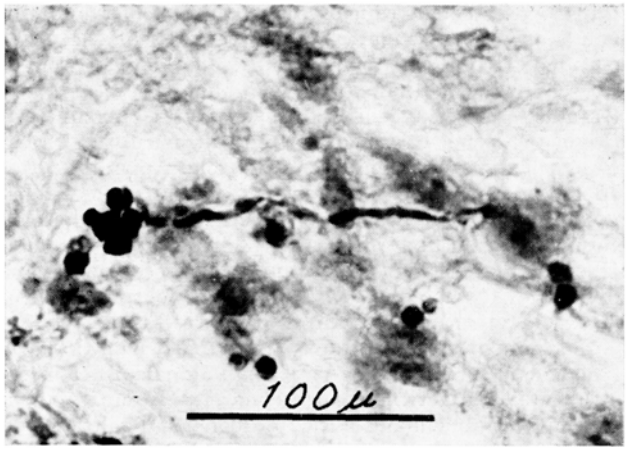

Fig. 1. Silver impregnated remnants of a neurone from the lateral cervical nucleus on the affected side. × 320.

with the exception that the sucrose had been omitted from the formalin solution. The further treatment of the material was the same that has been described elsewhere[4].

On the side, where degenerating fibres had been seen in the NAUTA sections, we found in the electron microscope numerous dendrites of various sizes in a state of degeneration. On the normal side no such structures were seen. The degenerating dendrites (Figure 2) were shrunken and electron dense, like the dense type of degenerating terminal boutons found in various parts of the central nervous system after axonal transection[5]. The mitochondrial profiles recognized within the degenerating dendrites occupied most of their cross sectional area. The dendrites were surrounded by boutons which looked normal. The synaptic contacts between the boutons and the degenerating dendrites were easily recognized.

The results presented here are of great general interest. The degenerative changes of dendrites as viewed in the NAUTA sections can be extended to the ultrastructural level. It will thus be possible to 'mark' selectively in electron micrographs dendrites belonging to a specific group of neurones. This means that it will be possible also to study the terminal boutons contacting dendrites of fully identified neurones. And thereby a new method for mapping in the central nervous system at the ultrastructural level has become available.

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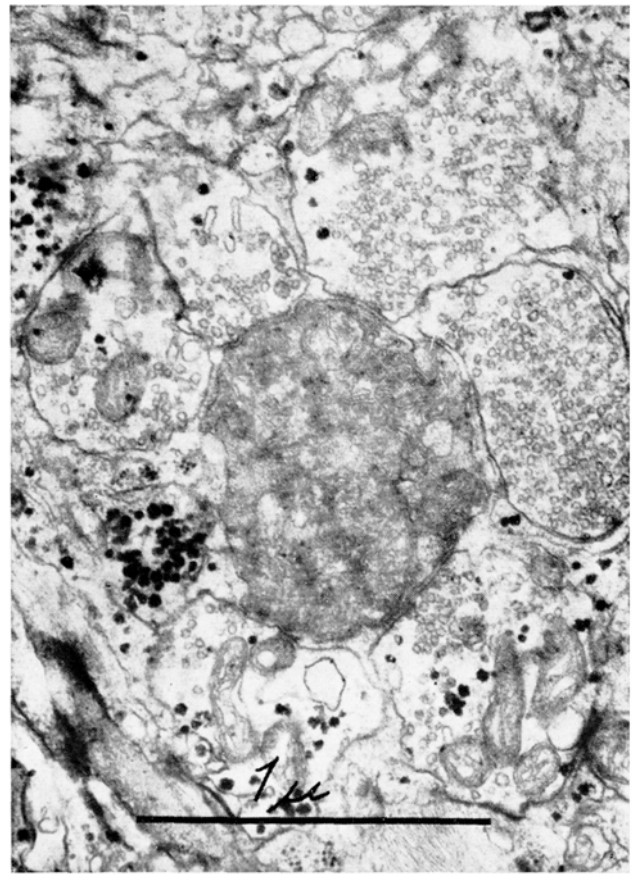

Fig. 2. Degenerating dendrite within the lateral cervical nucleus from the affected side. The dendrite is contacted by 6 terminal boutons, containing synaptic vesicles. Note the increased density of the dendritic profile when compared with the normal boutons surrounding it. × 45,000.

Zusammenfassung. Nervenzellen junger Katzen im Nucleus cervicalis lateralis wurden licht- und elektronenmikroskopisch nach Axon-Durchschneidung untersucht. Lichtmikroskopisch degenerierende Dendriten zeigten in denselben NAUTA-Präparaten auch ultrastrukturell eine Degeneration ähnlich wie bei den früher beschriebenen Endfüsschendegenerationen nach Axon-Durchschneidung. Damit wird eine neue Methode zur Markierung («napping») im Zentralnervensystem eingeführt.

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Lack of Endotoxin Effect on the Microcirculation after Pretreatment with Detoxified Endotoxin (Endotoxoid)

Endotoxin from *Serratia marcescens*, extracted by the BOIVIN method[1], was detoxified with potassium methylate (endotoxoid-2) by NOWOTNY[2]. It could be demonstrated that guinea-pigs survive lethal doses of endotoxin from *E. coli*, *P. vulgaris*, *S. marcescens* and *B. melitensis*, when pretreated with a single injection of this detoxified endotoxin (endotoxoid-2) 24 h before the challenge with the different endotoxins (URBASCHEK and NOWOTNY[3]).

