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THE PHYSIOLOGY OF NEUROMUSCULAR TRANSMISSION AND NEUROMUSCULAR BLOCK*

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When we talk to-day about neuromuscular transmission we have an advantage over anyone who spoke about this subject ten or even five years ago. Even five years ago we should have had to spend a substantial part on the question, Is acetylcholine the chemical transmitter from motor-nerve endings to skeletal muscle? We could, and certainly would, have made out an extremely strong case for acetylcholine, but the difference between then and now is not how strong the case has become in the meantime, but that the case, as a matter of dispute, is settled and no longer exists. I think it is no exaggeration to say that to-day the acetylcholine theory of neuromuscular transmission has been universally adopted.

What, however, about the other two transmission processes that have so often been grouped together with neuromuscular transmission—the transmission in a sympathetic ganglion and across the central synapse? For the sympathetic ganglion the position can be stated as follows. No one denies that here, too, a strong case can be made out for acetylcholine; but some ask, Is acetylcholine the whole story, or may the electrical variation of the nerve impulse be involved in the transmission process as well? This is what Bronk (1939) has called the pluralistic view. Recently Lorente de Nó and Laporte (1950) have published a monograph of nearly 200 pages on synaptic transmission in a sympathetic ganglion. They report mainly on their own experiments, but in fifteen lines they deal with the nature of the synaptic transmitter, or rather with the controversy on the nature of the transmitter. In their view the chemical theory in its present form cannot account for all their electrophysiological findings in the ganglion, and they therefore ask: Must we discard the chemical theory or can it be elaborated so as to explain the new findings? They conclude, "It is undoubtedly best to be thoughtful." So, whatever my personal views on this matter, for some at least the case is still pending.

When we come to the central nervous system the opposition against the acetylcholine theory is even stronger. About two years ago I spoke about this problem.† The conclusion was reached that the evidence so far available did not allow us to assume that acetyl-

choline was the universal central synaptic transmitter, but that it suggested that acetylcholine exerted such a function at numerous central synapses. What I actually did was to put before you all the evidence I could find in favour of acetylcholine as a transmitter in the central nervous system. In other words, I stated the case for the defence of acetylcholine.

I think the general acceptance of the acetylcholine theory in neuromuscular transmission is based not only on the overwhelmingly positive evidence, such as, for instance, that acetylcholine is released on motor-nerve stimulation, that injections of acetylcholine can imitate all details of motor-nerve stimulation, and that there is at the neuromuscular junction a concentration of the enzymes which synthesize and destroy acetylcholine; but also on what I may call the negative evidence—that is, whenever the possibility existed to try out whether the nerve impulse could cause contraction without acetylcholine the answer was no, the nerve impulse was unable to do so.

1. Evidence that the Nerve Impulse without Acetylcholine is Apparently Unable to Cause Contraction

What is so interesting is the fact that what I termed "the negative evidence" had actually been provided long before the acetylcholine theory had been suggested. In 1904 Langley and Anderson united different kinds of nerves and studied the results of nerve regeneration. They connected, for instance, to the distal stump of the sciatic the central end of the cut sensory saphenous nerve, which, as we now know, is a non-cholinergic nerve. Thirteen months later they stimulated electrically the distal end of the sciatic stump and observed a reflex response in the animal but no contractions of the muscles supplied by the sciatic. The reflex response proves that the sensory-nerve fibres must have grown into the sciatic, but the absence of contractions shows that they had been unable to form functional connexions with the muscle. At that time the various results obtained by Langley and Anderson must have been rather bewildering. For instance, why, if a sensory fibre on regeneration into a motor stump was unable to achieve functional innervation with a muscle, could a preganglionic sympathetic fibre do so; and why, on the other hand, could this same fibre not make functional connexion with the post-ganglionic sympathetic fibre?

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†The contents of the lecture have been published elsewhere (Feldberg, 1950).

It was left to Dale (1935) to show what these results meant. Langley and Anderson had found that nerve fibres grow into any nerve stump, but can make functional connexions only if they are of the same nature—that is, only cholinergic fibres can make functional connexion with cholinergic fibres, sensory only with sensory, adrenergic only with adrenergic, not sensory with cholinergic, nor cholinergic with adrenergic, and so on.

It may be asked how we know that in Langley and Anderson's experiments the sensory fibres had actually reached the muscle fibres, so that we are justified in concluding that the nerve impulse had got to the muscle fibre but could not make it contract. Such histological evidence was given by Weiss (1935) and by Weiss and Edds (1945).

Weiss, working with toads, implanted the cut ends of sensory roots direct into denervated muscles. Histological examination showed that the sensory fibres had spread throughout the muscle fibres. Nevertheless, in most of their experiments electrical stimulation of the sensory nerve did not cause contraction of the muscle. When it did, the intrusion into the wound of stray motor fibres of uncontrollable origin could be made responsible for it. This complication was later avoided in the experiment of Weiss and Edds. They worked with rats and used a special method of uniting sensory and motor nerves. Union was effected without suture by inserting the opposing nerve ends into fitting sleeves made from an artery of another, freshly killed, young rat. They established, without doubt, that sensory-motor union does not enable the sensory regenerating nerve fibres to make the muscle contract, although histological examination proves that they have grown in normal fashion through the motor stump and have pervaded the muscle with extensive arborizations which would provide ample opportunity for the establishment of myoneural junction if, as we may say, these fibres would only liberate acetylcholine.

