(*10*) reported an increase in the P_{50} in two subjects with pyruvate kinase deficiency and speculated that this increase could be a consequence of either the elevated concentrations of 2,3-diphosphoglycerate or the presence of a young red cell population. The finding of a disparity in oxygen-hemoglobin dissociation curves in two patients with similar degrees of anemia and reticulocytosis suggests that the concentration of 2,3-diphosphoglycerate is the factor responsible. These findings indicate that not only does the blood oxygenation affect red cell metabolism by altering the concentration of the 2,3-diphosphoglycerate in the red cells but also that intrinsic defects of red cell glycolysis can influence the affinity of hemoglobin for oxygen.

MARIA DELIVORIA-PAPADOPOULOS
FRANK A. OSKI
ARLAN J. GOTTLIEB
University of Pennsylvania
School of Medicine,
Philadelphia 19104

References and Notes

1. R. Benesch and R. E. Benesch, *Biochem. Biophys. Res. Commun.* **26**, 162 (1967); A. Chanutin and R. R. Curnish, *Arch. Biochem. Biophys.* **121**, 96 (1967).
2. F. Oski, W. Miller, M. Delivoria-Papadopoulos, A. Gottlieb, *Proc. Soc. Pediat. Res.* **39**, 46 (1969).
3. C. Lenfant, J. Torrance, E. English, C. A. Finch, C. Reynafarje, J. Ramos, J. Faura, *J. Clin. Invest.* **47**, 2652 (1968); J. W. Eaton, G. J. Brewer, R. F. Grover, *J. Lab. Clin. Med.* **73**, 603 (1969).
4. F. A. Oski, A. J. Gottlieb, M. Delivoria-Papadopoulos, W. Miller, *N. Engl. J. Med.* **280**, 1165 (1969).
5. W. Valentine, F. A. Oski, D. E. Paglia, M. A. Baughan, A. S. Schneider, J. L. Naiman, *ibid.* **276**, 1 (1967).
6. W. Schroter and H. Heyden, *Biochem. Z.* **341**, 387 (1965); I. Kirmsky, in *Methods of Enzymatic Analysis*, H. U. Bergmeyer, Ed. (Academic Press, New York, 1963), p. 238.
7. G. W. Severinghaus and A. F. Bradley, *J. Appl. Physiol.* **13**, 515 (1958).
8. G. G. Nahas, *ibid.*, p. 147.
9. J. W. Eaton and G. J. Brewer, *Proc. Nat. Acad. Sci. U.S.* **61**, 756 (1968).
10. A. Mourdjinis, C. Walters, M. J. Edwards, R. D. Koler, B. Vanderheinden, J. Metcalfe, *Clin. Res.* **17**, 153 (1969).
11. Supported by research career development awards K3-HD 38600 (F.A.O.), KO4-AM42392 (A.J.G.), and special fellowship award IF 3HD40661 (M.D.P.), and PHS grants HD01919, AM11984, and R01-HD-1860. We thank E. Brigandi for her technical assistance.

15 May 1969

Neuroglia Electrically Coupled to Neurons

Abstract. *Applied electric current is transmitted between mammalian glial cells grown in tissue culture. A similar electrical coupling exists between certain neurons as well as between neuroglia and neurons. Although this phenomenon may be a peculiarity of mammalian neural cells maintained in culture, it may, on the other hand, represent a phenomenon with greater neurophysiological significance, a process whereby neurons can become silent.*

Electrical coupling occurs between cells of tissue from various sources (*1*). Kuffler and his colleagues have described low resistance pathways that link glial cells in the nervous system of the leech (*2*) and of amphibians (*3*). It would be of interest to know whether similar connections occur between mammalian glial cells in order to establish a general characteristic of the cells, as well as to generate investigations of metabolic transport over what may be specialized membrane structures linking these cells together.

Since records from neighboring glial cells cannot be taken under visual control in the mammal, cultures of nervous tissue containing neurons and glia were chosen, especially since such cultures have been used successfully in the past for similar experiments that led to wider inquiries into the properties of glial cells in the intact brain (*4*). Explants were taken from the midbrain and cerebellum of rats 72 hours old and maintained in a tissue culture. Mammalian neural tissue that was grown on "flying cover slips" spread out into a thin layer of cells in which neurons and neuroglia became visibly distinct in 3 to 6 weeks. At a magnification of × 500 with phase optics, and with careful focusing, cell types and structures could be clearly defined in areas two or three cell layers thick (Fig. 1).

