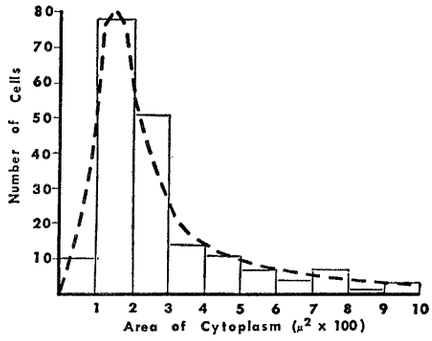

Fig. 2. A histogram showing the distribution of cell sizes in the mouse ventral horn. The dashed curve is a plot of the number of ventral root units responding (ordinate) to increasing muscle stretch stimulus [abscissa; derived from Henneman's (*1*) data on the cat].

wise noted, all the data refer to the normal side of the spinal cord.

The histogram in Fig. 2 describes the spectrum of neuronal sizes in the mouse ventral horn. Since the nucleus occupies a disproportionately large area in small neurones relative to large ones, the cytoplasmic area is used as a measure of cell size. The dashed curve superimposed upon the histogram plots the number of ventral root units responding to muscle stretch against the increasing stimulus threshold derived from Henneman's data (*1*) on the cat. The fit agrees with Henneman's notion of a relation between neuronal size and threshold. The cells with areas of 400 to 1000 μ^2 represent the large, alpha motor neurones; those with areas of 0 to 300 μ^2 represent smaller, oval cells which are either small motor neurones or interneurones. On the contralateral side, where the sciatic nerve had been sectioned, most of the smaller neurones were enlarged and had less heavily stained Nissl substance. Since clear-cut chromatolysis is difficult to observe in rodents, I offer this only as suggestive evidence that the majority of the cells with areas of 0 to 300 μ^2 are motor neurones.

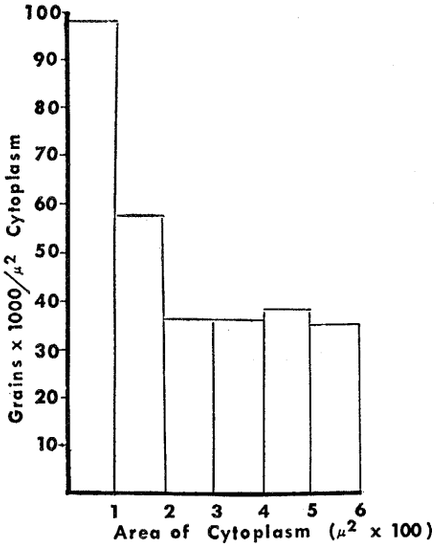

Fig. 3. The rate of protein synthesis per unit area among ventral horn neurones of different size groups. The ordinate represents grains over the cytoplasm above background $\times$ 10^3 per square micron of cytoplasmic area.

Figure 3 is a histogram describing the rate of protein synthesis (grains/per square micron of cytoplasm) in neurones of increasing sizes. The significance of the differences between the means of the first and second and between those of the second and third columns is in both cases $P < .0001$. There is clearly an inverse relationship between neuronal size and rate of uptake of H^3-phenylalanine per square micron of cytoplasm.

My data confirm that there is an inverse relation between neuronal size and firing rates and between size and rate of protein synthesis per square micron of cytoplasm. It could be suggested that there is a direct relation between the rate of firing and that of protein synthesis. However, there is also an inverse relation between neuronal size and the ratio of the neuronal surface area to volume. If the rate of protein synthesis were governed by the ratio of neuronal surface to volume, then one would expect increased rates of protein synthesis to be accompanied by decreased cell size (that is, an increased ratio of surface to volume). On the contrary, when neurones produce more protein during regeneration (*2*) or increased stimulation (*3*) their size actually increases. Therefore, of these two possibilities, I would suggest that it is the rate of firing, and not the ratio of surface to volume, which determines the rate of protein synthesis in neurones.