I am aware that one can argue against this conclusion, and that it can be said that the cause for the failure of transmission is not lack of acetylcholine but an inadequacy of structural connexions between the sensory endings and muscle. I wonder, however, if this objection has any real meaning. The sensory fibres have reached the muscle and we must realize that there is no difference in electrical excitability between different parts of a muscle fibre, not even between motor end-plate and muscle fibre, as was shown by Kuffler (1945). If lack of acetylcholine is the determining factor, any structural connexion without acetylcholine can be called inadequate.

Another instance which illustrates that a nerve impulse may reach a muscle without exciting it is found in crustacea. In these animals some limb muscles have a double innervation, motor and inhibitor; both nerves run closely together, and their common course can be followed down to fine intramuscular branches. The motor response is abolished or prevented by stimulation of the inhibitory axon. We do not know what the transmitter of even the motor axon is. In fact, the evidence so far available speaks against acetylcholine (see Katz and Kuffler, 1946; Welsh and Schallek, 1946; Bacq, 1947; Katz, 1936, 1949; Hunt and Kuffler, 1950b). The fact of interest for our argument is that when the inhibitory axon is stimulated to a resting muscle no muscular contraction occurs. The only visible response is a small intramuscular nerve spike, looking exactly like a motor-nerve spike, but this intra-

muscular nerve spike does not excite the muscle, or, expressed in more electrophysiological terms, the current spread of the nerve impulse is ineffective on the muscle (Katz and Kuffler, 1946).

One more example, to show that current spread from the nerve endings is apparently unable to produce the change at the motor end-plate which leads to excitation of the muscle fibres. This change is a depolarization. There is a definite but extremely small time-lag between the arrival of the nerve impulse and the depolarization of the end-plate—the so-called neuromuscular or end-plate delay. In the past this delay played a prominent part as an argument against the acetylcholine theory, the argument being that this time was far too short to account for the release of acetylcholine and its subsequent action on the end-plate. But now the opposite view is taken. Kuffler (1948) measured this delay in frog muscle. He stimulated, not the nerve trunk at some distance from the muscle, but the terminal nerve region only about 0.5 mm. away from the end-plate. If spread of current were the transmitter of the nerve impulse, depolarization of the end-plate should have been effected within a fifth of a millisecond. But it took about the whole of a millisecond. Four-fifths of a millisecond remained unaccounted for, and these four-fifths of a millisecond are now presented as evidence against the electric current from the nerve terminals being the transmitter agent.

I shall once more return briefly to this problem, and give another instance of indirect evidence that a brief electrical shock is apparently an ineffective mechanism for depolarization of the end-plate.

2. Action of Acetylcholine at the Motor End-plate

As acetylcholine is the transmitter of motor-nerve impulses, any condition which affects neuromuscular transmission must be interpretable in terms of acetylcholine. Obviously, a clear understanding of its action is necessary.

Acetylcholine has three actions on skeletal muscle. First, it may cause contraction; secondly, it may produce depression—that is, neuromuscular block; and, thirdly, it may cause a contracture. And all three actions are apparently the result of one effect of acetylcholine—that of depolarizing the motor end-plate. Acetylcholine has no action on the muscle fibre itself, or, I should be more cautious and say, no action which concerns neuromuscular transmission, because effects of acetylcholine on muscle metabolism have been observed (Gollwitzer-Meyer, 1950).

The Acetylcholine Contraction

For describing the action of acetylcholine on the motor end-plate a simple diagram like that shown in Fig. 1 is helpful. The acetylcholine released from the nerve endings depolarizes the membrane of the motor end-plate as indicated in the diagram. This depolariza-

FIG. 1.—Diagram of muscle fibre with end-plate. Nerve fibre ends on end-plate, which becomes depolarized (indicated by dots) by the released acetylcholine.

tion is the appropriate stimulus to excite the muscle fibre, so that an excitation wave travels along the fibre and elicits the contraction. The acetylcholine is quickly hydrolysed, and therefore the motor end-plate is no longer depolarized when the excited muscle fibre has come out of its refractory state, so a single nerve impulse elicits no second wave of excitation. That, however, occurs after eserine, or with an injection of a small dose of acetylcholine into the muscle artery. In other words, the response of the eserinated muscle to a single nerve impulse is no longer a twitch, but a summated contraction, a short tetanus; and the contraction due to an arterial injection of acetylcholine is also a short tetanus, but this time an asynchronous one. The tetanus in both cases results from persistence of acetylcholine at the one

FIG. 2.—Different stages in the development of the plates of an electric organ. (a) The early stage: muscle fibres with faint cross-striation are still predominant. Five muscle fibres with end-plate regions on the upper end of the four fibres. (b) Later stage: a single fibre. End-plate region has increased in size. (c) Fully developed single plate. Rudiment of muscle fibre still discernible: in many fully developed plates it is completely absent. From the arrangement of the muscle fibres and plates at (a) the final arrangement of the plates in columns built up of superimposed plates becomes evident. (After Ewart.)

end-plate region long enough to keep it depolarized for re-excitation of the muscle fibre after it has come out of its refractory state.

The depolarization of the end-plate is not a theory, but it can be demonstrated; it is termed the motor end-plate potential. End-plate potentials can be recorded from the end-plate region of a muscle, or we may use a preparation which consists of end-plates only. Such a preparation is the electric organ of the torpedo fish.

