It would seem, from these observations, that there is a structural basis for the muscle wasting to be seen in some myasthenics and for the muscle weakness refractory to Prostigmin: And that the term "myasthenic myopathy" might appropriately be applied to such cases, of which the following is an example:

K. B., boy aged 13. The first symptoms occurred six months before death and took the form of diplopia and bilateral ptosis. Shortly after, his gait changed. He would walk leaning backwards with the knees bent. Within two weeks he was bedridden, dysphagic and with indistinct speech. Prostigmin produced dramatic improvement and he was discharged from hospital able to walk and free from diplopia and ptosis. He relapsed some two months later and ceased to respond to Prostigmin. He came under my care just over two weeks before he died, suddenly and unexpectedly, in an iron lung. He was, at that time, grossly emaciated, dysarthric and dysphagic with facial paresis but no ophthalmoplegia (but he was still receiving Prostigmin). There was generalized symmetrical paresis of the proximal limb muscles and trunk. The tendon-jerks were absent and he had no contractures.

Post-mortem revealed congestive heart failure, acute bronchitis and bronchopneumonia and, in addition there was widespread muscular atrophy which involved the digestive tract, heart and skeletal muscles. The affected muscles showed histological changes corresponding to Russell's Types 1, 2 and 3 lesions (Figs. 1 and 2).

The terminal neural apparatus was perfectly normal in this case; also the acetylcholinesterase end-organ.

REFERENCES

ADAMS, R. D., DENNY-BROWN, D. E., and PEARSON, C. M. (1953) Diseases of Muscle. London.
BATTEN, F. E. (1909–10) *Quart. J. Med.*, **3**, 313.
BRANDT, S. (1950) Werdnig-Hoffmann's Infantile Progressive Muscular Atrophy. Copenhagen.
CONEL, J. L. (1938) *Arch. Neurol. Psychiat., Chicago*, **40**, 337.
—— (1940) *Arch. Path.*, **30**, 153.
DALLDORF, G., and SICKLES, G. M. (1948) *Science*, **108**, 61.
ELLIOTT, F. A. (1939) *Proc. R. Soc. Med.*, **32**, 876.
FORD, F. R. (1952) Diseases of the Nervous System in Infancy, Childhood and Adolescence. 3rd ed., Oxford; p. 323.
FUCHS, E. (1890) *Arch. Ophtal., Paris*, **36**, 234.
GOWERS, W. R. (1902) *Brit. med. J.*, ii, 89.
GREENFIELD, J. G., and STERN, R. O. (1927) *Brain*, **50**, 652.
HUTCHINSON, J. (1879) *Med.-chir. Trans.*, **62**, 307.
KILOH, L. G., and NEVIN, S. (1951) *Brain*, **74**, 115.
MACLEOD, J. G., and MACDONALD, R. M. (1942) *Arch. Dis. Childh.*, **17**, 30.
MCMULLEN, W. H., and HINE, M. L. (1921) *Brit. J. Ophthal.*, **5**, 337.
MARTIN, J. P. (1939) *Proc. R. Soc. Med.*, **32**, 876.
NATTRASS, F. J. (1955) *Brain*, **77**, 549.
NEVIN, S. (1936) *Quart. J. Med.*, **5**, 51.
NIKITIN, M. P. (1929) *Z. ges. Neurol. Psychiat.*, **120**, 575.
RABINOVITCH, R., GIBSON, W. C., and MCEACHERN, D. (1951) *J. Neurol. Psychiat.*, **14**, 95.
RUSSELL, D. S. (1953) *J. Path. Bact.*, **65**, 279.
SANDIFER, P. H. (1946) *J. Neurol. Psychiat.*, **9**, 81.
SHY, G. M., and MCEACHERN, D. (1951) *J. Neurol. Psychiat.*, **14**, 101.
SPILLER, W. G. (1914) *Brain*, **36**, 75.
TURNER, J. W. A. (1940) *Brain*, **63**, 163.
—— (1949) *Brain*, **72**, 25.
WALTON, J. N., and NATTRASS, F. J. (1954) *Brain*, **77**, 169.
WELLANDER, L. (1950) *Acta psychiat., Kbh.*, Suppl. 59, 133.
WILDBRAND, H., and SAENGER, A. (1921) *Neurologie des Auges. Munich;* **8**, 121.

The Pathology of the Lower Motor Neurone in the Light of New Muscle Biopsy Techniques

By A. L. WOOLF, M.D., and KENNETH TILL, F.R.C.S.

