Dünnschichtplatte sind unter UV-Licht 0,05 µg Protoporphyrin-ME und mit *o*-Tolidinreagens 0,001 µg Hämin-ME noch gut erkennbar.

(4) Überführung von Eisenprotoporphyrin-di-ME in Protoporphyrin-di-ME: Die Eisensulfatmethode[15] ist auch auf die Hämin-ME anwendbar, wobei die Lösung der Hämin-ME und die Extraktion der Protoporphyrin-ME in Chloroform erfolgt. Die Methylesterbindung wird während der kurzen Säureeinwirkung (10 min) nicht oder nur in geringem Umfang angegriffen.

Resultate. Die gesamte X-Faktoraktivität des Kulturüberstandes von *A. metalcaligenes* wird durch LKB-Ultrafiltration in der makromolekularen Fraktion (Molekulargewicht >30000) konzentriert. Die auf Sephadex-Säulen raschlaufenden, biologisch aktiven Fraktionen des Überstandes von Kulturen im synthetischen Medium weisen eine schwache, die später eluierten, biologisch inaktiven Fraktionen zum Teil starke Rotfluoreszenz auf.

Die dünnschichtchromatographische Trennung der aus dem lyophilisierten Rückstand hergestellten Porphyrin-Methylester zeigte, dass nur die biologisch aktive Fraktion ein Dicarboxylporphyrin sowie wechselnde Mengen einer peroxydase-positiven Substanz enthält, die biologisch inaktiven Fraktionen dagegen relativ grosse Mengen von Uroporphyrin führen. Die Porphyrine und die peroxydase-positive Substanz können auch durch Ausschütteln mit Äthylacetat-Essigsäure 4:1 (V/V) leicht aus ihrer Assoziation mit dem makromolekularen Anteil gelöst werden. Der Porphyrin-Methylester aus der aktiven Fraktion konnte aufgrund des chromatographischen Verhaltens (Figur 1) und der Absorptionsmaxima (Figur 2)[17] mit Protoporphyrin-IX-dimethylester identifiziert werden. Die peroxydase-positive Substanz verhielt sich im Chromatogramm und spektrophotometrisch wie Eisenprotoporphyrinmethylester; das daraus gewonnene metallfreie Produkt erwies sich ebenfalls als Protoporphyrindimethylester.

Die Darstellung der Protoporphyrindimethylester (Veresterung, Extraktion, Chromatographie, Elution) wurde quantitativ überprüft; es ergab sich eine Ausbeute von 89%. Die im biologischen Test ermittelten Werte für Protoporphyrin und Protohämin korrelieren mit den aus dem aktiven Material durchgeführten Analysen (Tabelle). Daraus ist die Folgerung berechtigt, dass der Protoporphyrin- und Eisenprotoporphyrin-Gehalt des Präparats für die gesamte biologische Aktivität aufkommt.

Die entsprechende Aufarbeitung eines Peptonkulturfiltrats von *A. metalcaligenes* ergab eine biologisch aktive, makromolekulare Fraktion, die Protoporphyrin, neben Spuren von Uroporphyrin und Hämin, enthielt. Da der Hämin-Gehalt des aus dem komplexen Medium gewonnenen Präparats vernachlässigt werden kann, ist Protoporphyrin – oder seine reduzierte, nichtfluoreszierende Vorstufe, Protoporphyrinogen (cf. [5,7,8]) – als die physiologische Ausscheidungsform des aktiven Prinzips zu betrachten.

Summary. A macromolecular fraction from the culture fluid of *Achromobacter metalcaligenes* showing the activity of the Haemophilus-X factor was investigated for its content of porphyrin derivatives. Porphyrin methylesters were directly prepared from the lyophilized material and then separated by thin layer chromatography. Protoporphyrin and iron protoporphyrin could be isolated in amounts which correlated with the growth-promoting activity for *Haemophilus influenzae.*

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Autoradiographic Study on Incorporation of Leucine-H^3 in Peripheral Nerves During Regeneration

The proximal stumps of regenerating nerves show various characteristic changes. Apart from swelling, the axons show an increased activity of oxidative and hydrolytic enzymes[1–5]. The morphological substrate of these histochemical changes, on the electron microscopic level, is an impressive accumulation of mitochondria, lysosomes, vesicles and tubular structures[6–11]. The massive occurrence of these organelles and structures raises the question as to whether they are transported by axoplasmic flow mechanisms or arise from local processes[9,12,13]. A local build up of these structures, if it is possible, would be only by a high local protein synthesis. Such an hypothesis can be tested by fine resolution autoradiography using leucine-H^3 as a precursor of protein synthesis, and allowing very short survival time after injection of the radiochemical in order to eliminate the possibility of axoplasmic flow. The purpose of this investigation was to localize the site of protein synthesis in regenerating nerves and to evaluate differentially such a protein metabolism.