The Depolarizing Action of Acetylcholine on the Electric Organ of the Torpedo

The organ is an accumulation of modified hypertrophied end-plates derived from skeletal muscle but

FIG. 3.—Diagram of an electric organ in the torpedo with its large extrinsic nerves.

lacking the contractile element. Before the organ is fully developed it consists of striated muscle fibres which have their end-plate not in the middle of the fibre, as usual, but at one end. This end grows until a fully developed plate is obtained, whereas the small muscle fibre remains either unchanged, diminishes, or disappears. In Fig. 2 are shown the different stages in the development of a plate, whereas Fig. 3 gives a schematic illustration of the arrangement of the whole electric organ. It is formed by a great number of columns arranged side by side, each column being built up of several hundred superimposed plates. Large efferent nerves enter the plates from the ventral sides, covering them with a terminal nerve net. Impulses are sent down from the central nervous system along the nerves, causing a brief discharge of the organ. A photograph of a torpedo fish on which an experiment was done is given in Fig. 4; the left organ has been removed and is shown at *b* with its large nerves. The blood vessels run with the nerves and the organ can be perfused from its cannulated artery.

Marnay (1937) and later Nachmansohn and Lederer (1939) had found that the electric organ is extremely

FIG. 4.—(a) Torpedo fish with left electric organ removed. (b) Removed electric organ with large extrinsic nerves.

FIG. 5.—Depolarizing effect of 10, 5, and 2.5 μ g. of acetylcholine (at I, II, and IV) injected arterially into the perfused electric organ of the torpedo. At III mechanical effect of a saline injection. (After Feldberg and Fessard, 1942.)

rich in cholinesterase, and Feldberg and Fessard (1942) could show that it is also extremely rich in acetylcholine, and that acetylcholine is released by stimulating the nerve to the perfused organ. Fessard and I then proceeded to find out if acetylcholine has an electrogenic effect, and for that purpose we injected acetylcholine arterially into the perfused organ. The result is seen in Fig. 5, which shows the strong depolarizing or electrogenic effect of small doses of acetylcholine. The small deviation downwards, when saline alone is injected (at III), is due to the mechanical disturbance of the injection. The potentials due to the depolarizing action of the acetylcholine occur after a short latency.

I can now give the additional evidence I mentioned before—why, in my opinion, a short electrical shock is an ineffective stimulus for depolarization of the end-plates.

FIG. 6.—Photomicrograph of a living single muscle fibre with its nerve supply. A, Low magnification. B, Higher magnification. Dark area to the right of the nerve-entrance is a small air bubble. (After Kuffler, 1942.)

The isolated electric organ of the torpedo can be stimulated artificially, for instance, by pressing on it with an ivory rod, or by an electric current. The response is a discharge which, on electrical stimulation, is clearly distinguishable by a short latency from the artifact of stimulation. But the position is different when the nerves to the organ have been severed and have degenerated. Then a discharge is no longer obtainable whatever stimulus we apply. Even an electric shock gives only the artifact of the stimulus without subsequent discharge. This was known to Garten (1910), and has been confirmed more recently by Auger and Fessard (1938).

The following explanation is suggested. In normally innervated organs any stimulus, mechanical or electrical, will excite the intraplatal nerve fibres; this leads to release of acetylcholine, which, in its turn, depolarizes the ventral surfaces of the plates. In the chronically denervated organ the nerve fibres have degenerated, no acetylcholine is present, therefore no depolarizing substance is released, and the electric shock itself is ineffective in depolarizing the plates. To prove this theory Fessard and I made arrangements to inject acetylcholine arterially into the chronically denervated perfused organ to see if acetylcholine, the appropriate physiological stimulus, would produce its electrogenic effect. The outbreak of the war prevented us from carrying out the experiment, about the outcome of which, however, I have little doubt.

End-plate Potentials

The most elegant demonstration of end-plate potentials in muscle, which are essentially the same potentials as those in the electric organ, is that of Kuffler (1942, 1943). He worked on isolated nerve muscle fibres of the frog, and used the following ingenious method. A single nerve-muscle fibre preparation, like that seen in Fig. 6, is brought into a trough containing a saline solution, on top of which paraffin is floating. The preparation is lifted into the paraffin. One electrode makes contact with the end of the muscle fibre, whereas the other can be moved with the help of a microadjustment to different positions along the fibre. Kuffler used a little glass rod terminating in a hook, in the loop of which this second electrode is fixed. The loop also holds a fine droplet of diluted solution of acetylcholine (Fig. 7.) Under direct microscopical observation, the hook is lowered to the end-plate region. The result is a local depolarization of this region which, if strong enough, leads to propagated

FIG. 7.—Diagram illustrating local application of acetylcholine to an isolated muscle fibre. M (transverse section). G, Glass rod terminating in a fine hook. Shaded area shows droplet of acetylcholine which is brought into contact with the muscle fibre by lowering the glass rod (arrow). (After Kuffler, 1942.)

impulses along the muscle fibre. Fig. 8 is taken from the paper by Kuffler and shows the effects of acetylcholine applied in this way to the end-plate region. At *a* the depolarization by acetylcholine sets up four propagated impulses, but at *b* depolarization is too weak to excite the fibre; only the slow motor end-plate potential

FIG. 8.—Local application of acetylcholine to the end-plate region of a single nerve-muscle fibre preparation. Recording is done from the point of application. *a*, 10^{-6} Acetylcholine sets up four propagating muscle impulses. *b*, A weaker concentration of acetylcholine produces a local negative potential only. Time scale: 1 div. = 10 msec. (After Kuffler, 1942.)

is recorded. Such a potential can be obtained only when led off directly from the end-plate region; when the leading-off is a few millimetres away we get only propagated impulses as illustrated in Fig. 9. Further, acetylcholine produces no effect when applied to the muscle fibre away from the end-plate region.

