extract (Fig. 4*a*), but the peafowl heart extract did not react even when the antiserum concentration was raised. By contrast, antiserum to H_4 reacted strongly with the peafowl heart extract as well as with chicken H_4 LDH, but showed no reaction with the peafowl breast extract (Fig. 4*b*).

The immunological, catalytic, and thermostability experiments permit us to identify peafowl breast LDH as an M_4 isoenzyme and peafowl heart LDH, despite its low electrophoretic mobility (*24*), as an H_4 isoenzyme. Had only electrophoretic criteria been employed, the peafowl isoenzymes might have been incorrectly identified.

RICHARD G. ROSE
ALLAN C. WILSON
Department of Biochemistry, University of California, Berkeley

References and Notes

1. Number 1.1.1.27 in *Enzyme Nomenclature*, Recommendations of the International Union of Biochemistry (Elsevier, New York, 1965).
2. Random combination of LDH subunits appears to take place in many but not all vertebrates, as shown by S. N. Salthe, O. P. Chilson, N. O. Kaplan, *Nature* **207**, 723 (1965).
3. Abbreviations used in this paper: LDH (lactate dehydrogenase); NAD (nicotinamide adenine dinucleotide); NADH, the reduced form of NAD; AcPyAD (3-acetylpyridine adenine-dinucleotide); EDTA, ethylenediaminetetraacetate; BSA, bovine serum albumin.
4. E. Appella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961); C. L. Markert and H. Ursprung, *Develop. Biol.* **5**, 363 (1962); C. L. Markert, *Science* **140**, 1329 (1963).
5. R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
6. T. P. Fondy, A. Pesce, I. Freedberg, F. Stolzenbach, N. O. Kaplan, *Biochemistry* **3**, 522 (1964); A. Pesce *et al.*, *J. Biol. Chem.* **239**, 1753 (1964).
7. A. C. Wilson, S. N. Salthe, N. O. Kaplan, in preparation.
8. C. L. Markert and I. Faulhaber, *J. Exp. Zool.* **159**, 319 (1965).
9. We thank C. Baldwin (San Fancisco Zoo), K. Lint (San Diego Zoo), G. Spalding (Los Angeles County Aboretum), L. Poisal (Pleasanton, California), H. Abplanalp (Univ. of California, Davis), and R. Paynter (Harvard Univ.) for assistance in obtaining specimens.
10. A. C. Wilson, N. O. Kaplan, L. Levine, A. Pesce, M. Reichlin, W. S. Allison, *Fed. Proc.* **23**, 1258 (1964). We thank E. Stoltzenbach and A. Pesce who characterized and purified H_4 and M_4 in N. O. Kaplan's laboratory.
11. Oxidation of NADH was followed by measuring decrease in absorbance at 340 mμ, with a Zeiss model PMQII spectrophotometer, in potassium phosphate buffer (0.1*M*, *p*H 7.5) at 23°C. Initial concentrations were: NADH, $1.4 \times 10^{-4}M$; sodium pyruvate, $3.3 \times 10^{-1}M$; LDH, approximately 3×10^{-2} μg per milliliter; in a final volume of 3.0 ml. As used in this paper a unit of LDH activity produces an absorbance change of 1.0 per minute in a 3-ml reaction volume at 23°C and is approximately equivalent to 1 μg of LDH or 1 international unit (at 30°C).
12. I. H. Fine and L. Costello, *Methods Enzymol.* **6**, 958 (1963).
13. I. Smith, *Chromatographic and Electrophoretic Techniques*, vol. 2, *Zone Electrophoresis* (Interscience, New York, 1960), p. 95.