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References and Notes

1. E. Henneman, G. Somjan, D. O. Carpenter, *J. Neurophysiol.* **28**, 561 (1965).
2. S. O. Brattgard, J. E. Edström, H. Hydén, *Exp. Cell Res. Suppl. 5*, p. 185 (1958).
3. M. Murray, *Anat. Rec.* **154**, 392 (1966); R. Ortman, in *Handbook of Physiology*, J. Field, Ed. (Williams and Wilkins, Baltimore, 1960), vol. 2, sec. 1, p. 1039.
4. Supported by PHS grant NB-05038. I thank Miss B. Bruser for technical assistance and Dr. A. Morrison for his continued interest.

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Minchinia nelsoni n. sp. (Haplosporida, Haplosporidiidae): Causative Agent of the Delaware Bay Oyster Epizoötic

Abstract. *Since 1957, oyster populations of the Middle Atlantic coast have been ravaged by a new sporozoan parasite that has been called "MSX." This parasite is identified as a new species of* Minchinia *that invades oysters through epithelial tissues of gill, palp, water tubes, and, occasionally, of the digestive tract. Multinucleate plasmodia are recognized in fresh and fixed preparations.*

In the spring of 1957 approximately half the oysters planted on New Jersey oyster grounds in Delaware Bay died within 6 weeks. The pattern of losses and the continuing mortalities later that summer and fall indicated disease as the cause. Histological examinations of oysters in the spring of 1958 revealed a microorganism previously unreported from oysters (Figs. 1 to 5). In the late summer that year the new organism was associated with the high mortalities that extended throughout the lower bay. The organism was called "MSX" from the multinucleate spherical plasmodium and has been so designated (*1*).

To expedite application of research to problems in industry, results have been freely circulated through conferences and manuscript reports (*2*). The purpose of this report is to establish the taxon and thus facilitate release of accumulated information to the published literature.

Minchinia nelsoni n. sp.: plasmodial stages in blood spaces of all tissues of the eastern oyster, *Crassostrea virginica* (Gmelin); also in epithelium of gills, water tubes, and at times in all epithelia of gut. Type locality. Oyster grounds of Delaware Bay, New Jersey; range extends to Great Bay, New Jersey, Great South Bay, Long Island, Chincoteague, and lower Chesapeake Bay of Maryland and Virginia. The species is named for T. C. Nelson.

Plasmodial stage. Roughly spherical plasmodia usually from 4 to 30 μ in diameter. Occasionally as large as 50 μ; one to more than 60 nuclei from 1.5 to 7.5 μ in diameter. Nuclei show a prominent peripheral endosome and all except the smallest sizes have the intranuclear bar (Figs. 1 and 2) described by others in *Minchinia* species (*3*, *4*).

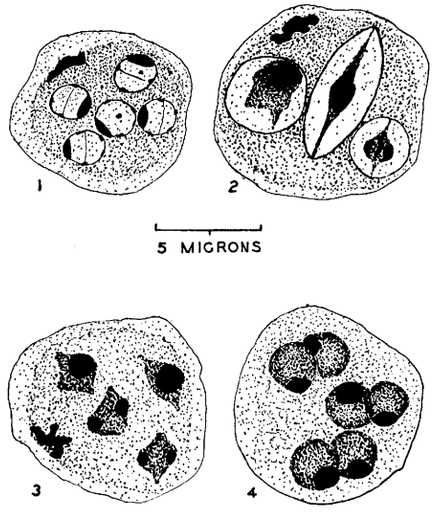
These nuclear details appear both in

fresh preparations stained with 0.1 percent methylene blue and in sectioned materials fixed in Zenker's or Davidson's fluids and stained with Harris' or iron hematoxylin. Frequently the nuclei appear to lie in a central cytoplasmic zone surrounded by a less densely staining cytoplasmic zone. The plasmodial membrane is usually distinct and frequently irregular. Two nuclear types are recognizable: (i) small nuclei, 1.5 to 1.6 μ in diameter with densely staining caps, in plasmodia concentrated in and under epithelium of gills, palps, and epibrancial chambers in newly infected oysters; (ii) large nuclei, 2.5 to 3.0 μ in diameter, with intranuclear bar may be found in plasmodia throughout blood spaces of the oyster within 10 days after the first appearance of infection. This type persists throughout the year in plasmodia, with the cytoplasm frequently vacuolated and granular. Frequently interspersed with these throughout the year are plasmodia with very large nuclei ranging up to 7.5 μ in diameter. These very large nuclei may be either spherical or ovoid and invariably have the prominent intradesmose (Fig. 2). Occasionally large and very large nuclei are found in the same plasmodia.

