verabreicht. Compound 48/80 ist eine synthetische mastzelldegranulierende Substanz (vgl. [13]); chemisch handelt es sich um ein Kondensationsprodukt aus *p*-Methoxyphenyläthylmethylamin und Formaldehyd.

*Ergebnisse.* Die Ergebnisse sind in der Tabelle zusammengestellt. 24 h nach der Mastzelldegranulierung tritt eine statistisch signifikante Erhöhung der AP im Harn ein. Die Erhöhung beträgt im Durchschnitt das Vierfache des Ausgangswertes.

Ein anaphylaktoider Schock führt zu einer deutlichen Zunahme der beiden aus den Nierenepithelien stammenden Enzyme AP und LAP. Bei Ausbleiben einer Schockreaktion nach Verabreichung von Compound 48/80 im Zustand der Mastzellerschöpfung (z.B. durch Wiederholung einer Injektion von Compound 48/80 nach 48 h) tritt keine Erhöhung der AP- und LAP-Ausscheidung ein. Ob die im anaphylaktoiden Schock vergleichsweise wesentlich stärkere Zunahme der LAP-Ausscheidung als Beweis für eine zusätzliche extrarenale Herkunft dieses Enzyms gewertet werden darf, kann nicht mit Sicherheit ausgesagt werden. Wie an anderer Stelle noch ausführlicher berichtet werden soll, findet nach Auslösung eines anaphylaktoiden Schocks keine Erhöhung der AP und LAP im Serum statt. Die vermehrte Ausscheidung dürfte also ausschliesslich durch schockbedingte Nierenveränderungen bedingt sein; neben dem Blutdruckabfall ist vor allem die reflektorische Vasokonstriktion (vgl. [1]) für die Hypoxie und die hierdurch verursachte Schädigung der empfindlichen Tubulusepithelien verantwortlich.

*Summary.* Alkaline phosphatase activity was determined in rat urine under normal conditions (80 animals) and following mast cell depletion (30 animals). A statistically significant increase in urinary APA was found after administration of the mast-cell depleting compound 48/80. This fourfold increase over normal activity is due to renal changes caused by shock.

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## Demonstration of Sarcoplasmic Reticulum of Skeletal Muscle by Hematoxylin

The sarcoplasmic reticulum structure of skeletal muscle was demonstrated by the early histologists, using the technique of gold impregnation. Such an intermyofibrillar network pattern can be demonstrated clearly by staining with hematoxylin.

Skeletal muscle from human biopsies, from rats and from dogs were rapidly frozen on a chuck with 5% Tragant (gum tragacanth) by plunging into an acetone-dry ice mixture. Frozen sections were cut 10 $\mu$ thick in a cryostat at $-$ 25°C, picked up on coverslips, allowed to dry at room temperature, and then stained 5 to 60 min in hematoxylin solution (Caraci's hematoxylin was ordinarily used for 20 min). After being stained, the tissues were washed briefly in distilled water, dehydrated and mounted in Canada Balsam.

Material in intermyofibrillar regions was clearly stained blue. This blue intermyofibrillar staining was not prevented by prior washing of the section for 24 h at 4°C in normal saline or 30 min at 37°C in saliva or normal saline. The blue-staining of intermyofibrillar network was prevented by prior washing of the section in 100% ethanol for 30 min, and imperfectly prevented by prior washing in acetone and 10% neutral formaline for 24 h at room temperature. The blue intermyofibrillar network was not removed by alcohol or xylene during dehydration. The intermyofibrillar network pattern demonstrated by gold impregnation was prevented imperfectly by prior washing in 100% ethanol for 30 min. Cellular intermyofibrillar components of striated muscle fibre include the mito-

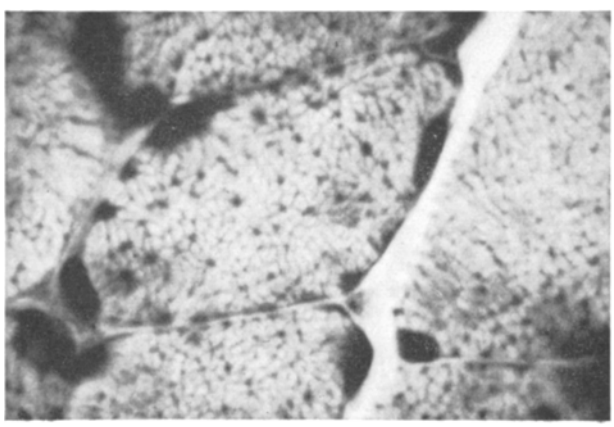

Fig. 1. Intermyofibrillar network of the anterior tibial muscle of rat. Cross section. Fibres with 'Fibrillenstruktur' (centre) and fibre with 'Felderstruktur' (right) are distinct. Hematoxylin, 1000 ×.