To record end-plate potentials in mammalian muscle either by acetylcholine given artificially or by the nerve

FIG. 9.—Potentials recorded from single nerve-muscle-fibre preparation. *c*, Acetylcholine applied to end-plate, while the recording is done from point of application (as Fig. 8, but faster sweep). *a*, Impulses initiated at end-plate are recorded 5–6 mm. away from it. Note the absence of a negative potential preceding or following the spikes. *b*, Base line. (Redrawn from Kuffler, 1942.)

impulse has become simple by the use of curare preparations for this purpose, introduced by Eccles and his co-workers (Eccles and O'Connor, 1938, 1939; Eccles, Katz, and Kuffler, 1941). D-Tubocurarine reduces the acetylcholine effects on the end-plates so that depolarization is insufficient to excite the fibres but still sufficient to be recorded. Fig. 10 illustrates end-plate potentials produced by a single nerve volley in curarized cat's muscle. Again, in order to obtain the potentials it is necessary to lead off directly from the end-plate region, because this depolarization is not propagated, or, as it is

termed, it spreads decrementally for a few millimetres only on either side of the end-plate.

The events which occur on the arrival of the motor-nerve impulse at its terminals thus are: nerve impulse—release of acetylcholine—depolarization of the end-plate—excitation of the muscle fibre. The excitation is

FIG. 10.—Action potentials to a single nerve impulse from end-plate region of cat's soleus muscle. (1) Full curarization: shows the slow end-plate potential alone. (2) Less curarization: at *a* the spike response at the end-plates; at *b* no potential when led off 2 cm. away from the end-plates. No contraction. (After Eccles and O'Connor, 1938.)

propagated and leads to contraction of the contractile elements. When we discuss contraction of muscle fibres we have to distinguish, as emphasized by Katz and Kuffler, between the two processes—excitation of the muscle membrane, the action potential, and activation by it of the contractile substance, the contraction.

Release of Acetylcholine from the Resting Nerve

There is this problem: is acetylcholine released only by the arrival of the nerve impulse, and is there a completely resting condition between nerve impulses? I do not think so.

We may compare the release of acetylcholine with what happens at a sluice or lock, where there is always some leakage even when the lock is closed. The amounts of acetylcholine released at rest, however, are far too small to produce effective transmission so long as the mechanism for the destruction of acetylcholine is intact. This idea is based on the effect of eserine, which causes fascicular twitching and fibrillation even after cutting the nerve. The fascicular twitchings may be accounted for by the release of acetylcholine by random impulses passing down the cut nerve. The fibrillations cannot be explained in this way. They might be due to continuous release and resynthesis of minute amounts of acetylcholine from the endings of the resting nerve, so slight as to have no effect in the absence of eserine (Feldberg, 1945). There is a fact which could be strikingly explained on this assumption: eserine loses its ability to produce these motor effects one to two days after cutting the nerve, although the motor end-plate remains sensitive to acetylcholine and the nerve still conducts. The only change which has occurred at this time is an impairment of the nerve fibre to synthesize acetylcholine. The acetylcholine content of the nerve has dropped and the tissue synthesizes acetylcholine in small amounts only. Therefore even after eserine no effect is to be expected.

The analogy of the sluice has recently found a more scientific and exact explanation. Fatt and Katz (1950) inserted a micro-electrode immediately under the motor-nerve ending of a frog muscle fibre and observed, without stimulating the nerve, small action potentials which followed one another at random intervals and were of the size of 1% of the normal end-plate potential. These were increased by neostigmine and abolished by D-tubocurarine; they were termed "miniature end-plate potentials." Fatt and Katz gave the following explanation of their origin. The fine nerve terminals are excited by spontaneous fluctuations of their membrane potential; the nerve terminals are so thin that this local excitation will not lead to a propagated impulse along the whole nerve, the area affected being too small; but it will lead to the release of a minute amount of acetylcholine, which then produces the miniature end-plate potential which, again, is subthreshold to excite the muscle fibre. So the idea of a resting state at the nerve endings has to be relinquished.

Depression by Acetylcholine

The depression obtained with acetylcholine occurs either when large doses of acetylcholine are injected into a muscle or when the nerve to an eserinizated muscle is frequently stimulated. Fig. 11 illustrates a tension

FIG. 11.—Cat under chloralose anaesthesia. Contractions of tibialis anterior in response to maximal shocks to nerve at frequency of either 6 or 180 a minute. At arrow, intravenous eserine 0.5 mg. per kg. body weight. The dark patches are caused by overlapping of the individual contractions during high-frequency stimulation. (After Bacq and Brown, 1937.)

record of a cat's muscle stimulated from its motor nerve either with single impulses placed at 10-second intervals or with frequent stimulation. At the arrow, eserine is given. The response to the single impulses is augmented and converted into a short tetanus, as mentioned before, but tetanic stimulation is reduced. Fig. 12 illustrates the reduction in the response of single nerve impulses

FIG. 12.—Spinal cat. Contractions of gastrocnemius muscle in response to single maximal shocks to nerve at 10-second intervals, after arterial injection of 0.1 mg. of the anticholinesterase miotine. A, Arterial injection of 10 µg. of acetylcholine. B, Short tetanic stimulation of nerve. (After Bacq and Brown, 1937.)

when an arterial injection of acetylcholine or a short tetanus is interpolated. The depression in both experiments is the result of too much acetylcholine, and has in the past often been called the "curare-like action of acetylcholine"—a classification which, as we shall see, is misleading.

