durant 15 h dans un milieu semblable contenant de l'orangé d'acridine (1 µg/ml). Avant l'expérience, elles sont remises dans un milieu sans colorant. b) Des volumes connus d'une suspension d'amibes (1500/ml) et de cellules de *Tetrahymena* (800/ml) sont mélangés dans une fiole conique de 15 ml. Une aliquote est aussitôt transférée sur une lame à puits et observée au microscope en fluorescence. Le pourcentage d'amibes contenant un phagosome (spot fluorescent), ou plus, est déterminé à divers temps.

Résultats. L'activité phagocytaire de l'amibe en présence de neuraminidase subit une réduction de 15 et 30%, après 10 min seulement, aux concentrations respectives d'enzyme de 3 à 6 unités/ml (figure). Au bout de 15 min l'inhibition de la phagocytose peut atteindre facilement 50%, à 6 unités/ml.

Une étude du mouvement de l'amibe, à ces concentrations (3 et 6 unités/ml), rélève un comportement et un déplacement tout à fait normal des cellules, si on les compare aux témoins (sans enzyme). Ce comportement se traduit par la formation de pseudopode et l'adoption d'un mouvement polarisé. D'ailleurs il a été démontré qu'à des concentrations en neuraminidase beaucoup plus élevées (25 unités/0.1 ml) et pour une durée d'immersion beaucoup plus longue (60 min), les amibes conservaient une apparence normale[2].

Discussion. L'effet inhibiteur de la neuraminidase sur l'activité phagocytaire, à des concentrations qui n'affectent pas le mouvement ou l'apparence de l'amibe, met en relief un rôle important que semblerait jouer le glycocalyx de l'amibe dans le processus de capture et d'ingestion de ses proies. Des inhibitions analogues ont été observées chez les leucocytes affectés par le virus de l'influenza[14–17] et l'on a établi que la neuraminidase, en libérant les acides neuraminiques de la surface cellulaire, provoquait un affaissement des charges négatives qui se traduit par une baisse de la phagocytose.

Egalement chez *A. proteus*, il y aurait lieu de croire que l'intégrité du glycocalyx et surtout la présence d'acides sialiques pourrait jouer un rôle de premier ordre dans la perception des substances chimiotactiques libérées par les proies naturelles de l'amibe[9,13,18,19].

1 G.D. Pappas, J. Sci. *54*, 195 (1954).
2 P.K. Ray et S. Chatterjee, Experientia *31*, 1075 (1975).
3 S. Chatterjee et P.K. Ray, Indian J. exp. Biol. *13*, 294 (1975).
4 J.M. Marshall et V.T. Nachmias, J. Histochem. Cytochem. *13*, 92 (1965).
5 P.W. Brandt et G.D. Pappas, J. Biophys. Biochem. Cytol. *8*, 675 (1960).
6 P.W. Brandt, Exp. Cell Res. *15*, 300 (1958).
7 C. Chapman-Andresen, C. r. Trav. Lab. Carlsberg *33*, 73 (1962).
8 J.E. Brewers et L.G.E. Bell, J. Cell Sci. *4*, 17 (1969).
9 J.E. Brewers et L.G.E. Bell, J. Cell Sci. *7*, 549 (1970).
10 R.E. Lindberg, J. Protozool. *21*, 444 (1974).
11 D.M. Prescott et F.F. Carrier, dans: Methods in cell physiology, vol. I, p. 85. Academic Press, New York 1964.
12 J.L. Griffin, dans: The biology of *Amoeba*, p. 83. Ed. K.W. Jeon. Academic Press, New York 1973.
13 P. Marsot et P. Couillard, Can. J. Zool. *50*, 745 (1972).
14 D.J. Merchant et H.R. Morgan, Proc. Soc. exp. Biol. Med. *74*, 651 (1950).
15 T.N. Fisher et H.S. Ginsberg, Virology *2*, 656 (1956).
16 E.J. Ambrose, dans: Cell Electrophoresis. Symp. Br. Biophys. Soc. J.A. Churchill Ltd, 1965.
17 M.F. Tsan et P.A. McIntyre, J. exp. Med. *143*, 1308 (1976).
18 P. Marsot et P. Couillard, Can. J. Zool. *56*, 1497 (1978).
19 K.W. Jeon et L.G.E. Bell, Exp. Cell Res. *38*, 536 (1965).

Triple cones found in retinas of 3 fish species

B.A. Collins and E.F. MacNichol, Jr[1]

Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole (Massachusetts 02543, USA) 17 May 1978

Summary. Triple cones were found in the retinas of 3 species of fishes indigenous to the Woods Hole area. The function of these triple cones can not be deduced from the behavior patterns of these fishes.

