Fig. 1. Photomicrograph of a pollen grain of *Ephedra*, found as fossil in Pleistocene deposits on Banks Island. Longest axis of the pollen grain measures 48 μ.

Phytogeographical studies also would benefit by further palynological investigations, for these investigations greatly help to trace the migrations of plants during the past.

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References

1. S. T. Andersen, *Danmarks Geol. Undersøgelse II*, No. 80 (1954).
2. H. C. Cutler, *Ann. Missouri Botan. Garden.* 26 (1939).

17 October 1955

Conduction Block in Peripheral Nerve Produced by Oxygen at High Pressure

Paul Bert demonstrated inexcitability of frog sciatic nerve-muscle preparations that were exposed to 15 "superoxygenated atmospheres" for 2 hours (*1*). Hill and Macleod (*2*) and Bean and Bohr (*3*) extended these studies, confirming the toxic action of oxygen at high partial pressure (OHP) on the nerve-muscle preparation. Since the toxic effects noted in the nerve-muscle preparations (progressively decreasing contractile response to stimulation of the nerve and disappearance of the treppe phenomenon) could be attributed to either muscle, myoneural junction, or nerve dysfunction, singly or in combination, they offer no proof of neuronal poisoning by OHP. Accordingly, we have investigated the effects of OHP on peripheral nerve alone as a functioning unit.

Twenty-six experiments were performed on frog sciatic nerve. The nerves were excised, placed in a phosphate-buffered Ringer's solution (*4*), and suspended 1 hour later on an array of silver electrodes in a plastic block. This was placed in a pressure chamber (2 in. in diameter by 4 in. long) that was provided with electric feed-throughs for stimulating, voltage recording, and thermocouple leads. The nerves were kept moist by placing gauze sponges that had been soaked in Ringer's solution over the plastic block.

Usually the nerves were continuously stimulated throughout an experiment at 20 pulses/sec, 0.1 msec duration, beginning as soon as the chamber was sealed. Stimulus strength was just supramaximal for alpha fibers (generally 0.5 v), and the amplifier was adjusted so that the amplitude of each nerve action potential was displayed initially at 77 mm on an oscilloscope.

Control and experimental nerves were unpaired and selected at random, and the spike amplitude was measured every 5 minutes. After an observation period of 30 minutes, the chamber was flushed with the gas being investigated for 2 minutes, sealed, and brought to pressure in 5 to 10 minutes. Experiments were conducted at two levels, 1 and 12 atm (gage). The temperature during compression never increased more than 3°C and returned to precompression levels within 5 minutes. Figure 1 illustrates the extent of conduction block produced in peripheral nerve by pure oxygen under these conditions (complete conduction block being defined as the absence of the action potential in a nerve when stimulated with the initial electric stimulus parameters).

A standard t test (*5*) for significant differences in the means of the two groups (6 control and 9 experimental nerves) indicated that $p < 0.02$ at 3½ hours and $p < 0.01$ at 4 hours.

In a series of nerves exposed to a 5-percent CO_2–95-percent O_2 mixture, the stimulus threshold rose immediately upon compression; but if, after the chamber reached 12 atm, the stimulus voltage was increased approximately 100 percent (to about 1 v) so that the precompression spike amplitude was obtained, the nerves were blocked in the same fashion as ones at 12 atm of oxygen but with a significantly shorter latent period ($p < 0.01$). Mean time for block with 5-percent CO_2–95-percent O_2 mixture at 12 atm was 3 hours, compared with 4.5 hours for oxygen at 12 atm. Evidence was obtained that hyperoxic conduction block occurs at 10 atm and lower pressures with longer latencies, but a complete spectrum of pressures was not investigated.

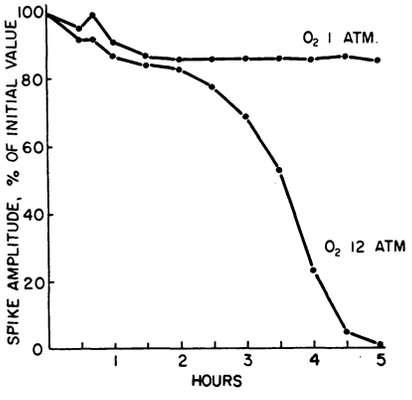

Fig. 1. Time course of conduction block produced in frog sciatic nerve stimulated with 0.5 v, 0.1-msec duration, 20/sec pulses in 12 atm of oxygen, compared with controls in 1 atm of oxygen that were stimulated in the same fashion. Spike amplitudes are mean values.

