est administré par la même voie. Les animaux sont sacrifiés au bout de 30 min (sauf les rats T 60 qui sont soignés à la 60e min).

Dès le sacrifice des animaux, les reins sont excisés, puis de petits fragments corticaux (1 mm de côté) sont prélevés, puis plongés dans le glutaraldéhyde à 2,5% en milieu tamponné. Après 1 h de fixation, les pièces sont rincées dans du tampon-phosphate dilué au quart. Les pièces sont incluses dans de l'Epon 812, sectionnées à l'ultramicrotome, contrastées à l'acétate d'uranyle et au tétroxyde d'osmium, puis examinées au microscope électronique RCA-EMU 3G.

Les grains de ferritine sont comptés à la loupe sur les micrographies, toutes au même grossissement (×62500). La densité des grains est définie au niveau de la membrane basale glomérulaire (délimitée par les membranes des cellules endothéliales et épithéliales) par le nombre de grains comptés par unité de surface de structure à l'aide de petites fenêtres carrées de 4 cm de côté, découpées dans du carton.

Résultats. Sur les micrographies (rats T 30), la densité est de 5,08 ± 1,51 grains/cm². – Sur les micrographies témoins (rats T 60), la densité passe à 18,30 ± 3,21 grains/cm², soit une augmentation de 260%. – Sur les micrographies théophylline (rats Th 30), la densité passe à 17,11 ± 3,9 grains/cm², soit une augmentation de 236%.

Discussion. Malgré son haut poids moléculaire (500 000), le choix de la ferritine présente par rapport aux autres traceurs deux avantages majeurs. C'est une macromolécule qui présente, à la fois, l'avantage par rapport aux dextrans[4,5] d'être une protéine, donc une molécule chargée dont le comportement se rapproche des protéines sériques, tout en possédant de petits grains (d'hydroxyde ferrique) bien individualisés et aisément comptables.

Si l'on compare les densités de grains de ferritine se trouvant au sein de la membrane basale 30 et 60 min après l'injection du traceur protéique, nous remarquons une très nette augmentation (+ 260%), qui traduit l'arrivée progressive du traceur au niveau des structures filtrantes. De même, si l'on compare les densités de grains présents au sein des membranes basales de reins perfusés avec du sérum physiologique et de la théophylline, nous remarquons une très nette augmentation (+ 236%), qui met en évidence une intensification du passage du traceur protéique sous l'influence du réactif xanthique. Cette observation est confirmée par l'augmentation de 73,7% de l'excrétion protéique urinaire notée chez le rat[6].

Ainsi, par cette méthode, nous pouvons évaluer quantitativement les modifications du passage transglomérulaire d'un traceur protéique, soit sous l'influence du facteur temps, soit, à temps constant, sous l'influence d'un réactif physiopharmacologique modifiant les paramètres hémodynamiques de la filtration glomérulaire.

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Correlation of histochemical and physiological properties of muscle units in the striped skunk[1]

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Summary. Single motor units in striped skunk medial gastrocnemias were isolated and physiologically characterized. Individual muscle unit fibres tagged by glycogen depletion were analyzed histochemically and found to have histochemical profiles consistently correlated with the physiological findings.

The direct correlation between the histochemical and physiological properties of mammalian muscle fibres has been demonstrated with a technique which employs selective glycogen depletion following physiological measurements to 'tag' the component fibres of a single muscle unit[4,5]. Once tagged by this method the individual fibres of a muscle unit can be identified in frozen sections stained for glycogen. These same fibres can then be traced through serial sections stained for myofibrillar-ATPase and other enzymes used in the histochemical typing of muscle fibers.

This technique has been used on the m. soleus of the laboratory rat[4] and the gastrocnemius and soleus of the domestic cat[5]. The results showed that there is almost complete histochemical uniformity in the muscle fibres within a muscle unit and that there are 3 histochemical fibre types which consistently correlate to 3 physiological muscle unit types. Results, however consistent, from only 2 muscles in 2 species can only support cautious generalizations about the correlation of histochemical and physiological properties in other species. We sought to expand these generalizations by using these same techniques on another species with locomotor behavior and morphological characteristics divergent from the rat and the cat.

Our earlier work with the striped skunk (*Mephitis mephitis*) left us curious about the correlation between the histochemical and physiological properties of limb muscles from these animals. Studies of the physiological properties of striped skunk motor units, revealed only fatigue resistant units with a broad range of contraction times (30–95 msec)[6]. Subsequent histochemical analysis was supportive of the motor unit findings in that only fast oxidative glycolytic (FOG, type II A) and slow oxidative (SO, type I) fibres were present[7]. These unusual findings

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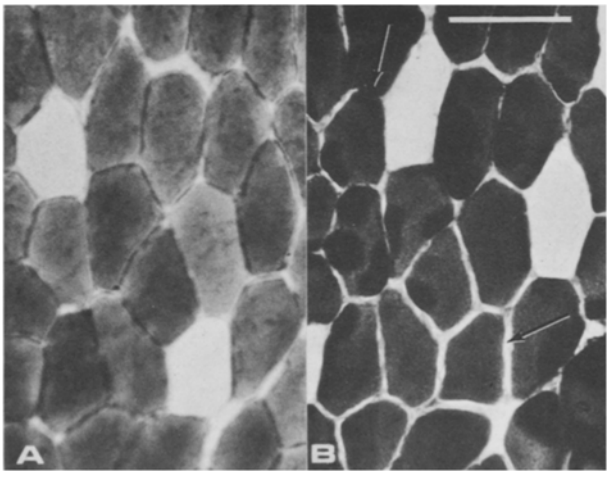
along with the divergent phylogeny and the different morphology and locomotor behavior of striped skunks made them a logical choice for the further testing of the direct correlation of muscle unit histochemical and physiological properties.

