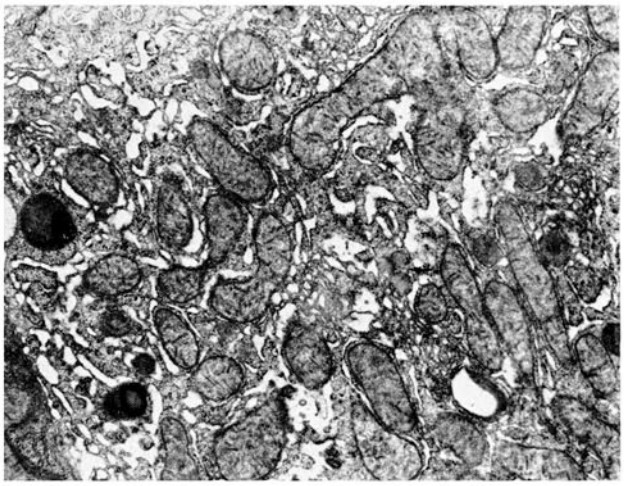

Fig. 3. Hepatocyte, periphery of the lobule, at 6 h. Note dilatation of endoplasmic reticulum. Liposomes at arrow. ×11,846.

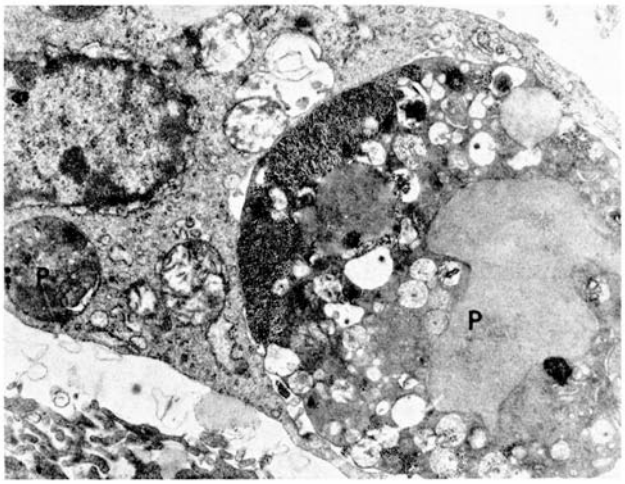

Fig. 4. Kupffer cell at 6 h. Large phagosomes (P) in the cytoplasm. ×12,923.

normal appearance, the increase of fat droplets notwithstanding.

It should be noted that no significant changes in the appearance of hepatocellular nuclei were seen, the necrotic cells notwithstanding. Intercellular bile canaliculli and sinusoids were occasionally dilated. Swelling of microvilli on both sides of liver cells was frequently observed, but was considered due to glutaraldehyde fixation and was encountered in control animals as well. Bile ductular cells did not undergo any significant changes.

The main target organelle affected by tetracycline is the endoplasmic reticulum. Tetracycline inhibits the binding of aminoacyl-sRNA to ribosomes and the protein synthesis[6]. This is in part reflected in the dilatation of endoplasmic reticulum and the disaggregation of the ribosomes from the membranes. Blockade of acceptor protein (apoprotein) synthesis leads to inhibition of formation and excretion of lipoproteins and acounts for the accumulation of fat in the hepatocytes. The ultrastructural changes induced by tetracycline are in this respect similar to those induced by other inhibitors of protein synthesis, such as orotic acid[10] or ethionine[11].

The affinity of tetracycline for mitochondria was demonstrated previously[7,12]. WRUBLE et al.[8] have considered the swelling of mitochondria as the main ultrastructural change in the liver biopsy in a clinically suspected case of tetracycline toxicity. Our study indicates that binding of tetracycline to mitochondria is accompanied by changes which could be interpreted as the morphological equivalent of biochemical derangements.