As in oxygen poisoning of muscle (*3*), peripheral nerve usually shows a partial recovery from hyperoxic conduction block when it is returned to air at 1 atm provided that the block is not maintained more than a few minutes. No complete recoveries occurred in our series. Nerves in which conduction was blocked by 5-percent CO_2–95-percent O_2 showed partial recovery sooner (within 10 minutes) and more completely upon decompression than those that were blocked by oxygen alone.

Nerve activity resulting from continuous stimulation neither seems to modify nor to be essential for the production of conduction block. A group of nerves was exposed to 12 atm of oxygen and stimulated every 30 minutes (for no more than 30 sec, just long enough to measure the spike amplitude). A second group of nerves was exposed to 12 atm of oxygen and continuously stimulated. No significant difference was observed between the two groups with respect to the time course of conduction block.

Hyperoxic conduction block in nerve may result from a direct toxic action of oxygen on the oxidative processes that are essential for maintenance of the membrane potential and impulse transmission. Inactivation of thiol (-SH group) enzymes (*6*) by increased formation of oxidizing free radicals (*7*) is a recently advanced explanation of this direct toxicity.

It has been reported that carbon dioxide decreases the latency of central nervous system manifestations of OHP (*8*), and it is interesting to note that it also decreases the latency of hyperoxic conduction block in peripheral nerve. Whether its efficacy in potentiating the block consists in changing the pH, increasing the membrane permeability, or involves some more obscure mechanism remains unknown. As yet, there is no evidence that OHP produces conduction block in the central nervous system, but hyperoxic conduction block may well play a role in the neural insult that initiates hyperoxic convulsions. The length of exposure and the pressure required to produce even mild nerve block in these experiments are greater than

those required to produce hyperoxic seizures, but it is conceivable that conduction deficiencies may develop more quickly and with lower pressures of oxygen in tissue with a circulation.

Oxygen at high pressure (12 atm) produces conduction block in frog peripheral nerve. The block is partially reversible if it is not maintained more than a few minutes. The block caused by 5-percent CO_2–95-percent O_2 has a shorter latency, and its reversibility is more pronounced than that of the block produced by oxygen alone. The presence or absence of activity in nerve caused by continuous stimulation does not affect the time course of hyperoxic conduction block (*9*).

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References and Notes

1. P. Bert, *La pression barométrique* (Paris, 1878), translated by M. A. and F. A. Hitchcock (College Book Co., Columbus, Ohio, 1943), p. 840.
2. L. Hill and J. J. R. Macleod, *J. Hyg.* 3, 401 (1903).
3. J. W. Bean and D. F. Bohr, *Am. J. Physiol.* 124, 576 (1938).
4. R. Lorente de Nó, *A Study of Nerve Physiology* (Rockefeller Institute for Medical Research, New York, 1947), p. 17 (Corrected by personal communication). Buffer formula: Ringer stock: 65 g NaCl, 1 g KCl, 12 ml 10-percent $CaCl_2$ solution, and sufficient distilled water to make 1 lit. Buffer stock: 0.92 g $NaH_2PO_4 \cdot H_2O$, 3.765 g Na_2HPO_4, and sufficient distilled water to make 500 ml. Use 10 ml of Ringer stock to 100 ml of distilled H_2O for normal Ringer. Use 5 ml of buffer stock to 100 ml of normal Ringer for *p*H 7.3.
5. G. W. Snedecor, *Statistical Methods* (Iowa State College Press, Ames, ed. 4, 1948), p. 81.
6. F. Dickens, *Biochem. J. London* 40, 145 (1946).
7. R. Gerschman *et al.*, *Science* 119, 623 (1954).
8. L. A. Shaw *et al.*, *Am. J. Physiol.* 108, 652 (1934).
9. Opinions and assertions in this report are the author's private ones and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

27 October 1955

Basis for After-Discharge in the Median Giant Axon of the Earthworm

The ventral nerve cord of the earthworm (*Lumbricus terrestris*) contains three giant axons, many smaller nerves, and a complex neuropile. The giant axons are endowed with many synapses that range from endings of smaller nerves on the giant axons to the segmental transverse septa that interrupt the substance of the latter (*1*). Because of this abundance, the giant axons are interesting material for the study of some fundamental properties of interneuronal relationship (*2*).

Isolated ventral nerve cords were immersed in an earthworm saline (*3*) and stimulated with a pair of surface electrodes. Responses were simultaneously recorded with a pair of electrodes on the entire nerve cord and one or two prefilled capillary microelectrodes (*4*) in the median giant axon. Action potentials recorded from the intracellular loci ranged from 80 to 100 mv; these were 25 to 35 mv beyond the resting potential of 50 to 70 mv. Svaetichin (*5*) has reported the same limits for the resting potential obtained with an axially inserted microelectrode.