Spore. The spore is not identified.

Differentiating characters. The only other *Minchinia* described in oysters *M. costalis* overlaps the range of *M. nelsoni* along the seaside of Virginia and Maryland. It occurs rarely in Delaware Bay. *M. costalis* progresses from plasmodia to spores from late winter through spring to July, while *M. nelsoni* is found only in plasmodial stage throughout the year. The stage of *M. nelsoni*, after it has invaded the host, closely resembles smaller plasmodia of *M. costalis*, but the nuclear membrane of *costalis* is frequently indistinct; the endosome is smaller and gives the impression of a dense peripheral dot. In contrast, the nuclear membrane of *M. nelsoni* is distinct and the prominent endosome usually appears as a polar cap. Close examination also reveals smaller peripheral endosomes. Beyond the smaller plasmodial stages *M. nelsoni* is distinguished by larger nuclei with distinct membranes. The plasmodial membranes of *M. costalis* are comparatively indistinct with a characteristic "frayed-out" appearance.

Paratypes. Permanent, stained slides have been sent to numerous marine laboratories on the East Coast from Prince Edward Island to North Carolina, and to the College of Fisheries, Seattle, Washington.



Figs. 1 to 4. Plasmodial stages of *M. nelsoni* commonly seen in Delaware Bay oysters.

The close correspondence in structural detail of this new oyster parasite to plasmodial stages of several species of *Minchinia* as revised by Sprague (*4*), especially to *M. limnodrili* (Granata) and *M. cernosvitovi* (Jirovec), has led us to place it provisionally in this genus, though there is some uncertainty about this decision until the spore is identified. Although over 10,000 oysters with *M. nelsoni* infections have been examined from Delaware Bay alone, only two (one in July 1961 and one in November 1961) have been found with typical large *Minchinia* spores. This large spore (larger than *M. costalis*) was seen earlier in an oyster from the lower Chesapeake Bay and more recently in an oyster from the Maryland Chincoteague Bay (*5*). In all four of these oysters typical *M. nelsoni* plasmodial stages are also present and there are clearly distinguishable sporulation stages in epithelia of the digestive caeca. This extremely low incidence of spores leads us to suspect, however, that they are not *M. nelsoni* spores but rather indicate double infections as discussed by Canzonier (*6*). Since late 1958 all attempts to transfer infection to healthy oysters in the laboratory have failed. Failures in effecting transmission and rarity of oysters with sporulation stages point strongly to an alternate or reservoir host for the parasite.

Barrow (*7*) reviewed the question of sexuality in the genus *Minchinia* (*Haplosporidium*), and in his studies of *M. pickfordae* failed to find evidence for autogamy or reduction division. We also fail to find clear-cut evidence for gametic fusion, although we have abundant materials in which both types of nuclei described above are seen in pairs (Fig. 4). Our small dense plasmodia with small nuclei, typically found in newly infected oysters, would appear to correspond to the plasmotomy stages of Pixell-Goodrich (*4*) and perhaps the pre-autogamy stages of Debaisieux (*9*).

Patterns of infection and mortality in susceptible stocks of oysters in Delaware Bay have been worked out in detail. Intense epizoötic kill begins within