Originally, I had the idea that this effect, which occurs when the muscle is, so to speak,

flooded with acetylcholine, was an action of acetylcholine on the muscle fibre (Feldberg, 1950). This idea may be wrong. Acetylcholine apparently acts only on the end-plate, as recently shown by Burns and Paton (1950). But when the acetylcholine persists here long enough, then not the acetylcholine but the depolarization current spreads to the adjacent parts of the muscle fibre, which also become depolarized, and when part of a muscle fibre becomes depolarized, not for a brief moment but for some time, this part becomes electrically inexcitable—that is, an excitation wave cannot pass this region, therefore a nerve can no longer excite the muscle fibre—neuromuscular block has been established.

Burns and Paton have measured the depolarization by acetylcholine in the cat's gracilis muscle, which is a simple muscle with localized end-plate regions and nerve-free distal parts. They used the following technique. The electrode sweeps to and fro over the muscle. Normally there is no change in the state of depolarization during such a sweep, but when acetylcholine has previously been injected into the muscle artery—or, better, another substance of which we shall speak more later on, decamethonium—then each time the electrode approaches an end-plate region a depolarization current is recorded. When the sweep is repeated a few minutes later it is found that the depolarized region has become larger: the depolarization apparently has spread along the muscle fibre on either side of the end-plate; thus the region depolarized is no longer confined to the end-plate. This is the most likely explanation of the depressant action of acetylcholine. But we cannot exclude the possibility that persistent depolarization of the end-plate region itself produces a condition of depression which, for instance, occurs even in the electric organ of the torpedo after eserine, although this organ lacks the muscle fibres.

The Acetylcholine Contracture

In mammalian muscle, depolarization of the muscle fibre on either side of the end-plate leads only to depression. In the frog such depolarization is associated with another phenomenon—contracture. From the point of excitation, contractures—that is, non-propagated contractions—and depression are the same. In both conditions the muscle is electrically inexcitable at the depolarized region. This, in mammalian muscle, is the sole effect, and the cause of neuromuscular block. In frog muscle it produces a slow shortening of the muscle, the contracture. But essentially there is no difference between the normal mammalian muscle and the frog muscle. After motor denervation of a mammalian muscle, depolarization of the muscle membrane by acetylcholine produces strong contractures in this muscle also. It is possible that even in the normal muscle persistence of acetylcholine produces a very slight local contracture of the muscle fibre directly near the motor end-plate region, but too small to be recorded with our normal tension records; it will, however, naturally produce block. In fact Brown, Burns, and myself (1948) observed that an arterial injection of acetylcholine after diisopropylfluorophosphate produced, on a number of occasions, not only the quick twitch-like contraction but in addition a secondary, slow development of tension similar to but much smaller than the contracture occurring in denervated muscle. The difference between the normal mammalian muscle and the denervated or the frog muscle is therefore probably quantitative only.

There is one important difference: whereas depression in mammalian muscle by too much acetylcholine is not a physiological phenomenon, contractures in frogs are physiological events which may take place instead of propagated contractions, as is shown below.

There is a problem on which opinions differ. Can the same muscle fibre show the phenomenon of contracture and of propagated contraction? Hunt and Kuffler believe that in frog muscle these two abilities are properties of separate fibres, which are distributed differently in different muscles. For instance, the sartorius contains only muscle fibres which give contraction, the rectus only muscle fibres which give contracture. On the other hand, Brecht and Feneis (1950), in Germany, have examined the effect of acetylcholine on single muscle fibres of different muscles in the frog and obtained apparently both effects in the same muscle fibre (see Figs. 13 and 14). The position may vary in different muscles, but the experiments of Brecht and Feneis seem to show that both effects can be obtained from the same muscle fibre. How does acetylcholine produce the double effect? Does it first depolarize the end-plate region which excites the muscle fibre, and

then, when the depolarization spreads to the adjacent part of the fibre, contracture sets in? The record of Fig. 13 could well be explained in this way, particularly because, when we again reduce the action of acetylcholine by D-tubocurarine, the slow contraction disappears first (Fig. 13). D-Tubocurarine, as we shall see, counteracts the depolarizing effect of acetylcholine. On the other hand, in the record of Fig. 14 quick contractions occur during the development of the slow shortening—that is, at a time when we should expect the muscle fibre on either side of the end-plate to be inexcitable. How, then, can the fibre be excited in this condition from a depolarization of the end-plate? There might be a simple explanation. Katz and Kuffler (1941) have shown that a single muscle fibre may have more than one motor end-plate. We can thus imagine that depolarization of the one causes the local contracture, depolarization of the other producing propagated contraction. If this muscle fibre should contain only one end-plate, we may assume that the spreading depolarization at first excites the adjacent part of the fibre before it goes itself into the state of local contracture and electrical inexcitability. At present we must wait for further experiments before a definite answer can be given on this problem.

