

sue cells capable of reproduction than monocytes. We still find that 50% of the final fibrocytic population in our diffusion chambers is of monocytic origin.

In actual repair or inflammation, a continuous influx of hematogenous cells is likely to occur, whereas our 'model granulation tissue' only results from one initial cellular inoculum. Where no membrane hinders immigration of new monocytes, their participation in the final fibrocytic population should exceed that observed in our diffusion chambers.

The observation that fibrocytes did not grow if explanted alone in diffusion chambers is surprising and quite at variance with observations *in vitro*. The fact that infiltrating or added leucocytes induce abundant growth of the same tissue leads us to similar conclusions as CARREL¹⁶. On the basis of observations made on cultures *in vitro*, he suggested that leucocytes contain growth promoting substances called 'trephones', stimulating fibrocytic reproduction.

It was astonishing, anyhow, that the growth-promoting action of leucocytes surrounding the chamber was not transmitted through the Millipore filters with its pore-size of 0.45 μ . One has to assume, therefore, that any growth-promoting substance was present only in very low concentration and only active in cell-to-cell contact, or that its particles were of too large a size to pass through the pores of the filter. In this relationship consideration might be given to the observations of DUMONT⁸ on the reutilization of leucocytic DNA by proliferating cells in areas of inflammation. The growth-stimulating properties of embryonic extract, on the other hand, have been found in a nucleoprotein fraction (KUTSKY and FEICHTMEIR¹⁷). One might assume, therefore, that the growth-promoting action of leucocytes is associated with reutilized leucocytic DNA transferred in larger particles.

One might argue that *in vivo* blood vessels will contribute a substantial part of the perivascular fibrocytes. This element is not represented in our combined cultures. In earlier comparative tissue culture studies (ALLGÖWER¹), cultures of adult small blood vessels did not show any better growth than ordinary connective tissue. Further-

more, explants harbouring capillaries show no substantial difference in outgrowth from cultures taken in an avascular area.

On the basis of our investigations, we can conclude that the contribution of hematogenous monocytes to fibrocytic repair is considerable and appears to be in the order of magnitude of 50% or more of the final fibrocytic population¹⁸.

Zusammenfassung. Monocyten des strömenden Blutes können *in vitro* die morphologischen und funktionellen (Hydroxyprolinbildung) Kriterien von Fibrocyten erwerben. Die quantitative Bedeutung dieses Phänomens für den reparativen Bindegewebsaufbau wurde mit folgender Versuchsanordnung geprüft: Vergleichbare Inokulate von Monocyten (enthalten in der Leucocytenhaut des zentrifugierten Blutes) und von Fibrocyten (aus subcutanem Bindegewebe) von Kaninchen verschiedenen Geschlechts, wurden in Millipore-Kammern eingeschlossen und für 14–21 Tage in das Abdomen eines Kaninchens implantiert. Die Auszählung des Sex-Chromatins in den fibrocytären Kernen am Ende der Zuchtungsperiode ergibt, dass ca. 50% monocytären Ursprunges sind. Es wird gefolgert, dass dieses Phänomen bei der eigentlichen Wundheilung noch bedeutungsvoller sein dürfte, da der Zustrom teilungsfähiger Monocyten in einem Wundgebiet kontinuierlich vor sich geht und nicht auf ein initiales Inokulum beschränkt ist.

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¹⁶ A. CARREL, *J. exp. Med.* 36, 385 (1922).

¹⁷ R. J. KUTSKY and T. V. FEICHTMEIR, *Nature* 194, 1050 (1962).

¹⁸ *Acknowledgment.* This work was supported by a grant of the Schweiz. Nationalfonds.

Monoamines in Sympathetic Ganglia Studied with Fluorescence Microscopy

Biochemical and pharmacological studies have furnished evidence that the adrenergic transmitter, nor-adrenaline, is present in the sympathetic nervous system, not only in the peripheral terminals, but also in the cell bodies of the adrenergic postganglionic neuron¹. Hitherto, however, the cellular distribution of this monoamine in the adrenergic neuron has not been known. Recently, a fluorescence method has been developed, which makes it possible to study the localization of monoamines at the cellular level^{2–4}. Under different conditions, the content and localization of catecholamines in peripheral sympathetic neurons and the action of drugs on these neurons are now being investigated by this method. Some results are reported here.

