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Rates of Oxygen Uptake by Embryonic Anterior Horn Tissue Isolated at Various Developmental Stages[1] (38883)

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(Introduced by Sergey Fedoroff)

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Little information is available on the oxygen consumption of specific portions of the central nervous system during their initial stages of embryonic development (1, 2). The spinal cord motor horns of chick embryos are well suited for such a study since their development has been well described (3–15). Therefore, in order to follow changes in the respiration rate of a neural tissue as it develops from neuroepithelium into arrays of neurons we measured the oxygen consumption of fragments of the anterior motor horns of chick embryo spinal cords at various stages of development.

Methods. Fertile white Leghorn eggs were obtained from the Poultry Science Department, University of Saskatchewan, and were incubated at 38° until the desired embryonic stages (16) were reached, i.e., stages 11 ± 1, 17, 26 ± 1, 36, and 42 (approximately 1¾, 2½, 5, 10, and 16 days of incubation, respectively). The embryos were removed from the eggs, placed in Hanks' balanced salt solution and carefully staged. Under a dissecting microscope the spinal cord, brachial or presumptive brachial level, was dissected out of the embryo and transferred to incubation medium (150 m*M* NaCl; 3.0 m*M* KCl; 1.7 m*M* KH_2PO_4; 8.0 m*M* $Na_2HPO_4 \cdot 7H_2O$; 1.0 m*M* $CaCl_2 \cdot 2H_2O$; 0.6 m*M* $MgCl_2$; 7 m*M* D-glucose; pH = 7.4, 327 mOsm per kg water) lying over 2% "Special Agar-Noble" (Difco). Small cubes averaging approximately 10 × 10^5 μm^3 in size (range 5–30 × 10^5 μm^3) were cut out of the motor horns, or presumptive motor horns (see Fig. 1) by pressing finely sharpened tungsten needles through the cord into the agar. The actual dimensions of each cube were measured by means of a calibrated micrometer mounted in the ocular of the dissecting microscope and recorded. Each individual cube was sucked into a Cartesian microdiver together with a few microliters of incubation medium and its oxygen consumption measured over a 3-hr period as previously described (17, 18). The first 30 min of each experiment was allowed for equilibration of the microdiver and the measuring system. The cumulative oxygen uptake for each diver was calculated, plotted against time, and the amount consumed at standard 15-min intervals was read off. Since the size of the tissue fragments varied, these values were recalculated to show the oxygen consumption in nanoliters of oxygen per 10 × 10^5 μm^3 of tissue. These standardized 15-min values were then averaged for each stage and regraphed (Fig. 2).

Results. At stage 11 the chick embryo has only 13 somites. Thus, the brachial region, approximately somite 15–22, of the spinal cord has yet to be formed. The tissue excised for measurement of oxygen consumption at this stage was a short length of the right half of the presumptive spinal cord from a level near the future somites 18 to 19. This tissue consists almost solely of undifferentiated neuroepithelium (Fig. 1a). By stage 17 the alar and basal plates of the spinal cord have developed (Fig. 1b) and the first argyrophilic neurons have differentiated in the presumptive motor region. For respirometry, a short length of the right basal plate was excised. In stage 26 chick embryos the formation of motor neurons is virtually completed (3) and these neurons are grouped into a distinct anterior motor column (Fig. 1c). The subdivision of this primordial motor column and the differentiation of glial elements has not yet begun in the brachial levels of the spinal cord. The cube of stage 26 spinal cord tissue