Summary of the Action of Acetylcholine

Summarizing, we may say: depolarization of the end-plate by acetylcholine leads to propagated excitation, followed by contraction. If the depolarization of the end-plate, however, spreads to the muscle fibre, two other phenomena may be observed—depression, or contracture; whereas contracture is a physiological event, depression is not. The depression causes neuromuscular block. Thus a substance with acetylcholine-like action can, particularly if it is not quickly destroyed, be an effective neuromuscular blocking agent, and decamethonium—first synthesized by Barlow and Ing (1948) and later by Zaimis (1950b), and introduced as a new curare substitute by Paton and Zaimis (1949)—acts in this way.

Before we discuss neuromuscular transmission, another point is worth mentioning. How are we protected against the action of too much acetylcholine? The distribution of the true cholinesterase, which destroys the released acetylcholine, may give an answer to this question. In a smooth muscle innervated by a parasympathetic nerve, too much release of acetylcholine would have no other action but that of increasing the effect of nerve stimulation. In the skeletal muscle just the opposite effect might be produced, and I think it is for this reason that we find a fundamental difference in the distribution of the true cholinesterase in skeletal muscle and in parasympathetic innervated organs. Here the true cholinesterase is present in the nerve endings, and when the nerve degenerates the true cholinesterase decreases. It is not so with skeletal muscle; here the cholinesterase is also concentrated at the end-plate region. Therefore, no decrease occurs in the true cholinesterase content of muscle after motor-nerve degeneration (Marnay and Nachmansohn, 1937; Couteaux and Nachmansohn, 1938; Feng and Ting, 1938).

3. Neuromuscular Block

In terms of acetylcholine there are three possible ways by which neuromuscular block may be effected: the end-plate becomes less sensitive to the depolarizing action of acetylcholine, or too much acetylcholine is

FIG. 13.—Tension record from a single 52- μ -thick muscle fibre of frog's anconeus muscle (a) bathed in acetylcholine 1×10^{-5} ; (b) effect of acetylcholine 1×10^{-5} for 20 minutes in curare 1×10^{-5} . Ordinates = Tension in mg. Abscissae = Time in seconds. (From Brecht and Feneis, 1950.)

FIG. 14.—Tension record from a single 30-40- μ -thick muscle fibre of frog's anconeus muscle bathed in acetylcholine 1×10^{-5} . At the arrow, changing bath fluid to Ringer solution. Ordinates = Tension in mg. Abscissae = Time in seconds. (From Brecht and Feneis, 1950.)

released so that depolarization spreads beyond the end-plate region, or insufficient amounts of acetylcholine are released by the nerve impulse. All three forms of neuromuscular block have been observed.

D-Tubocurarine

D-Tubocurarine acts by reducing the depolarizing action of acetylcholine, so that the amounts usually released are insufficient to produce this required change at the end-plate. That explains why the anticholinesterases are such good antidotes to curare paralysis.

Decamethonium

In the action of decamethonium we have neuromuscular block produced by too much depolarization, and therefore anticholinesterases would intensify the block. In fact, although it sounds paradoxical, this block could be reduced by D-tubocurarine if given in the appropriate concentration. Recently Burns, Paton, and Dias (1949) have demonstrated the difference of the mechanism in producing neuromuscular block by these two substances on the cat's gracilis muscle. As D-tubocurarine affects only the end-plate, excitation of one nerve-free muscle-end will excite the whole length of the muscle fibre; the excitation will pass beyond the end-plate region, and can be led off from the other end of the muscle. On the other hand, during block produced by decamethonium, the muscle fibre can naturally be excited at the nerve-free ending and the excitation wave will travel along the muscle, but only to the end-plate region; it cannot pass the blocked area—block produced by too much depolarization and no excitation wave will appear on the other side of the motor end-plate.

In the laboratory there is a quick way, without the use of complicated apparatus, to find out if a new neuromuscular blocking agent acts more like D-tubocurarine or more like decamethonium: to test its effect on the frog muscle or on the denervated mammalian muscle. If it acts like decamethonium it will readily cause contracture, but not if it acts like D-tubocurarine, although under certain conditions D-tubocurarine may produce contracture preceding its actual paralyzing effect (McIntyre, King, and Dunn, 1945). Decamethonium contractures have been described by Paton and Zaimis (1949) and by Zaimis (1950a).

Botulinum Toxin

The third mechanism—neuromuscular block produced by the inability of the nerve endings to release acetylcholine—is shown in the condition of paralysis produced by botulinum toxin. Guyton and MacDonald (1947) showed that the toxin did not interfere with nerve conduction and that it left the muscle excitable to direct stimulation. Later, Burgen, Dickens, and Zatman (1949) analysed on the rat diaphragm the irreversible neuromuscular block produced by botulinum toxin. The muscle remains excitable to direct stimulation. The nerve still transmits the impulse to the endings. The motor end-plate shows its normal sensitivity to acetylcholine. This was shown by a rather ingenious method of a reversed arterial injection. It is not possible to inject the acetylcholine in such a preparation through the artery, but practically the whole diaphragm drains into a large vein, which empties into the inferior vena cava. They injected the acetylcholine, retrograde, through the vein, and the paralysed diaphragm responded like the normal diaphragm. But

when they studied the release of acetylcholine during stimulation they found a greatly reduced output of acetylcholine from the paralysed preparation. The neuromuscular block by botulinum toxin must be explained by an action of the toxin on the myelin-free nerve terminals which prevents release of acetylcholine.

It will be necessary in future to be more cautious in the use of the term curare-like action. This term should be reserved only for neuromuscular block produced by substances which, like D-tubocurarine, have themselves no depolarizing action on the motor end-plate but antagonize those of acetylcholine. If block is not produced in this way, it should be called neuromuscular block, and the mechanism by which it is effected—too much depolarization or too little acetylcholine—should be stated.