Sympathetic ganglia of male albino rats were freeze-dried, treated with formaldehyde gas, embedded in paraffin, sectioned and the sections mounted for fluorescence microscopy according to FALCK³.

By the formaldehyde treatment a specific green to yellow-green fluorescence developed in the perikarya of the majority of the ganglion cells, whereas the nucleus

was non-fluorescent. The fluorescence was rather intense in a minority of the cells, whereas it was of medium or low intensity in most of them. The larger processes of the nerve cells exhibited a very faint fluorescence. A small number of cells were completely devoid of this type of fluorescence. These cells may be cholinergic⁵. In some prevertebral ganglia, adrenergic nerve terminals (FALCK³) with typical varicosities were observed. These terminals are not related to the blood vessels. Sometimes the fibres were seen to terminate in close contact with nerve cell bodies; and thus, in all probability, they represent synaptic terminals. In a study of the sympathetic ganglia of the cat, very numerous adrenergic terminals were observed in the inferior mesenteric ganglion⁵.

¹ U. S. v. EULER, *Noradrenaline* (Ch. C. Thomas Springfield, Ill. 1956).

² A. CARLSSON, B. FALCK, and N.-Å. HILLARP, *Acta physiol. scand.* 56, Suppl. 196 (1962).

³ B. FALCK, *Acta physiol. scand.* 56, Suppl. 197 (1962).

⁴ B. FALCK, N.-Å. HILLARP, G. THIEME, and A. TORP, *J. Histochem. Cytochem.* 10, 348 (1962).

⁵ B. HAMBERGER, K.-A. NORBERG, and F. SJÖGVIK, *Biochem. Pharmacol.*, in press.

The ganglion cells in animals, treated with the monoamine oxidase inhibitor nialamide⁶ (500 mg/kg, i.p., 5½ h), showed a general slight increase of the fluorescence level. Particularly cells with a low or medium degree of fluorescence displayed increased intensity. However, even with this treatment, there was considerable variation in intensity among the cell bodies (see Figure) and some cells showed no fluorescence at all. Specific fluorescence did not occur in animals given reserpine⁶ (5 mg/kg, i.p., 2-6 h), and was of very low intensity after α -methyl-m-tyrosine (400 mg/kg, i.p., 24 h) and L-aramine⁶ (25 mg/kg, i.p., 6 h).

The results obtained in the experiments with the above-mentioned drugs strongly support the view that the fluorescence is due to a monoamine, probably a catecholamine (CARLSSON et al.²). Since the histochemical criteria (see FALCK³) were also satisfied, there seems to be little

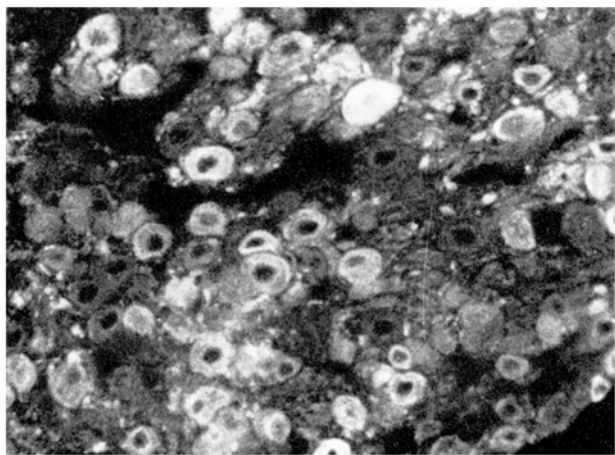
doubt that the fluorescent material is derived from a primary catecholamine. As most of the neurons in the sympathetic ganglia are adrenergic, it may be concluded that the amine, demonstrated in the cells, is, in fact, noradrenaline.