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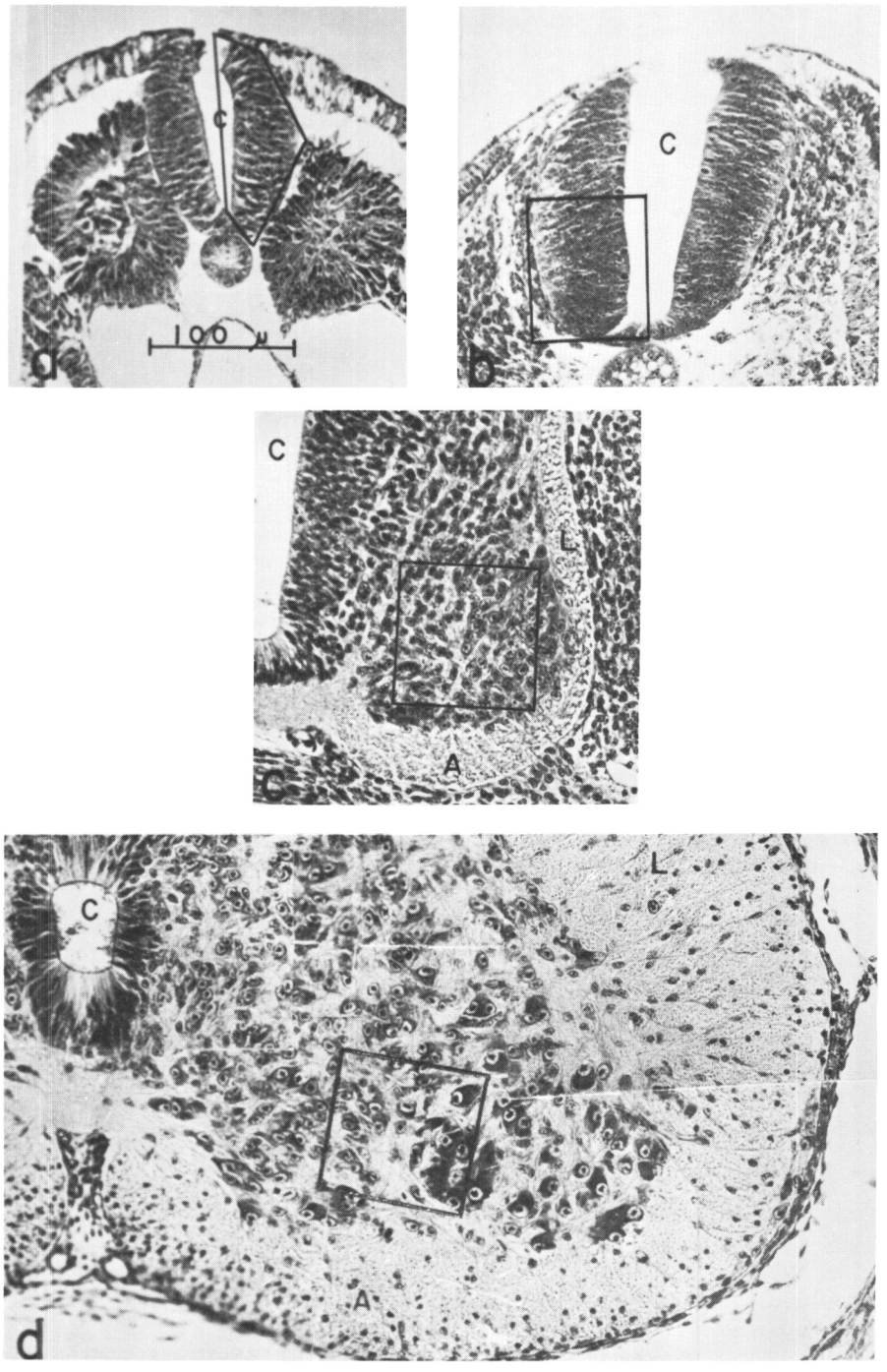

FIG. 1. Sections of chick embryo spinal cords. The location of the fragment excised for respirometry is indicated by the 100 × 100 μm squares (b, c, d). All photomicrographs are of the same magnification. (a) This stage 12 spinal cord consists of neuroepithelium. The section is from the presumptive posterior cervical level. (b) In this stage 17 spinal cord the alar and basal plates and the protodifferentiated motor horns are visible. (c) At stage 26 the motor horns are distinct as enlargements of the basal plates. (d) By stage 36 the cells of the brachial anterior horn are much less tightly packed. (C = central canal; L = lateral funiculus; A = anterior funiculus; hematoxylin and eosin stained).

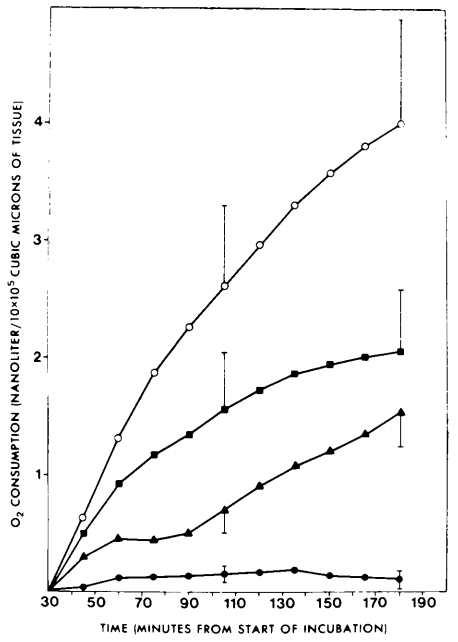

FIG. 2. Cumulative oxygen consumption of fragments of anterior motor horns (or presumptive motor horns), brachial level from chick embryos of various developmental stages. ● = Control, ▲ = Stage 11 ± 1, ■ = Stage 17, ○ = Stage 26 ± 1. Standard errors are given by vertical bars.

excised for respirometry (Fig. 1c) was composed primarily of motor neuron perikarya. The beginning of gliogenesis, the outgrowth of nerve fibers (dendritization), and nerve cell growth and migration result in a physical separation of the motor neurons in the anterior horns of stage 36 chick embryo spinal cords. The cube of tissue taken for oxygen consumption measurement was excised from the ventromedial portion of the right anterior horn (Fig. 1d). By stage 42 the spinal cord has developed to near maturity, both morphologically and functionally. A cube of tissue similar to that excised from stage 36 embryos was taken for measurement of oxygen consumption.

