassay of Hales & Randle (1963) using [^{125}I]insulin (Radiochemical Centre, Amersham) and 'insulin binding reagent' (Burroughs Wellcome). The standard used in the assay was crystalline ox insulin (Burroughs Wellcome).

At the end of the experiment the animal was killed with an overdose of pentobarbitone and the position of the catheters confirmed. The stomach, duodenum and small bowel were examined to ensure that no food had been present during the experiment.

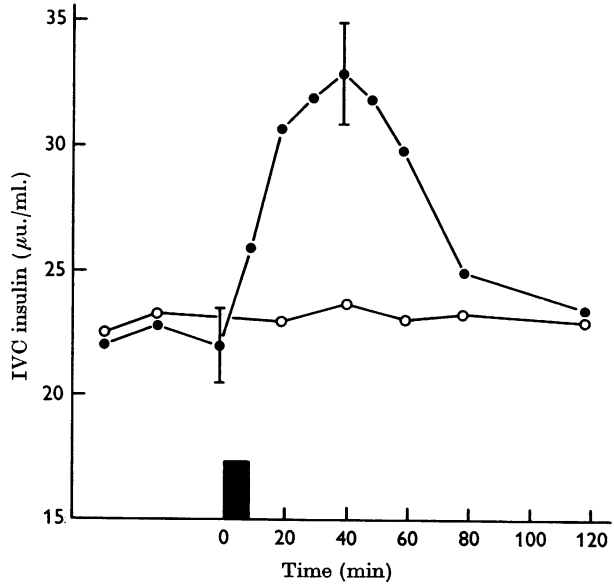
RESULTS

In the fourteen male baboons the mean resting concentrations of insulin in the blood of the IVC and splenic vein are shown in Table 1. Splenectomy had no effect on the resting levels of IVC insulin nor on insulin concentrations following stimulation.

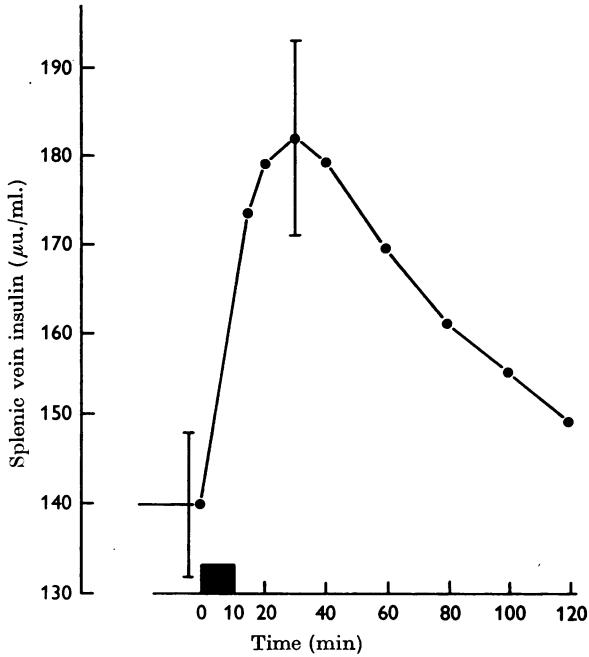
TABLE 1. The resting levels of insulin in IVC and splenic vein blood of fasting baboons anaesthetized with pentobarbitone sodium

Vein	No. of expts.	No. of samples	Mean insulin concn. in μu./ml.	S.E.M.
IVC	14	40	22	± 2
Splenic	7	24	140	± 9

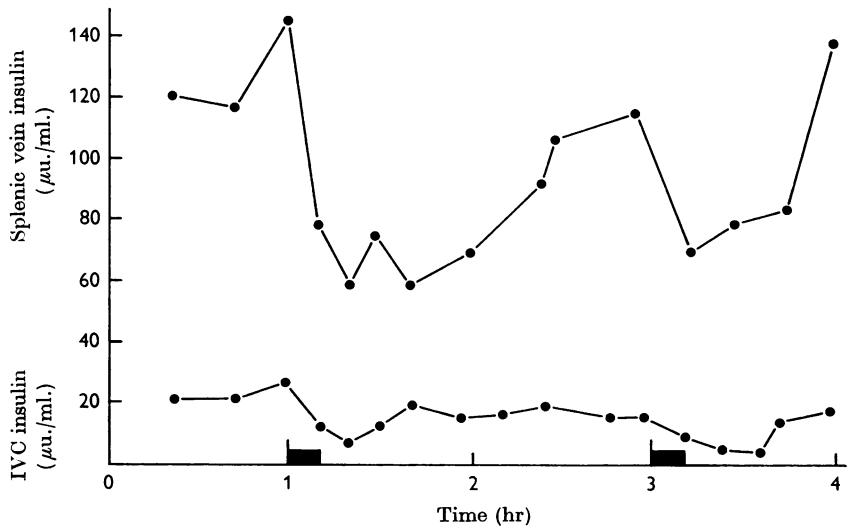
Effect of stimulation of the vagus nerve on insulin in the IVC. Stimulation at a duration of 1·5 msec at 10 impulses/sec for 10 min produced a mean rise in insulin concentration of about 10 μu./ml. in IVC blood (Text-fig. 3).



