tence of a replicon at the centromere with an abnormal base ratio, or one shorter than 2.5 μ, is not excluded.

DAVID E. COMINGS
Department of Medical Genetics, City of Hope Medical Center, Duarte, California

References and Notes

1. E. J. DuPraw. *Nature* **206**, 338 (1965); *ibid.* **209**, 577 (1966).
2. F. Jacobs, S. Brenner, F. Cuzin, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 329 (1963).
3. R. Baserga and K. Nemeroff, *Stain Technol.* **37**, 21 (1962).
4. D. E. Comings, *Cytogenetics*, in press.
5. C. Leuchtenberger, R. Leuchtenberger, A. M. Davis, *Amer. J. Pathol.* **30**, 65 (1954)
6. Based on the assumption that there is not a significant difference in the thickness of flattened fibroblasts and metaphase or anaphase spreads.
7. Estimation of 48 μ as the length of a replicon in humans was based on 96 minutes as the duration of replication of the heavily labeling, asynchronous and late-replicating chromatin of the inactive X chromosome (*4*) and 0.5 μ/min as the rate of replication of an extended DNA helix in HeLa cells (*8*).
8. J. Cairns, *J. Mol. Biol.* **15**, 372 (1966).
9. R. B. Painter, D. A. Jermany, R. E. Rasmussen, *ibid.* **17**, 47 (1966).
10. Supported in part by HE 3091 from PHS and general support grant, City of Hope Medical Center.

23 September 1966

Perineural Epithelium: A New Concept of its Role in the Integrity of the Peripheral Nervous System

Abstract. *A multilayered, squamous-celled epithelial cell membrane covering the individual nerve fasciculi of the entire peripheral nervous system (both voluntary and autonomic) including the sensory and motor end organs has been demonstrated in various species of animals, including man. This membrane is the direct continuation of the pia-arachnoid mater from the central nervous system. Functional significance of this membrane, especially as a diffusion barrier and as a protector of the peripheral nervous system, is briefly discussed.*

Key and Retzius (*1*) originally described what appeared to be a sheath that was composed of endothelial-like cells which surrounded nerve trunks; it has subsequently been referred to sporadically in the literature (*2*). This continuous layer of cells surrounds not only nerve trunks but all nerve fasciculi, and it underlies the connective tissue of the perineurium (Fig. 1c) (*3*). It completely surrounds the nerve fasciculi in frog, shark, whale, rat, cat, dog, guinea pig, rabbit, monkey, chimpanzee, and man.

In the frog, the membrane is two layers thick; in mammals it is from 5 to 12 layers thick, the number of layers decreasing with the decreasing size of the fasciculus. With the electron microscope, some fine collagen fibrils can be demonstrated between the layers (*4*, *5*). Each epithelial cell has a basement membrane on either side (Fig. 2). Small blood vessels ramify between the layers and carry a sleeve of epithelial cells from one of these layers some distance into the nerve fasciculus (*4*, *6*). In the same way, the blood vessels of the central nervous system carry a sleeve of leptomeninges.

The squamous shape of the cells and the multilayered nature of the membrane can be demonstrated (with the dissection microscope) when it is isolated and then stained to demonstrate the cellular borders, or when tangential sections are made of it. When transverse or longitudinal sections of a nerve fasciculus are cut as in standard histological preparations, the perineural epithelium appears as the lamellated structure that is described in some standard histological textbooks, but this appearance is due to the multiple layers of squamous cells. The fasciculi in sections of a nerve trunk close to the central nervous system show clear-cut and very thick lamellation because of the great number of cellular layers present, but small nerve fasciculi (terminal divisions) that are some distance from the central nervous system and are surrounded by only one or two layers of cells will not show any obvious lamellation under the microscope. Thus, the description of the perineurium as a lamellated structure cannot be applied to the perineural covering of all nerve fasciculi.