THE following observations were originally made in Paris and Antwerp and later augmented by studies at the Hospital for Sick Children, Great Ormond Street, and the Midland Centre for Neurosurgery, Smethwick. It is a particular pleasure to record here the enthusiastic co-operation received at the Children's Hospital and the Salpêtrière in Paris, where the first muscle biopsies were carried out, and from Dr. Ludo van Bogaert at the Institut Bunge, Antwerp, where the animal studies were made.

Nowhere is more difficulty encountered than in determining the effect upon electrical function of lesions of the different *intramuscular* components of the lower motor neurone. This is largely because anatomical studies upon these structures are almost completely lacking in the patients upon whom the electrical studies are made. Even where biopsies are taken, current histological technique has failed to reveal the condition of the structures

in whose function we are most interested. It was in order to remedy this defect that we have followed Coërs (1952, 1953*a*, and *b*) in applying to muscle biopsies the vital staining with methylene-blue, so successfully employed by Weddell and his collaborators (Weddell and Glees, 1941; Weddell and Zander, 1950), together with the acetylcholinesterase technique of Koelle and Friedenwald (1949) improved by Couteaux (1951).

The Normal Morphology of the Lower Motor Neurone

The main trunk of the motor nerve has an approximately fixed position in any given muscle and this position can be determined by faradic stimulation, the motor nerve apparently lying underneath the "motor point". This is of considerable importance in the siting of muscle biopsies, as the motor end-plates are not distributed haphazard through the muscle but, as Coërs (1953*c*) has shown, are distributed as a band along the line joining the geometrical centres of the muscle fibres. While the motor point does not, of course, overlie all the motor end-plates, it does seem always to overlie some of them and the chances of obtaining a portion of the neural apparatus in a biopsy are greatly enhanced by taking muscle from this situation. That part of the lower motor neurone which can be studied in muscle biopsies consists of the small, intramuscular, motor nerve fibres ending in the *sub-terminal* nerve fibres, the *terminal expansions* at the ends of these fibres and the *sub-neural, sub-sarcolemmal* apparatus, which surrounds these expansions and forms, together with them, the motor end-plate.

The sub-terminal nerve fibres can be stained by the Bielschowsky silver impregnation technique, when they appear as a relatively simple ramification, without any marked difference in form amongst the different members of the mammalia or in different muscles from the same species.

We have been unable to impregnate the terminal expansions with silver, but have been able to demonstrate them by *in vivo* staining with methylene blue. They vary considerably in form in any one muscle, but, in our experience, are sufficiently distinctive in many species to permit identification of the species from which the muscle was taken, provided that a sufficient number of end-plates are available for examination.

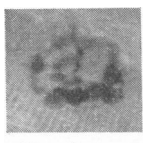

FIG. 1.

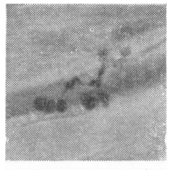

FIG. 2.

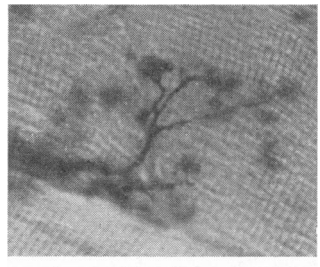

FIG. 3.

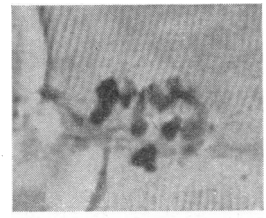

FIG. 4.

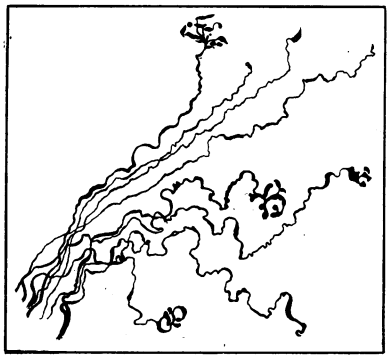

FIG. 5.

FIG. 1.—"Terminaison en plaque" from quadratus plantæ of cat, stained *in vivo* with methylene-blue. × 640.

FIG. 2.—"Terminaison en grappe" from same muscle of cat, stained *in vivo* with methylene-blue. × 640.

FIG. 3.—Terminal arborization from the interossei of the rabbit. Stained *in vivo* with methylene-blue. × 1,200.

