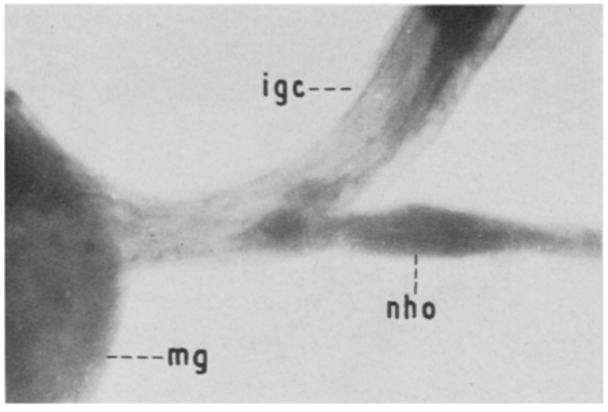

Fig. 1. Whole mount of mesothoracic neurohaemal organ (nho). mg, mesothoracic ganglion; igc interganglionic connective.

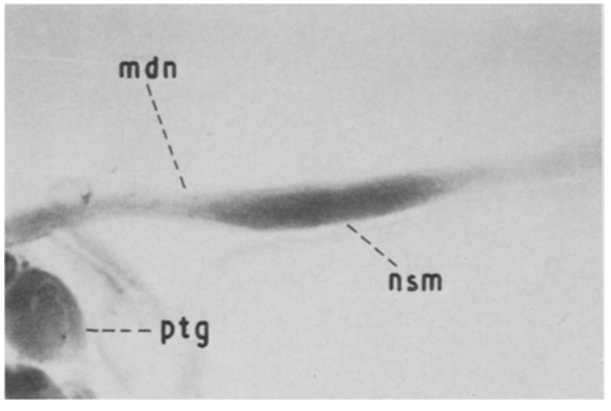

Fig. 2. A portion of mesothoracic dorsal nerve (mdn) to show the neurosecretory material (nsm). ptg, prothoracic gland.

by staining with reduced methylene blue solution in situ (0.5% ascorbic acid in 2% of aqueous methylene blue).

The base of the mesothoracic dorsal nerve of the larva of *Philosamia ricini*, which arises from the interganglionic connectives between the prothoracic and mesothoracic ganglia, bears a distinct dilation (Figure 1) representing the thoracic neurohaemal organ. Granules of neurosecretory material were found at 2 places in the mesothoracic dorsal nerve (Figures 1 and 2) which innervates the prothoracic gland.

HINKS[3] considered the presence of well-developed thoracic neurohaemal organs to be a primitive feature, probably because such organs are also reported in Blattaria[4]. He further reported that these organs might have been simply overlooked in other insects or been inconspicuous, but it was possible that some insects release thoracic neurosecretion from other end organs. DELPHIN[5] showed the neurosecretory transport along the ventral nerve cord, and BURGESS[6] found the presence of neurosecretion through the longitudinal axons of the ventral nerve cord of certain Diptera. CHALAYE[7] reported that one type of the neurosecretory product of the suboesophageal neurosecretory cells is transported to the corpora cardiaca or corpora allata and a second type, produced by other cells, passes back into the ventral nerve cord. HINKS[3] believes that thoracic and abdominal (lateral and medial) neurohaemal organs each stores and releases a single but different type of neorosecretion unlike the corpora cardiaca, which in all insects store and release several different secretions[8].

Résumé. Dans la larve de *Philosamia ricini*, le nerf dorsal mésothoracique possède à sa base un organe neurohémal contenant des éléments neurosécrétifs.

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The Effect of Lymphostasis on the Isolated Working Rat Heart

The importance of the mechanically induced restriction of the lymphatic circulation in relation to the damage of the myocardium has often been emphasized in recent years by FÖLDI et al.[1-3]. Their findings suggest that impairment of the cardiac lymph flow after ligation of the lymph vessels is followed by severe changes in the myocardium which are caused by cardiac lymphedema and by narrowing of the coronary arteries as a result of plasma imbibition of the vascular wall[4].

