head along the sutura saggitalis (1 cm) and siliceous acid (15 mg) was implanted s.c. Beginning at the time of implantation, at subsequent 8 h intervals the cross-circulation was arrested by an intestinal clamp while one member of each pair (acceptor-animal) was given an i.p. injection of unlabelled thymidine (0.01 mg/g body weight). Subsequently the other member (donor-animal) received a single dose of 1 μCi/g body weight ^{3}H-TdR (Amersham-Buchler, Braunschweig, specific activity: 5.0 Ci/mmol). 20 min later, the acceptor-animal received a further i.p. injection of unlabelled thymidine and then the clamp was removed. The animals were sacrified at 5 and 7 days post implantation by cardial perfusion of formalin. Embedding was made in paraffin. 5 μm sections of skin and subcutaneous granulation tissue, as well as of the small intestine, were coated with Ilford K2 and exposed for 14 days and for 21 days in darkness by + 4 °C. Some sections are stained by GÖMÖRI's silver impregnation technique for demonstration of reticulin fibres. The other sections are stained after the exposition by hematoxylin and eosin (HE).

While among the epithelia of the small intestine of the acceptor-animal no labelled cell – with the exception of rare intravasal blood cells – could be observed, nearly each epithelial cell of the donor-animal was labelled by ^{3}H-TdR (Figure 1 a, b). This examination was made to control whether labelled thymidine had left the donor animal by cross-circulation inspite of the clamp and the injection of nonlabelled thymidine.

Within the subcutaneous granulation tissues of the acceptor-animals, very many labelled mononuclear and polymorphnuclear cells could be seen. At the border of the granulation tissues, a net of reticulin fibres in combination with very many fibroblasts spread out. There, mononuclear cells could be observed which were found to be labelled up to 20–30%. Moreover fibroblasts could be seen which clearly showed reduced silver grains above the nucleus (Figure 1c). Such fibroblasts were seen up to 2–4%. On the sections which were stained by silver-impregnation before the autoradiographical process, some labelled fibroblasts could be identified, too.

Obviously Ross et al. could not find labelled fibroblasts because these authors made injection-procedures only once within 24 h. Moreover the number of labelled fibroblasts is too small when using electronmicroscopic autoradiographic methods. Only by accident could a labelled fibroblast have been seen. Further considerations are impossible because of the lack of distinct information about the dose of ^{3}H-TdR and about the exposition time of the sections for light microscopy.

In conclusion we have to state that by puls-labelling of non-parabiotic rats with ^{3}H-TdR after subcutaneous implantation of siliceous acid, nearly 25% of fibroblasts could be found labelled on the 5th and 7th day after operation. That means: the increase of fibroblasts will be mainly caused by local proliferation. Only additionally a transformation of mononuclear blood cells into fibroblasts can be observed.

Zusammenfassung. Es wird bei parabiotischen Ratten das Granulationsgewebe nach subcutaner Kieselsäureimplantation untersucht. Durch Bestrahlung der Hinterläufe einer der beiden parabiotischen Ratten erfolgt deren Blutversorgung über die Parabiosenaht zum Teil durch die andere Ratte; dieser anderen Ratte war zur Markierung der Leukozyten ^{3}H-Thymidin injiziert. Im Granulationsgewebe der bestrahlten Ratte finden sich typische Fibroblasten, deren Markierung auf ihren hämatogenen Ursprung hinweist. Die Pulsmarkierung bei Einzelratten nach Kieselsäure-Implantation ergab ferner eine DNS-Synthese der ortsständigen Fibroblasten als Hinweis für eine lokale Proliferation.

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Smooth Muscle of the Pancreatic Duct of the Cat and its Innervation

EBERTH[1] described the presence of a smooth muscle layer around the pancreatic duct of the cat in 1863 but it has received little attention since. Recent experiments by LENNINGER[2] have shown that vagal stimulation or parathympathomimetic drugs increase the resistance to perfusion flow in the pancreatic duct of the cat. Further studies showed that segments of the main pancreatic duct exhibit spontaneous contractions in vitro and the tension induced could be increased by certain drugs[3].

