from phagocytosing leucocytes give rise to a very remarkable oxygen uptake. Such an enormous difference makes meaningless, with regard to the situation in phagocytosing cells, any speculation[1,2,4] based on experiments with oxidase activities and their sensitivity to inhibitors in homogenates from resting cells only.

The addition of saponine induces a swelling of the granules, with no difference between the decrease in optical density of granule suspensions from resting or phagocytosing leucocytes (Figure 2). During this swelling, the $NADPH_2$-oxidase from phagocytosing cells appears strongly stimulated whilst the $NADH_2$-oxidase is not (Figure 1). This shows that the attachment of the 2 enzymes to the granules, or their structure-linked latency, cannot be considered in the same way.

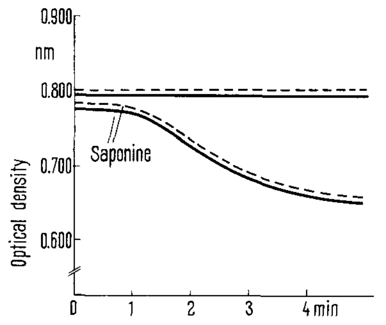

Fig. 2. Decrease in optical density of granules from resting (– – –) and phagocytosing (——) leucocytes. Upper curves: controls. Lower curves: effect of saponine, 1 mg/ml. Composition of the system as for Figure 1(a) except for the dilution of the preparations, adjusted to read approximately 0.800 at 520 nm.

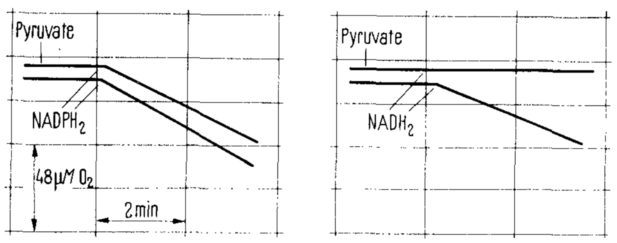

Fig. 3. Changes in oxygen uptake of a system containing 1 m*M* KCN and granules plus supernatant (see Figure 1(a)) from phagocytosing leucocytes. Upper curves: systems containing 1 m*M* pyruvate. Lower curves: controls without pyruvate.

The experiments presented in Figure 1 also show that the addition of saponine does not stimulate the oxidase activities of granules of resting leucocytes, which is relevant to the hypothesis[7] that during phagocytosis the oxidase activities of swollen granules are more active because of their swelling. It is evident that the saponine-induced swelling is not comparable to that caused by phagocytosis and which is associated with changes in the availability of the granule-bound oxidases. Further studies are needed to elucidate these questions.

The results presented in Figure 3 show that the interrelationships between the granular $NADH_2$- and $NADPH_2$-oxidases and the $NADH_2$- and $NADPH_2$-linked activities of the lactate dehydrogenase are such as to prevent the $NADH_2$-oxidase from being active in the presence of pyruvate.

These findings indicate that in the phagocytosing PMN leucocyte the $NADPH_2$ can be oxidized by oxygen, whilst the $NADH_2$ is preferentially oxidized by pyruvate through lactate dehydrogenase.

Riassunto. Si è dimostrato che l'attività $NADH_2$ e $NADPH_2$ ossidasica dei granuli di leucociti polimorfonucleati, misurata come consumo di ossigeno con elettrodo di Clark in presenza di cianuro 1 m*M*, è enormemente aumentata nelle preparazioni da leucociti in fagocitosi, mentre l'attività del sopranatante è inapprezzabile. La competizione della lattico-deidrogenasi con le ossidasi sui due coenzimi ridotti è tale da consentire soltanto alla $NADPH_2$ ossidasi un ruolo rilevante nel sostenere il consumo d'ossigeno dei leucociti in fagocitosi. Questi risultati chiaramente dimostrano l'infondatezza di speculazioni basate su esperimenti esclusivamente condotti con omogenati da leucociti in riposo[1-3].

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Unit Centre 'G. Vernoni' for the Study of Physiopathology and Institute of General Pathology, University of Padova (Italy), May 16, 1966.