Under these conditions, glass microelectrodes filled with 3*M* KCl, 1.0*M* NaCl, or 1.0*M* potassium citrate could be easily manipulated into neurons and neuroglia, and the resting membrane potential (RMP) could be recorded. A technique similar to that used in the past (*5*) for measuring membrane resistance was used to determine electrical coupling of cells. A recording microelectrode was placed in one glial cell and a current-passing microelectrode in a second glial cell at least 250 μm from the cell bearing the recording electrode. We measured the voltage changes recorded in the first cell, which were produced by passing current into the second cell. The current electrode was then brought closer to the first cell by sampling successive cells until finally both electrodes were in the same cell. With this technique, a standard pulse of 5×10^{-8} amp for a 5-msec duration produced measurable voltage changes at distances up to approximately 200

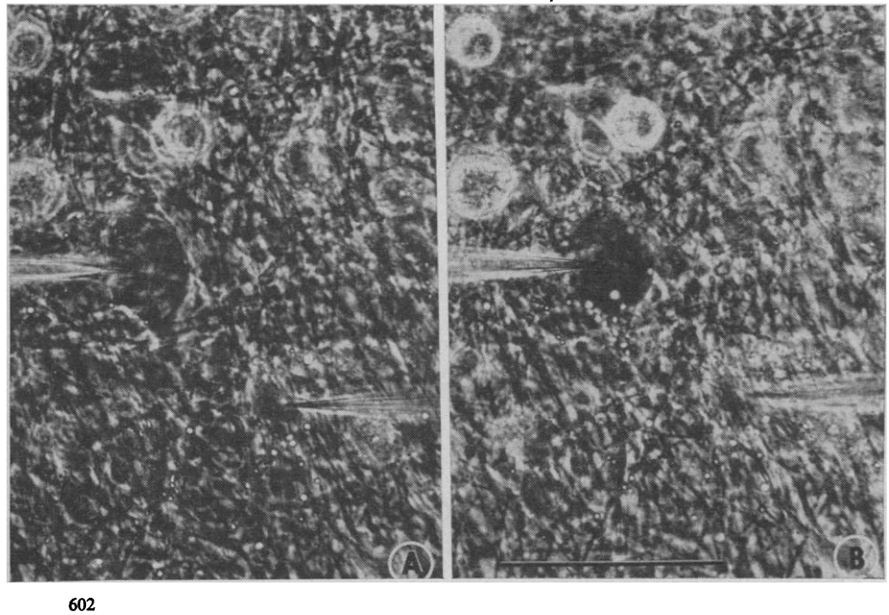

Fig. 1. (A) Phase photomicrograph illustrating placement of microelectrodes into the living cells of an explant being studied. The larger cell is a neuron and the smaller one is a neuroglia from a culture 28 days old. (B) Same field as in (A). The neuron has been stained blue (appearing dark within phase-contrast photomicrograph) after methyl blue was delivered electrophoretically into the cytoplasm. Bar is 50 μm.

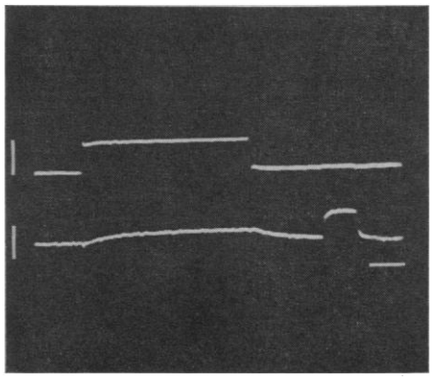

Fig. 2. Demonstration of electrical coupling of a neuron and glial cell. Upper trace represents a rectangular pulse of 4.5×10^{-8} amp for a duration of 5 msec. Pulse is measured as voltage drop across a resistor of 10 kilohms. Lower trace indicates voltage deflection recorded from the neuron resulting from current injected into the neuroglia. Both traces overlapped at the beginning of the experiment; therefore, RMP is indicated by the distance between the two traces measured by the lower vertical bar. The square wave at the end of the trace indicates the electrode resistance which in this case is 6 megohms. Horizontal bar is 1.0 msec; upper vertical bar is 5×10^{-8} amp; and lower bar is 10 mv.

μm between electrodes, provided there was no break in the contiguity of the chain of cells. The spread of current was observed to diminish and fail as either the cell injected with current or the cell from which the recording was taken showed signs of injury and changes in contour. Macrophages and fibroblasts lying close to glial cells did not participate in the transmission of applied current. Electrical coupling between glial cells was a constant finding in the cultures examined.

The same experimental arrangement was employed to investigate electrical coupling between neurons and neuroglia (Fig. 1). Electrical coupling does indeed exist between neurons and neuroglia (Fig. 2), as well as between neurons. However, the neurons that exhibited electrical coupling possessed other unusual properties. Action potentials could not be obtained from these cells, although they did not differ morphologically from neurons producing spikes. These quiet neurons had a low RMP of -20 to -40 mv and a low effective membrane resistance measuring approximately 0.3 megohm, a value in the same range as that obtained previously by Hild and Klee (*6*). Furthermore, this value is in the same low range of values of membrane resistance determined for neuroglia (*5*). Action potentials are frequently obtained from neurons grown in culture with the electrode touching the membrane or after penetration. These action potentials either occur spontaneously or can be driven by passing current through the recording electrode or through an extracellularly placed stimulating electrode. However, the cells that exhibited coupling not only failed to show action potentials but responded to an externally applied electric shock in the same manner as did neuroglia, that is, with a graded, long-lasting depolarization that decayed over several seconds (*4, 5, 7*).

