jects, the criteria (*4*) used were perhaps more rigorous than those employed by some other investigators (*5*). Concordance of the results of the selection tests could not, of course, rule out the possibility that the six deuteranopes and nine normal subjects were not truly representative of their respective populations in sensitivity and luminosity. In this regard it is of interest to note that Boynton's (*6*) normal subject was quite unlike Hsia and Graham's normal subjects and that his deuteranope and protanope showed virtually identical foveal thresholds, from 410 to 540 mμ, in marked contrast to the loss of sensitivity of deuteranopes relative to protanopes in this spectral region found by Hsia and Graham, but in excellent agreement with our (photopically determined) identity of this section for all subjects at all brightness levels.

GORDON G. HEATH
Division of Optometry,
Indiana University, Bloomington

References and Notes

1. The intensitive variations of receptor contributions are evident in the drastic color changes of lights of high brightness [E. Auerbach and G. Wald, *Science* **120**, 401 (1954)] and in the not-so-drastic color changes of the Bezold-Brücke phenomenon. This was also the explanation advanced by W. T. M. Forbes [*Am. J. Psychol.* 41, 517 (1929)] to account for L. L. Sloan's [*Psychol. Monograph No. 38*, (1928), p. 7] discovery of a hump which appeared on the red side of the luminosity curve at reduced intensities, a hump also found on foveal luminosity curves by H. V. Walters and W. D. Wright [*Proc. Roy. Soc. (London)* **B131**, 340 (1943)] and appearing as a prominent feature of our CFF-determined curves [G. G. Heath, *Science* **128**, 775 (1958)].
2. In addition to the evidence cited in (*1*), studies of dark adaptation with colored lights [A. Kohlrausch, *Arch. ges. Physiol. Pflüger's* **196**, 113 (1922); A. Chapanis, *Am. J. Physiol.* **146**, 489 (1946); ———, *J. Gen. Physiol.* **30**, 423 (1947); E. Auerbach and G. Wald, *Science* **120**, 401 (1954)] have provided direct evidence of the inapplicability of cone threshold data to studies of photopic luminosity curves, since differently colored test lights equated for brightness at photopic levels required quite different attenuations to reduce them to cone threshold.
3. S. Hecht and Y. Hsia, *J. Gen. Physiol.* **31**, 141 (1947); S. Hecht, *Documenta Ophthalmol.* **3**, 289 (1949).
4. In addition to appropriate performance on AO H–R–R pseudoisochromatic plates, tests and criteria were those described by G. L. Walls and G. G. Heath [*J. Opt. Soc. Am.* **46**, 640 (1956)], which included the Nagel anomaloscope, a neutral-point determination, and the Walls-Mathews three-light and RDP tests [G. L. Walls and R. W. Mathews, *Univ. Calif. (Berkeley) Publs. Psychol.* **7**, 1 (1952)].
5. For example, at least three of the seven "deuteranopes" and three of the seven "protanopes" reported by Zanen [J. Zanen, R. Wibail, A. Meunier, *Bull. mém. soc. franç. Ophtalmol.* **70**, 81 (1957)] were obviously no more than anomalous trichromates, as shown by their performances on the Farnsworth Dichotomous and the Farnsworth-Munsell 100-hue tests, and perhaps also on the anomaloscope, though their test procedure with the latter is not clear but seems unconventional. These faults, and the failure to determine neutral points, were pointed out by Dubois-Poulsen, and the possibility of misdiagnoses was admitted by Zanen in the discussion at the end of the paper.
6. R. M. Boynton, G. Kandel, J. W. Onley, *J. Opt. Soc. Am.* **49**, 654 (1959).

19 November 1959

Site and Mechanism of Tick Paralysis

Abstract. A neurophysiological investigation indicates that the paralysis produced in the dog by the wood tick, *Dermacentor andersoni* Stiles, is due to failure in the liberation of acetylcholine at the neuromuscular junction because of a conduction block in the somatic motor fibers produced by the tick "toxin."

