612.741.15:547.435-292

REACTIONS OF THE NORMAL MAMMALIAN MUSCLE TO ACETYLCHOLINE AND TO ESERINE

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(*Received May* 19, 1936)

Dale *et al.* [1936] have recently presented evidence that impulses reaching motor nerve endings on voluntary muscle fibres cause the liberation of acetylcholine at those endings. From analogy with cases earlier recognized, the suggestion was obvious that the acetylcholine so liberated might act as the transmitter of excitation from nerve ending to muscle end-plate. In this case, however, a difficulty was presented by the apparently low and irregular sensitiveness of normal, voluntary mammalian muscle to the artificial application of acetylcholine. There was evidence, indeed [Feldberg & Minz, 1931; Feldberg, 1933; Simonart & Simonart, 1935 *a*; Simonart, 1935 *b*], that quick contractions, or groups of such, could be elicited from the normal mammalian muscle by injections of acetylcholine; but the effects were rather irregular, the nature of the response not very clearly determined, and the doses required to elicit them so large as to make their physiological significance doubtful. To explain the fact that even such effects had escaped the notice of most earlier observers, the Simonarts suggested that they were readily suppressed by anæsthetics, and especially by ether. They observed the contractile responses most readily in the earlier stages of barbiturate anæsthesia, or in spinal preparations after complete removal of ether. Apart from this suggested deterrent action of anæsthetics, the evidence for which we shall discuss later, it appeared to us that the methods hitherto used, for the artificial application of acetylcholine to voluntary muscle, were not well calculated to display an action of the kind which it would have to produce, in transmitting the excitatory effect of a motor nerve impulse. If it performed this function, its release by the impulse must occur at the nerve ending, in close contact with

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the end-plate of the muscle fibre; and the quantity liberated by a single impulse, though extremely small, would be shed into a correspondingly minute volume of fluid, so as to produce a substantial concentration very abruptly. It was not, indeed, to be expected that any artificial method of application would reproduce such a sudden and simultaneous access of acetylcholine to the end-plate of every fibre of a muscle, as would be caused by a maximal volley of impulses in its nerve. It seemed possible, however, to make a more definite experimental approach to such conditions than had been attempted in earlier experiments. Arterial injections had already been used; but Simonart [1935 *b*], using a method already employed by Dale & Gasser [1926], made them into the lower end of the aorta without interrupting the circulation, so that the acetylcholine must have been distributed to an area much wider than the recording muscle (gastrocnemius), and must have reached the latter in quantities and at rates which were both variable and unknown. In the experiments in which Feldberg [1933] made the nearest approach to the methods here to be described, injecting into the lingual artery with the circulation stopped, only direct, visual observations were made of the responses of the tongue muscles.

In this paper we describe experiments, in which we have aimed at bringing doses of acetylcholine as rapidly and as nearly simultaneously as possible into contact with every fibre of a normal mammalian muscle. The results thus obtained have shown a remarkable contrast to the negative or irregular results earlier recorded by different observers, including some of our own number. We record also the results of some experiments made on the effects of curarine and of eserine, on the responses of mammalian muscle to artificial applications of acetylcholine and to motor nerve impulses.

Methods

Our experiments have been made on cats, and mostly on the gastrocnemius muscle. One experiment was made, for comparison, on the gastrocnemius of a dog. In most cases spinal animals have been used, kept under artificial respiration sufficiently long to remove the preliminary ether, but a few experiments have been made under ether for comparison.

Preparation of muscle. The gastrocnemius was completely exposed and freed almost to its origin from the femur, the calcaneum being divided so as to leave a segment attached to the Achilles tendon. The lower end of the femur, freed by ligature and section of the thigh muscles,

