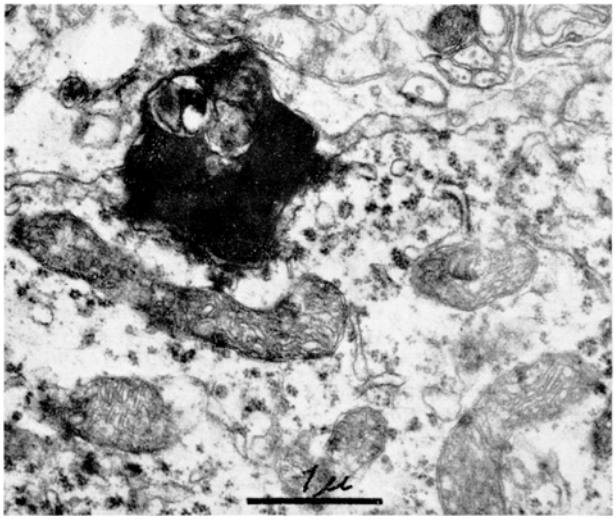

Fig. 2. Degenerating terminal bouton in the same case as in Figure 1. The bouton contacts a cell body. Note the high density of the bouton. × 18,000.

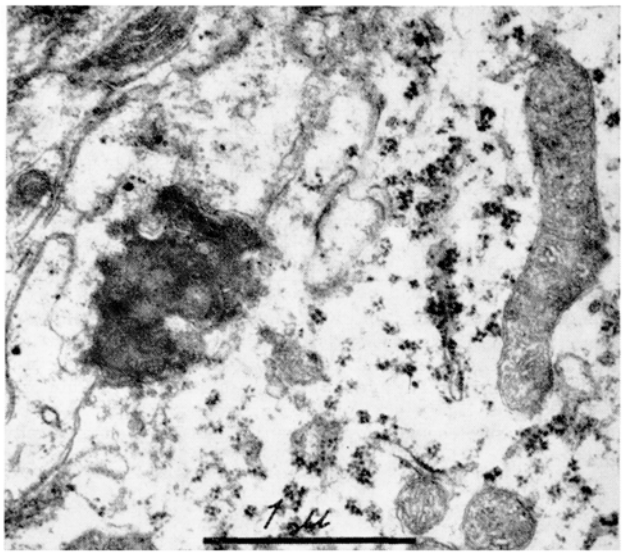

Fig. 3. Degenerating terminal bouton in the VPL 5 days after lesion of the LCN of the contralateral side. The bouton contacts a cell body (right). × 28,000.

same size and were only a small part of the total number of boutons in the area as in the DCN case.

The extreme density of the degenerating boutons indicates that the degeneration in the VPL described here is rather advanced already after 5 days[6]. That may be the reason why only the electron dense type of degeneration[5] and not the filamentous type[7] was seen. It has been proposed that the filamentous type is present only in the early stage of degeneration[8].

Degenerating boutons were seen in the VPL in synaptic contact with both nerve cell bodies and dendrites after lesions of the DCN as well as of the LCN. The same double localization has been found in most parts of the central nervous system after transection of afferent fibres[4,6,9,10]. In most cases the degenerating boutons on the dendrites are more numerous[6,9]. Probably the reason for this is the larger surface area of the dendrites compared to that of the cell body[11].

Zusammenfassung. Fünf Tage nach Zerstörung des Nucleus cervicalis lateralis in einer Katze und der Hinterstrangkerne in einer anderen, wurde das elektronmikroskopische Bild der degenerierenden Boutons in den kontralateralen VPL studiert. Die Boutons waren in beiden Fällen meistens in synaptischem Kontakt mit Dendriten, aber auch mit Zellkörpern und zeigten elektrondichten Typus von Degeneration. Die nicht degenerierenden Boutons waren viel zahlreicher als die degenerierenden.

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Some Structural Features of Isolated Mitochondrial Membranes

Subunits in the unit-membrane have been described by various authors. SJÖSTRAND[1] found an electron-transparent 'ultrastructural element' in sections of mitochondrial membranes and of the smooth endoplasmic reticulum, fixed with OsO_4 or $KMnO_4$; a similar subunit was also found by DEUTSCH and KRAUSE[2] in cross sections of isolated mitochondrial membranes treated with enzymes and fixed with OsO_4. Negatively-stained isolated membranes of the frog retina show an array of spherical particles (BLASIE et al.[3]). PEASE[4] has found electron-dense spherical particles in sections of membranes of some mitochondria. Similar particles, 'structural globular particles' (diameter about 60 Å), were observed by DEUTSCH and KRAUSE[2] in sections of isolated mito-

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chondrial membranes, fixed with $KMnO_4$. The object of the present investigation was to demonstrate the existence of 'structural globular particles' in unsectioned isolated mitochondrial membranes.