Although coupling was not observed between neuroglia and those neurons that produced action potentials, these studies do not exclude this possibility. The length of time during which discharges can be observed with an intracellular electrode is most often quite short, measured in seconds, and is reduced to even less by the manipulation of a second microelectrode into the explant. Could it be that the tip of the electrode observed to lie in a neuron lacking the usual electrical sign is actually in a vertically adjacent glial cell? The electrode was observed to dimple the neuronal membrane before impaling it. Later, during the time of recording, the neuron bearing the electrode tip could be observed to degenerate, which was correlated with a loss of RMP. Moreover, in order to answer this question definitely, numerous cells studied were injected electrophoretically with methyl blue (Fig. 1) by the method described by Thomas and Wilson (*8*).

It would appear, therefore, that in cultured mammalian tissue two functional varieties of morphologically identical neurons exist: one that responds in an all-or-none fashion and a second that is silent and has the characteristics usually attributed to neuroglia. There is no ready explanation for the different behavior of neurons in culture. Factors such as aging of the culture, its degree of flattening or spreading, and the physical relations between cells in thick or thin areas, among others, could be related to the different behavior but the real reason is unknown. Sheridan (*9*) has presented evidence for low-resistance junctions between various cell types in the developing chick embryo. Results obtained from tissue cultures may indicate that in this material a similar situation could obtain, suggesting a "physiological dedifferentiation" of elements in the nervous tissue, a phenomenon possibly peculiar to the in vitro environment.

It would be interesting to know whether similar low-resistance connections occur in the brains of animals. There is ample evidence that interneuronal "electrotonic transmission" exists in vertebrates (see *10*), and unresponsive cells have been described in the brains of animals (see *11*). The formation of low-resistance junctions as described above between neurons and neuroglia could represent a dynamic process whereby certain neurons become "turned off" or silent as current is shunted from a neuron to a neuroglia through a low-resistance pathway parallel to the membrane resistance. Such a process could be the mechanism accounting for some "silent cells" in vivo. Most of these have been shown to be neuroglia (*12*). However, the same process could account for the temporary silence of single cell activity (*13*), as well as the flattened cortical electroencephalogram and increased impedance observed during spreading depression.

FRANKLIN D. WALKER
Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis 46207

WALTHER J. HILD
Department of Anatomy, University of Texas Medical Branch, Galveston 77550

References and Notes

1. W. R. Loewenstein, *Ann. N.Y. Acad. Sci.* **137**, 441 (1966).
2. S. W. Kuffler and D. D. Potter, *J. Neurophysiol.* **27**, 290 (1964).
3. ———, J. G. Nicholls, R. K. Orkand, *ibid.* **29**, 768 (1966).
4. W. Hild, J. J. Chang, I. Tasaki, *Experientia* **14**, 220 (1958); I. Tasaki and J. J. Chang, *Science* **128**, 1209 (1958).
5. W. Hild and I. Tasaki, *J. Neurophysiol.* **25**, 277 (1962); ———, T. Takenaka, F. Walker, *Exp. Neurol.* **11**, 493 (1965).
6. M. R. Klee and W. Hild, *Pfluegers Arch.* **297**, 66 (1967); W. Hild and M. R. Klee, *Anat. Rec.* **160**, 366 (1968).
7. W. M. Wardell, *Proc. Roy. Soc. London Ser. B* **165**, 326 (1966).
8. R. C. Thomas and V. J. Wilson, *Science* **151**, 1538 (1966).
9. J. D. Sheridan, *J. Cell Biol.* **37**, 650 (1968).
10. M. V. L. Bennett, Y. Nakajima, G. D. Pappas, *J. Neurophysiol.* **30**, 161 (1967).
11. K. Krnjevic and S. Schwartz, *Exp. Brain Res.* **3**, 306 (1967).
12. J. S. Kelly, K. Krnjevic, G. K. W. Yim, *Brain Res.* **6**, 767 (1967).
13. N. L. Morlock, K. Mori, A. A. Ward, Jr., *J. Neurophysiol.* **27**, 1192 (1964); H. Collewijn and A. Van Harreveld, *Exp. Neurol.* **15**, 425 (1966).
14. Conducted in the Tissue Culture Laboratory, Department of Anatomy, University of Texas Medical Branch. Supported by grant NB-03114 from the National Institutes of Health. F.D.W. was supported by a special fellowship award, 1-F3-MH-37, 569-01 (APA).

15 April 1969