4. The Small-motor-nerve-fibre System to Skeletal Muscle

Recently a new problem in neuromuscular physiology has come into the foreground. It concerns the function of the small nerve fibres in motor roots. It has long been known that ventral roots contain, in addition to the well-known large-diameter fibres, a distinct group of small-diameter fibres (Eccles and Sherrington, 1930; O'Leary, Heinbecker, and Bishop, 1934). These have been described in amphibians and mammals, but their function was for a long time unknown. This is no longer so. We may say this fibre system is concerned with the maintenance of long-lasting, sustained contractions, but the mechanism by which these fibres achieve their effect is different in frogs and mammals. We must realize that the same applies to the phenomenon of muscle tone or sustained contractions, which can be brought about by different mechanisms in frogs and in mammals.

The skeletal muscle of amphibia can exhibit slow sustained contractions by its ability to respond to acetylcholine with contracture instead of with propagated summated contractions. The small-motor-nerve-fibre system in frogs is concerned with this form of localized contraction. On the other hand, the normal mammalian muscle, with a few exceptions, does not exhibit this kind of motor response. In mammals "muscle tone" is, as we know since Sherrington (1915) and Liddell and Sherrington (1924, 1925), entirely "reflex tone"; the myotatic reflex is the fundamental basis of postural tone. Myotatic reflexes, however, are practically absent in limb muscles of frogs. The small-motor-nerve discharge in mammals appears to be concerned with regulation of postural reflexes—that is, it acts, as termed by Hunt and Kuffler (1950a), as a muscle proprioceptor regulator. In detail the position in frogs and mammals is as follows.

Experiments on Frogs

The work on the frog is connected with the names of Tasaki and Mizutani (1944), Kuffler and Gerard (1947), Kuffler, Laporte, and Ransmeier (1947). Stimulation of the small motor fibres in frogs sets up local potential changes at the neuromuscular junction which resemble the motor end-plate potential and are called the small-fibre junctional potentials. These do not give rise, like the motor end-plate potentials, to propagated contractions but to localized contractures. But, in order to develop appreciable shortening by this mechanism, the small nerve fibres must be excited repetitively and at a sufficient frequency. Then the small-fibre-junction

potential builds up and a larger and larger part of the muscle fibre becomes involved in the localized contracture.

The small-motor-nerve-fibre system varies in its distribution in different muscles. There are none to the sartorius and adductor longus, but they supply most limb muscles, like gastrocnemius and pectoralis, and some, like the semitendinosus, especially richly. In some muscles the muscle fibres supplied by the small motor fibres form a distinct part—for instance in the ileo-fibularis, where Sommerkamp (1928) spoke of this part as "Tonusbündel." When we assay acetylcholine on the frog rectus abdominis muscle, the assay is made on small-nerve-innervated muscle fibres.

Kuffler, Laporte, and Ransmeier (1947) have shown that there exists in the spinal cord a distinct reflex mechanism, by which the small-motor-nerve-fibre system may be activated exclusively, and that reflexes through this system are widespread in upper and lower extremities. The small nerve fibres can maintain reflexly an appreciable tension, while the twitch system is not, or only slightly, active. The maintenance of long-lasting relatively slow muscle contractions by this mechanism compensates for the absence of myotatic reflexes in the limbs of this species.

Originally Kuffler and Gerard seemed to think that the same muscle fibre might have a double innervation, but recently Hunt and Kuffler (1950b) have expressed the view that the small motor nerve fibres of frog innervate separate muscle fibres from those supplied by the larger nerve fibre group. The observation of Brecht and Feneis that a single muscle fibre shares both responses to acetylcholine makes it necessary to reopen this question. If Hunt and Kuffler are right—and they know more than anyone else about this system—then there is a difference between the frog and a phylogenetically much lower order, Crustacea.

Experiments on Crustacea

In crustacea the same muscle fibre has the stability to exhibit "fast" and "slow" contractions—that is, propagated contractions and localized contractures. In most limb muscles both responses are set up in the same muscle fibre by one and the same nerve fibre, the determining factor being the number and frequency of stimulation (Blaschko, Cattell and Kahn, 1931). But in some muscles—for instance the closer of the claw—the two responses are integrated in a double nerve supply, as in the frog, but here to the same muscle fibre. One motor axon gives rise to large spikes and powerful twitches; the other to smaller end-plate potentials, which on repeated stimulation grow in size and are accompanied by a slow, well-maintained contracture (Pantin, 1936; Wiersma, 1941; Katz and Kuffler, 1946; Kuffler and Katz, 1946).

Experiments on Mammals

In mammals there are also numerous small motor fibres; for instance, about 30% of the efferent myelinated fibres to the muscle of the hind limb in cats are of small diameter. But, as said before, the mammalian muscle does not normally exhibit the phenomenon of local contracture. There have been attempts in the past to find any efferent nerve supply exerting some kind of what was called a tonic function on skeletal muscle. The sympathetic nervous system and a system called the parasympathetic, but transmitted efferently via the posterior roots, has been suggested to have such a function, but it has never been successfully proved.