As tetracycline accumulates predominantly in centrolobular hepatocytes[13], one can assume that only these cells are exposed to excessive amounts of the drug and therefore undergo irreversible changes. Disruption of liver cells and extrusion of damaged cytoplasmic organelles is accompanied by increased scavanger activity of Kupffer cells. High concentrations of tetracycline observed in Kupffer cells[13] probably reflect in part their participation in evacuating the cellular dentritus containing large amounts of the antibiotic.

Summarizing our findings, we should like to point out that the ultrastructural findings induced by tetracycline correlate and are in full accord with the biochemical data reflecting the action of this antibiotic on the liver cells. These changes are not specific and are mostly reversible within the first 24 h after injection. Focal centrolobular necrosis was sparsely encountered but represents the only irreversible change induced by a single large dose of tetracycline.

Zusammenfassung. Einmalige i.p. Gabe von Tetrazyclin in sublethaler Dose führt zur Verfettung der Rattenleber. Die Dilatation und Vesikulation des Endoplasmatischen Retikulums und die Quellung der Mitochondrien sind die auffälligsten, Veränderungen die der Verfettung der Leberzellen vorausgehen. Diese Veränderungen sind unspezifisch und reversibel.

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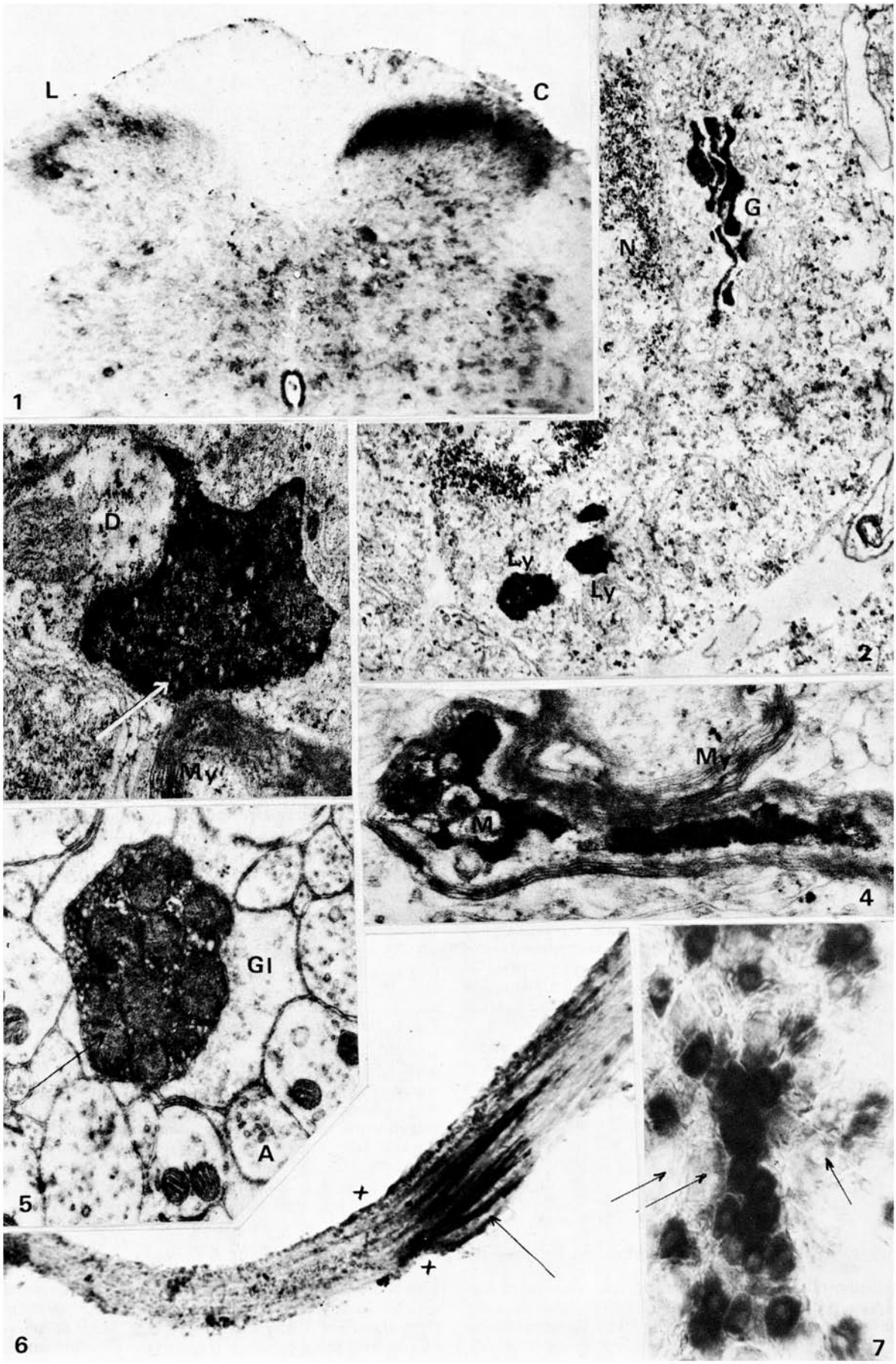
Fluoride-Resistant Acid Phosphatase System of Nociceptive Dorsal Root Afferents