At room temperature of 19° to 24°C, the internally recorded spike lasted 1 msec, but it was followed by a slight negativity that persisted for about 4 msec. Occasionally, with one brief stimulus, two or more responses occurred. The first direct response to the shock rose rapidly, while the later ones were often preceded by a prepotential that rose more slowly. The nature of the repetitive discharge could be resolved by applying two brief shocks in rapid succession. The second response elicited in the relative refractory period was followed by a sequence of small and additive potentials. These were highly variable in number, amplitude, frequency, and time and site of occurrence.

Figure 1 illustrates a case in which activity was recorded from three sites of a preparation. The entire nerve cord was stimulated at one end, and impulses were recorded from the other end with external electrodes. Two microelectrodes, 11.3 mm apart, were placed in the median giant axon in a stretch between the surface electrodes. Response to the first stimulus, which was threshold for the median giant axon alone, was recorded earliest at the two microelectrodes and, after the appropriate conduction time, on the external leads. The strong second stimulus that was applied in the relative refractory period reexcited the axon, but the internally recorded responses were smaller and the externally recorded response was markedly delayed. The external trace, but neither internal trace, also registered the spike of the lateral giants evoked by the strong stimulus. The trace of one of the microelectrodes, however, remained elevated above the base line and carried numerous small peaks. The trace of the other had only a brief additional potential soon after the spike. About 7 msec after the second stimulus another spike developed in the giant axon. Since the small potentials began during the absolute refractory period of the giant axon, they could not be produced by the same mechanism responsible for the electrically excitable spike. Owing to their local nonpropagating nature, low amplitude, frequency of repetition, and additiveness, these potentials are probably synaptic, developing at the terminations of small nerve fibers on the giant axon. This junctionally

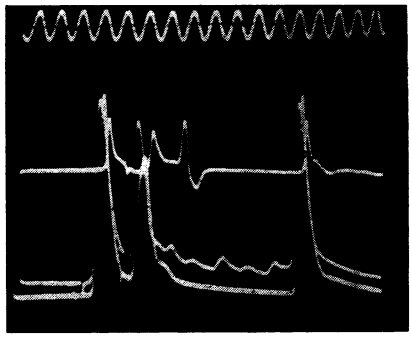

Fig. 1. Simultaneous recording from three sites of the nerve cord (external leads) and median giant axon (internal microelectrodes). The upper trace is the external recording, which is also the base line for the resting potentials. The latter are 72 and 74 mv; spike heights are 98 and 110 mv. The distance between the stimulating cathode and proximal microelectrode was 8.5 mm; between the microelectrodes, 11.3 mm; and between the distal microelectrode and the first external lead, 3.5 mm. The two directly coupled beams are not coincident. Time 1000 cycles.

elicited postsynaptic activity of the median giant axon is then responsible for reexciting the fiber.

Other evidence indicates that the activity of the small nerve fibers that excite the giant axon synaptically are themselves initiated, at least in part, by previous activity of the giant axon. Thus, small fiber activity, as well as synaptic potentials and late all-or-none discharges, disappears when the second stimulus is applied during the absolute refractory period of the giant axon. The evidence, therefore, suggests the presence of a closed circuit containing efferents from and afferents into the median giant axon. It also indicates that "reentry" is an important factor in the after-discharge following a brief stimulus to this preparation.

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References and Notes

1. H. B. Stough, *J. Comp. Neurol.* 40, 409 (1926); W. M. Smallwood and M. T. Holmes, *ibid.* 43, 327 (1927); T. H. Bullock, *J. Neurophysiol.* 8, 55 (1945); J. Stephenson, *The Oligochaeta* (Oxford Univ. Press, Oxford, 1930).
2. I am deeply indebted to H. Grundfest of the department of neurology, College of Physicians and Surgeons, Columbia University, for a generous loan of instruments. To him, and to C. McC. Brooks, I am grateful for many stimulating discussions.
3. W. A. H. Rushton, *Proc. Roy. Soc. London* B132, 423 (1945).
4. G. Ling and R. W. Gerard, *J. Cellular Comp. Physiol.* 34, 383 (1949); W. L. Nastuk and A. L. Hodgkin, *ibid.* 35, 39 (1950); C. Y. Kao, *Science* 119, 846 (1954).
5. G. Svaetichin, *Acta Physiol. Scand.* 24, Suppl. 80, 50 (1951).
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