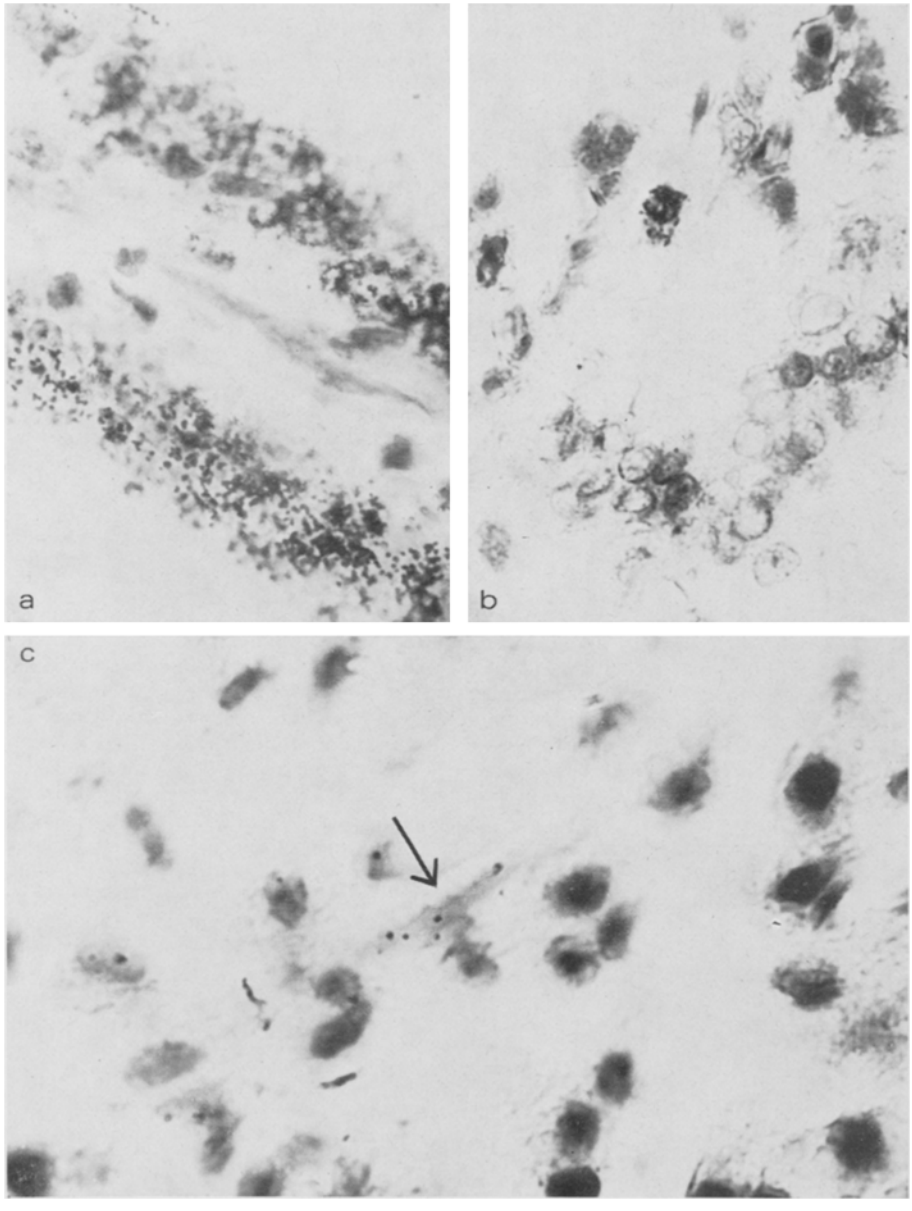
Demonstration of Hematogenous Origin of Fibroblasts by Parabiosis

Ross et al.[1] stated that all fibroblasts in healing wounds of skin arise from native connective tissue cells, not immigrant leucocytes. This was deduced from experiments in which ^{3}H-thymidine (^{3}H-TdR) was given to one partner of an irradiated parabiotic pair of rats dependent for their marrow function and survival on the shielded femur of the animal that was later injected. Labelled leucocytes were found in skin wounds of both but labelled fibroblasts only in the animal exposed to ^{3}H-TdR. This finding is in constrast to the results of other authors[2,3]. This difference has to be explained, especially because the model of irradiated parabioses was sucessfully used to obtain evidence of the hematogen origin of macrophages by VOLKMAN and GOWANS[4] and of microglia and pericytes by OEHMICHEN et al.[5].

8 New Zealand inbred rats (breed: A S 2-Max Planck Institute of Immunobiology, Freiburg, W.-Germany) – approximately 100 g each – were joined in pairs and placed in a parabiosis cage by the method of GRÜNINGER and OEHMICHEN[6]. 10 days after surgery, when the cross-circulation was established, 800 R of irradiation from a cobalt 60-machine were given to the femurs of 1 animal (acceptor-animal) while the rest of the bodies of the pairs was shielded by a lead-block which was 10 cm high. This dose has previously been shown to be sufficient to destroy essentially all of the hemopoietic tissues within the femurs[7]. 3 days after irradiation a skin incision was made on the

1 R. Ross, N. B. EVERETT and R. TYLER, J. Cell. Biol. *44*, 645 (1970).
2 L.-D. LEDER, *Der Blutmonocyt* (Springer, Berlin-Heidelberg-New York 1967).
3 R. VAN FURTH, *Mononuclear Phagocytes* (Blackwell Scientific Publications, Oxford – Edinburgh 1970).
4 A. VOLKMAN and J. L. GOWANS, Br. J. exp. Path. *46*, 50 (1965).
5 M. OEHMICHEN, H. GRÜNINGER, R. SAEBISCH and Y. NARITA, Acta neuropath. (Berl.) *23*, 200 (1973).
6 H. GRÜNINGER and M. OEHMICHEN, Expl Path. *6*, 343 (1972).
7 R. W. C. TYLER and N. B. EVERETT, Blood *28*, 873 (1966).



^{3}H-thymidine injection into one of a pair of irradiated parabiotic rats: Small intestine of the shielded (a) and not-shielded (b) parabiont; a labelled fibroblast (c) within the granulation tissue of the not-shielded parabiont. HE, a, b: ×600; c: ×800.

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

[1] C. J. EBERTH, Z. wiss. Zool. *12*, 360 (1863).
[2] S. LENNINGER, Acta physiol. scand. *82*, 345 (1971).
[3] S. LENNINGER, Acta physiol. scand. *84*, 134 (1972).
[4] A. C. LENDRUM, in *Recent advances in clinical Pathology* (Churchill, London 1947), chapt. 41, p. 452.
[5] M. J. KARNOVSKY, J. Cell Biol. *24*, 137A (1965).