NÉMETHY and SCHERAGA[14,15] have discussed changes of water structure. They referred to the production of "clusters" which may influence permeability in the fibre etc. We may add that the fact that sodiumperchlorate has an intensive influence on collagen tension production, while $NaClO_3$ is completely inactive, may lead one to question the role of oxydation of collagen by perchlorate.

If the collagen fibre is transferred from 0.85% NaCl to the 5 *M* perchlorate, the osmotic pressure difference will drive perchlorate into the fibre and result in the observed increase of fibre weight, as the experiments show.

It may be worthwhile to follow the role of the aldehyde activity on collagen. One action is that aldehyde diminishes the solubility of collagen and increases its insolubility remarkably. It prolongs and increases the mechanical tension also. Young fibres have mainly soluble collagen while in old animals' fibres only insoluble collagen is present.

While former studies showed that the tension of collagen fibres increases with the animals' age, it is now found that studies with $NaClO_4$ and aldehyde present farther possibilities in the analysis of tension-producing factors. This mechanism is not explained in our experiments, but one is reminded that it may have a relation to the observation that in ageing, under biological conditions, tension changes appear. It remains to follow up with research along these lines.

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Hypertrophy and Atrophy of Mammalian Extraocular Muscle Fibres Following Denervation

There are at least six different types of muscle fibres in mammalian extraocular muscles[1–3]. Some of them have single neuromuscular junctions (phasic muscle fibres), others have multiple innervation by diffuse en grappe nerve terminations (slow tonic muscle fibres). Reactions of muscle fibres to denervation may be caused by the lack of neurotrophic influence from their motor innervation and by passive stretch, which influences the inactive muscle during activity of its antagonists. According to data available on reactions to denervation of various vertebrate muscles, different responses of the single muscle fibre types composing extraocular muscles are to be expected. Avian slow tonic muscle fibres show a long-lasting hypertrophy after denervation, especially if an additional stretch is given (reviewed by[4]). Common skeletal muscles (phasic) are known to react to deprivation of their motor innervation by atrophy[5], the phasic muscle fibres of the denervated hemidiaphragm, however nndergo a transient hypertrophy[4,6].

So far no reports were published about calibre changes of muscle fibres of extraocular muscles following denervation. It will be shown that the reactions of the different types of fibres found in extraocular muscles were as varied as was to be expected, considering the results mentioned above.

Material and methods. After anesthetizing adult rabbits with barbiturates, their right orbitae were opened. The inferior oblique muscle was denervated by removing a 3–5 mm segment of the nerve bundle innervating the muscle (belonging to N. oculomotorius). The results of denervating this single muscle were assessed histochemically 7, 21, 28, 34, 46, 55 and 83 days after denervation. At these times, the rabbits were sacrificed and the inferior oblique muscles of both sides were removed. The de-

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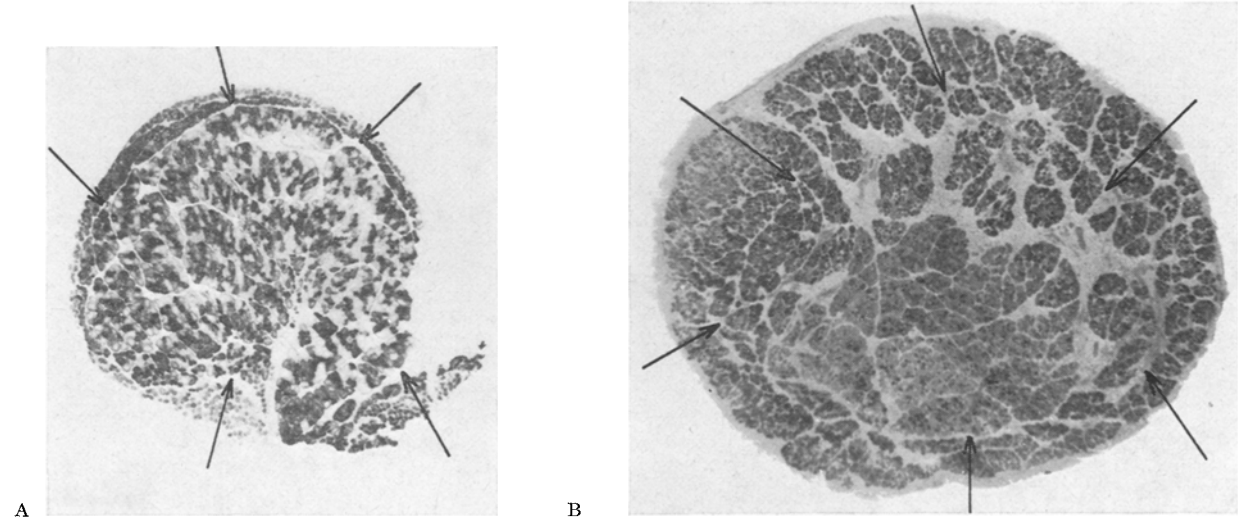