Häggqvist (1938, 1940) thought that the small motor nerve fibres might exert such a function. He systematically examined the distribution of these fibres in ventral roots of monkeys and further tried to obtain a condition in which the large motor fibres were destroyed. By occlusion of the aorta the cells in the spinal cord are destroyed, and he found that the large anterior horn cells, which give rise to the large-diameter fibres, are more susceptible to ischaemia than the cells of the small-diameter fibre. After 15–20 minutes' ischaemia only the large anterior horn cells were killed. The result was spastic paresis and rigidity. The merit of the experiments of Häggqvist was to draw our attention again to this motor system; but where he erred was to think that the efferent impulses in these fibres produce directly some kind of tonic effect on the muscle.

A clear insight of the functions of these fibres in mammals was given by Leksell (1945). He examined the effect of stimulating the small motor fibres without stimulating the large ones. For that purpose he eliminated conduction in the large fibres by applying a pressure block or a polarization block to the nerve trunk. For instance, an effective selective pressure block could be achieved by a simple spring clamp, padded with soft rubber cushions and tightened with a screw. The nerve was then stimulated proximal to the site of compression and only small fibres were found to conduct beyond the block.

These impulses did not produce contraction of the innervated muscle, but they had a very definite effect on the afferent discharge from the muscle proprioceptors. Provided that the muscle was under a certain stretch, stimulation of the small motor fibres considerably increased the discharge of the afferent nerves, and he definitely stated that these small motor fibres served as regulators of sensory activity originating in the muscle.

The problem has recently been taken up by Hunt and Kuffler (1950a, 1950b). They could confirm Leksell's statement that no detectable contractile response or propagated muscle impulse was produced. But these small motor nerve fibres, when excited, set up local potentials apparently arising from the muscle elements within the muscle spindles, the intrafusal muscle fibres, and this apparently increased the discharge in the afferent nerve fibres. They used an extremely elegant method—a preparation consisting of a single small efferent fibre, the components of a muscle spindle, and a single afferent fibre. Stimulation of the small nerve fibre would cause a discharge in a receptor which had been quiescent, or could increase an existing discharge rate. The effect was influenced by existing muscle stretch being increased at higher muscle tension. This is not a new conception, for Matthews in 1933, in his classical studies on nerve endings in muscle, had already stated quite clearly that the intrafusal fibres are contracted by impulses travelling down the small motor nerve fibres, and that the sensory endings in the spindle are then stimulated by the mechanical effect of the contraction.

We know the intrafusal muscle fibres have motor end-plates: no experiments have as yet been performed to show that they are depolarized by acetylcholine, or, in other words, that the small-motor-fibre system is cholinergic. It is unlikely, however, that it should be otherwise. On the assumption that all motor fibres in the ventral roots are cholinergic, there still remains this difference: whereas with the large motor system and

with the small motor system in frogs the contractile effect upon the muscle fibre is the final event, with the small motor system in mammals this is not so. Here the contraction presents an intermediary stage; it acts as a mechanical stimulus for exciting the nerve endings in the spindle and thus sets up an afferent discharge. We shall probably hear much more about this system in the future.

It may be felt that when discussing the small-motor-fibre system I should have confined myself to mammalian muscle. But we are not certain whether the more primitive mechanism of producing sustained contraction in frogs and crustacea may not exist in some mammalian muscle—for instance, in the eye muscle. Further, under abnormal conditions—for instance, after denervation—the mammalian skeletal muscle reverts to the more primitive form of response; that is, localized contracture. This phenomenon is understandable only under the wider phylogenetical aspect. But the main reason is that I found it so fascinating to see how Nature ingeniously adapts parts of an old system, the small-motor-fibre system, to a more complicated mechanism in the development of postural reflexes in higher animals, that I could not resist giving the full story so far as it has been unravelled by recent experiments.

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EOSINOPHIL AND OTHER LEUCOCYTE CHANGES IN BURNED PATIENTS

WITH SPECIAL REFERENCE TO ADRENO- CORTICAL ACTIVITY

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An injection of anterior corticotrophic hormone of the hypophysis (A.C.T.H.) produces a marked fall in the eosinophils in the blood, associated with lymphocytopenia and neutrophil leucocytosis: in patients with Addison's disease, however, cortisone, but not A.C.T.H., produces similar effects (Thorn *et al.*, 1947a; Dougherty and White, 1947; Forsham *et al.*, 1948; Hills *et al.*, 1948; Prunty, 1950). The leucocyte changes, therefore, are mediated through adrenocortical stimulation by the anterior pituitary. Selye (1950) maintains that the major endocrine response to stress is an increased secretion of A.C.T.H. by the pituitary, which induces glyco-corticoid secretion by the adrenal cortex. The cortical hormones act on the peripheral organs and produce eosinopenia, lymphocytopenia, and neutrophilia.

The initial polymorphonuclear leucocytosis in burned patients is well known (Locke, 1902; Dorrance, 1933; McIver, 1933; Brown, 1944), and reference has been made to eosinopenia (Schreiner and Pucsko, 1925; Crassweller *et al.*, 1950a). The purpose of this study was to follow the changes in number of the blood leucocytes of recently burned patients and to correlate the findings with the extent of burning and other factors. In this way it was hoped to gain information on adrenocortical activity.

The Burned Patients

Thirty-five patients (19 males, 16 female) were studied; they were all between the ages of 13 months and 80 years and the extent of burning varied from 0.1% to 80% of the body area (Table I). Twenty-seven were admitted to the Burns Research Unit within three hours of burning, most within an hour or two, and all within nine hours. Twenty-four were or became clinically shocked and 26 were transfused with plasma or dextran or both fluids.

Eight patients died—two were moribund on admission and died a few hours later, five died two to four days