Tick paralysis, an acute ascending flaccid paralysis which may terminate in respiratory failure, affects animals and humans. It is caused by the feeding of the female tick, which is believed to secrete a neurotoxin in its salivary glands (*1*). As the common wood tick, *Dermacentor andersoni* Stiles, has been responsible for most of the cases in North America, the disease has, accordingly, been largely confined to the northwestern United States and the adjoining southwestern region of Canada. Human cases have been recorded recently in the eastern and southern United States, due largely to the common dog tick *D. variabilis* Say (*2*). This indicates a more widespread distribution of the disease than is generally recognized. Over 300 cases of tick paralysis in humans have been recorded on this continent, with a mortality of approximately 12 percent (*3*). As removal of the tick ensures recovery except when the patient is moribund, early diagnosis of the disease is desirable to avoid fatalities.

In order to determine the site and the pathological mechanism of the paralysis, a neurophysiological study was carried out on mongrel dogs which were paralyzed by applying the ticks *D. andersoni* Stiles. The disease produced in the dog closely resembles that in the human; in the severely paralyzed animal the muscles are flaccid and the tendon reflexes are absent.

The anterior tibial muscle was selected for study because it was found to be involved early in the paralysis. The first significant observation was that this muscle responded to direct electrical stimulation but failed to contract when stimulated through the peroneal nerve (*4*). This finding indicated either that the motor nerve fibers could not conduct a nerve impulse or that there was a block at the neuromuscular junction. When the sixth lumbar ventral root was stimulated, an action potential was recorded from the peroneal nerve, indicating that motor nerve fibers were conducting an impulse (*5*). The muscle, which failed to respond to nerve stimulation, contracted when acetylcholine was injected intra-arterially directed into the muscle. Indeed, the paralyzed muscle exhibited an increased sensitivity to acetylcholine (*6*), a condition which occurs in denervated muscle. The response to acetylcholine, to antagonists of blocking agents, and to repetitive stimulation indicated that the paralysis did not resemble that produced by the known blocking agents —for example, curare or decamethonium—or by excessive doses of anticholinesterases, but was similar to that in botulinum toxin poisoning (*5*). The latter has been shown to be probably due to a block in the small terminal motor nerve fibers (*7*).

When a paralyzing dose of succinylcholine was injected into normal and tick-paralyzed anesthetized dogs and a wick electrode was swept across the under surface of the anterior tibial muscle, small areas of depolarization could be consistently detected and located; these represented depolarized end-plate regions. When the peroneal nerve was stimulated intermittently in normal dogs, each nerve impulse produced a transient potential (end-plate potential) which could be recorded from each located end-plate region; in tick-paralyzed dogs, however, end-plate potentials could not be detected at located end-plate regions during nerve stimulation, which suggested that acetylcholine was not being liberated at the nerve terminal (*6*).

In order to prove conclusively that tick paralysis is due to failure in the liberation of acetylcholine, paralyzed and normal anterior tibial muscles were perfused with Ringer's solution and the acetylcholine liberated into the perfusate was estimated. While acetylcholine was liberated by the normal muscle (6×10^{-12} gm per nerve volley) during nerve stimulation, none was liberated by the paralyzed muscle on stimulating either the nerve or the muscle directly (*8*). Emmons and McLennan (*9*) have shown recently that the muscles of the perfused hind leg of the tick-paralyzed ground hog also fail to liberate acetylcholine when the sciatic nerve is stimulated.