FIG. 4.—"Terminaison en plaque" from brachioradialis of man. *In vivo* methylene-blue staining. × 800.

FIG. 5.—Drawing of sub-terminal fibres and "terminaisons en grappe" in man. *In vivo* staining with methylene-blue. × 163.

Thus, in the cat we observed a pale staining, "glomerular", "terminaison en plaque" (Fig. 1) and a darkly staining, cherry-like, "terminaison en grappe" (Fig. 2). In the rabbit, the terminal expansions more closely resembled a tree bearing fruit (Fig. 3). In the rat, Coërs (personal communication) has shown the expansions to have a plexiform arrange-

ment. None of these types of expansion could possibly be confused with those found in man which do, however, fall broadly into pale and darkly staining "terminaisons en plaque et en grappe" (Figs. 4 and 5).

The form of the terminal *neural* expansion finds expression in the structure of the *sub-neural, sub-sarcolemmal* apparatus. This apparatus is apparently composed of acetylcholinesterase and can easily be demonstrated by Koelle's histochemical technique or by vital staining with Janus green B. With either method their structure appears the same. It was studied by Couteaux in the mouse and by Coërs in man who have demonstrated the lamellated arrangement of the acetylcholinesterase and the synaptic groove into which the terminal expansion appears to fit (Figs. 6, 7). It is because of this arrangement that the form of the sub-neural apparatus is complementary to that of the terminal expansion. It seems probable that the form of the sub-neural apparatus varies, not only with the species, but also with different muscles in the same animal. The *species* differences are most marked between the rat or rabbit and man (Fig. 8), while the differences between the form of the sub-neural apparatus in *different muscles* are most marked in the small muscles of the paw and the large rectus abdominis muscle in the cat (Figs. 9 and 10). These differences may be related to the differences in the reaction of certain muscles to *d*-tubocurarine, recently demonstrated by Paton and Zaimis (1951).

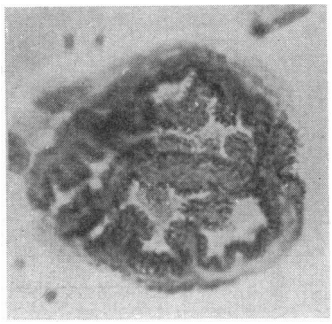

FIG. 6.—Sub-neural apparatus from a large muscle of the cat forelimb. Modified Koelle's method. × 1,200.

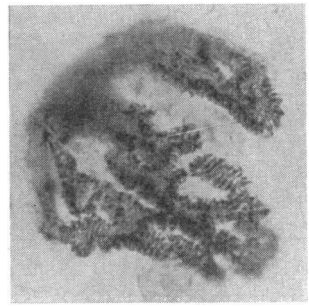

FIG. 7.—Sub-neural apparatus from the gastrocnemius of a rabbit. Modified Koelle's method. × 1,600.

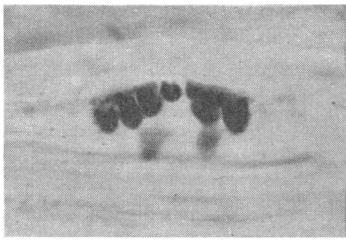

FIG. 8.—Sub-neural apparatus from a human case of "myasthenic myopathy". Modified Koelle's method. × 800.

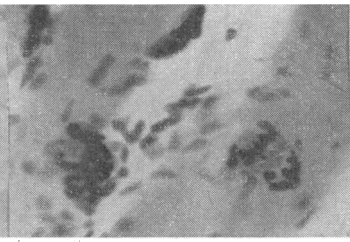

FIG. 9.—Sub-neural apparatus of the quadratus plantæ of the cat. Modified Koelle's method. × 330.

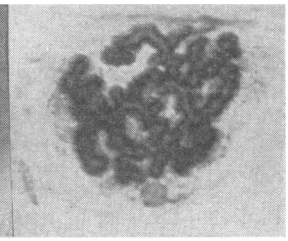

FIG. 10. — Sub-neural apparatus from the rectus abdominis of the cat. Modified Koelle's method. × 640.

