THE INNERVATION OF PANCREAS OF THE RAT, CAT AND RABBIT AS REVEALED BY THE CHOLINESTERASE TECHNIQUE

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INTRODUCTION

The early works of Cajal (1891) and Müller (1892) demonstrated a rich innervation of the mammalian pancreas. Nerve fibres and multipolar cells were observed in association with blood vessels and acinar tissue, but no mention was made of the innervation of the islets of Langerhans. Later, however, Gentes (1902) and Pensa (1905) extended these findings and described a well-marked network of nerve fibres in association with islet tissue in the rat, cat and dog.

De Castro (1923) described in detail the innervation of the pancreas of the mouse, cat, dog and guinea-pig and confirmed and extended the observations of Gentes (1902) and Pensa (1905). De Castro observed both sympathetic and vagal fibres in the pancreas which formed a plexus along the walls of blood vessels and in association with the islets of Langerhans. Scattered groups of multipolar neurones were observed in the interlobular and intralobular connective tissue and occasionally in association with the islets of Langerhans.

Van Campenhout (1927) made a detailed study of the association between neurones and pancreatic islet tissue in a variety of mammals and introduced the concept of the 'neuro-insular' complex. This work was further extended by Simard (1942), who described three types of 'neuro-insular' complexes according to the relative numbers of the islet cells and associated neurones. De Castro (1923) considered that these neurones were postganglionic parasympathetic elements, whereas Simard (1942) referred to the whole complex as a 'metasympathetic' structure which he likened to the vagal and sympathetic paraganglia.

Earlier work relied mainly upon silver impregnation techniques for the demonstration of nerve fibres. It has recently been shown by Koelle & Friedenwald (1949), Koelle (1950, 1951, 1955), Couteaux (1951) and Coërs (1953) that nerve fibres can be revealed by the use of the cholinesterase technique, and the present work relates to the application of this histochemical method to the pancreas. The cholinesterase technique has advantages over metallic impregnation techniques in that thicker sections can be used, that reticular fibres usually give a negative reaction, and that the method gives readily reproducible results; it has an advantage over methylene-blue techniques in that fixed tissue may be used and hence better sections obtained.

METHODS

Adult rats (Sprague-Dawley strain), cats and rabbits have been used. The animals were killed by an intravenous or intraperitoneal injection of Nembutal and the pancreas removed immediately after death and fixed in cold 10% neutral formalin

or formol-saline for 4–18 hr. Frozen sections were cut at 30–40 μ, washed in saline for 30–45 min. and then mounted on slides and allowed to dry. Sections were incubated 4–48 hr. in a modification of the Koelle (1951) and Coërs (1953) substrates, which was made up of the following ingredients:

(1) 0·6 ml. copper-glycine (3·75 g. glycine, 2·5 g. $CuSO_4.5H_2O$, distilled water to 100 ml.).

(2) 0·6 ml. magnesium chloride (9·25 g. $MgCl_2$, distilled water to 100 ml.).

(3) 5 ml. N/5 acetate buffer, pH 5 or 5·6.

(4) 7·6 ml. 40% anhydrous sodium sulphate in water.

(5) 1·2 ml. acetyl thiocholine or butyryl thiocholine made up according to the method of Koelle (1951).

The sections were then rinsed in water, treated for 1–2 min. with freshly prepared dilute ammonium sulphide, rinsed in water, dehydrated and mounted in neutral balsam. Slides were allowed to dry at room temperature. Some sections were counterstained with 0·5% safranine. The optimal pH was found to vary with species. The most satisfactory demonstration of nerve fibres were obtained at pH 4·6–5 in the rat and cat and at pH 5·6 in the rabbit. The use of a higher pH resulted in a greater background reaction due to the presence of tissue esterases, while a lower pH resulted in an incomplete demonstration of the nerve net, which was then limited to the sites of maximal enzyme activity—probably to cholinergic types of fibres (Koelle, 1955). The presence of sodium sulphate resulted in the formation of a finer particulate deposit than did incubation in the absence of this substance; this therefore improves the localization of the method. The site of maximal cholinesterase activity was assessed by the use of a lower pH (rat and cat pH 4, rabbit pH 5) and by a reduction of the time of incubation.