In contrast to these findings, other investigators did not observe any myocardial changes after ligation of the lymphatics[5,6]. Therefore the importance of the lymphostatic myocardial damage was disputed and doubts arose whether an impaired lymph flow would actually result in cardiac failure[7]. In comparison with the coronary flow, there is a very low cardiac lymph flow of about 4.8 ml/g heart tissue/24 h at its maximum[8]. On the other hand, the velocity of the cardiac lymph flow as shown in the isolated heart by india ink injection is considerable, which suggests the presence and effectiveness of lymphovenous anastomoses[9]. In our studies, it was of special interest to establish whether a partial restriction of the lymph flow was a factor limiting the performance of the heart (as measured by the minute output of the aorta), and if so, after which period of time functional and morphological changes would occur.

Materials and methods. Hearts of male albino rats with an average weight of 230 g were prepared and perfused by a modified MORGAN technique[10]: perfusion at 37 °C, pH 7.4, arterial pO_2 646 mm Hg, venous pO_2 360 mm Hg (Langendorff) and 200 mm Hg (working heart). The isolated hearts were subjected to retrograde perfusion

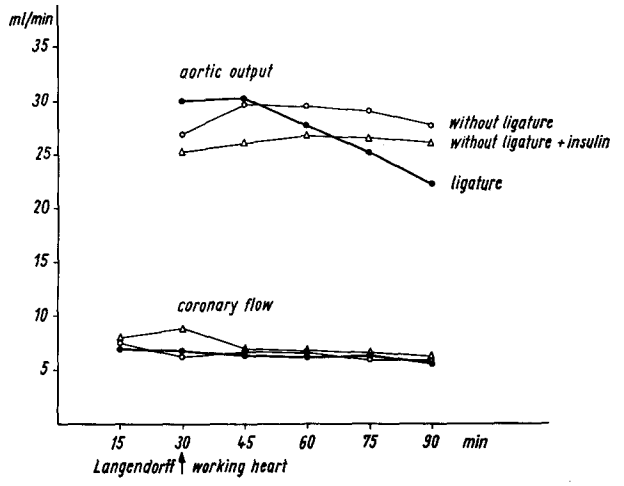
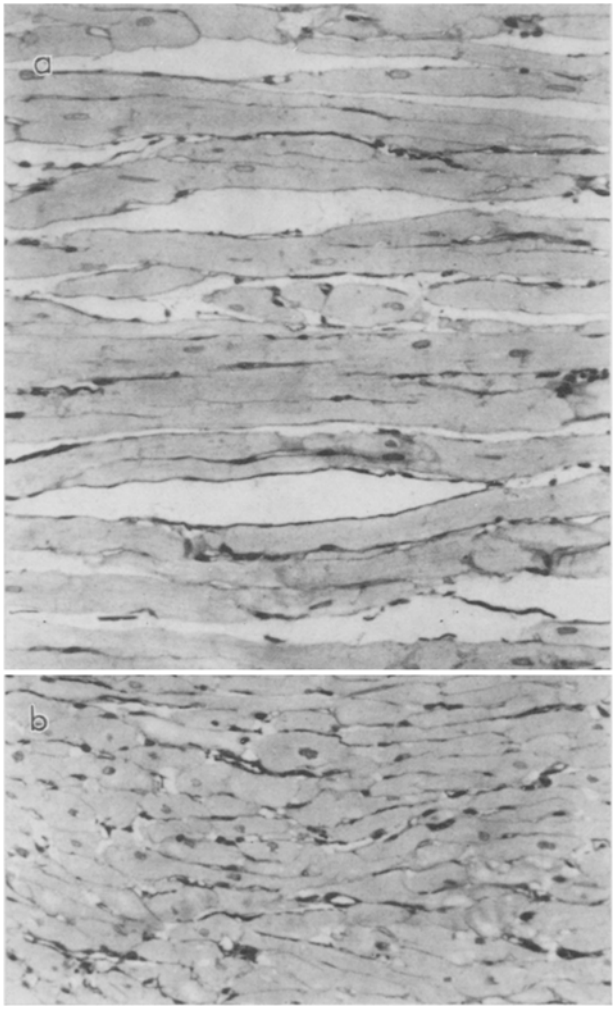