On Short Adrenergic Neurons in the Accessory Male Genital Organs of the Bull

The vas deferens and the internal male genital glands of different mammals have recently proved to be innervated by short adrenergic neurons[1-5], which have a very dense terminal distribution in the various organs. Further, certain functional parameters of the isolated noradrenalin granules of the seminal vesicle (or, vesicular gland) and the vas deferens of the bull have in several respects been found to occupy an intermediary position between bovine splenic nerve granules and adrenomedullary granules[6,7]. In order to establish from which cell system these noradrenalin granules originate, it is necessary to obtain histochemical information on the catecholamine-containing structures of the internal male genital organs in this species.

Material and methods. Pieces from various internal male genital organs (proximal and distal parts of the seminal vesicle, ampulla of the vas deferens, and the prostate body) were removed from 5 adult bulls within 10 min

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[3] B. Falck, Ch. Owman, and N. O. Sjöstrand, Experientia *21*, 98 (1965).
[4] Ch. Owman and N. O. Sjöstrand, Z. Zellforsch. *66*, 300 (1965).
[5] N. O. Sjöstrand, Acta physiol. scand. *65*, suppl. 257, 1 (1965).
[6] U. S. v. Euler and F. Lishajko, Life Sci. *5*, 687 (1966).
[7] L. Stjärne and F. Lishajko, J. Neurochem., in press (1966).

of slaughtering. The specimens were immediately quenched to the temperature of liquid nitrogen, freeze-dried, treated in formaldehyde gas at + 80 °C (1 h) for the histochemical demonstration of certain monoamines[8–11], embedded in paraffin and sectioned at 6 μ thickness for fluorescent microscopical analysis, all according to the description given by FALCK and OWMAN[12].

Results. Fluorescence microscopy of the internal male accessory genital organs of the bull revealed the presence of only 2 structures that emitted an intense green fluorescence characteristic of primary catecholamines: nerves and mast cells.

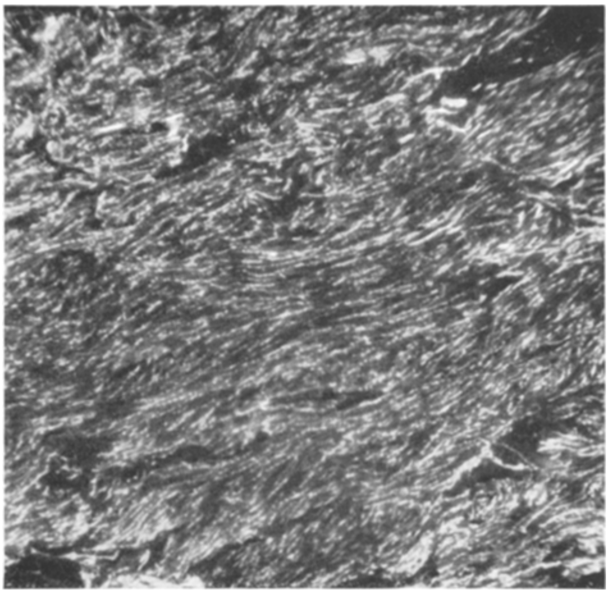

Fig. 1. Rich distribution of varicose adrenergic nerve terminals in the circular muscle layer of the proximal portion of the seminal vesicle. × 90.

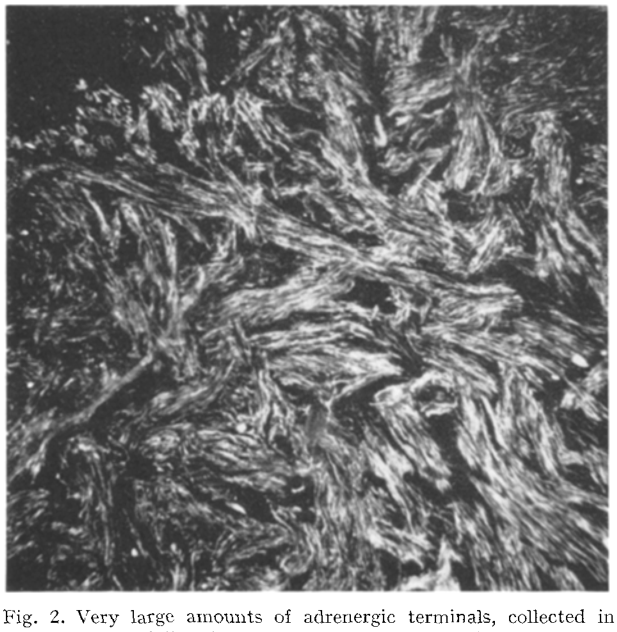

Fig. 2. Very large amounts of adrenergic terminals, collected in groups, run in all directions in the smooth muscle wall of the ampulla of the vas deferens. × 60.