TECHNIQUE OF MUSCLE BIOPSY

In order to obtain as much information as possible from a biopsy, we have used the following technique, which is largely based upon that used by Coërs. If local anæsthesia is used, adrenaline should not be added to the Novocain, as this may interfere with the staining with methylene blue. General anæsthesia is more convenient in children and the motor point may be marked on the skin and the strength-duration curve made at the same time. The incision is made in the line of the muscle fibres and with two-thirds of it below the motor point, as the motor point on the exposed muscle is always distal to that on the skin. The fascia of the muscle is opened and the motor point sought directly on the skin using the same apparatus as is used for the strength-duration curve, though the current required is, of course, very small. As stimulating electrode, a trimmed piece of cotton-wool held in a wired Spencer Wells forceps and moistened with physiological saline, is quite

satisfactory. A long strip of muscle, with the motor point at its centre, is removed and placed in 10% formol saline to be stained later by the Koelle acetylcholinesterase technique. Frozen sections from this specimen can be stained with Bielschowsky combined, if desired, with the Koelle technique, and the remaining tissue used for paraffin or celloidin embedding. The motor point of the remaining muscle is again determined and with this as centre, methylene blue is injected and a piece of injected muscle is removed and treated as described by Coërs (1952). Neither Coërs nor ourselves have seen any disability result from this excision of muscle at the motor point, the reason being that only the muscle lying superficially is excised, while the main branch or branches of the motor nerve lie deeply.

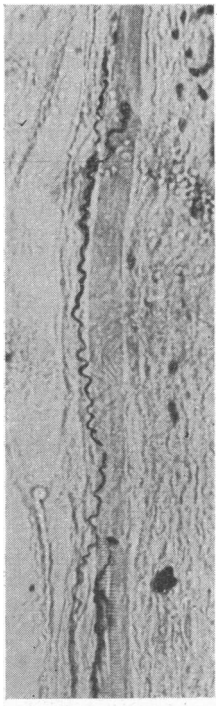

FIG. 11.

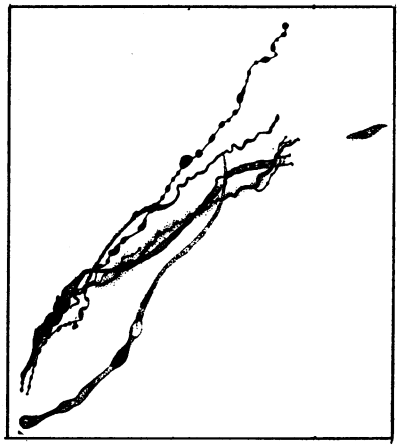

FIG. 12.

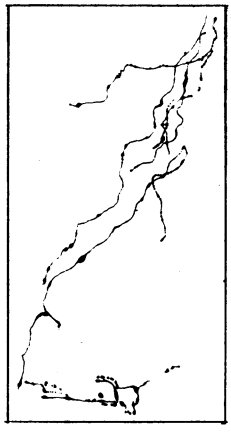

FIG. 13.

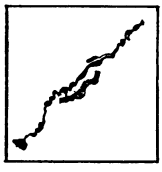

FIG. 14.

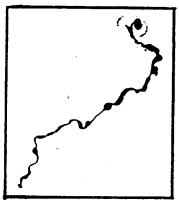

FIG. 15.

FIG. 11.—Wandering reinnervating nerve fibres from a case of Guillain-Barré syndrome. Note two poorly formed end-plates on one muscle fibre. *In vivo* methylene-blue staining. × 220.

FIG. 12.—Drawing of bundle of fibres from same case showing beading and branching reinnervating fibres. The broadest fibres are myelin sheaths. *In vivo* methylene-blue staining. × 163.

FIG. 13.—Drawing of terminal arborization from a case of Werdnig-Hoffmann's disease. *In vivo* methylene-blue staining. × 223.

FIGS. 14 and 15.—Drawings of poorly formed terminal expansions in a case of Guillain-Barré syndrome. *In vivo* methylene-blue staining. × 163.

CHRONIC TERMINAL MOTOR NEURONOPATHY (*see* TABLE I)

There is one finding which is of especial interest, and which does not seem to have been described before. We have seen it in conditions varying greatly in their natural history, though showing in common a flaccid paralysis (Table I). We have referred to the change as "chronic terminal motor neuronopathy" because there is evidence of a chronic disease of the terminal part of the motor neurone. It is, of course, true that in a muscle biopsy only the terminal part of the neurone can be visualized and that in our cases there may be changes, demonstrable at autopsy, in the proximal parts of the neurone. This was, indeed, true for Case I which showed loss of anterior horn cells (Mme. Bargeton). There is, however, some evidence that in Case VI (to be reported in detail elsewhere) the more proximal parts of even the *intramuscular* nerve fibres are largely free from any demonstrable structural abnormality.