Fig. 1. Behaviour of the aortic output and coronary flow of the isolated Langendorff and working rat heart. One group with lymphostasis (by ligature) and 2 groups without lymphostasis (controls), but perfused with an insulin-containing or insulin-free medium (glucose 5.5 m*M*). The performance of the working heart is rapidly decreased during lymphostasis, while the controls do not indicate changes of the aortic output.



through the aorta for 30 min by the method of Langendorff, followed by an orthrograde perfusion via the left atrium. To produce a lymphostasis, the lung hili being ligated including the pulmonary artery and pericardium and moreover the periaortal connective tissue just above the aortic cannula, taking into consideration the course of the great lymph vessels in the dog heart[2, 11, 12], because it is very difficult to isolate the lymphatics in the rat and ligate them completely. Lymphostasis was demonstrated by injecting india ink into the wall of the left heart. Retention of the indian ink injected was only found in animals with ligature of lung hili and periaortal connective tissue. To compare the functional data with the morphological findings, the animals were divided into 3 groups: group 1 included animals with complete ligature of the lung hili and the periaortal connective tissue (lymphostasis group), group 2 and group 3 consisted of animals without obstruction of the lymphatics (controls), but in the 3rd group insulin (10 IU of insulin/l) was added to the medium.

For light microscopical examination, paraffin-embedded stage cut cross sections (6 μm in thickness) and metacrylate-embedded semithin sections (0.5–1 μm) were prepared and stained with haematoxylin and eosin (HE) or Movat for silvering basement membranes.

For electron microscopical examination, small pieces (1×1 mm) of the myocardium (right ventricle and inner layer of the left ventricle) were used, fixed with cacodylate buffered osmium tetroxide, and embedded in Vestopal W. Ultrathin sections were obtained by means of LKB ultratome and staines sections were examined using the Elmiskop Ia (Siemens AG).

Results and discussion. A progressive decrease in the aortic output of the ligated group was noted after a period of 15 min, while the coronary flow was unchanged (Figure 1). Histologically, interstitial and cellular edema were found in the hearts with a reduced lymph flow, most frequently accompanied with dilatation of the subepi-

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Fig. 2. Enlargement of the interstitial spaces and dilatation of the lymph capillaries, as well as severe edema of the muscle cells, are shown after long-lasting perfusion of the working rat heart (a). The same findings are noted in animals with cardiac lymphostasis, but they are lacking in the controls or short-time perfused hearts (b). Semithin sections, Movat's silver staining. ×320.

cardial lymph vessels even after 60 min of perfusion in the working heart. The interstitial spaces as well as the lymph capillaries were enlarged. These findings are identical with those after long-time perfusions (Figure 2a and b). Electron microscopically, the muscle cells showed an edema especially in the subsarcolemmal space, a loss in mitochondrial granules and severe changes in the cell structure such as swelling of the mitochondria partly with great electron-dense flocculent granules, contraction bands of the myofilaments, disintegration of the Z-bands, and fibrillolysis (Figure 3). Edema of the capillary endothelium with a narrowing of the lumen of the blood capillaries was usually recognized. Focal necrosis of the muscle cells was observed only in the working left heart. The changes described above did not occur in animals without blocking the lymph flow, except some small changes in the muscle cells and blood capillary endothelium. In all groups, enlarged lymphatics have been seen partly with 'open endothelial junctions'[13] increasing with the severity of edema[14] (Figure 4). Comparing the