Inhibitors were used in order to demonstrate true and pseudocholinesterase activity. Eserine was added to the incubating solution in a final concentration of 10^{-5} M. Some slides were immersed for 20 min. in a solution of DFP, 10^{-6} or 10^{-7} M in saline, prior to incubation. Eserine inhibited the activity of both true and pseudocholinesterase. DFP inhibited the activity of pseudocholinesterase. Koelle (1955) reported that DFP was not a satisfactory inhibitor of pseudocholinesterase in the rabbit; in the present work this statement has not been confirmed and DFP has been found to be an adequate inhibitor in all three species.

RESULTS

Rat

Acinar and islet cells give a negative reaction. Nerve fibres and neurones give a positive reaction which is due to the presence of both true and pseudocholinesterase. The smooth muscle cells in both ducts and large arteries give a positive reaction for pseudocholinesterase.

Nerve fibres are scattered throughout the gland. They enter along connective tissue septa as discrete bundles or as perivascular plexuses from which branches pass to the glandular tissue. Fine nerve fibres may be traced from both the nerve bundles and the perivascular plexuses to acinar and islet tissue. Nerve fibres form a nerve net between the acini which extends throughout each lobule. The most marked

collections of nerve fibres are, however, found in association with the islets of Langerhans (Pl. 1, fig. 1). This takes the form of a peri-insular plexus (Pl. 1, figs. 2, 5), from which fibres extend inwards along the intra-insular blood vessels and form a fine plexus about these structures; some may be traced between the islet cells. No discrete nerve endings have been observed. The fibres appear to form a terminal network which is intimately associated with small cells; these are usually observed at the site of junction of three or more strands of the nerve net and considered to be interstitial cells (Pl. 1, figs. 3, 4). Neurones are frequently observed in the interlobular connective tissue in association with the discrete nerve bundles and much less commonly in association with the islets of Langerhans (Pl. 1, fig. 5). In the latter situation they may be in contact with islet cells or a short distance away from these structures, but in both cases fibres may be traced from the neurones to the insular plexus.

Cat

Nerve fibres give a positive reaction for both true and pseudocholinesterase. Smooth muscle cells in the walls of arteries and the larger ducts give a positive reaction for pseudocholinesterase. The acinar and islet cells are negative.

The general distribution of nerve fibres resembles that observed in the rat. Nerve fibres enter the gland as discrete bundles and in the form of perivascular plexuses. A diffuse nerve net, without discrete nerve-endings, pervades the acinar tissue and is united to both the discrete nerve bundles and the perivascular fibres. The islets are more heavily innervated than is the acinar tissue (Pl. 1, fig. 6, Pl. 2, fig. 7) and in association with these structures nerve fibres form a peri-acinar plexus from which branches may be traced into the interior of the islet. A close association between intra-insular nerve fibres and blood vessels is not observed in the cat.

The acinar nerve net is made up of both fine and moderately coarse fibres (Pl. 2, fig. 8), which are occasionally associated with interstitial cells. Collections of nerve cells are frequently observed along the course of discrete nerve bundles which run in the connective tissue septa and rarely in proximity to islets of Langerhans.

In some sections thick nerve fibres may be traced to Pacinian corpuscles. The latter give a strong positive reaction for both true and pseudocholinesterase over the nerve filament and central core (Pl. 2, fig. 9), while the lamellar portion is entirely negative. Nerve fibres form a rich plexus in the walls of both blood vessels (Pl. 2, fig. 10) and the pancreatic duct (Pl. 2, figs. 11, 12).

Rabbit

Nerve fibres give a positive reaction for both true and pseudocholinesterase. The smooth muscle cells of the larger ducts and blood vessels give a positive reaction for pseudocholinesterase. The acinar cells are negative. The islet cells give a strong positive reaction for both true and pseudocholinesterase (Pl. 2, fig. 13).

The general distribution of nerve fibres resembles that in the rat and cat. Nerve nets exist in relation to both acinar and islet tissue. No discrete nerve endings are observed. Nerve fibres reach the islets of Langerhans by passing from the perivascular plexus of nearby vessels and directly from the acinar tissue (Pl. 2, fig. 14); they then run in an irregular fashion between the individual cells.