It was therefore decided to examine the pancreatic duct of the cat by histology, histochemistry and electron microscopy to assess the arrangement and innervation of any smooth muscle that may be present.

Methods. The principal ducts from the head and tail of the pancreas have been removed from 16 cats. 3 ducts were fixed whole in 4% formaldehyde in 0.08 *M* cacodylate buffer containing 7.5% sucrose. Blocks of tissue were embedded in paraffin and sections were cut from all areas of the ducts and stained by Lissamine Fast Red and tartrazine[4] to show smooth muscle. 3 other ducts were fixed by a formaldehyde-glutaraldehyde mixture[5], post-osmicated, embedded in araldite and ultrathin sections from many areas were examined electron microscopically after staining with lead citrate. From the remaining animals small segments from different parts of the duct were rapidly frozen in iso-pentane in liquid nitrogen. Half these blocks were freeze dried, treated with formaldehyde vapour and sections were examined for formaldehyde induced fluorescence. The adjacent blocks were used for demonstrating acetylcholinesterase (AChE) activity. Cryostat sections were postfixed in the formaldehyde-sucrose mixture and incubated with substrate[6] in the presence of iso-OMPA 3×10^{-6} *M*.

Results. The paraffin sections confirmed the presence of smooth muscle around the duct (Figure 1). The lining of smooth muscle surrounded the entire length of the main duct of both the head and the tail of the pancreas. A layer of lax connective tissue separated the muscle layer from the epithelium of the duct. The muscle coat

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was composed of a number of poorly defined separate bundles of smooth muscle cells, which appeared to accompany the duct in longitudinally directed spirals. Large main branches of the duct appeared to take a muscle layer with them but the majority of ducts branching off the main duct simply passed between the muscle bundles and were unaccompanied by muscle cells.

Cholinesterase staining showed that AChE-positive nerves were associated with the ductal smooth muscle throughout its length, with possibly an increase in their number towards the duodenal end. No adrenergic nerves were detected in association with the ductal smooth muscle. Infrequent fluorescing nerves were seen accompanying some blood vessels by the duct.

Electron microscopy supported the light microscopical description of the ductal smooth muscle. It showed that not only were the bundles ill defined but, within the bundles, the muscle cells were loosely arranged and interspersed with connective tissue. Nexus es were present between groups of muscle cells within the bundles and one gained the impression that they were forming myogenic units. Non-myelinated axons in Schwann-axon bundles were present in the muscle layer; some appeared to be passing between bundles others were present within bundles. The number of nerves was never very extensive but probable neuro-effector sites[7] were readily found. These consisted of vesicle-containing axons in close proximity to smooth muscle cells (Figure 2). The vesicles in the axons were of the agranular small vesicle type plus some larger dense-cored vesicles, which supports the idea that most if not all of these nerves were cholinergic. Occasional myelinated axons were seen passing between the muscle layer or in the connective tissue between it and the ductal epithelium. These were probably afferent fibres but their terminal arrangements have not been ascertained.

Discussion. The present study has confirmed the presence of a muscle layer around the principal pancreatic ducts of the cat. The evidence indicates that it has a cholinergic innervation, which supports the findings of the in-vivo and in-vitro physiological studies aforemen-

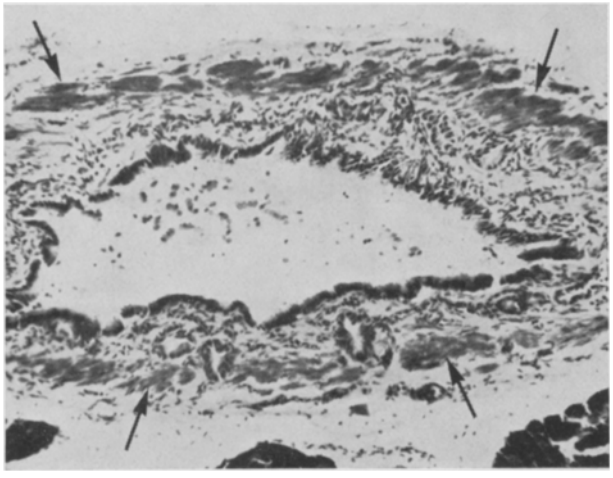

Fig. 1. Main pancreatic duct showing irregular bundles of smooth muscle cells (↑) arranged around the periphery of the duct and separated from the ductal epithelium by thick layer of lax connective tissue (Lendrum's stain[4]), ×112.