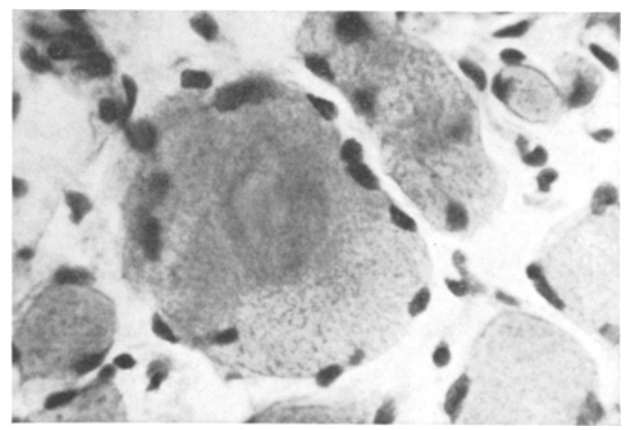

Fig. 2. Target appearance of a denervated muscle fibre (centre). Amyotrophic lateral sclerosis. Central portion of the fibre is lost the regular network appearance. Hematoxylin, 400 ×.

chondria, sarcoplasmic reticulum, aqueous sarcoplasm and lipid droplets. From the above tests, the blue-stained intermyofibrillar network is most likely a membranous component: mitochondria or sarcoplasmic reticulum or both, as suggested by ENGEL and CUNNINGHAM[1] for the red intermyofibrillar material seen with the modified trichrome stain.

Fibres of 'Fibrillenstruktur' and 'Felderstruktur'[2] were distinct in cross section, as shown by the pattern of the network surrounding the myofibrils after gold impregnation and after hematoxylin staining (Figure 1). In longitudinal section, this network appeared as longitudinal streaks between myofibrils or showed weak transverse banding due to transverse appearance of dots.

The changes of the intermyofibrillar network were observed in skeletal muscles from patients with neuromuscular diseases. The target appearance in denervated muscle fibres[3] was demonstrated as a central unstained region surrounded by a circle deeply stained with hematoxylin (Figure 2). The affected small fibres in neuropathy or myopathy showed an irregular pattern of the intermyofibrillar structure as shown by electronmicroscopy. It is possible to demonstrate the endplates and the intermyofibrillar network patterns in the same section by applying hematoxylin after cholinesterase histochemistry by Koelle's technique[4].

*Zusammenfassung.* Cytologische Untersuchung des Sarkoplasmas von Skelettmuskulatur an Hämatoxylin-gefärbten Gefrierschnitten. Es wird auf bestimmte Veränderungen des sarkoplasmatischen Reticulums bei neuromuskulären Erkrankungen hingewiesen.

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*July 7, 1965.*

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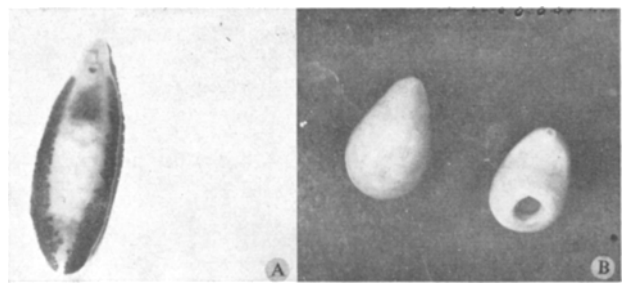
## Egg-Shell in Paramphistomatidae (Trematoda: Digenea)

The mechanism of egg-shell formation is one aspect of the physiology of reproduction in helminths that has been receiving increasing attention in recent years. The egg-shell in trematodes should be conceived as a device for the protection and transport of the miracidium. The eggs are released singly inside the host and then passed into the external environment. The miracidium developing inside the eggs requires to be protected from chemical action of the exudates from the tissues of the host as well as a multitude of fluctuations in the external environment. It is now well known that the egg-shell is highly resistant to many types of chemical attacks and other disturbances in the medium. The miracidium enclosed by such an egg-shell is thus amply protected against many vicissitudes. A comparable situation occurs in pseudophyllidean cestodes where the larva is a coracidium.

So far as it is known, in several trematodes, in which the chemical nature of shell material has been studied, the egg-shell is a quinone-tanned protein. It is characteristically amber coloured and is derived from precursors synthesized and stored in the vitelline cells. These precursors inside the vitelline cells have been histochemically identified as consisting of proteins rich in tyrosine and phenolase[1-5]. During my studies on egg-shell formation in trematodes, it was observed that in members of the family Paramphistomatidae the chemical nature of egg-shell differs significantly from this fundamental pattern. A remarkable feature, as differing from other trematodes, noticed in several members of this family (*Diplodiscus mehrai* Pande, 1937; *Paramphistomum cervi* (Zeder, 1790); *Gastrodiscus secundus* Looss, 1907; and *Carmyerius spatiosus* (Brandes, 1898)) is that the egg-shell is colourless and transparent without any indication of amber colour at any stage. The shell precursors inside the vitelline cells have also been found to be histochemically distinct. They gave positive reactions for proteins (Bromophenol blue reaction[6]), while tests for phenols (Fast Red Salt B and Argentaffin reactions[7,8]) and phenolase (Catechol reaction[2]) were entirely negative. Histochemical and other tests pointed out that the egg-shell in amphistomes is a keratin type of scleroprotein where the protein is stabilized by disulphide (–S–S–) linkages.

It has thus been revealed for the first time that at least two types of mechanisms are operating to stabilize




*A, Artyfechinostomum mehrai.* *B, Paramphistomum cervi.*

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