It is not suggested that all the cases included in Table I were suffering from the same disease, but merely that all showed a similar change in the terminal part of the motor neurone. The changes are strikingly demonstrated by the *in vivo* methylene-blue technique,

but only crudely indicated with Bielschowsky silver impregnation and could, indeed, easily be missed with this method.

TABLE I.—CASES OF FLACCID PARALYSIS SHOWING CHRONIC TERMINAL MOTOR NEURONOPATHY

Case No.	Clinical diagnosis	Age at biopsy	Remarks
I	Oppenheim's disease	4 months	Progressive weakness of trunk and proximal limb muscles since birth
II	Amyoplasia congenita	4 months	Poor development of all muscles. Little movement of limbs. Deformed chest
III	Werdnig-Hoffmann's disease	1 year 7 months	Walked at 8 months then regression
IV	Amyotonia congenita	7 months	Floppy child. Kicks weakly. Arm held like Erb.
V	Guillain-Barré syndrome	13 years	Poor recovery from acute attack four years previously
VI	Myasthenia gravis	40 years	Good response to Prostigmin

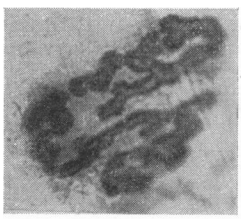

FIG. 16.

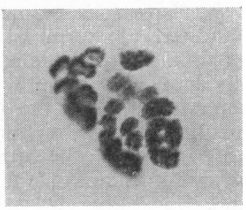

FIG. 17.

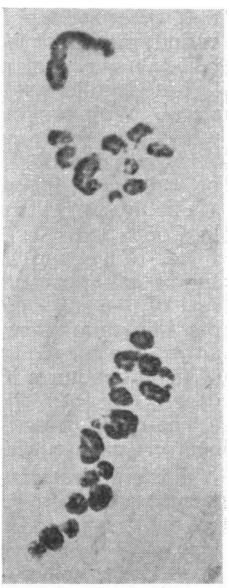

FIG. 20.

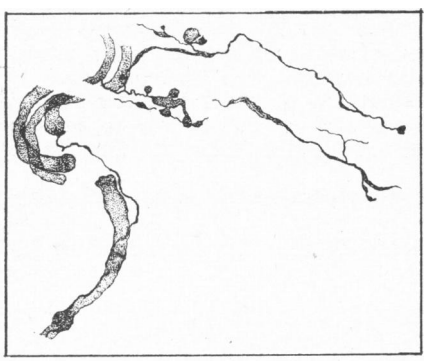

FIG. 18.

FIG. 19.

FIGS. 16 and 17.—Sub-neural apparatuses from unoperated and operated sides in a rabbit whose sciatic nerve had been clamped on one side two and a half months previously. Modified Koelle's method. × 640.

FIG. 18.—Drawing of the nerve fibres serving a muscle spindle from a case of Oppenheim's disease. *In vivo* staining with methylene blue. × 163.

FIG. 19.—Drawing of unusually simple, possibly unduly immature sub-neural apparatuses from a floppy child aged 2¾ years. Modified Koelle's method. × 245.

FIG. 20.—Unusually large sub-neural apparatus from a case of motor neurone disease with very marked fasciculation. Modified Koelle's method. × 800.

In cases showing "Chronic Terminal Motor Neuronopathy", the sub-terminal, neural fibres pursue a long, wandering course in isolation (Figs. 11 and 12), show beading of various degrees of coarseness, give rise to side-shoots, which are often of great delicacy and show irregular swellings, some of which appear to make contact with a muscle fibre as an inadequate end-plate (Figs. 13, 14 and 15). Many of the larger fibres terminate on the surface of muscle fibres in an unusually elaborate terminal arborization, ending in turn in bizarre, often diminutive terminal expansions. There may be as many as three end-plates on one muscle fibre, all the plates being in the same vicinity. The sub-neural apparatuses may show, in acetylcholinesterase preparations, an excessive number of very small, plate-staining units spread over a larger area than normal. To sum up, there is, in this condition, an extravagance instead of the normal economy of nervous tissue. These appearances recall Hoffman's (1953) hyperneurotized, reinnervated rat muscles, but in our cases the

attempts at reinnervation are abortive, as they are made by nerve fibres, themselves attacked by the blight, which has already destroyed the fibres they are about to replace.