When this membrane is traced back along the nerve trunk to its point of origin in the central nervous system, it can be seen to surround the whole nerve trunk and to pass through the vertebral foramen where it becomes continuous with the pia-arachnoid membrane of the central nervous system (Fig. 1, a and b). The cells of this membrane are identical in shape and size, in nuclear structure, and in enzyme histochemistry with the cells of the pia-arachnoid mater (Fig. 1, a–c). We believe they are the same kind of cell and that this cellular sheet is embryologically derived from the same ectodermal cells as the pia-arachnoid. If the latter is regarded as a product of the ectoderm, then this membrane is an epithelium—a multilayered squamous epithelium. However, the name is not as important as its structure and relationships.

The capsule of the dorsal root ganglion is simply a reflection of the perineural epithelium around the ganglion. The epithelium also surrounds the sympathetic ganglia and the sympathetic nerve trunks and their divisions (*6*); it also surrounds the fasciculi of the vagus nerve (*7*), as well as those of other cranial nerves.

As the peripheral nerves branch, the perineural epithelium branches with them; as the branches get smaller, the number of layers of cells in the epithelium becomes less, until finally the terminal nerve fiber is surrounded by a single-layered, continuous sheet of cells (Fig. 2) which accompanies the fiber to its termination.

At the termination of the fiber, the perineural epithelium forms the capsule of the end organs. In the motor end plate it constitutes the bell mouth of Henle which envelops the end plate and attaches directly to the sarcolemma (*8*) (Fig. 3). In the Pacinian corpuscle, the various lamellae of the corpuscle are simply additional layers of cells of the epithelium which surround the naked nerve ending (*9*) (Figs. 1e, 3, and 4) as they are in the morphologically similar structure, the Herbst corpuscle of the duck. The capsule of the muscle spindle (Fig. 3) is a sculptured or molded perineural epithelium (*10*) as are the capsules of the various nerve endings in the skin (Fig. 3). In the eye, the choroid is continuous with the pia-arachnoid membrane (perineural epithelium) of the optic nerve (Fig. 1d), the cells of the two structures being morphologically and histochemically identical (*11*).

The choroid is continuous with the endothelial cells of the anterior-chamber angle meshwork and with the "endothelium" underlying the cornea, so that the retina and the contents of the eye are completely enclosed by a membrane continuous with the pia-arachnoid membrane of the optic nerve (perineural epithelium) (*11*), and the choroid is presumably derived from the same embryological origin as the pia-arachnoid and the perineural epithelium covering the nerve endings.

Because of its complexity, no attempt has been made to homologize the structures of the inner ear with the perineural epithelium; to do this, detailed embryological study is needed.

If a nerve is sectioned, the perineural epithelium remains unchanged in the parts of the nerve both distal and proximal to the point of section (*12*). Thus, it forms a tube inside which the degenerating and regenerating components of nerve fasciculi are protected and isolated. In keeping with their relationship to this epithelium, the sheath of the bell mouth of Henle and the capsule of the muscle spindle are unaffected, although the former collapses on top of the remains of the motor end plate (*8, 12*). The perineural epithelium, therefore, functions as an additional guide for regenerating nerves, and serves to lead the nerve back precisely to the end organ.

Our studies show that the peripheral nervous system has its own equivalent of a "blood brain barrier" in which it is encased from its origin up to, and including its termination and is, therefore, isolated from the body fluids. This barrier, like the pia-arachnoid membranes from which it is derived, does not have very high activities of oxidative enzymes, but does have considerable activity of dephosphorylating enzymes, especially of adenosine triphosphate (ATP), thus indicating that it can function as a metabolically active diffusion barrier (*3, 13*). Removal of the sheath covering the nerve fasciculus causes its swelling, increase in its weight, and gradual change in the fast part of the electrotonus (*14*).