Acid phosphatase activity in the nervous system is mainly, but not exclusively, associated with the lysosomes of nerve cells. It has been shown by GEREBTZOFF et al.[1-3] that in Lamina II of the posterior column (Rolando substance) in the rat spinal cord, there exists a peculiar, conspicuously strong acid phosphatase activity differing from the trivial lysosomal localization. This exceptionally intense acid phosphatase activity of the Rolando substance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is, in striking contrast to the lysosomal acid phosphatase in neuronal perikarya, resistant to 0.3 m*M* sodium fluoride (Figure 1, right-hand side).

Our electron histochemical investigations[4] revealed that in nerve cells, acid phosphatase is located in lysosomes and in Golgi substances, representing localization of the fluoride-sensitive iso-enzyme (Figure 2). On the other hand, nerve fibers and axon terminals constituting large groups amongst Rolando cells contain a fluoride-resistant acid phosphatase (FRAP). This iso-enzyme is dispersed in the axoplasms of thin myelinated nerve fibers and within terminal enlargements of the axons; mitochondria and synaptic vesicles are unstained (Figures 3 and 4).

We sought to trace the nerve cells from where FRAP-active Rolando terminals derive. It could be shown that these axons originated from dorsal root ganglia, since degeneration of FRAP-active dark terminals ensued shortly after transection of the dorsal roots (Figure 5).

Ligature of the dorsal root results in an accumulation of FRAP in some of the dorsal root nerve fibers, proximal to the ganglion (Figure 6). At the same time, FRAP-activity of the Rolando substance decreases on the operated side (Figure 1, left-hand side).

What kind of dorsal root ganglion cells give rise to FRAP-active Rolando terminals? Heterogeneity of dorsal root ganglion cells is a century-old puzzle in neurohistology: in between large, pale pseudo-unipolar cells there are dispersed small, darkly stained ones. The number of such 'dark cells' is high in the rat[5] and, though in smaller amounts, they are present in other mammals, too[6]. In spite of numerous efforts, no specific function could be ascribed to the samll dark cells[7]. We found that, while virtually every nerve cell in the rat dorsal root ganglion exert acid phosphatase activity, only the small dark cells contain a fluoride resistant iso-enzyme (Figure 7).

In our opinion, explanation of the above experiments is as follows: Small dark pseudounipolar nerve cells within the dorsal root ganglion contain FRAP in their cytoplasms; the enzyme, like other macromolecules, is transported via axonal flow to the central endings of these dorsal root axons, i.e. to the FRAP-terminals in the Rolando substance (Figure 8).

Since the Rolando substance is the site of primary analysis of nociceptive impulses, FRAP-active neurons appear to be associated with nociception. The role of acid phosphatase in their central terminals is not clear as yet.