14. Since no individual variation was detected in the electrophoretic mobility of the LDH's of 44 peafowl, subsequent investigations were carried out with heart and breast extracts from one or two individuals.
15. This buffer contained: 0.14*M* NaCl, 0.01*M* tris, $5 \times 10^{-4}M$ magnesium sulfate; $1.5 \times 10^{-4}M$ calcium chloride; and bovine serum albumin (1 mg/ml). The *p*H was 7.5.
16. A. C. Wilson and N. O. Kaplan, in preparation.
17. F. Kubowitz and P. Ott, *Biochem. Z.* **314**, 94 (1943).
18. The relative lack of pyruvate inhibition shown by the M_4 enzyme is thought to facilitate rapid anaerobic glycolysis, such as occurs in large skeletal muscles. The H_4 enzyme is strongly inhibited by pyruvate and has a high affinity for lactate. Since this enzyme predominates in tissues capable of sustained aerobic metabolism, such as the heart, it is though to be concerned with aerobic metabolism. See, for example, N. O. Kaplan and T. L. Goodfriend, *Advance. Enzyme Regulat.* **2**, 203 (1964); D. M. Dawson, T. L. Goodfriend, N. O. Kaplan, *Science* **143**, 929 (1964); A. C. Wilson, R. D. Cahn, N. O. Kaplan, *Nature* **197**, 331 (1963); C. L. Markert and H. Ursprung, *Develop. Biol.* **5**, 363 (1962).
19. N. O. Kaplan, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 243.
20. We have been unable to confirm a recent report that M_4 LDH becomes strongly inhibited by pyruvate at 37°C [E. S. Vesell, *Science* **150**, 1590 (1965)].
21. N. O. Kaplan, M. M. Ciotti, M. Hamolsky, R. E. Bieber, *Science* **131**, 392 (1960); N. O. Kaplan and M. M. Ciotti, *Ann. N.Y. Acad. Sci.* **94**, 701 (1961).
22. D. Stollar and L. Levine, *Methods Enzymol.* **6**, 848 (1963).
23. E. Wasserman and L. Levine, *J. Immunol.* **87**, 290 (1961).
24. Two factors may account for the reversal of the relative mobilities of the peafowl H_4 and M_4 isozymes, (i) the unusually high isoelectric point (approximately 7 to 8) of this H_4 enzyme and (ii) a high histidine content in the M_4 enzyme. The M_4 enzymes of six avian species, representing three diverse orders, have been subjected to amino acid analysis. Every one contains about 60 moles of histidine per mole of LDH, whereas every avian H_4 enzyme analyzed contains about 30 moles of histidine per mole of LDH [see *6* and *10*, and T. P. Fondy and N. O. Kaplan, *Ann. N.Y. Acad. Sci.* **119**, 888 (1965)]. At *p*H 7, many histidyl residues bear a positive charge but, at *p*H 8.6 or higher, most of them are uncharged [J. Steinhardt and S. Beychok, in *The Proteins*, H. Neurath Ed. (Academic Press, New York, 1964), vol. 2, p. 223]. Because of their histidine content, avian M_4 enzymes would be expected to increase markedly in anodic mobility when the *p*H is raised from 7 to 8.6. Such an increase was observed, as is evident in Fig. 1, not only for the chicken M_4 enzyme, which has a high histidine content (63 moles per mole of LDH), but also for the peafowl M_4 enzyme, whose histidine content has not been determined.
25. Supported in part by NSF grant to A.C.W. from NSF.