A B

Fig. 1. M. obliquus inferior, rabbit, SDH-activity, cross section. × 20. A) control. B) 55 days after nerve section. The border between superficial (orbital) and central (global) layers are marked by arrows.

Characteristics of muscle fibre types in extraocular muscles of the rabbit and typical denervation responses

Location	Orbital		Global			
Muscle fibre type	1	2	3	4	5	6
Diameter (μm)	10–25	10–20	30–55	20–40	15–30	20–40
SDH	++++	+++	+	++	+++	+/++[a]
Sudan	+++	++	+	++	+++	(+)
ATPase	+++	++	+++	+++	+++	+
Innervation[b]	Focal	Multiple	Focal	Focal	Focal	Multiple
Diameter changes after denervation	Hypertrophy	Hypertrophy	Atrophy	Different changes	Transient hypertrophy	Hypertrophy

[a] Special pattern of formazan deposits. [b] After[2].

nervated and the contralateral muscles (the latter serving for control) were loaded with 10 g and frozen with cooled Hexan at a temperature of −60 °C. They were then transferred to a cryostat (Dittes-Duspiva) where transverse serial sections (14 μm thickness) were cut at a temperature of −20 °C. These were fixed to glass slides and stained for succinic dehydrogenase (SDH; EC 1.3.99.1), adenosine triphosphatase at pH 9.4 (ATPase, pH 9,4; EC 3.6.1.3), and lipids by Sudan Black B, (for details, see[1]). The diameter of muscle fibres was determined by an ocular micrometer; 50 fibres of each fibre type were considered. Fibres are identified as representing a special fibre type by their location and staining reactions. After denervation, all muscle fibres undergo a progressive decrease in SDH reaction intensity. As the staining reaction of ATPase does not change much, the original fibre types may usually be distinguished even after denervation. Comparable fibres of the experimental and the contralateral muscles are collated.