Site of maximum cholinesterase activity

It has been shown by Koelle (1955) that cholinergic nerve fibres give a stronger positive cholinesterase reaction than do either adrenergic or sensory nerve fibres; this was confirmed in the case of the superior cervical ganglion of the cat by Snell (1957). The site of maximal activity may be found by either short incubation in the substrate at the optimal pH for the species or by more prolonged incubation at a lower pH, i.e. in the case of the cat and rat at pH 4, in the rabbit pH 5. The application of both these methods in all three species in the present work reveals the strongest activity in the large bundles of fibres which enter the gland along connective tissue septa, and which are probably vagal in origin (De Castro, 1923) and in the collections of neurones which are associated with these fibres. A strong reaction is also given by some, but not all, fibres of the general nerve net which is associated with acinar tissue, by the peri-insular and central fibres of the islets of Langerhans and some perivascular fibres. The plexus in both islet and acinar tissue as revealed in this way is never as complete as that observed after incubation in conditions of optimal time and temperature. These findings would be in keeping with an adrenergic and cholinergic innervation of the pancreas, the adrenergic fibres only being revealed by incubation at optimal time and temperature, the more reactive cholinergic fibres being demonstrated at a lower pH or reduced incubation time. The fact, however, that the central core of the Pacinian corpuscle and its attendant nerve fibre also give a strong positive reaction at a reduced pH, indicates that cholinesterase exists in high concentration in nerves not usually regarded as being 'cholinergic' in type and hence the ability of the technique to distinguish between the various functional types of nerve fibres is in doubt.

DISCUSSION

The general arrangement of nerve fibres in the pancreas of the rat, cat and rabbit as revealed by the use of the cholinesterase method is similar to that described and drawn by De Castro (1923), who used a silver impregnation technique. Nerve fibres enter the gland along connective tissue septa as discrete bundles and in the form of perivascular plexuses. From these branches may be traced to both acinar and islet tissue, the latter being the most richly innervated. In the region of the islets of Langerhans nerve fibres may be traced from nearby perivascular plexuses, from the acinar nerve net and from discrete nerve bundles to the peri-insular region where they form a plexus. From this, fine fibres penetrate the islet passing between the cells. In the rat the intra-insular fibres are closely associated with the blood vessels and form a very fine perivascular plexus along these, in the cat and rabbit this association is not observed.

Neurones are frequently seen in association with nerve fibres and, as observed by De Castro (1923), usually lie in the connective tissue septa. Although these structures have also been observed in contact with or in close proximity to islets of Langerhans, this arrangement is relatively rare and Simard's (1942) type II and III neuro-insular complexes, in which large numbers of neurones are intimately associated with islet cells, have not been observed. Islets of Langerhans are only occasionally associated

with neurones, and from the present work there is no indication that these elements are any more than peripherally placed terminal parasympathetic neurones, as suggested by De Castro (1923); no evidence has been found which would support Simard's (1942) concept that they are part of a metasympathetic system. Incubation of sections at a lower than optimal pH demonstrates only part of the nerve net in both acinar and islet tissue; this may indicate that some fibres are adrenergic (weak reaction) and that some are cholinergic (strong reaction) as was suggested by De Castro (1923). Such an interpretation of the findings is, however, open to doubt as a result of the strong reaction given by Pacinian corpuscles and associated nerve fibres.

The islet cells of the rabbit give a strong positive reaction for both true and pseudocholinesterase. The significance of this is difficult to assess, but the finding is of interest when considered in conjunction with the rich innervation of the islet cells in all three species and with earlier work on the nervous control of the islets of Langerhans. Eppinger, Falta & Rudinger (1908) suggested the possibility of a vagal control over the islets of Langerhans; the subsequent works of De Corral (1918), Clark (1926, 1927, 1931), Britton (1925), Zunz & La Barre (1928), produced conflicting results, some indicating that stimulation of the right vagus increased the output of insulin, while others suggested that it had an inhibitory effect. All investigators were, however, in agreement that the sympathetic system had no direct effect on the islets.

SUMMARY

The nerve fibres of the pancreas may be revealed by the cholinesterase technique.

A general nerve net pervades the acinar tissue. Fibres are associated with occasional interstitial cells. No discrete nerve endings are observed.

The islets of Langerhans are associated with a peri-insular plexus from which fibres may be traced into the interior of the islet. In the rat the internal fibres are closely associated with blood vessels.