The presence or absence of a diffusion barrier in the sheath covering the nerve fasciculus has been the subject of controversy for a long time. Lorente de No (*15*) showed that sodium (small molecular weight and strong electrolyte) and cocaine (large molecular weight and strong electrolyte) were able to diffuse freely and rapidly through the epineurium. Therefore, he states that the view held by other workers that the epineurium is an effective diffusion barrier is erroneous. On the other hand, Feng and Gerard, and Feng and Liu (*16*), by using an assortment of agents such as inorganic ions, alkaloids, isotonic solutions of different organic substances, organic solvents, metabolic poisons, and methylene blue, have conclusively shown that the connective tissue sheath of the amphibian sciatic nerve is an effective diffusion barrier. Causey and Palmer (*17*), using P^{32}, investigated the transport of phosphate ions in nerve and concluded that the epineurium is a barrier to diffusion of phosphate ions in vivo. Krnjevic (*18*), using a variety of compounds ($BaCl_2$, $CuCl_2$, RbCl, NH_4Cl_2, $HgCl_2$, $CaCl_2$, and so forth), demonstrated that the perineurium is a diffusion barrier. The sheath covering the nervous system in insects also acts as a diffusion barrier by allowing the normal functioning of the nerve in spite of the great variations in the potassium concentration of the blood (*19*). It is very likely that this sheath is the equivalent of the perineural epithelium of vertebrate nerves. The sheath covering the nerve fasciulus, not the epineurium, is the limiting factor in the entry and exit of experimental substances in the intact sciatic nerve in vitro (*20*). That it acts as a diffusion barrier for ions, is suggested by the work of Vorontsov (*21*) who demonstrated that the fast part of the electrotonus of nerves is lost if the perineurium, but not the epineurium, is removed. Vorontsov stated that this effect could result from the perineurium acting as an obstacle to the passage of both anions and cations. It appears,