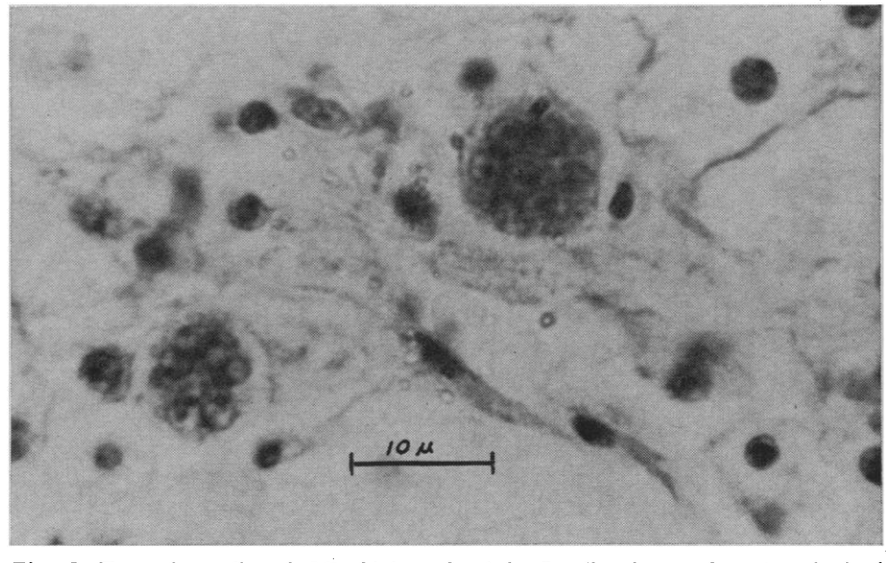


Fig. 5. Two plasmodia of *Minchinia nelsoni* in Leydig tissue of oyster dredged from ground 471 C, Delaware Bay, N.J., 15 April 1958. This was the oyster in which *M. nelsoni* was first recognized. Bouin fixation; hematoxylin-eosin.

3 weeks after first appearance of plasmodia in gills. Immediately prior to the kill 70 to 75 percent of living oysters have been found infected with *M. nelsoni*. Incidence in gapers (recently dead oysters) during the epizoötics is commonly 100 percent.

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References and Notes

1. H. Haskin, *Proceedings 1st National Coastal and Shallow Water Research Conference* (NSF, 1961, p. 207); J. L. Wood and J. D. Andrews, *Science* **136**, 710 (1962); J. D. Andrews, *Proc. Nat. Shellfish. Assoc.*, 1962, p. 65; C. A. Farley, *J. Invert. Pathol.* **7**, 144 (1965); H. Haskin, W. J. Canzonier, J. Myhre, *Ann. Rept. Am. Malacol. Union*, 1965, p. 20; J. D. Andrews, *Ecology* **47**, 19 (1966).
2. In January 1959, representatives of university, state, and federal laboratories and agencies of oyster-producing regions of the United States and Canada discussed at Rutgers University the problems of the new oyster epizoötic. As "MSX" has been associated with successive mortalities in Delaware, Maryland, Virginia, and New York, annual mortality conferences have been continued with a free-sharing of research materials and results among the cooperating laboratories.
3. L. Granata, *Arch. Protistenk.* **35**, 47 (1914); O. Jirovec, *ibid.* **86**, 500 (1935). H. Pixell-Goodrich, *Proc. Zool. Soc. London* **1915**, 445 (1915).
4. V. Sprague, *J. Protozool.* **10**, 263 (1963).
5. We thank Dr. J. L. Wood of the Virginia Institute of Marine Science for the first slides of this spore, taken November 1960, and Dr. V. Sprague of the Chesapeake Biological Laboratory for materials collected in June 1965. One additional oyster with sporulation stages was obtained by the Virginia Institute of Marine Science in each of the the years 1963, 1964, and 1965.
6. W. J. Canzonier, *Proceedings of the 5th Annual Shellfish Mortality Conference*, Oxford, Maryland, 1963, p. 36.
7. J. H. Barrow, *Trans. Amer. Microscop. Soc.* **80**, 819 (1961).
8. P. Debaisieux, *La Cellule* **30**, 291 (1920).
9. Supported by U.S. Bureau of Commercial Fisheries since 1958, and through grants from the Division of Shellfisheries of the New Jersey Department of Conservation and Economic Development. We thank W. J. Canzonier, J. L. Myhre, D. R. Kunkle and W. A. Richards for technical assistance. Mr. Myhre prepared our illustrations.