[6] G. Gomori, *Microscopic histochemistry* (Chicago Univ. Press, Chicago 1952).

[7] G. Burnstock, in *Smooth Muscle* (Edward Arnold, London 1970), p. 1.

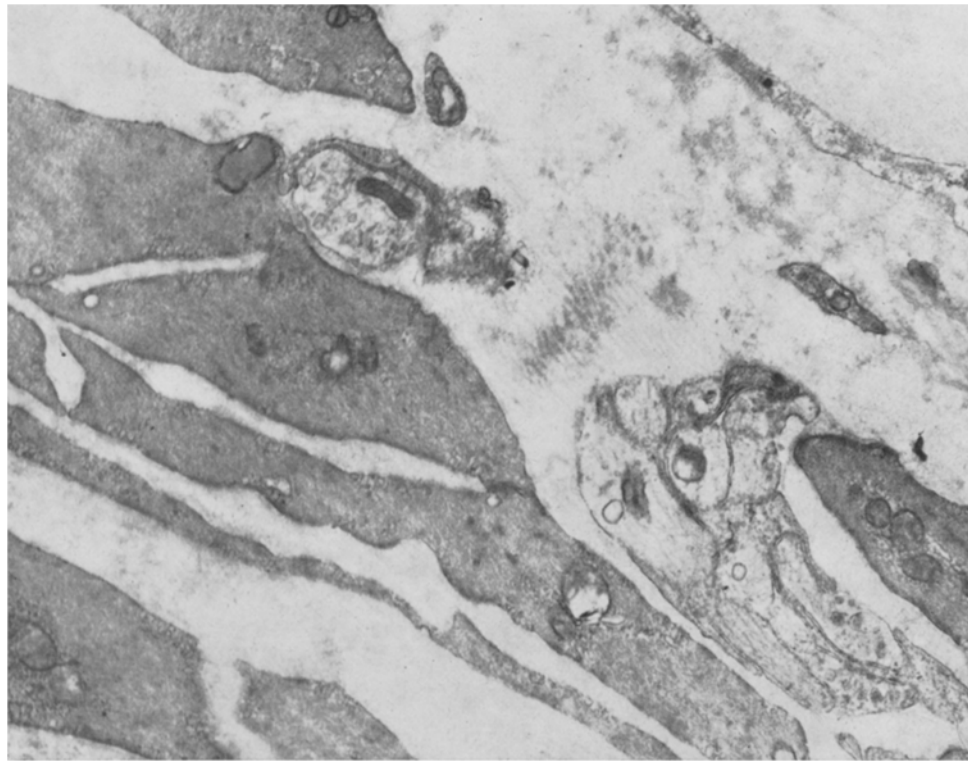

Fig. 2. Electron micrograph of main pancreatic duct showing axons, containing vesicles, in close proximity to smooth muscle cells. Nexus es are evident between the muscle cells, ×19,000.

tioned[2,3]. To this should be added that EKSTRÖM and LENNINGER[8] have shown that there is choline acetyltransferase in the ductal tissues. The cholinergic nerves probably induce contraction of the muscle layer. The perfusion studies indicated that nerve-induced luminal narrowing may impede flow but it is not known whether this occurs under physiological conditions. It is thought unlikely that under normal conditions a big back pressure against secretion would be induced by the muscle contractions, but this may occur under pathological influences. The function of the muscle layer is obscure. In vitro the muscle exhibits rhythmical activity which was considered to be myogenic[3]. If such activity occurs in vivo it may have a milking effect on the contents. But what of nerve induced contractions? These possibly arise in response to reflex stimuli and the effect may be to impart a greater rigidity to the duct. Furthermore a strong contraction may help to hinder a sudden rise of pressure in the main duct from passing out to the parenchyma, for it seems likely that the smaller branch ducts will be occluded to some extent by a strong contraction of the muscle layer.