Acute experiments were performed on 5 adult striped skunks. Details of preparation and mounting for the motor unit analyses were as given elsewhere[6]. Briefly, the skunks were deeply anesthetized with pentobarbitol sodium (20 mg/kg b.wt) after an initial injection of acepromazine (1 mg/kg). Single alpha axons were isolated by ventral root dissection following a laminectomy from L_3 to S_2. A mineral oil pool maintained at 36 $\pm$ 3 °C was formed around the medial m. gastrocnemius following dennervation of the other hindlimb muscles. The muscle was tied to a strain ring attached to a rack and pinion. Confirmation of functional isolation of a single alpha axon was provided by: a) an all-or-none twitch and/or tetanic response following filament stimulation at voltages 2–5fold above threshold; b) a corresponding all-or-none EMG record from the muscle that was unchanging with increased voltage, and c) record of an all-or-none action potential from the spinal nerve filament following stimulation of the entire muscle nerve distally.

A lenth-active tension curve corresponding to normal physiological limits was generated for the whole muscle and isolated unit. All motor unit tests were performed at the shortest lenth corresponding to maximum tetanic tension. To determine the contraction time, each was stimulated at 100 Hz for 600 msec. 2 sec later a twitch was generated. This procedure was repeated at 5-sec intervals until the twitch had grown to a peak amplitude and twitch time had increased to its maximum. Twitch contraction time was measured from the onset of the EMG to the peak of twitch tension. To determine each unit's profile during an unfused tetanus (sag test), it was stimulated repetitively for 600 msec with interstimulus interval at 125% of the potentiated twitch contraction time. Muscle unit fibres were depleted of glycogen by 1 of 2 methods. The first 2 units were stimulated at 40 Hz for 2-min periods alternating with 2-min rest periods until no recovery of tension was observed. The remaining 2 units were depleted during muscle ischaemia following the method of Kugelberg[8]. Each unit was stimulated continuously at 40 Hz. Following 2 min of stimulation, ischaemia was produced by clamping the artery to the muscle in the groin for 2 min. This procedure was repeated 4 or 5 times or until no tension remained.

The whole medial m. gastrocnemius was removed and cut into 4 1–1.5-cm-thick discs, mounted for transverse sectioning and quick frozen in isopentane cooled to −160 °C in liquid nitrogen. The muscle samples were then sectioned (15 μm) and stained for the presence of glycogen using the periodic acid-Schiff (PAS) method[9]. Subsequent serial sections (10 μm) were stained for myofibrillar-ATPase (MF-ATPase)[10]; reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR)[11], and menadione mediated α-glycerophosphate dehydrogenase (α-GPD)[12] to facilitate the typing of the component fibres of the muscle unit as either fast oxidative glycolytic (FOG) or slow oxidative (SO)[13].

4 muscle units were successfully isolated and depleted. Their physiological and histochemical properties are summarized in the table. The figure shows a sample of



Serial sections from striped skunk medial gastrocnemius subjected to the glycogen depletion regimen. Fibres shown in *A* are stained for glycogen by the PAS method and those in *B* for MF-ATPase. 2 component fibres of muscle unit 4 (a FR unit) are shown in *A* as the relatively unstained fibres. These same fibres are indicated by arrows in *B*. Scale in *B* indicates 100 μm.

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Interrelated physiological and histochemical properties of muscle units from skunk medial m. gastrocnemius

	Muscle unit 1	2	3	4
Physiological properties				
Twitch contraction time (msec)	64	53	50	36
Maximum tetanic tension (TT) (g)	20	37	30	37
% of total maximum TT	1.6	3.1	2.6	1.8
Twitch tension (g)	4.5	8	10	15.5
% of total twitch tension	1.5	2.2	3.6	3.1
Resistance to fatigue	High	High	High	High
Post-tetanic potentiation (PTP)	0.89+	1.125	0.90+	0.71+
Sag in unfused tetani	0	+	0	+
Motor unit classification	S	S	S	FR
Estimated innervation ratio	950	750	600	350
Histochemical properties				
Mean fibre area (μm²)*	4791.9	5017.8	4090.4	3989.9
MF-ATPase	Low	Low	Low	High
NADH-TR	High	High	High	High**
α-GPD	Low	Low	Low	Intermediate to high
Fibre type classification	SO	SO	SO	FOG

*Taken from the cross-sectional area of 10–45 representative depleted fibres. Individual fibres in cross-sections near the middle of the muscle's length were projected and their outlines drawn. A planimeter was used to calculate the area within each fibre outline.