Text-fig. 3. Concentrations of insulin in IVC blood before and after vagal stimulation, using long and short impulse durations. ● 1·5 msec impulses, ○ 0·5 msec impulses. There were nine stimulations (six animals) with the longer impulses and four stimulations (four animals) with the shorter pulses. Both sets of stimuli were at 10 impulses/sec, 5 V for 10 min. Black rectangle represents period of stimulation; vertical lines S.E.M.; the difference between resting and peak levels is significant ($P < 0{\cdot}005$).



Text-fig. 4. Mean concentration of insulin in splenic vein blood after stimulation of the right vagus (impulse duration 1·5 msec, 10/sec for 10 min). Mean of eight stimulations. Black rectangle represents period of stimulation; vertical lines: S.E.M.; the differences between resting and peak values are significant ($P < 0{\cdot}01$).



Text-fig. 5. Paradoxical experiment. Female baboon. Insulin concentration in splenic blood (upper curve) and IVC blood (lower curve). Two periods of stimulation were given, shown by black rectangles. Although both resting concentrations are in the normal range, vagal stimulation inhibited insulin release, in striking contrast to the results seen in the other experiments (Text-figs. 3 and 4).

This rise reached its peak at about 40 min and returned to normal levels after 2 hr. Stimulation at an impulse duration of 0·5 msec, the other parameters being unchanged, produced no effect on the concentration of insulin in the IVC.

Effect of stimulation of the vagus nerve on insulin in the splenic vein. The scatter of splenic insulin levels was large. Vagal stimulation produced a significant rise in the insulin in the blood of the splenic vein which reached its highest point at 40 min, corresponding to a mean rise of 30 % above the resting level. This was from a mean level of 140 μu./ml. to 182 μu./ml. The concentration of insulin returned virtually to resting levels within 2 hr (Text-fig. 4).

A paradoxical result. In one monkey (the only female in the series) vagal stimulation produced a *fall* in the insulin concentration both in splenic blood and in IVC blood (Text-fig. 5).

Venous glucose levels. Glucose estimations were performed on splenic vein and IVC blood during the last six experiments. The mean glucose levels in these animals were:

Splenic vein	89·3 mg/100 ml. ± 2·5 (S.E.M.) (30 samples)
IVC	79·4 mg/100 ml. ± 2·0 (S.E.M.) (30 samples)

The difference between these levels is significant ($P < 0{\cdot}005$). There was no significant change in glucose concentration in either vein throughout the period following vagal stimulation.

DISCUSSION

Immunological methods for the assay of hormones are, in general, highly specific and sensitive. The insulin immunoassay used here, for instance, can distinguish between samples differing by 1 μu./ml. which is $0{\cdot}4 \times 10^{-7}$ mg of insulin. The insulin standard used in each assay was crystalline ox insulin, for monkey insulin was not available at the time. Although the results obtained are theoretically not absolute values, any changes in insulin levels found represent real changes. In spite of this proviso with respect to insulin standards, the resting levels of insulin in the blood of these baboons are similar to those found in other animals when homologous insulin has been used as standard.

Results of vagal stimulation

The concentration of insulin in the blood of the IVC and of the splenic vein was raised in all the experiments, except in one paradoxical experiment.

Changes in IVC insulin. It can be seen from Text-fig. 3 that vagal stimulation produced a mean rise of 10 μu./ml. (i.e. about 50 %) over the

resting levels in IVC blood. Following the stimulation there was no increase in blood glucose levels, so that the increased insulin was not secondary to glucose being released (e.g. from the liver) by vagal stimulation. Should this extra amount of insulin secreted have caused a fall in blood glucose? This is not an easy question to answer, but the work of Zierler & Rabinowitz (1964) is relevant. These authors perfused the brachial artery of fasting human subjects with insulin at a rate of 10 μu./kg./min. This raised the level of insulin in the arterial blood, returning to the arm from the rest of the body, by 38 μu./ml., but did not alter blood glucose, in spite of striking effects on free fatty acid and potassium levels. Thus the mean increase in the insulin level of 10 μu./ml. in our experiments should not have produced an effect on blood glucose levels, although it is possible that the free fatty acid or potassium concentrations might have changed.

