knowledge of animal anatomy, and his natural bent for sculpture gave an artistic quality to all his work. The modern taxidermist, instead of stuffing the skin of an animal with straw and rags, mounts it on a waterproof manikin made whenever possible from a plaster cast of the body. The animal is seldom placed on display without a stage setting suggesting its proper habitat. It is a far cry from the Ward's Natural Science Establishment of Akeley's youth to the great institution at Central Park West and Seventy-seventh Street, but in numerous respects Ward's led the way.—*The New York Sun.*

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR WORKING ON THE TERMINAL NERVE-MUSCLE UNIT

THE method here described[1] makes possible a direct experimental study of the terminal motor axone with its attached muscle fiber. Thus, in one field and focal plane with active circulation, an ultimate member of the motor-unit can be identified, operated upon and selectively stimulated under the compound microscope.

A technique employed by us for recording *in situ* by reflected light the mechanical response of muscle fibers in the retrolingual membrane (*membrana basihyoidea*) of the frog has recently been reported.[2] On noting in this structure the frequent presence of terminal nerve filaments (from *n. hypoglossus*) we have since met the requirement of transmitted light by a procedure similar to that devised by Richard Thoma for the observation of leucocyte migration and published at Heidelberg[3] in 1873. Both nerves and muscle fibers are mentioned by Thoma, who points out the many conditions fulfilled by the preparation in the study of living tissue elements.

The intrinsic lingual muscle fibers (*stratum arcuatum* Gaupp) which from either side enter this delicate membrane bounding dorsally the extended tongue form a transversely, somewhat sparsely disposed branching and anastomosing system. The fibers, though suggesting thus an interconducting syncytium, are nevertheless partitioned into discrete members each of which is of the striated-voluntary type. Since Thoma's observations these fibers have from time to time been an object of interest to workers on muscle structure and function, notably Ranvier[4] and Kahn,[5] and have come recently under special notice in a fruitful examination of the credentials of the all-or-none law.[6]

[1] *Cf.* abstract, F. H. Pratt and M. A. Reid, *Proc.* Am. Physiol. Soc. (Chicago meeting, March 28–29, 1930); *Am. J. Physiol.*, 93: 681, 1930.

[2] F. H. Pratt, *Am. J. Physiol.*, 93: 9, 1930.

[3] Verlagsbuchhandl. v. Fr. Bassermann. Buchdruck., G. Otto, Darmstadt.

[4] L. Ranvier, *Compt. Rend. Acad. Sci.*, 110: 504, 613, 1890.

[5] R. H. Kahn, *Zentralbl. f. Physiol.*, 17: 745, 1903–04.

[6] E. Fischl and R. H. Kahn, *Pfl. Arch.*, 219: 33, 1928; F. H. Pratt and M. A. Reid, *Proc.* XIII Int. Physiol. Cong., *Am. J. Physiol.*, 90: 480, 1929; S. Gelfan, *Am. J. Physiol.*, 93: 1, 650, 1930; H. Hintner, *Pfl. Arch.*, 224: 608, 1930; F. H. Pratt, *loc. cit.*

The *motor-unit,* a term introduced by Sherrington[7] to denote the nerve fiber with the muscle fibers governed by it, is known to be adapted in its pattern to the directional demands on the muscle in developing tension. Thus in the sartorius[8] the motor-unit is linear in disposition, involving formation in files of the "squad"[9] of muscle fibers under command of the neurone. This close formation of a group intimately bound into the muscular matrix offers little feasibility of detailed inspection *in vivo.* With the retrolingual membrane, however, the tension requirement is highly diffuse;[10] it is correlated with a dispersion or deployment of the muscle-squad in essentially one plane, the fibers being none the less completely integrated through the nerve strands passing along and across the intervals formed by the divergence and loose intertexture of the musculature (Fig. 2) as it invades the membrane from the arcuate layer. It should therefore be possible, with proper illumination and control, to take practical advantage of this natural isolation of what may be termed respectively a motor sub-unit (branch system subordinate to the motor-unit) and the motor-terminal (ultimate sub-unit—the innervated muscle fiber).