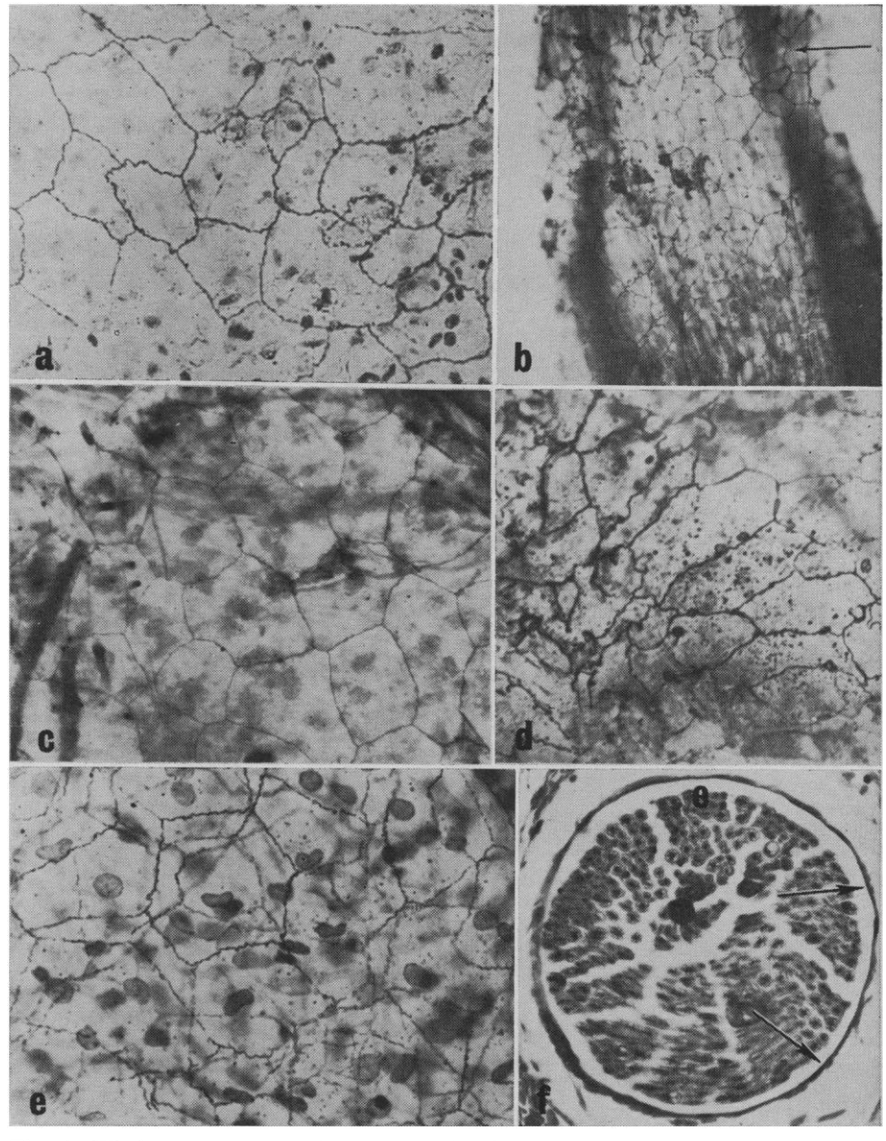

Fig. 1. Whole-mount preparations (a to e) of the covering membranes of various parts of the central and the peripheral nervous system, isolated under the dissection microscope and stained to show cellular borders. Two (c and e) are counterstained to show the nuclei in these cells. (a) Leptomeninges (pia-arachnoid mater) covering the spinal cord of the rat; (b) leptomeninges (pia-arachnoid mater) covering the root (arrow) of the spinal nerve of the cat; (c) perineural epithelium covering the cat sciatic nerve; (d) choroid of the rat eye; (e) perineural epithelial lamellae covering the Pacinian corpuscle of the cat; (f) section of rat trigeminal nerve fasciculus showing adenosine triphosphatase activity in the perineural epithelial membrane (arrows).

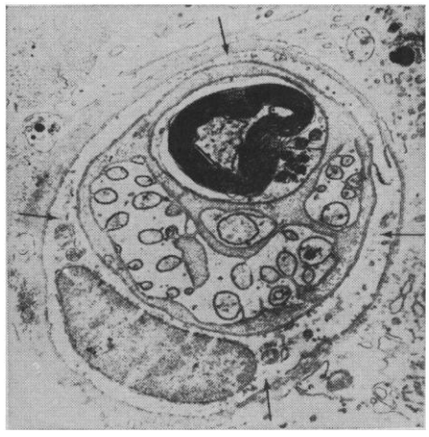

Fig. 2. Electron micrograph of a nerve in the rat bone marrow showing the perineural epithelial cell (arrows) covering the whole nerve fasciculus. Note the basement membrane on both sides of this cell (courtesy of Dr. O. P. Jones, × 23,400).

therefore, that one of the physiological functions of this membrane is to keep a proper concentration of ions in the neighborhood of the nerve fibers. The presence of considerable adenosine triphosphatase activity in its cells would provide the energy for active transport and would permit the membrane to assume such control of ion diffusion. Electron microscopical studies have shown that this membrane acts as a diffusion barrier for ferritin (*22*).

We believe that the lack of proper understanding concerning the structure of the epineurium and perineurium is responsible for the diversity of opinions about the existence or nonexistence of a diffusion barrier in the nerve sheath. Our studies on various parts of the peripheral nervous system (voluntary and autonomic) have shown that the epineurium is composed only of a lacework of connective tissue fibers, but that the perineurium has two components, a perineural connective tissue made up of a connective tissue network, and a perineural epithelium immediately below the perineural connective tissue of multiple layers of continuous squamous cells. Since the epineurium and perineural connective tissue are made up of a lacework of connective tissue components, they cannot act as effective diffusion barriers. It is the perineural epithelium, containing continuous sheets of cells, that acts in this way. If substances are injected under the epithelium, there will be no diffusion; if the injection is made only into the connective tissue of the perineurium, diffusion will be evident. This concept may help investigators control their approaches to the subject of diffusion barriers in nerve sheaths.

This epithelium probably also functions as a protective membrane against infection. This membrane has been found histologically intact even in badly infected areas where it is surrounded by pus, and there has been no inflammatory change in the nerve fasciculus within this membrane. The rabies virus ascends the nerves underneath the perineural epithelium and in association with the Schwann cells (*23*). The presence of this epithelial cell layer under the perineurium, which acts as a barrier to the penetration of antibiotics and antitoxins, may account for the difficulties of treating neural infections.