The motor horns of stage 36 and 42 embryos are much larger than those of younger embryos. In addition, the neurons have coalesced into various motor columns (Fig. 1). Thus, the inclusion (or exclusion) of a motor column in the tissue cube excised could result in an enriched (or depleted) proportion of neuronal perikarya in the fragment. In order to aid in the randomization of the site of origin of the tissue cube excised, the stage 36 and 42 motor horn fragments were usually made slightly larger than those taken from younger embryos. No correlation was observed between either the site of origin or the size of the specimen and the rate of oxygen consumption per unit tissue volume.

The cumulative oxygen uptakes of fragments from embryos of stage 11, 17, and 26 are shown in Fig. 2. The curves for stages 36 and 42 are not shown since they overlap the stage 17 curve (cf. Table I). At all embryonic stages the spinal cord fragments consumed oxygen. The rate of oxygen consumption was relatively well maintained throughout the incubation period with a tendency for a decline in the rate during the last hour. Since the curves are nearly rectilinear during the period of 60–120 min (Fig. 2), the slopes of the cumulative uptake curves during this period were taken for comparison of the rates of oxygen consumption at the various embryonic stages (Table I). The controls, prepared with only incubation medium from the operating dishes, had a negligible rate of oxygen consumption. Stage 11 spinal cord fragments consumed oxygen at a low but statistically significant ($P < 0.01$) level (Fig. 2, Table I).

The rate of uptake was greater at stage 17 and was significantly augmented by stage 26 ($P < 0.01$). At stage 36, the rate of oxygen consumption was significantly reduced from that of stage 26 ($P < 0.01$). The oxygen

TABLE I. RATE OF OXYGEN CONSUMPTION OF 10 × 10^5 μm^3 FRAGMENTS OF CHICK EMBRYO SPINAL CORDS AT VARIOUS DEVELOPMENTAL STAGES.

Stage	Number	Oxygen consumption[a] (nl/hr)
11 ± 1	9	0.47
17	6	0.80
26 ± 1	7	1.61
36	9	0.58
42	8	0.76
Control	11	0.05

[a] Calculated by method of least squares during the second hour of incubation.

consumption of stage 42 anterior horn fragments was similarily decreased to about the level of the stage 17 pieces (Table I).

Discussion. The neurons of the anterior motor horns are known to develop from the neuroepithelial cells of the early spinal cord (3–6, 9, 10). The cells of the whole spinal cord of stage 10–12 embryos become the cells of the basal plates of stage 17 embryos. The anterior motor horn neurons in older embryos develop from the cells of the stage 17 basal plates. As early as the second day (stages 13–19) of incubation, the fate of the cells in the spinal cord becomes fixed. Thus, in the present study the tissue cube excised from stage 11 embryos is the direct developmental progenator of the stage 36 and 42 spinal cord motor horns. This study, therefore, follows the metabolic pattern of a specific nervous tissue through its development.

From stage 11 through 17 to stage 26 the rate of oxygen uptake per unit tissue volume was found to increase (Fig. 2, Table I). Since the motor neurons differentiate from the neuroepithelium and the cell size, as evidence by nucleus size, increases during this period (Fig. 1), it is reasonable to suggest that as the neurons differentiate their rate of oxygen consumption increases. A similar metabolic increase with differentiation has been observed by Dittmann *et al.* (19) and Nissen *et al.* (21, 22) in their cultures of cells from 7-day chick embryo brains and from neuroblastomas. However, Romanoff (2) found no differences in the oxygen consumption by chick embryo brains between the 4th and the 21st day of incubation.

The reduced rate of oxygen uptake by the stage 36 and 42 anterior horn fragments compared to that of the stage 27 fragments appears to contradict the suggestion that the rate of consumption increased as neuronal differentiation proceeds. However, in the stage 36 brachial spinal cord, gliogenesis and neuronal process formation have begun (2, 3, 6, 7, 11, 15), resulting in an increased spacing of the neurons in the stage 36 and 42 anterior motor horns (Fig. 1). Neuronal processes and glial cells seem to have a lower rate of oxygen consumption than the neuronal perikarya (20, 23). It is, therefore, reasonable to suggest that the reduction in oxygen consumption by stage 36 and 42 motor horns is due to a "dilution" of the neuronal perikarya in the tissue fragments excised and that the rate of oxygen uptake by mature (stage 36 to 42) neuronal perikarya is at least as high as that measured at stage 27, i.e., about 1.6 nl/10 $\times$ 10^5 μm^3/hr or 70 μmoles/g wet wt/hr. This value is lower than the average neuronal perikaryon respiration of 300–1000 μmoles O_2/g/hr (20). However, spinal cord gray matter shows a lower respiratory intensity than gray matter from the brain (24, 25).