In our laboratory, a histological survey of the retinas of fishes indigenous to the Woods Hole area is in progress. Many histological slides have been screened in choosing the tissue that best represents the retina of a particular species of fish to be included in this survey. The age and sex of the fishes used in this study are not determined. The tissue is prepared so that transverse and tangential sections through some region of the retina can be obtained. While scanning the numerous slides that have been made for this survey, we have found triple cones in 3 fish species, out of approximately 30 species examined to date. Since we were not looking expressly for unusual cone formation, many more local fish species may have triple cones than those we have, by chance, observed. Perhaps it is significant that triple cones have been found in nearly 10% of the fish species observed in this survey.

Ali and Anctil[2] report on several fish species in which triple cones have been found. Approximately 170 families of fish are described in their text, of which only 8 species, including the yellowtail, are described as having triple cones. Most of these 8 species can be found in the waters surrounding Woods Hole. We have seen these unusual photoreceptors in the yellowtail, the white hake and the American pollock.

Materials and methods. These 3 fishes were caught in the waters of the Woods Hole area. The eyes were removed, the lens and cornea were dissected away, and the remaining eye cup was immersed in 3% glutaraldehyde in 0.066 M phosphate buffer (pH 7.4). The eye cup was then post fixed in 2% osmium tetroxide in 0.14 M Veronal-acetate buffer (pH 7.4). The retinal tissue was embedded in Epon and approximately 10-µm thick sections were cut. The sections were unstained, because the contrast offered by the osmium is adequate when a Zeiss Nomarski Differential Interference-Contrast Microscope[3] is used to view the sections. The tissue sections were photographed with Polaroid Positive/Negative Land Film, type 665, through a ×12.5 eyepiece installed in a Polaroid camera. A ×40 objective was used on the Nomarski microscope.

Results. Triple cones were seen in 3 of more than 30 fish species we have examined so far. In the yellowtail, Ali and Anctil[2] found triple cones in a confined spot near the area retinae. We found these unusual photoreceptors near the optic nerve in the pollock. Because no attempt was made to

determine from which part of the retina the tissue used for the histological survey came, we do not know if the triple cones of the white hake are confined to a particular retinal area.

The photographs of the tangential sections through the photoreceptor layer of these 3 fishes (figures 1–3) are the same magnification. The ellipsoid bodies of the double cones of the white hake and pollock are nearly the same width, approximately 15 µm. The width of the double cone ellipsoids of the yellowtail is smaller, 8 µm. The triple cones are correspondingly larger through the widest area, 18 µm for the hake and pollock and 10 µm for the yellowtail.

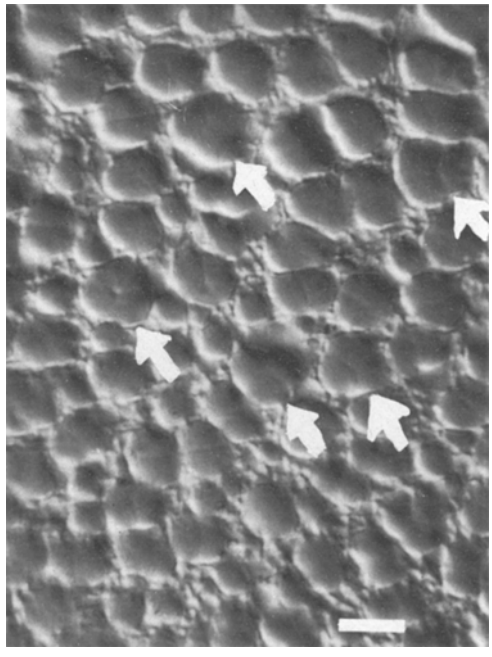

Fig. 1. Tangential section through the photoreceptor layer of the retina of the yellowtail *(Limanda ferruginae)*. Arrows point to some of the triple cones in the photograph. Double and single cones may also be seen.

Discussion. Because the function of unusual sensory receptors can sometimes be inferred from the behavior of an organism, we considered the habitat and feeding patterns of these 3 fishes.

As are most flatfishes, the yellowtail *(Limanda ferruginae)* is sluggish. It is known to eat small fish, but is not likely to catch these often. Stomach contents show it to be omnivorous, but it feeds chiefly on crustacea, shellfish and worms. The yellowtail is a bottom dweller, taking to the bottom after the fry stage. The fry swims on edge like any other fish. However, shortly before the flounder descends to the bottom, the migration of the eye takes place. Once it reaches the bottom, the flounder does not lie on its belly, but on its side. During the development of this fish, the skull twists so that the eye, which was originally fated to be underneath, migrates around the head until both eyes come to lie close together on the side that is uppermost as the fish lies on the bottom. The mouth retains its original position so that it is often described as opening sideways[4].