The more peripheral the nerve, the fewer the number of layers of cells; at the terminal nerve fibers the epithelium is only one layer thick, and the epineural and perineural connective tissue is scanty (Fig. 2); thus, it would be easier for infection, edema fluid, or toxic substances to penetrate to the fiber in these regions. This fact probably explains the distal distribution of peripheral neuritis (*24*). The role of the perineural epithelium of the optic nerves in the development of sympathetic ophthalmia has been discussed (*24*).

We have described the similarity in structure and histochemistry of the perineural epithelium of peripheral nerves and the pia-arachnoid of the brain and spinal cord. We are not just homologizing these membranes, we have shown by direct dissection that they are continuous with each other. Like the pia-arachnoid, the perineural epithelium is a squamous-celled multilayered mem-

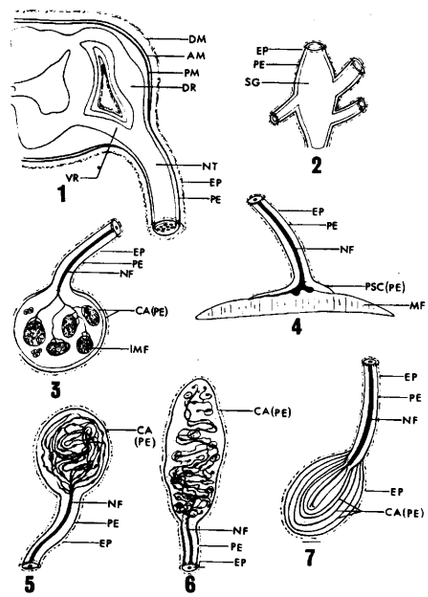

Fig. 3 (left). Diagrammatic representation, based on our studies, of various membranes and connective tissue sheaths of the central and peripheral nervous system including various sensory and motor end organs. (1) The spinal cord along with its membranous coverings and their relationship to peripheral nerve. The dura mater (*DM*) continues with the epi- and perineural connective tissue (*EP*) of the nerve trunk (*NT*). The pia-arachnoid mater covering the dorsal root and its ganglia (*DR*) as well as the ventral root (*VR*) continue as perineural epithelium (*PE*) of the nerve trunk (2–7). These drawings demonstrate the relationship of membranes of the peripheral nerve and the supply nerve (*NF*) of various sensory and motor end organs and the relationship of the membranous coverings of the supply nerve to the coverings of various sensory and motor end organs. (2) Sympathetic ganglion with its rami communicans and its branch; (3) T.S. of a muscle spindle and its nerve of supply; (4) motor end plate and its nerve of supply; (5) Krause end bulb for cold sensation and its nerve of supply; (6) Meissner's corpuscle for touch sensation and its nerve of supply; (7) Pacinian corpuscle for pressure sensation and its nerve of supply. *AM*, arachnoid mater; *PM*, pia mater; *CA*, capsule made up of extension of perineural epithelium covering the nerve fiber supplying the end organ; *PSC*, perisynaptic cell; *MF*, muscle fiber; *IMF*, intrafusal muscle fiber.

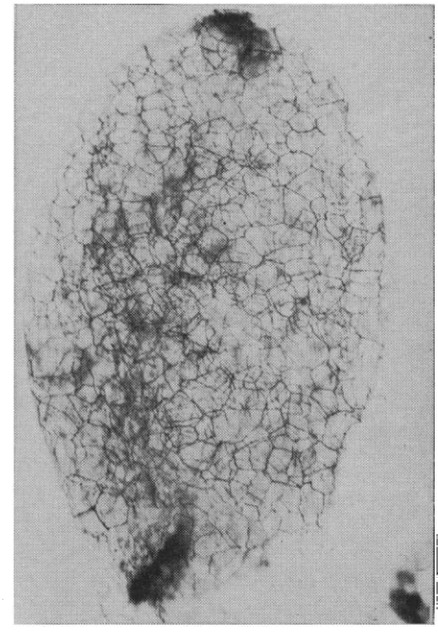

Fig. 4. Whole-mount preparation of a cat Pacinian corpuscle stained to show the cellular borders. The whole corpuscle is covered by squamous-cell lamellae laid on top of one another.