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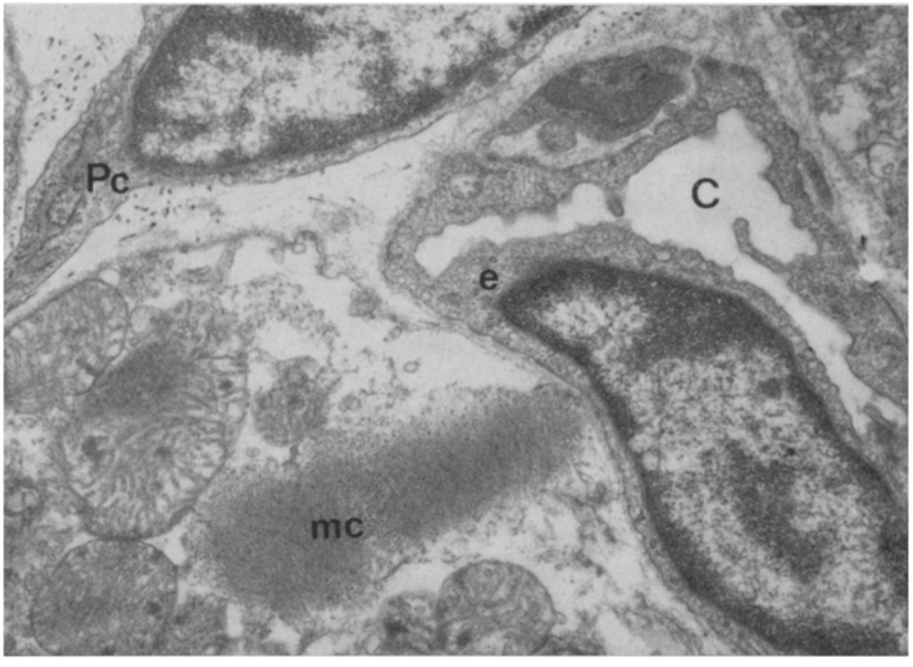

Fig. 3. Part of a muscle cell (mc) of the isolated working rat heart (left ventricle) with edema, fibrillolysis, and flocculent dense granula within swollen mitochondria. Beside a blood capillary (c) with enlarged endothelial cell (e) and a normal pericyte (pc). Group 1 (lymphostasis). Electron microsc. 12,000, total magnification 24,000.

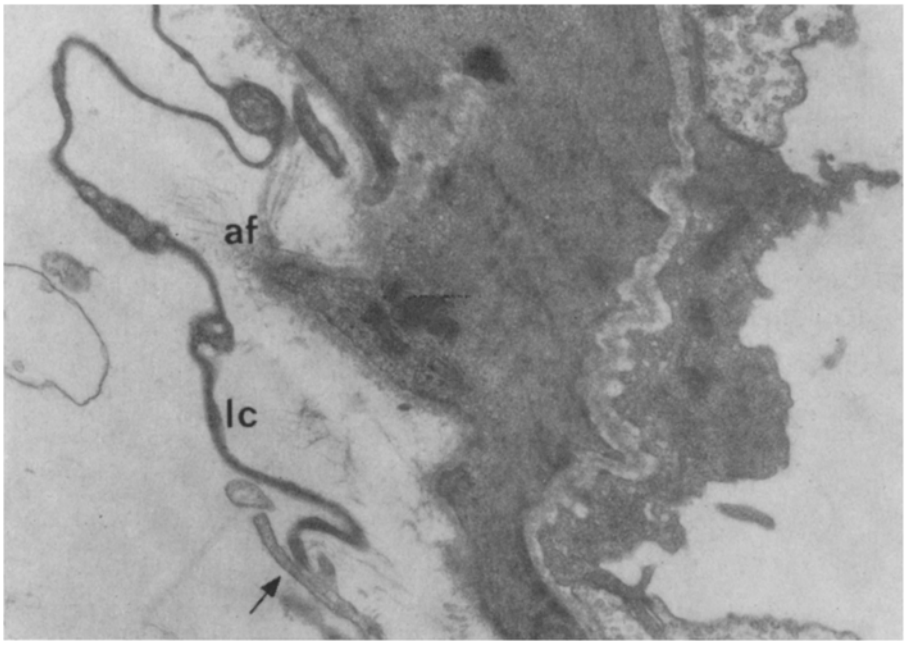

Fig. 4. Wide opened lymphatic capillary (lc) without basement membrane beside an arteriole in the left ventricle of the isolated working rat heart. Overlap (→) of an endothelial cell surrounded by anchoring filaments (af). Group 2 (control). Electron microsc. 12,000, total magnification 24,000.

2 control groups, an interesting fact was noted: in the hearts perfused with an insulin-containing medium, in agreement with the functional findings, no damage to the muscle cells or enlargement of the sarcoplasmatic reticulum (T-tubuli) was found as seen in long-time Langendorff-preparations[15].