The respiratory rates for brains of 4- to 21-day chick embryos given by Romanoff (2) can be recalculated to about 95 μmoles O_2/g wet wt/hr. This value is only slightly higher than the maximum value of 70 μmoles/g wet wt/hr at stage 26 (5 days) found in the present study. Since the brain is a nonhomogenous tissue and since it develops earlier than the spinal cord (26, 27), Romanoff's value of 95 μmoles/g/hr for whole brains should be compared to that (0.7 nl/10 $\times$ 10^5 μm^3/hr or 30 μmoles/g wet wt/hr) of spinal cords at later stages. Thus, Romanoff's finding of a constant rate of oxygen uptake by embryonic brains at various developmental stages is consistent with our observation of identical respiratory rates of spinal cord motor horn fragments at stage 36 and 42.

Summary. Neuroepithelial cells of the presumptive spinal cord (stage 11) consume oxygen, albeit at a low rate. As neurons differentiate in the presumptive motor horns the rate of oxygen consumption increases to approximately 70 μmoles/g wet wt/hr by stage 26. It is suggested that the rate of oxygen consumption per unit volume of neuron then remains constant as subsequent development ensues but since the neurons become more widely spaced the oxygen consumption per unit volume of anterior horn tissue decreases.

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1. Needham, J., "Chemical Embryology" Vol. II, Cambridge University Press, Cambridge (1931).
2. Romanoff, A. L., "The Biochemistry of the Avian Embryo." Wiley-Interscience, New York (1967).
3. Hamburger, V. J., J. Comp. Neurol. **88,** 221 (1948).
4. Wenger, E. L., J. Exp. Zool. **114,** 51 (1950).
5. Wenger, B. S., J. Exp. Zool. **116,** 132 (1951).
6. Hughes, A. F. W., J. Embryol. Exp. Morphol. **3,** 303 (1955).
7. Lyser, K. M., Develop. Biol. **10,** 433 (1964).
8. Lyser, K. M., Arch. Anat. Microsc. Morphol. Exp. **53,** 37 (1966).
9. Langman, J., and Haden, C. C., J. Comp. Neurol. **138,** 419 (1970).
10. Lyser, K. M., In Vitro **8,** 77 (1972).
11. Foelix, R. F., and Oppenheim, R. W., *in* "Studies on the Development of Behaviour and the Nervous System," (G. Gottlieb, ed.), Vol. 1, p. 103, Academic Press, New York (1973).
12. Pfeiffer, S. E., "Tissue Culture of the Nervous System" (G. Sato, ed.), p. 203. Plenum Press, New York (1973).
13. Provine, R. R., *in* "Studies on the Development of Behaviour and the Nervous System" (G. Gottlieb, ed.), Vol. **1,** p. 77, Academic Press, New York (1973).
14. O'Connor, T. M., and Wittenbach, C. R., J. Cell Biol. **60,** 448. (1974).
15. Skoff, R. P., and Hamburger, V., J. Comp. Neurol. **153,** 107 (1974).
16. Hamilton, H. L., and Hamburger, V., J. Morphol. **88,** 49 (1951).
17. Zeuthen, E., J. Embryol. Exp. Morphol. **1,** 239 (1953).
18. Hertz, L. J. Neurochem. **13,** 1373 (1966).
19. Dittmann, L., Hertz, L., Schousboe, A., Fosmark, H., Sensenbrenner, M., and Mandel, P., Exp. Cell Res. **80,** 425 (1973a).
20. Dittmann, L., Sensenbrenner, M., Hertz, L., and Mandel, P., J. Neurochem. **21,** 191 (1973b).
21. Nissen, C., Ciesielski-Treska, J., Hertz, L., and Mandel, P., Brain Res. **39,** 264 (1972).
22. Nissen, C., Ciesielski-Treska, J., Hertz, L., and Mandel, P., J. Neurochem. **20,** 1029 (1973).
23. Hertz, L., and Schousboe, A., Int. Rev. Neurobiol. **18,** (in press) (1975).
24. Hertz, L., and Clausen, T. Biochem. J. **89,** 526 (1963).
25. Ridge, J. W. Biochem. J. **105,** 831 (1967).
26. Levi-Montalcini, R., *in* "The Nature of Biological Diversity" (J. M. Allen, ed.), p. 261, McGraw-Hill, New York (1965).
27. Romanoff, A. L., "The Avian Embryo", MacMillan, New York (1960).

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