In Thoma's method[11] the everted tongue is stretched over a glass plate after an opening for transmission of light has been made in what is now the floor of the superficial lymph sac (*sinus basihyoideus*). This admits of continued circulation, the preparation being kept moist by irrigation. With the present modification, however, it is unnecessary to stretch the tissues since a glass disc or cylinder supports the membrane and fills the opening beneath it, with the further advantage that manipulation is afforded without deranging the focal position; as, for example, in the mechanical blocking of impulses in single nerve fibers. Even with extensive pithing the capillary circulation in the preparation may be highly active and persistent, all tissues under observation being immersed.

[7] C. S. Sherrington, *Proc. Roy. Soc.* B, 105: 332, 1929; J. C. Eccles and C. S. Sherrington, *ibid.*, 106: 326, 1930.

[8] S. Cooper, *J. Physiol.*, 67: 1, 1929.

[9] E. L. Porter, *Am. J. Physiol.*, 91: 345, 1929.

[10] R. H. Kahn, *loc. cit.*

[11] R. Thoma (illustrated description), Abderhalden's Handb., V, 4, 1924, p. 1928.

A disc made from a segment of glass rod (about 5 x 4 mm for *R. pipiens*) is cemented with balsam to a glass plate (*a*, Fig. 1). The tongue of the pithed or narcotized frog is everted and an incision made through its mid-region as far as the membrane, avoiding undue hemorrhage from the rich bilateral vascular supply. The preparation is now adjusted (prone position) so that the tongue, *b*, rests on the plate above mentioned, with the disc penetrating the open-

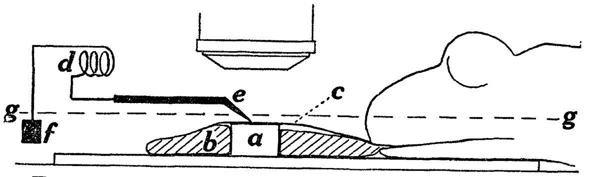

FIG. 1. *a*, glass disc cemented to slide; *b*, tongue (mid-sagittal section); *c*, retrolingual membrane; *d*, secondary coil; *e*, active electrode; *f*, indifferent electrode; *g–g*, submersion level.

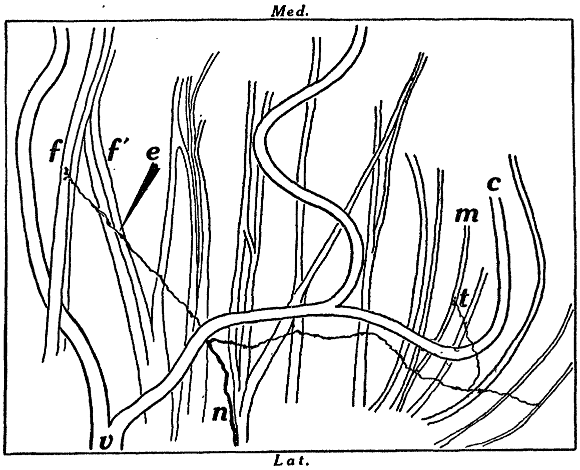

FIG. 2. Outline based on a typical nerve-containing field, retrolingual membrane, $\times 50$: *v*, vein; *c*, capillary; *n*, myelinate nerve fiber; *t*, one of its terminal filaments; *m*, one of a sub-unit group of muscle fibers that respond with the motor-unit to stimulation by *e*, the active electrode (axone reflex). If the nerve be cut or compressed between the contact of *e* and the structures central to it, and again stimulated, only the muscle fibers (*f*, *f′*) distal to the block respond. If the electrode be moved slightly from the nerve the fiber *f′* alone responds, but at a higher threshold. Being thus directly excited it may, especially if touched, show partial, gradable contractions as described by Gelfan and by Hintner (*loc. cit.*) and, for the sartorius, by Brown and Sichel.[15] The outline is semi-diagrammatic and slightly composite. No morphologic interpretation is here to be placed upon detail indicating contacts between nerve and muscle.