The seminal vesicle had a rich innervation of varicose adrenergic nerve terminals, most of which ran circularly in the muscle coat (Figure 1). The axons occurred singly or in thin bundles of 2–6 fibres. The density of fluorescent nerves was extremely high in the smooth muscle wall of the ampulla vas deferens (Figure 2), and somewhat lower in the prostate; the nerves, usually collected in groups, coursed more irregularly in all directions. In all organs, a large number of nerve terminals could be followed in the smooth muscle trabeculae of the lamina propria without entering the epithelium. Thick bundles of adrenergic nerves, both of the varicose and of the smooth type, ran contiguous to the different organs, issuing smaller branches into the muscle tissue. The arteries and arterioles supplying the internal genitals were enclosed by fairly dense adrenergic nerve plexa.

The amount of fluorescent adrenergic nerves present in the different regions corresponds well to the high levels of noradrenalin that can be measured fluorimetrically in the organs[5].

In the muscle wall of the prostate, as well as immediately outside it, several large masses of nerve-cell bodies

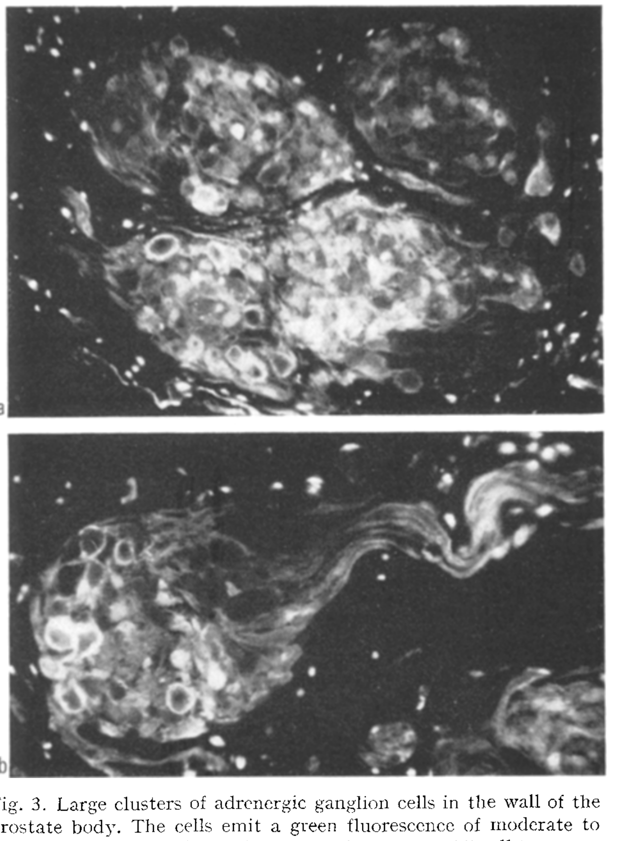

Fig. 3. Large clusters of adrenergic ganglion cells in the wall of the prostate body. The cells emit a green fluorescence of moderate to high intensity. Some few cells are non-fluorescent. All cell types are often surrounded by varicose terminals suggestive of a synaptic arrangement. Small mast cells carrying dopamine outside the ganglia. (a) Large ganglion composed of 3 groups of nerve cells. × 60. (b) Thick bundle of smooth adrenergic fibres of moderate fluorescence intensity leaves an adrenergic ganglion. × 65.

[8] B. FALCK, Acta physiol. scand. *56*, suppl. 197, 1 (1962).
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[11] H. CORRODI and N.-Å. HILLARP, Helv. chim. Acta *47*, 911 (1964).
[12] B. FALCK and CH. OWMAN, Acta Univ. Lund II *7*, 1 (1965).

were recognized. Some of the ganglion cells were non-fluorescent, others showed a low to fairly high green cytoplasmic fluorescence (Figure 3). In many instances both types of nerve cells were closely surrounded by nerve terminals indicative of a synaptic arrangement. Thick bundles of smooth, moderately green-fluorescent nerves were seen to leave the ganglia (Figure 3b).