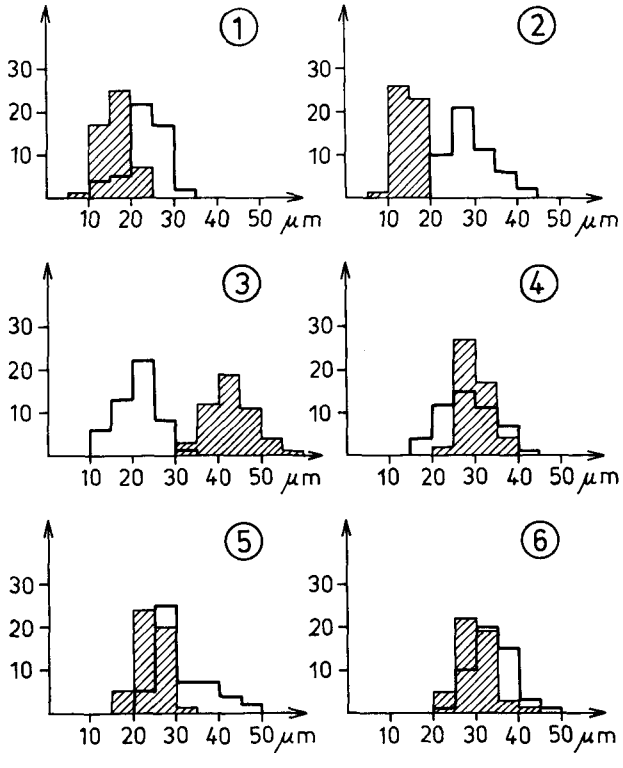

Fig. 2. Histograms of muscle fibre diameters in the inferior oblique muscle of the rabbit. Counts of 50 muscle fibres from a muscle after 34 days of denervation (boldface border) and from uninjured contralateral muscle (hatched). Numbers in this figure correspond to fibre types in the Table.

Results and discussion. After section of its motor nerve, the inferior oblique muscle of the right eye shows a distinct increase in volume (Figure 1A and B). This hypertrophy reaches its maximum about 4–6 weeks after denervation and is still evident after more than 9 months – provided there is no reinnervation. This phenomenon is caused by changing diameters of the muscle fibres, most of which grow thicker. How much and for how long a single muscle fibre will undergo hypertrophy will depend on the type of fibre.

In the Table the types of muscle fibres in normal extraocular muscles are summarized. The criteria for this distinction between fibre types are: calibre of muscle fibres, histochemical staining reactions, and type of innervation. In extraocular muscles two layers can be distinguished[1–3]. The superficial layer (usually 'orbital layer' because it is sometimes missing near the globe) is composed of 2 types of small-diameter muscle fibres (Table, type 1 and 2). Type 1 fibres are said to have single endplates, type 2 fibres have a distributed innervation[2]. The central layer (usually 'global layer' because it looks towards the eye ball if the superficial layer is missing there) contains mainly large-diameter muscle fibres. Besides a continuum of phasic muscle fibres (Table, type 3–5), there are fibres which show an extremely low ATPase staining reaction (type 6). The latter are supposed to be slow tonic (diffusely innervated) muscle fibres[1, 2].

The differentiated and often major change in fibre diameter subsequent to nerve section has been followed in its time course, using 7 animals at different times after denervation. 4 weeks after denervation, the changes reach a state which may be documented by the results of the animals sacrificed at the 28, 34 and 46 day in the same way. Figure 2 shows histograms of the diameters of the 6 fibre types of the inferior oblique muscle measured 34 days after surgery where 50 muscle fibres are compared in each case to the respective fibres of the uninjured contralateral muscle. The fibres of the superficial orbital layer (type 1 and 2) have significantly hypertrophied. This causes the big changes of the superficial layer of extraocular muscle seen in Figure 1. 34 days after nerve section in the global layer, the thick phasic muscle fibres (type 3) have atrophied; however, the thin phasic muscle fibres which are rich in mitochondria (type 5) exhibit hypertrophy – a transient hypertrophy as is shown by the specimens after long-lasting denervation. Type 6 muscle fibres show a clear-cut but limited hypertrophy.

The hypertrophy of fibre types 1, 2 and 6 was preserved for more than 3 months.

Extraocular muscles include muscle fibre types which are quite different from those to be found in common skeletal muscles. A long-lasting hypertrophy is shown by fibres which are considered to be tonic muscle fibres (types 2 and 6), subsequent to denervation. In particular, type 2 fibres react in a way which is known to be typical of avian slow tonic fibres[7,8]. The transient hypertrophy of the phasic muscle fibres which are comparatively rich in mitochondria (type 5) resembles the reaction described for diaphragm muscle fibres[4,6]. The results confirm the distinction made between no less than 6 different fibre types in extraocular muscles.

Summary. Extraocular muscles show a striking hypertrophy subsequent to denervation. This is due to hypertrophy of special histochemically characterized muscle fibre types.