Changes in splenic vein insulin concentration. There is little evidence in the literature that vagal stimulation produces any change in splenic blood flow, but it was felt that the possibility of change added a variable, so that in our experiments the spleen was removed. This meant that the splenic vein catheter was sampling pure pancreatic venous blood (Text-fig. 2). The mean resting concentration of insulin found, 140 μu./ml., must therefore be higher than that in the splenic vein of an animal with a spleen present, since there is no blood coming from the spleen to dilute the pancreatic blood.

Apart from the paradoxical result, vagal stimulation caused a consistent increase in insulin concentration in the splenic vein (see Text-fig. 4). The rise was of the order of 30% (a mean elevation of 50 μu./ml.) reaching its peak about 40 min after the beginning of the stimulus. The total output of insulin during this time is probably higher, for vagal stimulation is said to produce an increase in pancreatic blood flow (Gayet & Guillaumie, 1930). Since blood flowing through the islets only accounts for a very small proportion of pancreatic flow, it is clear that insulin concentrations in pancreatic venous blood could be greatly altered by changes in flow in the exocrine part of the gland. (The results of seven experiments in which insulin in the portal vein was measured gave results which we cannot at present interpret. Further experiments are being carried out.)

Neural factors influencing insulin secretion

The discovery of glucose as the most potent stimulus for insulin release has shifted interest away from neural stimuli. Standard text-books usually suggest cautiously that there might be a neurogenic mechanism, and then change the subject. Such comments are usually based on a series of papers by Zunz & La Barre in the late 1920's. These authors showed that by transfusing pancreatic venous blood from a donor dog to the

jugular vein of a recipient dog, and then stimulating the right vagus nerve of the donor dog, a fall in blood sugar occurred in the recipient animal. However, it seems unlikely that these authors demonstrated a neural release of insulin, since the maximum fall occurred in the recipient dog 2–2½ hr after the pancreatic blood from the donor dog had been disconnected (see La Barre, 1927). If the fall were due to insulin it is very difficult to see why it should be so delayed.

There is some evidence, derived from catecholamine fluorescence studies (Cegrell, Falck & Hellman, 1964) that the islets receive an adrenergic as well as a cholinergic innervation. If this is the case then stimulation of these adrenergic fibres might be expected to inhibit insulin release either by vasoconstriction of islet vessels or by a direct effect on β cells. Such a mechanism might explain the striking inhibition of insulin release seen in our paradoxical experiment (Text-fig. 5), where vagal stimulation caused a fall in both splenic vein and IVC concentrations. This would involve either adrenergic vagal nerve terminals, sympathetic fibres travelling in the subdiaphragmatic vagus, or inhibitory vagal fibres. As far as we are aware the effect of sympathetic stimulation, i.e. stimulation of the splanchnic nerves, on insulin secretion has not been studied. Furthermore, the liver is clearly of paramount importance in the metabolism of insulin; so it is just possible that vagal stimulation might alter the liver's ability to degrade the hormone.

Krulich (1961) perfused an internal carotid artery of anaesthetized rabbits and conscious dogs with 5% glucose solution. Identical infusions when put anywhere else into the circulation had no effect on systemic glucose levels, and, by inference, on insulin levels. After carotid infusion systemic blood glucose fell by 20–30 mg%, reaching its lowest point after about 20 min. Such an interval would be quite compatible with insulin release, but Krulich neither measured insulin concentrations nor tried the effect of vagotomy on the hypoglycaemic response. His results do suggest the presence of glucose receptors in the central nervous system (as does the work of many other authors) but they give no clue as to the nature of the efferent pathway, which could theoretically be via the pituitary, splanchnic nerves and adrenals or the vagus and pancreas. Chowers, Lavy & Halpern (1966) showed that insulin introduced into the cerebrospinal fluid of dogs did not enter the circulation, but that it did produce a fall in the glucose level of peripheral blood. Vagotomy significantly reduced this fall.

If there is a central mechanism which modifies insulin secretion by the pancreas, then localization of the sites of action of glucose or of insulin within the brain are likely eventually to solve the problem, but direct vagal stimulation offers answers that are less ambiguous. The parameters

of stimulation that we have used are probably not identical with the pattern of impulses passing down the vagus to the pancreas in the normal animal, but the existence of a neural mechanism for insulin release has been demonstrated.