brane. It follows all peripheral nerves (including the autonomic nerves) to their termination, being reflected over the various ganglia on the way to produce their capsules. At the termination of the nerve fibers it forms or completely surrounds the end organs, thus ensuring that the peripheral nervous system is completely enclosed in the equivalent of a blood brain barrier. The pia-arachnoid covering (perineural epithelium) of the optic nerve extends into the eye as the choroid, which, with the endothelial cell layer underlying the cornea, encloses the components of the eye in a cellular membrane, just as the other end organs are enclosed. The perineural epithelium appears to have important functions in the regeneration, physiology, and pathology of peripheral nerves.

T. R. SHANTHAVEERAPPA
GEOFFREY H. BOURNE
Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322

References and Notes

1. A. Key and G. Retzius, *Studien in der Anatomie des Nervensystems und des Bindegewebes* (Samson and Wallin, Stockholm, 1876), vol. 2, p. 102.
2. K. Krnjevic, *Quart. J. Exp. Physiol.* **39**, 55 (1954); H. J. Lehmann, *Nature* **172**, 1045 (1953); P. Rohlich and U. M. Weiss, *Acta Morphol. Budapest* **5**, 335 (1955).
3. T. R. Shanthaveerappa and G. H. Bourne, *J. Cell Biol.* **14**, 343 (1962); ———, *J. Anat., London* **96**, 527 (1962); ———, *Nature* **197**, 702 (1963).
4. T. R. Shanthaveerappa, J. Hope, G. H. Bourne, *Acta Anat.* **52**, 193 (1963).
5. J. D. Robertson, *J. Biophys. Biochem. Cytol.* **2**, 381 (1956); ———, *Amer. J. Phys. Med.* **39**, 1 (1960); P. Rohlich and A. Knoop, *Z. Zellforsch.* **53**, 299 (1961); D. C. Pease and W. Pallie, *J. Ultrastruc. Res.* **2**, 352 (1959).
6. T. R. Shanthaveerappa and G. H. Bourne, *Z. Zellforsch.* **61**, 742 (1964).
7. ———, *Acta Anat.* **52**, 95 (1963).
8. ———, *International Review of Cytology* (to be published by Academic Press, 1966), vol. 21.
9. ———, *Acta Anat.* **60**, 199 (1965); ———, *Nature* **209**, 1260 (1966); ———, *Amer. J. Anat.* **112**, 97 (1963).
10. ———, *Acta Anat.*, in press.
11. ———, *ibid.* **61**, 379 (1965).
12. ———, *Anat. Rec.* **150**, 35 (1964).
13. ———, *Amer. J. Anat.* **118**, 461 (1966).
14. R. Lorente de No, *Cold Spring Harbor Symp. Quant. Biol.* **17**, 299 (1952); A. M. Shane, *J. Cell. Comp. Physiol.* **41**, 305 (1953).
15. R. Lorente de No, *J. Cell. Comp. Physiol.* **35**, suppl. 195 (1950).
16. T. P. Feng and R. W. Gerard, *Proc. Soc. Exp. Biol. Med.* **27**, 1073 (1929-1930); T. P. Feng and Y. M. Liu, *J. Cell. Comp. Physiol.* **34**, 1 (1949).
17. C. Causey and E. Palmer, *J. Anat., London* **87**, 30 (1953).
18. K. Krnjevic, *J. Physiol.* **123**, 338 (1954).
19. G. Hoyle, *J. Exp. Biol.* **30**, 121 (1953).
20. A. M. Shane, *J. Cell. Comp. Physiol.* **43** suppl. 87, 99 (1954).
21. D. S. Vorontsov, *Fed. Proc.* (Trans. Suppl.) **22**, T241 (1963).
22. J. D. Waggener, S. M. Bunn, J. Beggs, *J. Neuropath. Exp. Neurol.* **24**, 430 (1965).
23. G. M. Baer, T. R. Shanthaveerappa, G. H. Bourne, *Bull. World Health Organ.* **33**, 783 (1965).
24. T. R. Shanthaveerappa and G. H. Bourne, *Nature* **199**, 577 (1963).
25. Supported in part by NIH grant FR 00165.