The islet cells of the rabbit give a strong positive reaction for both true and pseudocholinesterase.

Collections of neurones are occasionally associated with islets of Langerhans constituting the 'neuro-insular' complex. This association is, however, rarely observed, and the majority of neurones lie in the connective tissue septa along the course of nerve bundles which give a strong positive cholinesterase reaction.

A strong positive reaction for true and pseudocholinesterase has been observed in nerve fibres and neurones which are probably 'cholinergic' in type; a similarly intense reaction has, however, been observed in Pacinian corpuscles in the cat.

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EXPLANATIONS OF PLATES

PLATE 1

Tissues incubated 20 hr. in acetyl thiocholine. Rat and cat at pH 5, rabbit at pH 5·6.

Fig. 1. Rat pancreas. Fine nerve fibres may be seen in association with the acinar tissue and the islets of Langerhans. Distinct bundles of nerve fibres give a stronger reaction and are associated with swellings which are composed of groups of ganglion cells. ×40.

Fig. 2. Rat pancreas. Nerve fibres form perivascular plexuses and may be traced from these to the islets of Langerhans. In the vicinity of the islet a well-marked peri-insular plexus is formed while other fibres are associated with intra-insular blood vessels. ×100.

Fig. 3. Rat pancreas. Nerve fibres of two insular plexuses appear to be continuous. ×160.

Fig. 4. Enlargement of central region of Pl. 1, fig. 3, showing a cell (arrow) lying at the junction of four nerve fibres. ×400.

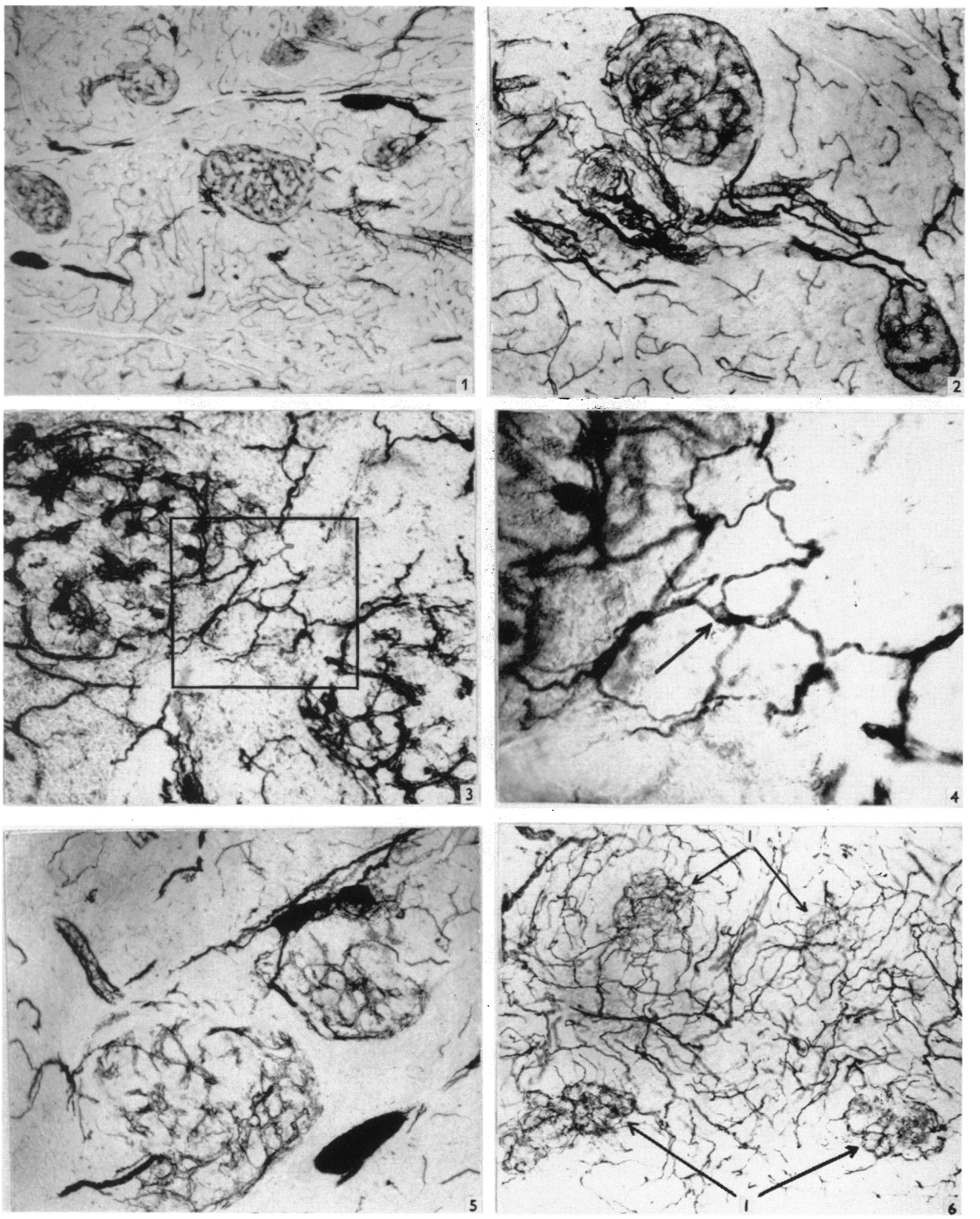
Fig. 5. Rat pancreas. The intense cholinesterase reaction adjacent to the upper islet overlies a group of neurones. Nerve fibres can be traced from this area to the insular plexus. ×90.