Our failure to produce hypoglycaemia may be due to the mild stimuli we used, causing only a small release of insulin; earlier writers (e.g. Britton, 1925) produced hypoglycaemia, but only after stimulating the vagus for periods of up to 2 hr.

Whether the rise in insulin concentration in peripheral blood produced by vagal stimulation is brought about by changes in blood flow to the islets, or by a direct effect on the β cells, or by some other mechanism, is unknown—as is the role of such a mechanism in the homeostasis of the normal animal.

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REFERENCES

ANDERSON, E. & LONG, J. A. (1948). Hormonal influences on the secretion of insulin. In *Recent Prog. Horm. Res.* **2**, 209–227.

BRITTON, S. W. (1925). Studies on the conditions of activity in endocrine glands (XVII). The nervous control of insulin secretion. *Am. J. Physiol.* **74**, 291–308.

BROWN, J. C., HARPER, A. A. & SCRATCHERD, T. (1967). Potentiation of secretin stimulation of the pancreas. *J. Physiol.* **190**, 519–530.

CEGRELL, L., FALCK, B. & HELLMAN, B. (1964). Monoaminergic mechanisms in the endocrine pancreas. In *The structure and Metabolism of the Pancreatic Islets*, ed. BROLIN, S. E., HELLMAN, B. & KNUTSON, H., pp. 429–435. Oxford: Pergamon Press.

CHOWERS, I., LAVY, S. & HALPERN, L. (1966). Effect of insulin administered intracisternally on the glucose level of the blood and the cerebrospinal fluid in vagotomized dogs. *Expl Neurol.* **14**, 383–389.

CLARK, G. A. (1931). The influence of the vagus nerves on the secretions of insulin. *J. Physiol.* **73**, 297–304.

COUPLAND, R. E. (1958). The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. *J. Anat.* **92**, 143–149.

DE CASTRO, F. (1923). Contribution à la connaissance de l'innervation du pancréas. *Trav. lab. Rech. Biol. Madrid* **21**, 423–457.

GAYET, R. & GUILLAUMIE, M. (1930). Les réactions vasomotrices du pancréas étudiées par la mesure des débits sanguins. *C. r. Séanc. Soc. Biol.* **103**, 1106–1110.

GELLHORN, E. (1943). *Autonomic Regulations.* New York: Interscience Publishers.

GENTES, M. (1902). Note sur les terminaisons nerveuses des ilots de Langerhans du pancréas. *C. r. Séanc. Soc. Biol.* **54**, 202–203.

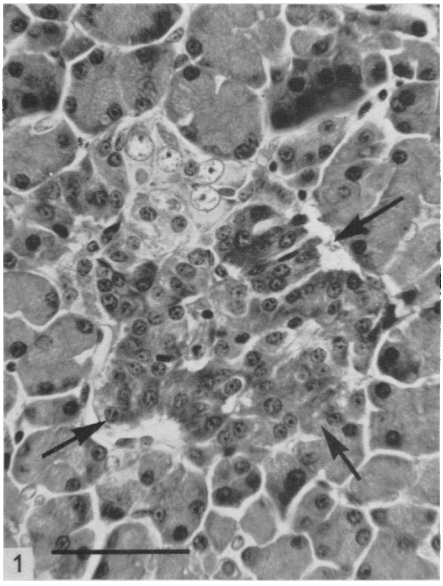
HALES, C. N. & RANDLE, P. J. (1963). Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**, 137–146.

KOELLE, G. B. & FRIEDENWALD, J. S. (1949). A histochemical method for localizing cholinesterase activity. *Proc. Soc. exp. Biol. Med.* **70**, 617–622.

KRULICH, L. (1961). Hypoglycaemic reaction to glucose infusion into the carotid artery. *Physiologia bohemoslov.* **10**, 393–401.

LA BARRE, J. (1927). Sur l'augmentation de la teneur en insuline du sang veineux pancréatique après excitation du nerf vague. *C. r. Séanc. Soc. Biol.* **96**, 193–196.