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Sleep Deprivation and Brain Acetylcholine

Abstract. *Rats deprived of D-state sleep (and, to some extent, of slow-wave sleep) for 96 hours show a significant fall in brain acetylcholine in the telencephalon; there were no significant changes in the diencephalon and brain stem. Restraint stress and activity wheel stress produced no significant change in acetylcholine levels in any of these regions; the telencephalic response to sleep deprivation, therefore, cannot be attributed to nonspecific stress. The effects of D-state deprivation and the psychoactive anticholinergic drugs on telencephalic acetylcholine levels are similar.*

The neurochemical consequences of prolonged sleep deprivation have not been identified. While the precise behavioral effects of sleep deprivation vary somewhat among species, some of the psychophysiological effects represent an ill-defined state of "activation." In man prolonged sleep deprivation results in signs of task-induced activation (*1*). Specific deprivation of those regularly recurrent periods of sleep referred to as REM, paradoxical sleep, activated sleep, or the D-state is followed by a "pressure" toward D-state (*2*). This is manifested by an apparently compensatory increase in activated or D-state sleep. These periods, associated with dreaming in man, are characterized in most mammals by signs of increased activity in various physiological measures [cortical electroencephalogram (EEG), heart rate, respiration, temperature, extraocular movements] and relaxation of certain groups of muscles of the head and neck (*3*). Since the amount of acetylcholine in the brain varies predictably along the crude dimensions of overall brain activity, from sleep or anesthesia (elevated acetylcholine) to seizures (lowered acetylcholine) (*4*), it appeared probable—given measures in the appropriate brain regions and adequate procedures for assuring D-state deprivation—that changes in acetylcholine could be observed. In a study in which rats were partially sleep-deprived for 48 hours on a slowly moving wheel in a water tank, there were no changes in amount of acetylcholine in whole brain (*5*); such procedures, however, do not reliably prevent D-state sleep, and estimation of acetylcholine in whole brain alone could mask significant regional changes.

To assure deprivation of the D-state component of sleep, male rats (200 to 275 g) were isolated for 96 hours on wooden blocks (5 cm square) in 5 cm of water (*6*); the animal does not get wet as long as it does not relax the muscles of the neck and head. It was first confirmed in 5 hours of early afternoon recording of a group of rats with implanted electrodes that these rats normally have about 15 periods of D-state sleep, each lasting 3 to 4 minutes, and 165 minutes of slow-wave sleep. The deprivation procedure did not permit any periods of D-state sleep (evident both by observation and EEG monitoring), although the animals were able to obtain numerous brief periods of slow-wave sleep. Recordings of two animals after 24 hours of D-state deprivation revealed an increased length and frequency of D-states. Accordingly, in the present studies the animals were deprived almost completely of D-state and perhaps to some extent of slow-wave sleep.

Experimental animals were removed every 4 hours for feeding and watering for a 15- to 20-minute period during which they were kept awake. At the end of the 96-hour deprivation period animals were taken immediately from the island and killed, and the brains were rapidly removed and dissected by a modified near-freezing technique (*7*) in which a mixture of acetone and dry ice was used in place of liquid nitrogen. Three regions of the brain were dissected: telencephalon (cortex, hippocampus, and caudate); diencephalon (thalamus, hypothalamus); and caudal brain stem (posterior to the colliculi and rostral to the obex and without the cerebellum). Control animals were of closely matched weights and ages. Acetylcholine was extracted with two portions of citrate buffer (*8*). Samples were frozen and assayed within 72 hours with LSD-25–stimulated ($10^{-7}M$) clam heart (*9*). Control values were in good agreement with those recently reported for comparable rat brain regions measured by the clam heart assay (*7*).

A total of 19 D-state deprived animals were studied in three separate experiments (Table 1). The telencephalon of the experimental animals showed a significantly lower mean acetylcholine level—a decrease of 35 percent—compared with controls. The difference between the mean acetylcholine values for control and sleep-deprived groups was significant ($P<.01$) for each experiment as well as for the pooled data. There was a slight but insignificant change in the same direction for the diencephalon and no change in the mean values for the caudal brain stem. To evaluate the specificity of this find-