4 June 1966

Cell Size and Rate of Protein Synthesis in Ventral Horn Neurones

Abstract. *Autoradiographic studies show that small ventral horn neurones synthesize protein at a greater rate per unit area of cytoplasm than do large ones. It is suggested that this is related to the faster rate of firing of the smaller neurones.*

Henneman *et al.* (*1*) have shown an inverse relation between the size of motor neurones and their excitability. Small motor neurones have a low threshold to excitation and fire more often than large motor neurones.

In order to determine whether the rate of protein synthesis in neurones is related to their firing rates, I have investigated the rate of uptake of H^3-phenylalanine into large and small ventral horn neurones of the mouse. If there is a direct relation, then the smaller neurones should show a greater rate of incorporation per unit area of cytoplasm than the larger.

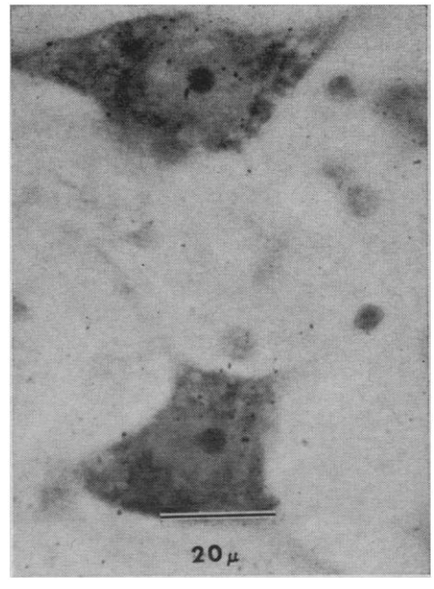

Fig. 1. An autoradiograph of two large motor neurones in the mouse lumbar spinal cord. In order to photograph cells and grains, both are slightly out of focus.

An adult mouse was injected with 100 μc of H^3-phenylalanine 4 days after the left sciatic nerve had been sectioned in the region of the midthigh. Five hours later the mouse, anesthetized with ether, was perfused through the left ventricle with 10 percent formal-saline. After several hours the lumbar enlargement was removed, fixed for 3 days, washed, dehydrated, embedded in paraffin, and serially sectioned at 10 μ. After the sections had been mounted and the paraffin removed, the slides were dipped in Kodak NTB-3 emulsion, and after a 3-week exposure were developed at 10°C in Kodak D-19.

I examined ventral horn neurones (Fig. 1) with discernible nucleoli at a magnification of 450× using a calibrated ocular micrometer. The area of the cell body was estimated by two measurements at right angles, usually the long and short axes of the cell. The nuclear diameter was also measured, and the nuclear and cytoplasmic areas calculated. Grains were counted over the cytoplasm and nucleoplasm separatedly, and the number of grains (above background) per square micron of cytoplasm was obtained. About 250 cells from several sections on two slides were examined in this manner. Unless other-

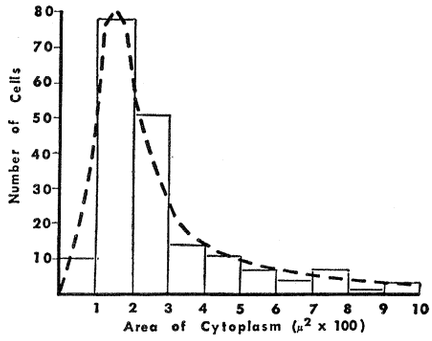

Fig. 2. A histogram showing the distribution of cell sizes in the mouse ventral horn. The dashed curve is a plot of the number of ventral root units responding (ordinate) to increasing muscle stretch stimulus [abscissa; derived from Henneman's (*1*) data on the cat].

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.

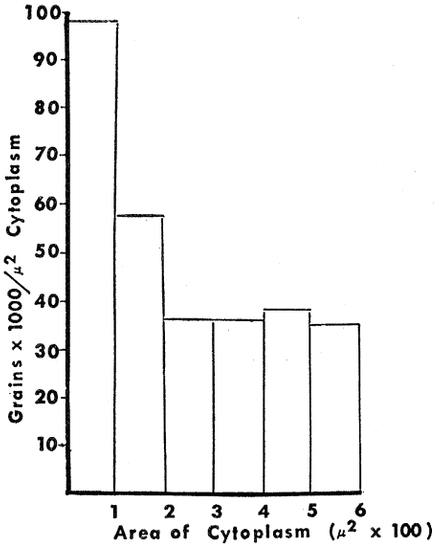

Fig. 3. The rate of protein synthesis per unit area among ventral horn neurones of different size groups. The ordinate represents grains over the cytoplasm above background $\times$ 10^3 per square micron of cytoplasmic area.

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.

R. Price Peterson
Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia 19104

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.

18 July 1966

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