The white hake *(Urophycis tenuis)* is also a sluggish swimmer. Only while food is in motion does the hake recognize it by sight. The hake swims close to the bottom, dragging the sensitive tips of its ventral fins along the ground, seeming to rely largely on its sense of touch or contact chemoreception in searching for food[5]. The hake, like the yellowtail, is a bottom dweller. After the fry stage, the hake takes to the bottom and remains a ground fish for the remainder of its life, rising sluggishly into the upper layers only in pursuit of food[6].

The American pollock *(Pollachius virens)*, unlike the yellowtail and white hake, is an active fish, living at any level between bottom and surface, according to the food supply and the season. The pollock is a ravenous feeder, appearing at the surface in schools devouring shrimp or small fish. It

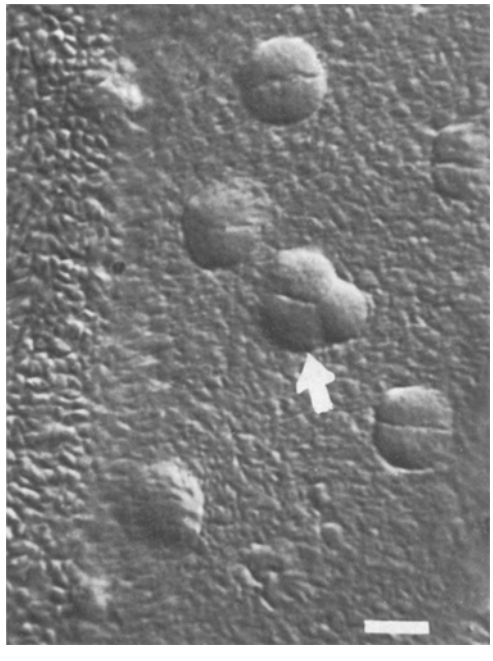

Fig. 2. Tangential section through the photoreceptor layer of the retina of the white hake *(Urophycis tenuis)*. The arrow points to a triple cone. Double cones may also be seen in the photograph.

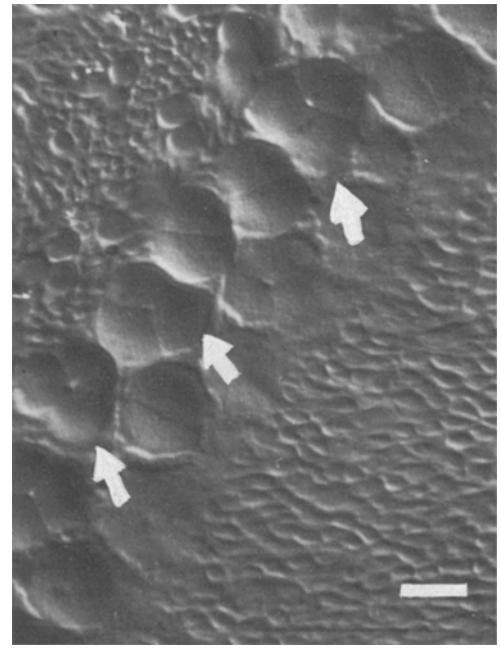

Fig. 3. Tangential section through the photoreceptor layer of the retina of the American pollock *(Pollachius virens)*. Arrows point to some of the triple cones in the photograph. Double cones may also be seen. Bars indicate 10 µm.

also feeds on bottom dwelling crabs and other crustacea. The distribution of prey governs the movement of pollock[7]. Experiments on fish kept in captivity at Woods Hole have shown that pollocks capture their food more by keen sight than by scent[5].

The behavior patterns of these fishes are obviously different. The yellowtail and hake are sluggish swimmers and are bottom dwellers in adult life. The pollock, in contrast, may be found in any part of the water column using its keen sight to actively pursue its prey. We conclude that the function of these triple cones cannot be deduced based on the behavioral patterns of these 3 fishes. It is, however, intriguing that many fishes in the Woods Hole area possess this unusual photoreceptor structure.

1 We wish to thank Kathleen French for her advice and criticism in the preparation of this paper.

2 M.A. Ali and M. Anctil, Retina of Fishes, An Atlas. Springer-Verlag, Berlin, Heidelberg and New York 1976.
3 R.D. Allen, G.B. David and G. Nomarski, Z. wiss. Mikrosk. *69*, 193 (1968).
4 H.B. Bigelow and W.C. Schroeder, Fishery Bull. Fish Wildl. Serv. U.S. *53*, 249 (1953).
5 C.J. Herrick, Bull. U.S. Fish Commn *22*, 258 (1904).
6 H.B. Bigelow and W.C. Schroeder, Fishery Bull. Fish Wildl. Serv. U.S. *53*, 221 (1953).
7 H.B. Bigelow and W.C. Schroeder, Fishery Bull. Fish Wildl. Serv. U.S. *53*, 213 (1953).