The mitochondrial membranes (rat liver) were isolated according to a method described in a previous paper (DEUTSCH and KRAUSE[5]). They were fixed with potassium permanganate (2 min in a 0.25% aqueous solution, buffered with sodium veronal/sodium acetate at pH 7). After fixation they were washed in distilled water and treated ultrasonically (1 h, 800 kc/sec, 5 W/cm^2), because it was thought that the effect of this treatment would tend to separate the subunits and make them more clearly detectable. Small droplets of the suspension were deposited on formvar-coated grids, backed with carbon. The specimens were allowed to dry, shadowed with platinum (angle 20°) and studied in a SEM 3 electron microscope (experimental resolution about 15–20 Å).

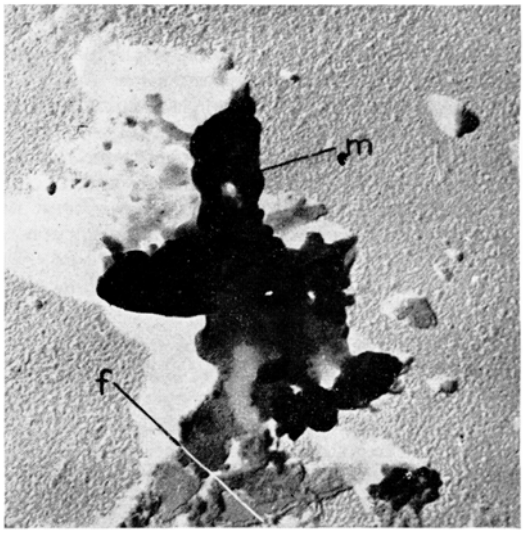

Fig. 1. Isolated mitochondrial membranes, fixed with potassium permanganate, treated ultrasonically, shadowed. Aggregation of membranes (m); indication of fibrillar structure (f). × 53,000.

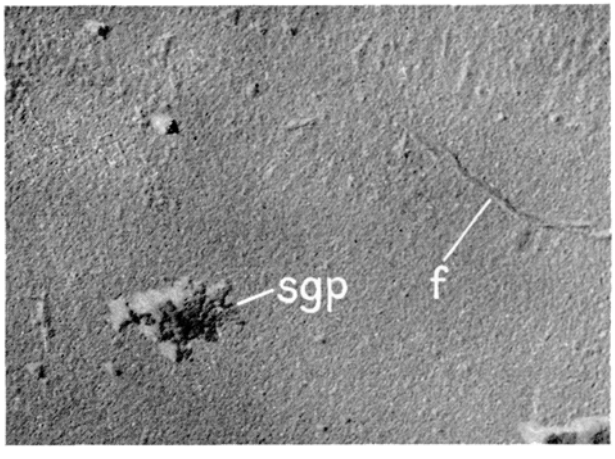

Fig. 2. Isolated mitochondrial membranes, fixed with potassium permanganate, treated ultrasonically, shadowed. Patch of disrupted membrane, consisting of 'structural globular particles' (sgp); fibril (f). × 53,000.

An inspection of the electron micrographs shows that there is a tendency for the membranes to aggregate (Figure 1), but often patches of broken-up membranes are separated and reveal structural details: they appear to consist of electron-dense spherical particles ('structural globular particles', diameter about 60 Å) (Figure 2). It should also be mentioned that occasionally very thin filaments (diameter about 40 Å) were found (Figure 2). There is some evidence that the membranes themselves contain fibrillar structures (Figure 1).

Unfortunately, it is impossible to study living cells in the electron microscope. Hence, electron micrographs are more likely to represent structural details of the living cell if they can be demonstrated with a variety of experimental methods. For this reason, the results reported in this paper appear to be valuable as they are in agreement with previous findings, but are based on another method. From all evidence available it appears extremely likely that the subunits seen on the electron micrographs are not artefacts, but that some unit-membranes, at least, consist of (or contain) subunits, presumably lipid protein complexes. But the more electron microscopy approaches the molecular level, the more difficult it becomes to avoid artefacts altogether; the picture of a unit-membrane and of its subunits, as seen on the electron micrographs, depends, to some extent, on the method of specimen preparation, and it is not always possible to decide which method is the most reliable one. Thus the arrangement of the lipid and protein molecules in the subunits is still uncertain; it may be different in different types of membranes, and it may even depend in the same membrane on the functional state of the cell (DEUTSCH and KRAUSE[6]). (For a more detailed discussion of these problems see KORN[7] and also DEUTSCH and KRAUSE[6]).

The origin of the fibrils, which are occasionally found, is rather obscure; they may constitute a structural unit of the membrane (possibly consisting of structural proteins, KORN[7]) or they may be formed as the result of specimen preparation. But they do not constitute a contamination, as they have not been seen in specimens prepared by other methods. Our findings, however, are in agreement with a theory proposed by some authors according to which the cell membranes form a lattice of fibrous proteins. This hypothesis is based on electron-microscopic investigations of erythrocyte ghosts (see, for example, GIESE[8]).

Zusammenfassung. Isolierte Membranen von Mitochondrien (Ratte) wurden mit Kaliumpermanganat fixiert, mit Ultraschall behandelt und dann bedampft. Elektronenmikroskopische Aufnahmen zeigen eine granuläre Struktur und gelegentlich auch Fasern. Diese Ergebnisse sind im Einklang mit denen einiger anderer Autoren.

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