The inability of nerve or direct muscle stimulation to liberate acetylcholine could be due to (i) failure of the terminal motor nerve fibers to conduct the nerve impulse, or (ii) defective storage, synthesis, or release of acetylcholine at the nerve terminals. When the paralyzed muscle was perfused with Ringer's solution containing four times the normal concentration of potassium, acetylcholine was liberated, indicating that it is available at the nerve terminals. Recent evidence indicates that choline acetylase, the enzyme required for acetylcholine synthesis (*10*), and probably acetylcholine (*11*) are produced in the nerve cell and migrate down the axon. In five normal dogs, the mean number of milligrams of acetycholine liberated per gram of acetone powder per hour

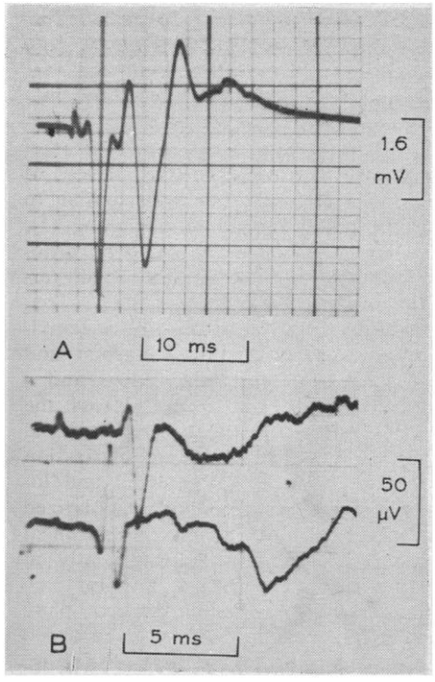

Fig. 1. Nerve action potentials recorded (*A*) in normal dog at sciatic-peroneal nerve, and (*B*) in moderately paralyzed dog at sciatic-peroneal (above) and peroneal nerve at fibula (below). Note difference in calibration between *A* and *B*.

(= choline acetylase activity), ± the standard error, for anterior motor roots and peroneal nerve, were 15.6 ± 0.7 and 1.3 ± 0.5, respectively. The respective values in nine paralyzed dogs were 13.6 ± 1.8 and 1.2 ± 0.2. The acetylcholine content (mean ± S.E.) in micrograms per gram of anterior motor roots and sciatic-peroneal nerve were 8.4 ± 0.9 and 2.0 ± 0.3, respectively, in four normal dogs, and 12.2 ± 3.1 and 2.5 ± 0.7, respectively, in three paralyzed dogs. No significant difference exists between normal and paralyzed animals in either case. In the estimation of choline acetylase activity, another enzyme system is included to produce acetyl coenzyme A, which supplies the active acetyl for the synthesis of acetylcholine from choline by choline acetylase. In order to exclude the possibility that deficiency of this cofactor and of the substrate are responsible for the paralysis, acetyl coenzyme A and choline were injected into a paralyzed dog, but they failed to counteract the paralysis (*8*). MacIntosh (*12*) has recently emphasized the importance of the calcium ion, carbon dioxide, and the chloroform-soluble dializable plasma factor for acetylcholine release. When calcium salts were injected and carbon dioxide increased on inducing asphyxia, and when part of the plasma of the tick-paralyzed dog was replaced by plasma from a normal dog, no lessening of the paralysis occurred. All this evidence indicated that tick paralysis is due neither to defective synthesis or storage of acetylcholine nor to absence of release factors, but apparently must be due to inability of the nerve impulse to traverse the terminal motor nerve fibers.

At this stage it was decided to reinvestigate conduction in motor nerve fibers, because in the initial study in 1956, although conduction had been demonstrated to be present, the measurements were not complete. In the reinvestigation (*13*) the sixth lumbar ventral root was stimulated and the nerve action potentials were recorded with silver-silver chloride surface electrodes at three sites. The sciatic-peroneal nerve in the thigh was laid on a triple pole electrode, the sciatic lying on the uppermost pole, and the peroneal on the lower two. Recording from the upper two poles was designated *S-P*; from the lower two, P_t. The peroneal nerve, where it winds round the neck of the fibula, was laid on a double pole electrode and this recording site was designated P_f. In six normal dogs the potentials recorded at any one of the three sites consisted of (i) an initial single biphasic potential of small amplitude (mean 0.7, 0.24, and 0.1 mv at *S-P*, P_t, and P_f, respectively), and (ii) subsequent multiple potentials of large amplitude (5 to 10 times that of the initial). In four moderately paralyzed dogs the mean amplitudes of the intial simple and subsequent multiple potentials were approximately ⅓ and 1/18, respectively, that of the normal, and the conduction velocity of the multiple potentials was significantly decreased (see Fig. 1). In four severely paralyzed dogs, only one biphasic potential of very small amplitude was recorded at each site.