LANGERHANS, P. (1869). Contributions to the microscopic anatomy of the pancreas. M.D. Thesis, Berlin Translated by MORRISON, H., pp. 1–12. Baltimore: The Johns Hopkins Hospital Press, 1937.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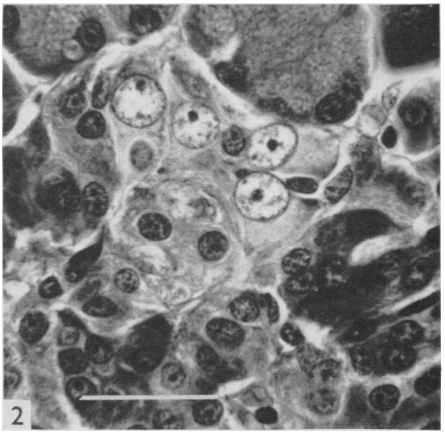
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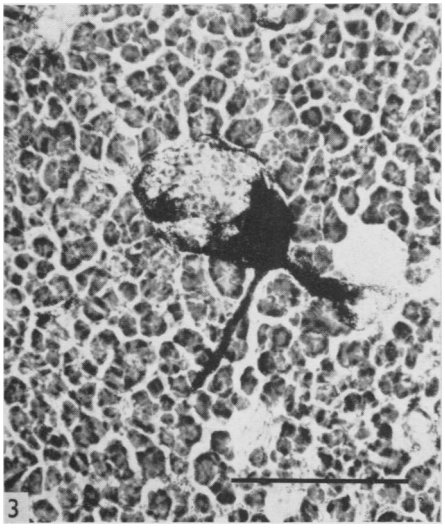
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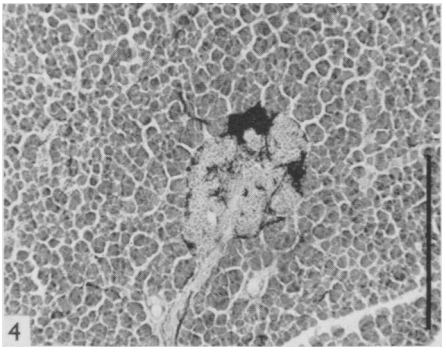
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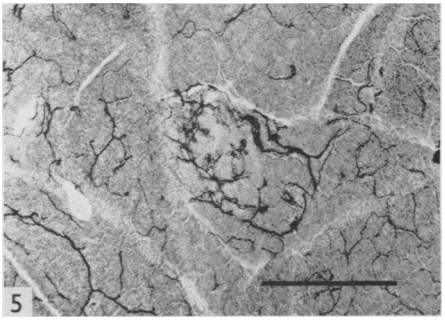
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RICHINS, C. A. (1945). The innervation of the pancreas. *J. comp. Neurol.* **83**, 223–236.

SIMARD, L. C. (1942). Le complexe neuro-insulaire du pancréas chez les mammifères adultes. *Revue can. Biol.* **1**, 2–49.

VAN CAMPENHOUT, E. (1927). Contributions à l'étude de l'histogenese du pancréas, chez quelques mammifères. Les complexes sympathico-insulaires. *Archs Biol., Paris* **35**, 121–171.

ZIERLER, K. L. & RABINOWITZ, D. (1964). Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action on potassium and free fatty acids without its effect on glucose. *J. clin. Invest.* **43**, 950–962.

ZUNZ, E. & LA BARRE, J. (1927). Sur la sensibilité des centres nerveux supérieurs à l'hyperglycémie provoquée par injection de dextrose. *C. r. Séanc. Soc. Biol.* **96**, 1400–1403.

EXPLANATION OF PLATE

Fig. 1. An islet of Langerhans (arrowed) from the pancreas of a baboon. Four big nerve cells with prominent nuclei and nucleoli may be seen at the top of the islet. Aldehyde fuchsin oil immersion. Bar is 50 μ.

Fig. 2. An enlargement of the four nerve cells in Fig. 1. They have what appears to be satellite cell nuclei around them. There are two α cells, with clear cytoplasm, immediately below. Darker β cells (aldehyde-fuchsin positive) can be seen throughout the lower half of the photograph. Bar is 25 μ.

Fig. 3. Section of a baboon's pancreas stained for cholinesterase and counterstained with haematoxylin. The clear oval area is an islet, the dark regions being strongly cholinergic areas corresponding with the groups of nerve cells seen in Figs. 1 and 2. These nerve cells contain mostly true cholinesterase. Substrate acetylthiocholine, 20 μ cryostat section. Bar is 200 μ.

Fig. 4. Section of a rhesus monkey pancreas showing one islet. Staining as in Fig. 3. 15 μ cryostat section. Bar is 300 μ.

Fig. 5. Section of a rat pancreas, showing one islet. Cholinergic nerve cell bodies are rare in the rat islet, but in this species there are many more fine nerves throughout the pancreas. These are especially numerous over the islets, as seen in this illustration. Acetylthiocholine substrate, 20 μ. Cryostat section. Bar is 300 μ.