Ultrastructure of muscle spindles in C57BL/6J dy^{2J}/dy^{2J} dystrophic mice[1]

N.T. James and G.A. Meek

Department of Human Biology and Anatomy, University of Sheffield, Sheffield, S10 2TN (England), 19 April 1978

Summary. The sensory organs of skeletal muscles, the muscle spindles, were examined using electron microscopy in dy^{2J}/dy^{2J} dystrophic mice. Despite widespread damage to the extrafusal (skeletomotor) fibres the intrafusal (spindle) fibres appeared normal and seemed resistant to the aetiological factors for murine dystrophy.

Muscular dystrophy is a term used to describe a variety of inherited progressive diseases of skeletal muscle. In the mouse, there exists a muscular dystrophy caused by an autosomal recessive gene (denoted *dy*). This well known muscular dystrophy has been extensively studied as it has been considered a model for human Duchenne[2,3] and extraocular[4] muscular dystrophies because of the similarities in muscle histology. More recently, a 2nd progressive hereditary mouse myopathy has been discovered in which neurological abnormalities are also present[5-7]. This disease is caused by a 2nd allele (denoted dy^{2J}) at the *dy* locus. Histochemical and morphological studies of extrafusal muscle fibres in dy^{2J}/dy^{2J} mice have recently been described[8,9].

Although the pathological changes in murine muscular dystrophy are commonly attributed to a primary abnormality of the muscle fibres, there is evidence to suggest that muscular dystrophy could be attributable to neuronal abnormalities[7,10-12] particularly in dy^{2J}/dy^{2J} mice. This became known as the 'neurogenic hypothesis'. Electron microscopical studies of the richly innervated sensory organs of skeletal muscles, the muscle spindles, in 129 ReJ *dy/dy* dystrophic mice have shown that, contrary to expectation, they appear normal. Consequently, partial rejection or modification of the neurogenic hypothesis was proposed[13].

The present study was undertaken to establish whether there are any ultrastructural changes detectable in the intrafusal fibres in muscle spindles of dy^{2J}/dy^{2J} dystrophic mice. Any evidence suggesting that intrafusal muscle fibres are capable of resisting the pathological processes that induce changes in the extrafusal fibres would be of considerable interest.

Specimens of extensor digitorum longus muscles were obtained from 6 clinically affected dy^{2J}/dy^{2J} dystrophic mice at 3 months of age and from 6 non-littermate controls and prepared for electron microscopy as described previously[13,14]. 15 spindles were found in dystrophic muscle. Ultrathin sections were cut from the spindles at intervals along most of their lengths. Some spindles were also examined in longitudinally-cut sections.

The dystrophic spindles contained an average of 4 intrafusal fibres ranging from 3 to 7 fibres. Nuclear bag fibres contained up to 3 nuclei and a peripheral rim of myofilaments in each equatorial cross section and could be clearly differentiated from the narrower and shorter nuclear chain fibres which contained singly occurring central nuclei. Both the major types of intrafusal fibre contained numerous myofilaments in their polar regions. Nuclear bag fibres tended to contain confluent masses of myofilaments interspaced with relatively infrequently occurring mitochondria of variable size. In a few nuclear bag fibres the mitochondria were of more regular size and more evenly dispersed throughout the fibre cross sections. These 2 types of nuclear bag fibre correspond to those previously designated as types 2 and 3 respectively[13,15]. Nuclear chain fibres contained groups of myofilaments which formed relatively discrete myofibrils often separated by numerous mitochondria. In both nuclear bag and nuclear chain fibres the number of myofilaments was markedly reduced in their equatorial regions compared with their polar regions. Numerous dilated terminal cisternae of triads typical of those previously reported in the mouse[12] and in other species[16] were also found.

Satellite cells were found lying between the basement membrane of the intrafusal fibres and their plasma membranes. They seemed inactive because their relatively small amounts of cytoplasm contained few ribosomes and little rough endoplasmic reticulum. All intrafusal fibres examined received a direct sensory innervation without intervening basement membrane.

Each spindle was seen to possess a prominent periaxial space containing a characteristic flocculent precipitate. Typically, aggregations of the precipitate could be found close to inner capsule cells on the side facing the intrafusal fibre. The periaxial space was bounded by a capsule consisting of up to 5 or 6 layers of perineural epithelial