These results indicate that tick paralysis is due to a defect in conduction of motor nerve fibers and that the tick "toxin" evidently exhibits a predilection for the slower conducting, smaller diameter fibers. Since direct stimulation of the perfused paralyzed muscle failed to liberate acetylcholine, a conduction block in the small-diameter terminal motor fibers must also be present (*14*).

MAURICE F. MURNAGHAN
Department of Pharmacology,
University of Ottawa,
Ottawa, Ontario, Canada

References and Notes

1. J. D. Gregson, *Proc. Entomol. Soc. Brit. Columbia* **48**, 54 (1952).
2. J. A. Costa, *Am. J. Diseases Children* **83**, 336 (1952).
3. I. Rose, *Can. Med. Assoc. J.* **70**, 175 (1954).
4. M. F. Murnaghan, *Rev. can. biol.* **14**, 273 (1955).
5. ———, *Proc. Xth (1956) Intern. Congr. Entomol.* **3**, 841 (1958).
6. ———, *Nature* **181**, 131 (1958).
7. A. S. V. Burgen, F. Dickens, L. J. Zatman, *J. Physiol. (London)* **109**, 10 (1949).
8. M. F. Murnaghan, *Proc. Can. Fed. Biol. Soc.* **2**, 48 (1959).
9. P. Emmons and H. McLennan, *Nature* **183**, 474 (1959).
10. C. O. Hebb and G. M. H. Waites, *J. Physiol. (London)* **132**, 667 (1956).
11. P. B. Sastry, thesis, McGill Univ., Montreal, Canada (1956).
12. F. C. MacIntosh, *Can. J. Biochem. and Physiol.* **37**, 343 (1959).
13. A paper describing the details is in preparation.
14. This investigation was aided by research grant EMR-54 from the Department of Agriculture, Ottawa, Canada.

9 September 1959

In vitro Culture of Ehrlich Ascites Tumor Cells

Abstract. The successful culturing and subculturing of ascites tumor cells for 80 odd days are described, as well as the growth pattern of these cells during this period. The cytological appearance of cells after 3 weeks in tissue culture and reinoculation into mice is also presented.

Following the successful culturing of both normal and abnormal cells by Puck and his associates, we have used the basic suggested medium of Marcus, Ciecura, and Puck (*1*), with a slight modification, to grow Ehrlich ascites tumor cells. The medium was modified by the substitution of horse serum for the calf serum and chick embryo extract employed by the above-named authors (*1*). It has not been necessary at any time to use spleen monocytes (*2*), explants of normal cells (*3*), or normal rat fibroblast cultures (*4*), or any adjunct in order to obtain a successful pure culture of Ehrlich ascites tumor cells.

To date, the Ehrlich ascites tumor cells have been maintained some 80 odd days and have been subcultured three times during that period; each culture is still active. The complete nutrient solution found to produce luxuriant growth on more than one occasion was made up of 45 percent Hanks' saline, 40 percent Puck's nutrient solution, and 15 percent horse serum. This is in contrast to Puck and Marcus' attachment solution of 40 percent nutrient solution, 5 percent calf serum and chick embryo extract, and 55 percent Hanks' saline (*1*).

The Ehrlich ascites tumor cells were aseptically harvested from a CF_1 male mouse bearing a 5-day-old intraperitoneal tumor and were transferred to a sterile 200-cm³ rubber-sealed, screw-capped milk dilution bottle gassed with 5 percent CO_2 and 95 percent air. The cultures were begun with a high population cell density, one culture with 5×10^7 cells and the other with 2.5×10^7 cells. The cells were maintained in a fluid culture without agita-