Fig. 6. Cat pancreas. Nerve fibres run through the acinar tissue and form a plexus in association with the islets of Langerhans (I). ×90.

PLATE 2

Fig. 7. Cat pancreas. Nerve plexus in association with an islet of Langerhans. ×180.

Fig. 8. Nerve fibres associated with the acinar tissue of the cat pancreas. ×400.



COUPLAND—INNERVATION OF PANCREAS OF THE RAT, CAT AND RABBIT

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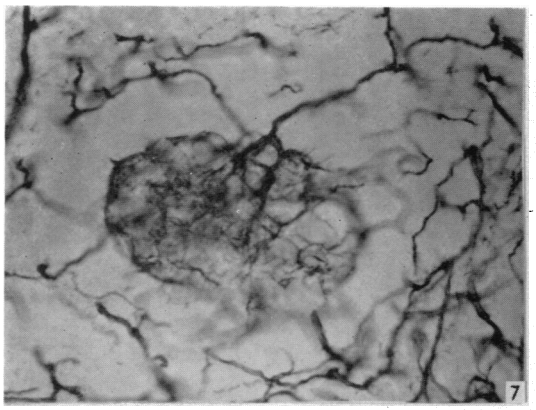
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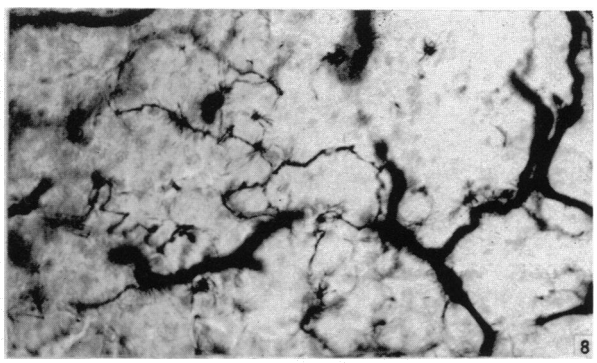
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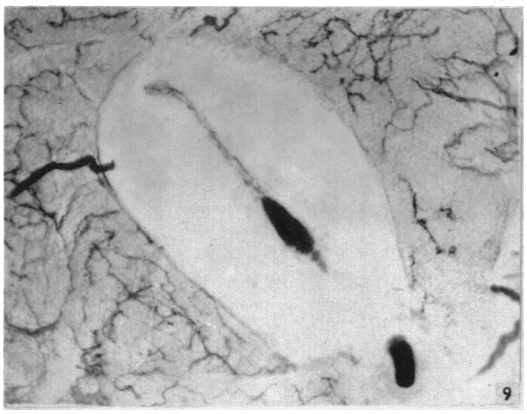
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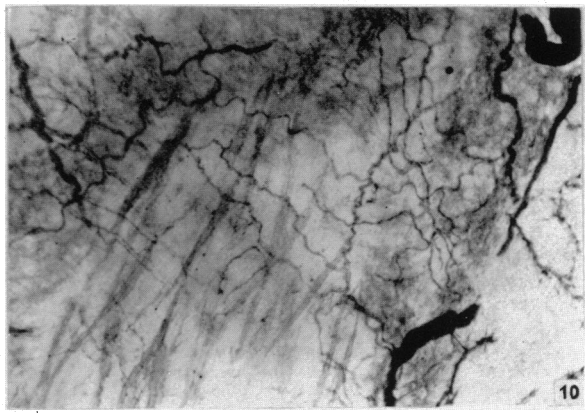
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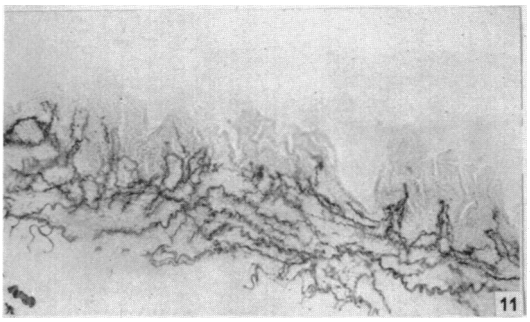
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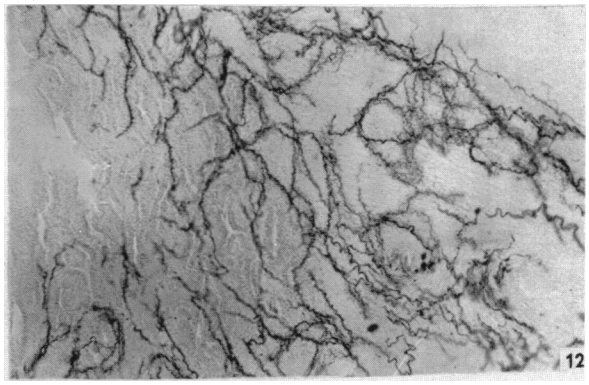