A small to moderate number of green-fluorescent mast cells (Figure 3), which in ruminants carry dopamine[13], were found at various sites in the genital organs. They occurred mostly in relation to the thick adrenergic nerve bundles close to the organs rather than in the actual smooth muscle tissue.

Discussion. In analogy with earlier denervation experiments on various species, including the ram[5], it seems reasonable to assume that also in the bull the very dense adrenergic innervation of the internal male accessory genital organs emanates from ganglia located close to the target organs as, for example, those presently demonstrated in the prostate wall. Furthermore, it is apparent that the bulk of noradrenalin granules isolated from these organs by EULER and LISHAJKO[6] and by STJÄRNE and LISHAJKO[7] derives from the terminal portions of *short* adrenergic neurons.

The mode of behaviour of these granules further supports the view that short adrenergic neurons constitute a special kind of peripheral adrenergic system, different from ordinary *long* adrenergic neurons and chromaffin cells. In this connection it is of interest that immunosympathectomy has little effect on the adrenergic innervation of certain organs[14,15] known to be innervated by short adrenergic neurons, such as the internal male genital organs[4,5], or on the adrenal medulla.

In view of the assumption that short adrenergic neurons represent a specific entity in the peripheral adrenergic system, it is tempting to suggest that also the wide-spread system of intensely green-fluorescent branching chromaffin cells[16] may constitute a special type of chromaffin tissue, having functional similarities with adrenergic neurons. Thus, they often occur in large numbers in adrenergic ganglia. Usually, one or more slender processes emerge from the cell. In many instances such a process can be followed for a considerable length in adrenergic nerve bundles or in the terminal adrenergic nerve plexus. A small, intensely fluorescent chromaffin cell, located in the rabbit uterus, has even been seen to directly issue 3 delicate processes with the typical appearance of varicose adrenergic nerve terminals[17].

These observations would then suggest that during development the autonomic ganglion primordia give rise to essentially 4 types of peripheral catecholamine-containing cells: on the one hand adrenal medullary cells and, on the other, *long* adrenergic neurons, in between which are found 2 step-wise transition forms, the branching chromaffin cells and the *short* adrenergic neurons. The properties of the specific noradrenalin granules in the accessory male genital organs further support the view that the short neurons from which these granules originate represent a special transition form between chromaffin cells and long adrenergic neurons.

Zusammenfassung. Basierend auf dem speziellen Betragen der isolierten Noradrenalingranula der inneren männlichen Geschlechtsdrüsen, die von kurzen adrenergischen Neuronen innerviert sind, werden Definitionen für 4 «Übergangsformen» der peripheren katecholaminenthaltenden Zellen diskutiert: chromaffine Zellen der Adrenomedulla, kleine verzweigte, nicht adrenale chromaffine Zellen, *kurze* adrenergische Neurone und *lange* adrenergische Neurone.

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Institute of Anatomy and Histology, University of Lund, and Department of Physiology I, Karolinska Institutet, Stockholm (Sweden), June 15, 1966.

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The Courting Habits in *Atherigona* spp. (Anthomyidae, Dipt.) and the Probable Role of the So-Called 'Clover Leaf Appendage'

The males of many species of *Atherigona* have at the end of the abdomen an appendage, the so-called 'Kleeblattanhang' or 'clover leaf appendage'. This is a flexible rod at the tip of which are 3 leaflets which are black in colour.

Upon inquiries taxonomists studying Diptera could not provide any information on the function of this organ; it is used as a taxonomic characteristic, since the leaflets differ in shape and proportions in each species[1]. The clover leaf appendage protrudes from beneath probably the 7th tergite. Its total length is about 1 mm, of which the petiole is about 800 μ and the leaflets about 200 μ each.

In the course of breeding the species of *Atherigona varia soccata* Rond. at Rehovot, a peculiar mode of courting was observed. Close inspection of the behaviour of the species in the breeding cage, using low-power binoculars, revealed that in the course of courting the clover leaf appendage is in function. When not in use, the appendage is hidden, curved over the hypopygium inside the tip of the abdomen, in a pocket the slit of which is beneath the 3rd sternite (considering the visible segments in the abdomen are 2–5) (Figure 2).

The male approaches the female laterally, with the last abdominal segments stretched out and the clover leaf appendage extended posteriorly. With the lower part of

[1] W. HENNIG, in: *Muscidae* (Ed. E. LINDNER, Stuttgart, Germany; 1955–1964) vol. VII, p. 493.