Our findings may be interpreted thus: The formation of cardiac edema is intensified by the ligation of lymph vessels in the isolated working heart. Moreover, an interference with microcirculation due to edema, swelling of the endothelial cells and closed or collapsed blood capillaries is increased by lymphostasis. Therefore, the irreversible damage to the myocardium is not only the result of the lymphedema but also due to ischemia, according to other investigators[16].

The reduced lymph flow causes an impairment of metabolism because oxygen transport, substrate utilization, and removal of metabolites may be hindered. If insulin is added to the medium, the substrate utilization is considerably improved, so that the disturbance of microcirculation is compensated for a certain period of time. Recent investigations[17-20] have shown that glucose and insulin have a protective effect on hypoxically induced injury of the heart. Whether a similarly favourable effect occurs in lymphostasis will have to be shown in further experiments.

Zusammenfassung. Die experimentelle Lymphstauung bewirkt am isolierten, arbeitenden Rattenherzen trotz konstantem Koronarfluss eine progressive Abnahme der Auswurfleistung des linken Ventrikels, die morphologisch unter anderem mit einem interstitiellen und intrazellulären Ödem, insbesondere der Herzmuskelzellen und Kapillarendothelien sowie mit partiellen Herzmuskelzellnekrosen einhergeht.

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Degeneration in the Adult Rat Spinal Cord Following Systemic Treatment with 6-Hydroxydopamine. Electron Microscopic Study

While there is now considerable evidence at the ultrastructural level to show that the systemic administration of 6-hydroxydopamine (6-OHDA) in adult animals causes degeneration of peripheral noradrenergic (NA) nerve terminals[1-9], the effect of the drug via this route on the central NA systems would appear to be much more severe if the animals treated were neonates than if they were adults[10-15]. This has been attributed to the greater permeability of 6-OHDA across the blood-brain barrier in the former than in the latter (see Thoenen and Tranzer[16] for discussion). On the other hand, in adult animals, when appropriate doses of the drug were injected either directly, intracisternally or intraventricularly, in several nuclei that are known to have a rich NA innervation, electron microscopy showed degeneration of nerve terminals[17-22]. This suggests that 6-OHDA can cause selective degeneration of central NA nerve terminals as well as peripheral ones. Recently, a review on the central effects of 6-OHDA suggests that in adult animals a little of the drug may cross the blood-brain barrier[23]. Whether this amount of diffusion of 6-OHDA would cause a sufficient degree of degeneration of central NA nerve terminals that can be detected ultrastructurally is not known. The present paper describes an electron microscopic study of this problem in the intermediolateral column (ILC) of the adult rat spinal cord, a nucleus that is known from fluorescence histochemical studies to be richly innervated by NA nerve terminals[24].

Adult albino rats weighing between 250–300 g were given a single i.v. injection through the saphenous vein of 100 mg/kg 6-OHDA HCl (25 mg/ml 6-OHDA HCl dissolved in a solution containing 1 mg/ml ascorbic acid). Control rats were injected i.v. with an equivalent volume of ascorbic acid. At 24, 48 and 72 h after injection of 6-OHDA, the spinal cord was fixed by intracardiac perfusion with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer containing 0.5 mg/l $CaCl_2$. The perfusion time was about 20 min. A short segment of the mid-thoracic spinal cord was then carefully dissected out and further sliced transversely into thinner segments. The latter were kept overnight in ice-cold fresh fixative, after which they were post-fixed in 1% osmium tetroxide[25], dehydrated with acetone and embedded in araldite. Semi-thin transverse sections of the spinal cord were stained with methylene blue and prepared for light microscopy. Ultra-thin transverse sections were stained with aqueous saturated uranyl acetate[26] and lead citrate[27] and examined in a Hitachi HS-8 electron microscope.

The observations were confined to the ILC of the adult rat spinal cord (Figure 1, inset). The results will be expressed in terms of those positive features that were present in the experimental material but were absent in the controls. The evaluation of the presence of degeneration was aided by reference to the paper of Glees and Hasan[28] and to previous studies on the degeneration of central NA nerve terminals following treatment with 6-OHDA[17-22].

A study of the 24 h material showed that scattered sparsely in the generally normal-looking neuropil were nerve terminals that were more electron-dense than others that were present (Figure 1). These electron-dense nerve terminals appeared collapsed with crowding of vesicles, the majority of which were of the small spherical