28 September 1966

Feedback of Speech Muscle Activity during Silent Reading: Rapid Extinction

Abstract. *Surface electromyograms of the laryngeal muscles were made while subjects read silently. Those who showed an increase in electrical activity over that at relaxation were provided with auditory feedback of the muscle activity. This treatment resulted in immediate and long-lasting cessation of the subvocalization. This method should prove valuable in treating some reading problems.*

The phenomenon of subvocal speech has been of great interest to educators concerned with the teaching of reading. It has, however, received little systematic study, with the exception of the work by Sokolov and by Edfeldt (*1*). Subvocalization is considered one of the most difficult problems to overcome in increasing reading speed. An individual who subvocalizes to any great extent is limited to a top reading speed of approximately 150 words per minute—a maximum attainable while reading aloud. We use the term subvocalization to include a wide range of activity, from inaudible articulations and vocalizations to audible whispering while reading. If subvocal activity includes movements of the lips and jaw, some corrective measures are possible. However, if the activity is limited to the vocal musculature, eliminating the response becomes more complex, especially since individuals are often not aware they are subvocalizing.

The initial study of subvocalization required the examination of several subjects with strong subvocalization patterns to determine whether subvocalization during silent reading could be detected by surface electromyograms recorded from the throat. A successful technique was developed with mesh electrodes placed over the thyroid cartilage. An ink-writing oscillograph was used to record the electromyogram (EMG). At maximum sensitivity of the oscillograph unit, the electrical activity of the vocal muscles can be detected while the subject is reading (if subvocalization is present), in contrast to a minimum signal (approximately 3 μv) obtained when the subject is relaxed, and to an extremely strong signal (approximately 1 mv) obtained when the subject speaks during normal conversation. To determine the presence or absence of subvocalization, the subject first selects reading material which he reads for 30 minutes, and during this time an oscillograph record of the EMG is obtained.

The presence of subvocalization is determined by asking the subject to stop reading, then to begin reading, and then to stop reading. Each time, the changes in the EMG record are noted. The presence of subvocalization can be detected quite reliably, there being a large increase in action potentials when

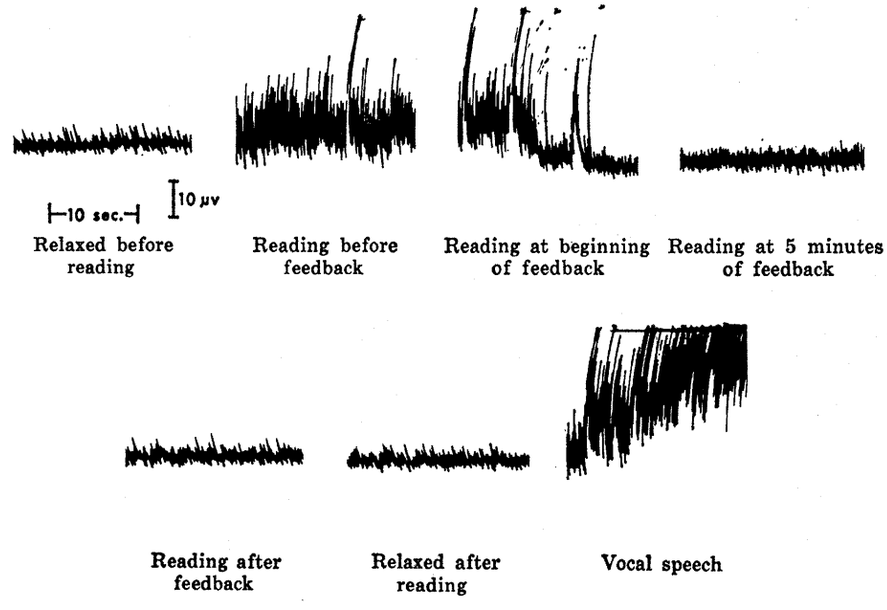

Fig. 1. Electromyograms recorded from the laryngeal muscles. This record is typical of those obtained from the 17 subjects who received feedback treatment.