**Subsarcolemmal aggregations and a clustering of mitochondria in the remainder of the sarcoplasm characterized muscle fibres in the unit. The fibres in the other slower muscle units showed a more even distribution of mitochondria.

the histochemical results. The units which were analysed are generally representative of the broad population of 47 motor units previously studied in skunk m. gastrocnemius[6]. Slow (3) and fast (1) contracting units with variable twitch tensions and fatigue characteristics were studied. The only exceptions to the typical nature (for the skunk) of the physiological results were the observations of 'sag' in a slow motor unit and the presence of post-tetanic potentiation in 2 of the slow twitch units. Motor unit 2 (contraction time = 53 msec) showed distinct sag, a property which has been used to separate fast from slow contracting motor units in the cat[14]. Another property normally associated with fast contracting units, post-tetanic potentiation[14], was found in slow units 1 and 3.

The histochemical properties of the component fibres of each muscle unit were remarkably uniform. Of the fibres studied in our 4 units only 3 were non-uniform. These all occurred in unit 4. These exceptional fibres were hypertrophied and atypically spheroid in shape, and they were characterized by low staining intensities for all enzymes. These fibres were similiar in many respects to the giant fibres previously reported in pig muscle[15]. It is our conclusion that the gylcogen depletion shown in these fibres is not the result of the stimulation regimen but simply reflects an overall lack of staining.

Our findings are in agreement with the direct correlation of contraction speed with myofibrillar ATPase activity[16] and with similar but indirect correlations found in whole muscles or portions of muscles with different mixtures of histochemical fibre types[17]. Further and most importantly our results are compatible with the patterns of physiological and histochemical interrelatedness reported in the rat[4] and cat[5] using the same techniques employed in this study.

These results lend strong support to the generalization that histochemical methods can resolve descrete types of muscle fibres which correspond to the muscle unit types generally found in mammalian muscle. The fact that this pattern is found in an animal as functionally and phylogenetically distinct, from the rat and the cat as is the skunk suggests that the observed correlation between histochemical and physiological properties is a trait which may be shared by most mammals.

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Visual pigment regeneration: Occurrence in frog retina upside down upon the pigment epithelium[1]

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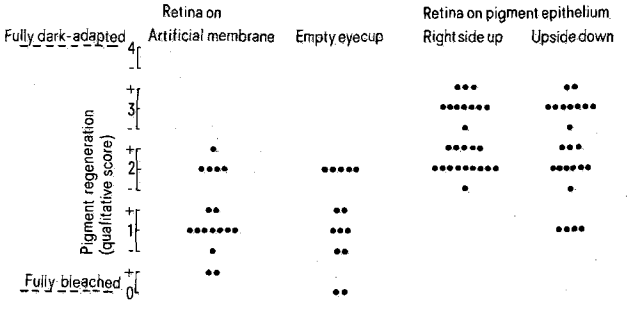
Summary. Visual pigment regeneration in the frog requires apposition between the retina and retinal pigment epithelium. However, regeneration occurs nearly as well with the retina upside down (i.e. with the inner limiting membrane facing the pigment epithelium) as right side up.

In 1877, Kühne[2] found that bleached frog retina would regenerate visual pigment only when it was in apposition to the pigment epithelium. Bridges[3] has suggested that the pigment epithelium may be necessary because vitamin A must be esterified during the regeneration cycle. We have had occasion recently to repeat some of Kühne's experiments, and made an additional observation that to our knowledge has not been reported: pigment regeneration occurs nearly as well with the retina upside down upon the pigment epithelium as right side up.



Qualitative assessment of pigment regeneration in the frog retina. The results are from 10 experiments in which placement of the retina right side up and upside down upon the pigment epithelium during dark-adaptation was compared. Each point represents 1 piece of retina from 1 experiment but the number of pieces in each category was not necessarily the same in every experiment.

Material and methods. Under dim red illumination, dark-adapted *Rana pipiens* were decapitated, sections of eyecup were prepared, and the retinas were gently peeled from the pigment epithelium and light-adapted for 15–30 min either as an isolated tissue or after replacement into the eyecups. The retinas were then dark-adapted for 3–5 h in a moist chamber at room temperature, under 1 of 4 conditions: 1. Isolated retina on a moist Nuclepore membrane. 2. Retina in bare eyecup (i.e. no choroid or pigment epithelium). 3. Retina in its normal position (i.e. right side up) upon the pigment epithelium in an eyecup. 4. Retina upside down (i.e. with the inner limiting membrane facing the pigment epithelium) in an eyecup. The retinal fragments averaged 5 mm², and were carefully placed right side up or upside down under direct visual control. They held their position well and became evenly colored after regeneration so that an edge effect could not have accounted for the results.

Results and discussion. The results after dark-adaptation were judged qualitatively, relative to the appearance of freshly peeled, fully dark-adapted retina ('4') or fully bleached retina ('0'). Color photographs of bleached and

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