Zusammenfassung. In der Wandung des *Ductus pancreaticus major* der Katze wird eine dünne Schicht von Muskelfaserzügen beschrieben. Histochemisch wird festgestellt, dass es sich bei fehlender adrenergischer um cholinerge Innervation dieser Muskelzellen handelt.

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The Institute of Anatomy and Histology, Lund, and the Institute of Physiology, Lund (Sweden), 29 January 1973.

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[9] J. R. GARRETT, wishes to express his gratitude to the Royal Society for travel grants that made this work possible.

Zinc Iodide-Osmium Tetroxide Reactive Substances in the Matrix of Granulated Vesicles in the Rat Pineal Nerves

In different works[1-3] it has been demonstrated that the mixture of zinc iodide-osmium tetroxide (ZIO) stains different types of synaptic vesicles. In the granulated vesicles of the pineal nerves, this technique reveals two components[4]: the central core and the matrix, which comprises the space between the vesicle membrane and the core. The matrix is more intensively stained than the core.

Studies made in our laboratory[5] demonstrated that ZIO reaction is temperature and time dependent. An increasing number of synaptic vesicles and other subcellular components appear positive when the temperature and time are increased. Granulated vesicles of different monoaminergic nerves do not react in the same way at a given schedule of fixation. Thus, when ZIO is used at 4 °C for 2 h (ZIO 4–2), the matrix of the granulated vesicles of the pineal nerves appears electron dense, whereas that of the granulated vesicles of the vas deferens nerves remains electron lucent[2]. If the fixation is done at 20 °C for 15 h (ZIO 20–15), the matrix of the granulated vesicles of the vas deferens nerves is also deeply stained[6]. The granulated vesicles of the pineal nerves have the same appearance with ZIO 20–15 as with ZIO 4–2. However the findings related to the vas deferens nerves suggested us that ZIO 4–2 and ZIO 20–15 could reveal different components of the matrix of granulated vesicles of the pineal nerves.

This posibility was explored by fixing with ZIO 4–2 and ZIO 20–15 pineal glands of rats treated with reserpine or *p*-chlorophenylalanine (*p*-CPA). Reserpine was administered i.p., at the dose of 10 mg/kg and the animals killed 4 h after the injection. Rats treated with *p*-CPA were given 2 × 300 mg/kg i.p. separated by a 48-h interval. They were killed 24 h after the last injection. Control rats received equivalent volumes of saline in both treatments.

ZIO 4–2 (Figure 1) reaction in the control pineal nerves of the rat has already been described[2]. Most of the vesicles show a heavily stained matrix. In a small proportion of vesicles the matrix is unstained and in them a dense core can be distinguished. With ZIO 20–15 (Figure 2) the appearance of synaptic vesicles is similar to that obtained after ZIO 4–2, but there is a greater reaction in the mitochondria and the cytoplasm.

In the glands treated with reserpine, the matrix and the core of small granulated vesicles become negative with ZIO 4–2, as shown previously[2]. With ZIO 20–15 (Figure 4) the matrix of a small number of vesicles remains but most of the vesicles are depleted. The membrane of the vesicles unstained is barely visible. As previously described[7] *p*-CPA depletes ZIO 4–2 reactive substances in the matrix and partially in the core (Figure 5). On the contrary, substances reacting with ZIO 20–15 (Figure 6) are not depleted by *p*-CPA and the aspect of nerve terminals is similar to that of the controls.

The finding here described confirms that *p*-CPA depletes ZIO 4–2 reactive material in the matrix of the granulated vesicles of the pineal gland of the rat and demonstrates that this drug does not affect ZIO 20–15 reactive material. On the contrary reserpine depletes both of them.

It is interesting that, while reserpine depletes catechol and indoleamines, *p*-CPA specifically inhibits the synthesis of serotonin in the pineal nerves[8,9]. ZIO 4–2 reactive material is also depleted by tyramine and oxypertine. It has been demonstrated with a histochemical approach that tyramine also depletes serotonin in the pineal

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