What the appearances are in human muscles reinnervated by *healthy* nerve fibres, is still unknown, but in rabbit muscles, deprived of their nerve supply two to three months previously and showing evidence of reinnervation, we have observed subneural apparatuses composed of a group of small units rich in acetylcholinesterase (Fig. 16) bearing little resemblance to the plexiform apparatus seen in the corresponding muscle on the unoperated side (Fig. 17).

Returning to "chronic terminal motor neuronopathy", we have seen, in some cases, that some of the nerve fibres in the muscle spindles are irregularly swollen or beaded (Fig. 18), The spindles are not destroyed and indeed appear more numerous rather than less, this, of course, being due to the diminished bulk of the muscle. In the absence of clinical evidence of sensory loss, we have assumed that it is only the motor fibre to the spindle that is degenerate.

It is interesting to note that Case VI responded well to Prostigmin. This is not surprising in view of the tenuous connexion between many of the nerve fibres and the muscle fibre. Probably little acetylcholine can be liberated at such end-plates. It may well be that some cases of myasthenia are diseases, not of the myoneural junction, but of the sub-terminal neural arborization. We say "some" cases, because, in at least one case of myasthenia, that described by Dr. Sandifer, the terminal neural and sub-neural apparatuses were entirely normal. We have found in patients and rats, that administration of Prostigmin, before removal of a specimen of muscle, prevents the motor end-plates from appearing in acetylcholinesterase preparations, after the normal period of incubation with the substrate. With prolonged incubation, however, or, if the dose was not too great, prolonged washing in water prior to incubation, the apparatuses appeared with the normal form. Whether this would be the case in the muscle from a patient resistant to Prostigmin would be well worth investigating.

Finally, we will refer to two other interesting findings. The first of these is the report by Coërs and Pelc (1954) of immature end-plates in a floppy child that did not sit up until 1 year of age and was diagnosed as amyotonia congenita. We also have seen very simple sub-neural apparatuses (Fig. 19) in 2 similar cases, one of which had a sister with the same sort of illness. We consider, however, that it would be unwise to lay too much stress on the immature appearance of the terminal arborization and sub-neural apparatuses in amyotonia congenita, until more numerous observations have been made of the appearance of these structures in individuals of the very young age at which patients with this disease usually present.

The other observation is of very extensive sub-neural apparatuses in a case of motor neurone disease with unusually severe fasciculation (Fig. 20).

Acknowledgments.—Thanks are due to M. Poolvoerde for help with the animal experiments and to Mr. Peter Cull for the drawings.

REFERENCES

COËRS, C. (1952) *J. Neurol. Psychiat.*, **15**, 211.
—— (1953*a*) *Rev. belge Path.*, **22**, 306.
—— (1953*b*) *Arch. Biol., Paris*, **64**, 133.
—— (1953*c*) *Arch. Biol., Paris*, **64**, 495.
——, and PELC, S. (1954) *Acta neurol. belg.*, **2**, 166.
COUTEAUX, R. (1951) *Arch. intern. Physiol.*, **59**, 526.
ERB, W. H. (1890) Quoted by BRAMWELL, E. (1926) *Brain*, **49**, 146.
HOFFMAN, H. (1953) *J. comp. Neurol.*, **99**, 331.
KOELLE, G. B., and FRIEDENWALD, J. S. (1949) *Proc. Soc. exp. Biol., N.Y.*, **70**, 617.
PATON, W. D. M., and ZAIMIS, E. J. (1951) *J. Physiol.*, **112**, 311.
RUSSELL, D. S. (1953) *J. Path. Bact.*, **65**, 279.
WEDDELL, G., and GLEES, P. (1941) *J. Anat., Lond.*, **76**, 65.
——, and ZANDER, E. (1950) *J. Anat., Lond.*, **84**, 168.

Electrodiagnosis in Motor Unit Dysfunction

By P. BAUWENS, M.R.C.S., L.R.C.P.

ELECTRODIAGNOSIS in dysfunction of the motor unit can be likened to an attempt at tracing a fault in a telephone system by applying a series of tests at the subscriber's end. It is a restricted form of investigation but it offers the advantage of being conducted on undisturbed living structures of which the normal patterns of reaction and behaviour are moderately well established and understood. In common with other systems which transmit signals and manifest them at the receiving end, the motor unit is heir to a variety of faults which may arise from: (1) Inability to accept signals. (2) Failure to propagate them. (3) Failure to transmit them to the effector, and (4) Failure of the effector to respond to them.