ing so as to support the endothelial surface of the membrane. The entire preparation is laid in a Petri dish and covered with Ringer's fluid to above the level of the membrane, which may now be transilluminated under the microscope and at the same time manipulated from above. Although low powers suffice for most experiments a high-power objective immersed in the solution gives excellent definition.

For stimulation the unipolar method is conveniently used. An indifferent terminal rests in the bath covering the preparation. The active electrode may take various forms; we have worked largely with quartz-covered platinum electrodes (diameters between 2 and 10 μ) of the type described by Taylor,[12] carried in a manipulator mounted integral with the carriage of the preparation. A micro-needle in a second manipulator is readily added to the apparatus. By means of this system single or faradic induction shocks are delivered to any point in the field, the intensity being graduated over a wide range with great delicacy by coarse and fine sliding rheostats—the former in series with the primary circuit, the latter on a shunt across the primary terminals in series with a further set of resistances variable up to 11000 ohms.

On exciting a single motor nerve fiber (see Fig. 2) it is very readily evident that the responding muscle fibers are those limited to innervation by the ramifying nerve filaments of the unit, that the nerve threshold is distinctly lower than the muscle threshold and that one has to deal with a system having a capacity of performance not only independent of changes in the intensity of stimulation but, so long as other factors are in abeyance, of unvarying character. Functional isolation of one motor-terminal is to be obtained by cutting or otherwise blocking the filaments passing to other muscle fibers of the unit, or by actual destruction of such fibers. The area of innervation (locus of "end-organ") appears with sufficient definition to enable the observer to manipulate the electrode with a considerable degree of selectivity. The persistence of thresholds, which are obtainable with great exactitude, appears to be correlated with the circulatory conditions. Frequently a muscle fiber with the innervating collateral is in immediate relation to an actively conducting capillary the walls of which are defined in optical section with extreme clearness. After circulatory failure or mechanical injury,[13] or in the course of fatigue or curarization,[14] the relative excitatory conditions of axone and muscle fiber are again readily investigated; the effect of nerve

[12] C. V. Taylor, *Proc. Soc. Exp. Biol. & Med.*, 23: 147, 1925.

[13] Our results are here in agreement with Hintner's (*loc. cit.*) observations on stimulation of the hypoglossal nerve in the excised tongue.

[14] F. H. Pratt, *Proc.* Am. Physiol. Soc. (Chicago meeting, March, 27–29, 1930); *Am. J. Physiol.*, 93: 608, 1930.

[15] D. E. S. Brown and F. J. M. Sichel, SCIENCE, 72: 17, July 4, 1930.

fiber on muscle fiber has so far proved consistently maximal.

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A NEW TECHNIQUE IN TREE MEDICATION FOR THE CONTROL OF BARK BEETLES[1]

THE possibility of injecting chemicals into the sap stream of living trees in order to inhibit the action of parasitic organisms or correct some pathological condition has appealed to botanists and entomologists for a number of years. Variations of this method of control have likewise been utilized by so-called "tree doctors" to reap no inconsiderable return from unsuspecting owners of valuable shade trees, usually without accomplishing the desired results. A number of valuable contributions in this field and on related subjects have been made as a result of careful experimental work.[2] That it is possible to introduce fluids into the sap stream has been conclusively demonstrated, but in most cases the results have been of limited practical value. This is because usually these substances are not well distributed through all portions of the tree. Good distribution, even to the leaves, has been reported in those portions of the tree in direct communication with the point of injection, but the lateral dissemination has usually been slight.