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In 8 young adult albino rats the right sciatic nerves were transected. The animals were sacrificed 14 and 26 h, 2, 3, 4, 7, 8 and 12 days after the operation. Each animal was injected i.p. with 2 mC of H^3-DL-leucine (specific activity 2.0 C/m*M*) 2 h before sacrifice. Under ether anaesthesia the proximal and distal stumps and the intact nerve from the control side were removed, fixed in formalin, and embedded in paraplast. Sections were cut 6 μ thick. The autoradiographic procedure was followed according to ALTMAN[14]. After the sections were deparaffinized and dried overnight, they were dipped in Kodak emulsion NTB3, packed in light-proof boxes, and stored at 4 °C for an exposure period of 8 weeks. The sections were then developed by usual procedure and post-stained with H & E.

Results. In normal sciatic nerves, leucine was incorporated only in the cytoplasma of Schwann cells, mesenchymal cells and in the cells of vessels. No label was detected in the myelin sheaths. In axons, grains were demonstrated only very rarely. In the *initial state of regeneration* 14, 26 and 48 h after operation, the proximal stumps showed typical torpedo-like enlargement of the axons, proliferation of Schwann cells and invasion of leucocytes and mesenchymal macrophages. Silver grains indicating incorporation of the radiochemical into proteins were detected in the perikaryon of the Schwann cells as well as in leucocytes and mesenchymal cells. The axonal enlargements show only very little label, essentially not more than that found in normal axons (Figure). In later stages of regeneration (3, 4 days) Schwann cells showed an increasing amount of activity. Their nuclei as well as their sheaths had a high degree of labelling. The axonal stumps, though swollen, presented very low incorporation of the radiochemical and in this respect differed very little from the control.

Proximal stump of transected sciatic nerve, 26 h after operation. Label is concentrated close to Schwann cell nuclei and the sheaths. Axons (darkly stained) show no radioactivity. The typical axonal enlargement (arrow) shows only few grains. H & E, × 350.

After 7, 8 and 12 days following the nerve transection, the proximal stumps showed a neuroma-like feltwork of proliferated thin axons between numerous Schwann cells. Exact localization of the labelling at this stage was not possible because in H & E preparations very thin fibres could not be discriminated with certainty from the densely packed proliferated Schwann cells. So far as resolution permitted, grains were localized mainly close to Schwann cell nuclei. The density of labelling in the Schwann cells of the regenerating stump was generally much higher than on the control side. However, only 1 cm above the point of cutting, the Schwann cells showed no striking increase in grain concentration.

Discussion. The distribution of the incorporated radiochemical demonstrated in our material shows Schwann cells as the main site of protein synthesis in a peripheral nerve during regeneration. Axonal swellings in the stumps show only a very weak incorporation of radioactive leucine. It is in the same range as in normal axons. The low level of incorporation of the radioleucine in the regenerating axons does not support the hypothesis of increase in local protein synthesis. From this we must assume that most of the subcellular elements which are so numerously accumulated in the proximal stumps of the axons are either transported by a proximo-distal flow mechanism in the axoplasma[1,2,15], or they are derived by transformation of pre-existent structures. The latter is discussed especially for endoplasmatic reticulum-like structures[11].

Schwann cells, on the other hand, show an increased rate of leucine incorporation, during early as well as later stages of regeneration. The importance of their role in regeneration has often been stressed. Recent electron microscopic studies give evidence for their participation in forming also the axoplasmic membranes of regenerating axonal sprouts[16]. Summarizing our results, we suggest Schwann cells to be the most important site for the increased metabolic processes during regeneration. We consider the accumulation of organelles in the proximal stump as well as in the distal stump to be more an accidental by-product of flow-mechanism rather than a meaningful mechanism in order to establish a supporting metabolic apparatus for the regenerating nerves.

Zusammenfassung. Durch Gabe von radioaktivem Leucin wurde festgestellt, dass im regenerierenden peripheren Nerven die Proteinsynthese in den Schwannschen Zellen deutlich erhöht ist. Verglichen damit ist die Eiweißsynthese in den Axonstümpfen, von denen die Regeneration ausgeht, geringfügig.

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