One possible explanation of the variation in fluorescence intensity among ganglion cells might be that the catecholamine content of some of the postganglionic neurons is in some way affected by means of preganglionic impulses. This, however, does not appear to be the case, since animals in which preganglionic denervation of the superior cervical ganglion had been performed 14-18 days before dissection, showed about the same degree of variation in fluorescence intensity among the ganglion cells, as did normal animals.

Zusammenfassung. Sympathische Ganglien wurden mit einer spezifischen und besonders empfindlichen histochemischen Methode zum Nachweis gewisser Monoamine studiert. Die meisten Zellkörper der Ganglienzellen enthalten im Cytoplasma mehr oder weniger einer primären Monoamine, wahrscheinlich Noradrenalin. In prävertebralen Ganglien wurden auch adrenerge Nevenendigungen nachgewiesen, die synaptische Verbindungen mit den Ganglienzellen eingehen.

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Superior cervical ganglion from a rat given nialamide 500 mg/kg, 5½ h previous to autopsy.

⁶ Thanks are due to the Swedish Ciba, Stockholm, for generous gifts of Serpasil (reserpine), to the Swedish Pfizer, Stockholm, for Niamid (nialamide), and to Merck Sharp and Dohme Research Lab., Rahway (N.Y., U.S.A.), for Aramine (methoxyhydroxy-norphenedrine).

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The Use of Lytic Enzymes of *Micromonospora* spp. to Prepare Protoplasts of Yeasts

In the past few years great progress has been made in the field of bacterial anatomy, due largely to the utilization of the cell wall lytic activity of various enzymes, mainly the egg white lysozyme. More recently, striking advances have been obtained with other groups of organisms due to the introduction in this practice of enzymes from the gut of the snail *Helix pomatia*¹ and from growth media filtrates of *Streptomyces* spp.^{2,3}

For the past months, studies have been made in the author's laboratory on cell wall lytic agents active against yeast and moulds. Microorganisms capable of lysing the cell walls of these organisms were isolated from soil samples. Some of the results obtained have already been described in previous papers⁴⁻⁸. So far as the authors' knowledge goes, the work reported here is the first specifically designed to obtain protoplasts of yeast using the lytic enzymes produced by species of *Micromonospora*. Recently a group of Japanese workers has reported in a preliminary note the lysis of BCG cell walls by extracts of *Micromonospora* sp.⁹

The method of obtaining a partially purified lytic enzyme preparation has been described elsewhere¹⁰. Further purifications of the enzyme(s) has been attempted using various absorbants, and although some improvement was obtained, satisfactory results have not yet been found. Further studies on this point are in progress.

¹ A. A. EDDY and D. H. WILLIAMSON, *Nature* 179, 1252 (1957).

² C. GARCIA MENDOZA and J. R. VILLANUEVA, *Microbiol. Espan.* 15, 139 (1962).

³ C. GARCIA MENDOZA and J. R. VILLANUEVA, *Nature* 195, 1326 (1962).

⁴ S. GASCON and J. R. VILLANUEVA, *Can. J. Microbiol.*, in press.

⁵ J. R. VILLANUEVA, S. GASCON, and I. GARCIA ACHA, *Nature* 198, 911 (1963).

⁶ M. J. R. AGUIRRE, I. GARCIA ACHA, and J. R. VILLANUEVA, *Exper.* 19, 82 (1963).

⁷ I. GARCIA ACHA and J. R. VILLANUEVA, *Can. J. Microbiol.* 9, 139 (1963).

⁸ I. GARCIA ACHA and J. R. VILLANUEVA, *Science*, in press.

⁹ S. KOTANI, K. HARADA, T. KITaura, Y. HASHIMOTO, T. MATSUBARA, and M. CHIMORI, *Biken J.* 5, 117 (1962).

¹⁰ S. GASCON and J. R. VILLANUEVA, *Biochim. biophys. Acta*, in press.