The question now raised is whether endotoxoid-2 is also able to prevent the disturbances on the microcirculation caused by endotoxins.

The effect of endotoxin from *E. coli* on the microcirculation, described elsewhere (URBASCHEK[4]; BRANEMARK and URBASCHEK[5]) was studied on the hamster cheek pouch and the guinea-pig mesenterium. In these experiments it was observed that some min after local

application of 50 μg or i.v. injection of 250 μg/100 g body weight of endotoxin from *E. coli*, the blood flow begins to slow down. The granulated periendothelial cells and the tissue mast cells are partially degranulated. A narrowing of the vessel lumen results from an increase in the volume of the endothelial cells and pericytes. At the same time the plasticity of the circulating blood cells changes. Adherence to the wall of the granulocytes precedes the formation of microthrombi. A dose-dependent blockade develops to varying degrees in the venules, capillaries and much later in the arterioles. An increase in the permeability of the vessels follows, which leads to microbleedings.

All these alterations of the microcirculation observed after the injection of endotoxin – in the same dose as described above – are prevented in guinea-pigs and hamsters by pretreatment with endotoxoid-2. The animals received by i.v. route 150 μg/100 g body weight of endotoxoid-2. Twenty-four hours later vitalmicroscopic observation of the guinea-pig mesenterium and the hamster cheek pouch showed that local as well as i.v. application of endotoxin from *E. coli* did not lead to any disturbance of the microcirculation.

It is of interest that a glucofuranosid derivative (Glyvenol®, CIBA Aktiengesellschaft, Basel) which was fed 2 h before the application of endotoxin protects the microcirculation of these animals in the same way (URBASCHEK, VERSTEYL and GÖTTE[6]).

Zusammenfassung. Die Endotoxin-bedingten Störungen an der Mikrozirkulation – geprüft an der Hamsterbackentasche und dem Meerschweinchenmesenterium – bleiben aus, wenn den Tieren 24 h zuvor detoxifiziertes Endotoxin verabreicht wurde.

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Induction of Antibodies Against Pure Proteins in *Xenopus laevis* Daud.

While many authors have long ago proved the formation of antibodies of the agglutinin and phagus-inhibiting type in Amphibia, both Anura[1,2] and Urodela[3], attempts to obtain from these animals antibodies of the precipitine-type against pure proteins always failed[4]. The proteins used as antigens were serum albumins or serum globulins of mammals, that is proteins with low molecular weight.

On this basis the author has used as an antigen the hemocyanine (Hc) from a Gasteropod, the *Viviparus ater* Cristofori et Jan, which is a protein with high molecular weight (6,760,000)[5], easily obtainable in the pure state.

Materials and methods. Three adult females of the clawed frog, *Xenopus laevis* Daud. were injected into the lymphatic sac, with 3 injections of 0.3 ml of pure Hc + 0.3 ml of adjuvant (complete Freund adjuvant Difco), each at a distance of a week after the previous one.

The hemocyanine, extracted from 10 specimens of *Viviparus* was purified by dialysis and ultracentrifugation[6]; its protein concentration, calculated with the biuret method, was 3.2 mg/ml.

Throughout the whole experiment the xenopus were kept at a constant temperature of about 25 °C and fed with minced ox-liver. Twenty days after the last injection 2 animals were killed and their blood drawn directly from the heart; from a third xenopus a small amount of blood was drawn from the marginal vein of the interdigital membrane, and afterwards it was injected with 0.5 mg (in 0.2 ml) of cortisone[7]. The animal was completely bled 12 h after the injection. The maximum dilution at which the sera are still active was calculated with the ring-test for all of them; this test was performed also on the serum after the cortisone treatment. The antigenic reaction was also tested with the method of the double diffusion on 1% agar microplates in 0.85 NaCl, against Hc of *Viviparus* and against Hc of *Potamobius fluviatilis* L. In order to study to which fraction of the globulins the antibody activity was correlated, the antiserum was analysed by electrophoresis on agar for 2 h, in veronal buffer (pH 8.4 i.s. 0.05) and then antibodies were looked for, placing the antigenic Hc on the migration plate.

Finally one of the 3 antisera was dialyzed for 12 h at room temperature against 2-mercaptoethanol (2-ME) 0.1 M[8], which neutralizes 19 s γ-globulins.

Results. The sera of the 3 xenopus after the third injection of Hc, gave a positive reaction against Hc of *Viviparus* (Figure 1), while no arc of precipitate was obtained when the antiserum was tested against Hc of *Potamobius* (Figure 2).

As was proved by the immunoelectrophoretic analysis (Figure 3), the antibody activity is correlated to the γ-globulins fraction.

With the ring-test method it was shown that the 3 antisera are effective up to a dilution of 1:30, while the serum of *Xenopus* treated with cortisone was active up to a dilution of 1:120. It was also observed (Figure 4) that

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