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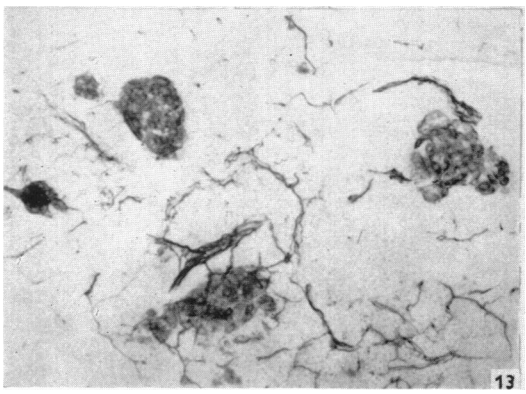
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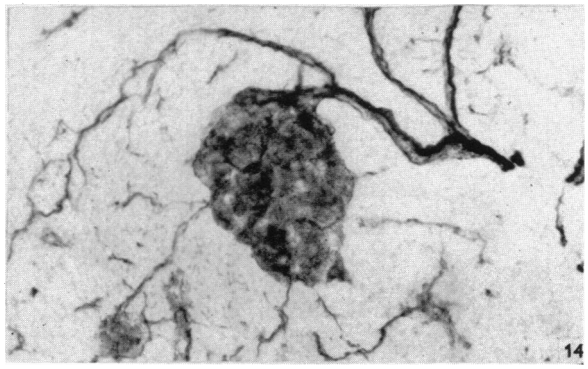
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Fig. 9. Pacinian corpuscle lying in the pancreas of a cat showing a positive reaction over the central core. Acinar tissue and associated nerve fibres surround the corpuscle. × 90.

Fig. 10. Cat pancreas. Nerve plexus lying in the wall of an artery. × 150.

Figs. 11, 12. Nerve plexus in the mucosa and submucosa of the main pancreatic duct of the cat viewed transversely (fig. 11) and in an oblique section (fig. 12). × 90.

Fig. 13. Rabbit pancreas. A positive cholinesterase reaction is given by both nerve fibres and by islet cells. × 70.

Fig. 14. Islet of Langerhans of a rabbit. Nerve fibres run to the islet from the vicinity of an adjacent blood vessel and from acinar tissue. Islet cells give a strong positive reaction. × 160.