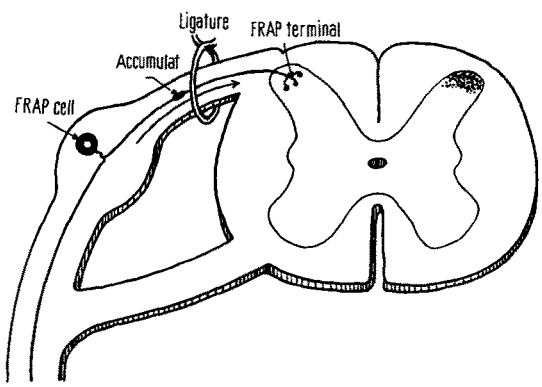

Fig. 8. Schematic drawing of the FRAP dorsal root system. Small dark pseudounipolar cells within the dorsal root ganglion contain a fluorid-resistant acid phosphatase that is transported via axonal flow to the FRAP-active Rolando terminals. Ligature of the dorsal root arrests axonal flow and results in an accumulation of FRAP in the dorsal root axon, proximal to the ganglion; accordingly, FRAP-activity decreases in the Rolando substance.

Zusammenfassung. Fluorid-resistente saure Phosphatase wird aus den kleinen (dunklen) Nervenzellen des Spinalganglions mittels axoplasmatischer Strömung durch die Hinterwurzeln in die Rolando-Substanz des Rückenmarkes transportiert, wo das Enzym in den Nervenendigungen eine freie axoplasmatische Lokalisation aufweist.

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Fig. 1. Light microscopic histochemical pattern of the Rolando acid phosphatase in the sacral lumbar cord of the rat, 7 days after ligation of dorsal roots L_6-S_3. Note the dramatic decrease of enzyme activity at the ligated (left-hand) side, and the conspicuously strong activity in the control (C) side. Method of BARKA and ANDERSON. ×100.

Fig. 2. Electron microscopic localization of acid phosphatase in the cytoplasm of a Rolando nerve cell. Enzyme activity is concentrated in the Golgi complex (G) and in lysosomes (Ly). N, nucleus of the nerve cell. Modified GÖMÖRI technique. ×30,000.

Fig. 3. Electron microscopic localization of acid phosphatase in a FRAP-active Rolando terminal. Enzyme activity is concentrated within the terminal axoplasm, sparing synaptic vesicles (white arrow) and mitochondria (M). The terminal is in a synaptic contact with a dendrite (D). My, myelinated nerve fibre. ×30,000. Modified GÖMÖRI technique.

Fig. 4. Electron microscopic localization of acid phosphatase in a preterminal (myelinated) FRAP-active Rolando axon. Activity is concentrated within the axoplasm, sparing mitochondria (M). Note the complete absence of any enzyme reaction in the axoplasm of a neighbouring myelinated nerve fiber (My). Modified GÖMÖRI technique. ×30,000.

Fig. 5. Degeneration of a dark Rolando terminal (identical with FRAP-active terminals), 2 days after transsection of dorsal roots L_5-S_3. Synaptic vesicles and mitochondria appear in the shape of a degenerative glomerulum (arrow), surrounded by glial profiles (Gl). Other axons in the vicinity (A) exhibit a normal ultrastructure. ×30,000.

Fig. 6. Accumulation of FRAP in some of the dorsal root axons 36 h after ligature of dorsal root S_2. Ligature was placed at the site marked by asterisks. Arrow points at the enzyme accumulation. In this picture, dorsal root ganglion is at the right-hand side and the spinal cord at the left-handside. Method of BARKA and ANDERSON. ×200.

Fig. 7. FRAP-activity of pseudounipolar cells in dorsal root ganglion L_4. A great number of nerve cells exert acid phosphatase activity after inhibition of the fluoride-sensitive iso-enzyme with 0.3 m*M* NaF. Large pale cells (arrows) do not show up in this picture, thoug they exert acid phosphatase activity in non-inhibited samples. Method of BARKA and ANDERSON, ×200.