In the summer of 1925, following the publication of results obtained by Lipman and Gordon,[3] the writers and Mr. J. A. Beal at Asheville, North Carolina, attempted to use the method therein described to destroy the developing broods of the southern pine beetle (*Dendroctonus frontalis* Zimm.) in shortleaf pine. It might be explained here, in reference to this insect, that these beetles attack the trees simultaneously in great numbers, bore through the bark and in about ten to fifteen days completely girdle the inner phloem and at the same time introduce blue-stain.[4] The trees after attack are necessarily doomed and, for the objective at hand, there was no effort made to save them. It was desired merely to kill the developing broods under the bark and thus prevent their escape and attack of other living trees. The control practices now in use for the bark beetles of the genus Dendroctonus consist in felling the tree and either peeling or burning the bark of the entire tree or other practices that destroy the broods, at a cost ranging from 75 cents to $5 per tree, depending on the size. It was hoped that these costs for treatment might be greatly reduced by some such method as that described.

Work was continued through 1926, 1927 and 1928. The results obtained were very conflicting. A high percentage of brood mortality occurred in some trees and with some chemicals, but in general the insects were killed only in a narrow strip above the point or points of injection. It was obvious that the idea might be practical but that the technique was poor; in other words, more thorough lateral distribution of the chemical was needed.

In the meantime several patents had been issued describing various methods of obtaining complete distribution of dye or preservatives in the tree. These were all too elaborate and expensive for the purposes intended. It occurred to the senior author that a combination of the technique already in use and the ringing practice used by orchardists to stimulate the setting of fruit buds might be more effective. Accordingly, during the summer of 1929 approximately two hundred trees were treated by the authors and R. W. Caird, the latter working chiefly on the physiological aspects of the problem.

The technique adopted was as follows.

(1) At a convenient working distance near the base of the tree the bark is first smoothed completely around the tree by the use of a wood rasp or draw knife to such a degree that it will permit a water-tight application.

(2) The next step consists in making a narrow (one eighth to one fourth inch) incision or notch completely around the circumference of the tree, by means of a saw or sharp knife, through the bark, and through two or more annual layers of wood, or through the entire sapwood, depending on the depth of penetration desired. This incision is located on that portion of the bark which has been smoothed.

(3) On one side of the tree an auger hole about

[1] Publication of the results obtained by the Bureau of Entomology at this time while the investigations are still in an experimental stage seems justified largely because a technique appears to have been developed which has some advantage over those previously described. It should be distinctly understood, however, that the Bureau of Entomology is not at this time advocating the use of this method for general application in the control of insects.

After this article had been prepared, Dr. Caroline Rumbold called our attention to the use of a somewhat similar technique by M. A. Boucherie, "Mémoire sur la conservation des bois," *Annales de Chimie et de Physique,*" 74: 113–157, 1840.

[2] J. Davidson and H. Henson, "The Internal Condition of the Host Plant in Relation to Insect Attack, with Special Reference to the Influence of Pyridine," *Ann. Appl. Biol.*, 16: 458–471, 1929; A. Muller, "Die innere Therapie der Pflanzen," Monograph angew. Ent. 8, pp. vi–206, illus., 1926 (abstract in *Rev. Appl. Ent.*, 14, Ser. A: 505); C. T. Rumbold, "The Injection of Chemicals into Chestnut Trees," *Am. Jour. Bot.*, 7: 1–20, 1920.

[3] C. B. Lipman and A. Gordon, "Further Studies on New Methods in the Physiology and Pathology of Plants," *Jour. Gen. Physiol.*, 7: 615–623, 1925.

[4] F. C. Craighead, "Interrelation of Tree-Killing Barkbeetles (Dendroctonus) and Blue Stains," *Jour. Forestry*, 26: 886–887, 1928; R. M. Nelson and J. A. Beal, "Experiments with Blue-stain Fungi in Southern Pines," *Phytopathology*, 19: 1101–1106, 1929.