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Richesse en lysosomes des préparations de synaptosomes du mésencéphale de rat
Lysosome-Rich Synaptosomal Preparations from Rat Mesencephalus

Depuis la découverte des lysosomes par de DUVE et al. en 1955[1] ces organites on fait l'object de nombreux travaux qui ont permis d'en préciser leurs caractères structuraux et biochimiques. Ces caractères sont réunis dans l'ouvrage de DINGLE[2].

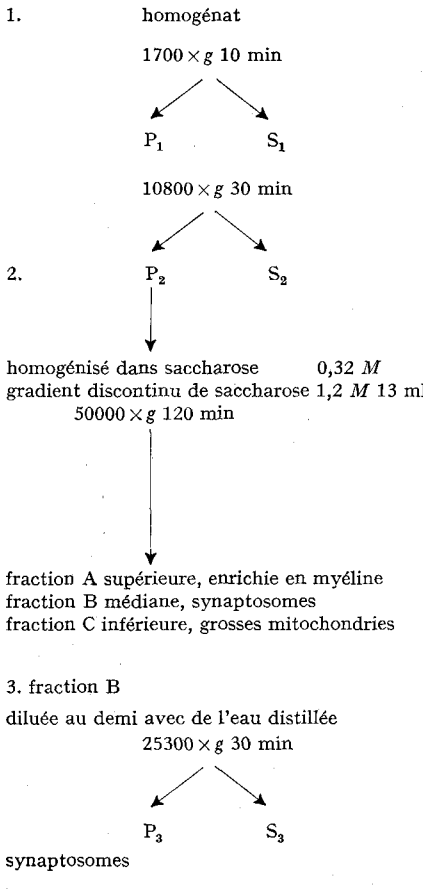
Les lysosomes peuvent être identifiés grâce à des «marqueurs» enzymatiques représentés par des hydrolases considérées comme leur étant spécifiques, notamment: phosphatase acide (EC 3.1.3.2.), β glucuronidase (EC 3.2.1.31.), ribonucléase (EC 2.7.7.16.).

Au cours de techniques de fractionnement des constituants cellulaires, les lysosomes, par leur taille et leur densité, sont le plus souvent associés aux mitochondries. D'autre part, les synaptosomes préparés en gradient discontinu, selon la méthode décrite par GRAY et WHITTAKER[3] montrent en microscopie électronique une «contamination» importante par les mitochondries. Ces deux phénomènes nous ont amené à étudier l'importance de la «contamination» par les lysosomes dans les différentes fractions décrites au cours de la séparation des synaptosomes, en mesurant l'activité phosphatase acide et en effectuant des photomicrographies au microscope électronique.

Matériel et méthodes. 1. Séparation des fractions. Des rats Sprague-Dawley, de 180–200 g, sont sacrifiés par décapitation, sans anesthésie. Après dissection de la boîte cranienne, on sépare les mésencéphales par la technique décrite par GLOWINSKY[4]. Les synaptosomes sont obtenus suivant la technique de GRAY et WHITTAKER[3] modifiée par ISRAEL et FRACHON-MASTOUR[5].

Les mésencéphales sont broyés à l'aide de l'appareil de Potter-B. Braun Melsungen, verre-teflon à 840 tours/min avec 10 aller-retours du piston (clearance 0,25 mm) pendant 1 min dans une solution de saccharose 0,32 *M* (homogénat à 10% p/v).

Les différentes fractions sont séparées selon le schéma suivant:



P_1, P_2, P_3 représentent les culots de centrifugation; S_1, S_2, S_3 représentent les surnageants.

Activité phosphatase acide au cours des différentes étapes de la séparation des synaptosomes du mésencéphale de rat

Fractions	Activité totale (mU/ml)	Protéines totales (mg/ml)	Activité spécifique (mU/mg)	Captation du GABA-I ^{14}C (n*M*/mg/min)
Fraction P_2	321	9,80	32,75	0,70
Fraction A	29	1,95	14,87	0,00
Fraction C	125	3,90	32,05	0,63
Fraction S_3	17	0,49	34,69	0,00
Fraction P_3	150	5,00	30,00	2,50