was transfixed by a stout drill attached to a rod, which was clamped to a steel pillar fixed to the operating table. The leg below the knee, to the exclusion of the gastrocnemius, was held in full extension by a string or accessory clamp, so as not to affect the movements of the gastrocnemius. The Achilles tendon was attached by a copper wire or a steel hook to the tension recording lever. This was in most cases a flat steel spring of the type used by Hill & Hartree [1920], the record being made on a smoked drum by a light aluminium lever moving in the vertical plane. In the earlier experiments the muscle was extended horizontally, so as to pull on a short vertical rod attached to the flat spring. Later, for reasons later discussed, the femur was fixed in such position as to allow the muscle to be extended vertically, and to pull downwards on a short horizontal rod attached to the spring. In some experiments isometric myograms were recorded optically, together with the action potential of the muscle. The drill immobilizing the femur was attached to a heavy iron plate attached through insulators to a cast-iron table. The tendon was connected through a steel hook, interrupted by an insulating fibre segment, to a torsion wire myograph of the type described by Eccles & Sherrington [1930]. The lead-off electrodes were either silver pins, the earth lead thrust into the belly of the muscle and the grid lead into the tendon, or a concentric needle electrode [Adrian and Bronk, 1929] in the belly of the muscle. The action potentials were amplified by two or three resistance capacity coupled stages connected to four pentodes in parallel, driving the coils of a Matthews oscillograph. The coupling condensers were of such size as to give a deflection falling to half its original value in 0·52 sec., when a rectangular potential was applied to the input.

In most experiments the exposed and extended muscle was kept warm and moist by draping it with a film of cotton-wool wet with warm saline, and continuously irrigating the surface with a jet of saline at about 38° C., which flowed down over the cotton draping into a drainage tray. For electrical recording this arrangement was unsuitable, and the muscle was, for this purpose, protected from evaporation by enclosing it in a loose wrapping of cellophane tied round the tendon and the femur, and was then kept warm by a beam from a suitable source of radiant heat.

Arrangements for injection. The object, as indicated, was to introduce the acetylcholine directly into the blood vessels of the muscle as rapidly as possible, and therefore through the shortest practicable length of artery, with the circulation arrested, and with the minimum of blood or other fluid in the vessels. Two methods were used.

(i) *Artificial perfusion with Locke's solution.* The popliteal artery and vein being exposed, all branches, even the finest, except those supplying or draining the gastrocnemius, were tied off in the popliteal space, to give a sufficient length of free vessels for the insertion of cannulæ. The tibial vessels, emerging from the tibial surface of the gastrocnemius between its heads, were then cleared and ligatured at the most proximal point attainable without damaging the muscle, care being especially needed to secure and tie branches to the knee joint. With the vessels thus prepared, an injection, made through a cannula tied into the popliteal artery close to the muscle, would pass immediately and entirely into the vessels of the gastrocnemius. The perfusion cannula was a three-way nickel-plated tube, with a central two-way tap, and with the peripheral limb ending in a fine cannula. One proximal limb was attached to a stout rubber tube leading from a Dale-Schuster pump, and including a glass bulb T-piece to accommodate a thermometer and serve as a bubble-trap. The temperature of the bath enclosing the pump was such as to maintain at 36–38° C. the Locke's solution reaching the muscle through the perfusion cannula. The other proximal limb of the cannula was open, and provided with an attachment for the injection syringe. At any moment the perfusion could be interrupted by turning the two-way tap, and, with the muscle drained of perfusion fluid through the vein, the injection could then be made directly into its vessels through the free limb of the cannula. A by-pass was provided in the system, so that the pump could work without interruption during this brief arrest of the flow for injection. The artery, having been tied and clipped, was incised at the nearest convenient point to the muscle, the cannula was tied in, the clip removed, and the perfusion opened up to the full rate. When the fluid in the vein became nearly clear, the vein was opened to give it free exit, and tied above the opening; in some cases a cannula was tied into the vein, so that the rate of venous outflow could be checked. The arterial cannula was then firmly clamped in a position enabling injections to be made conveniently through its free limb. When an injection was to be made, this free limb was filled, as far as the tap, with the solution to be injected. The syringe with the measured volume of solution was attached, the tap turned, the muscle allowed to drain through the vein for a few seconds, and the syringe then discharged as quickly as possible. The part of the cannula peripheral to the tap, down to the opening of the nozzle, had a volume of only 0·06 c.c.; this dead space was allowed for by a corresponding addition to the volume injected.

(ii) *Natural circulation.* A few of the early experiments were made by tying off all the side branches of the external iliac, superficial femoral and popliteal arteries down to the gastrocnemius muscle, as well as both the internal iliac arteries, and making the injection through a cannula tied into the central end of the other external iliac artery, the aorta being clamped just above the bifurcation while the injection was made. The effects obtained were somewhat irregular, for reasons discussed below, and we later found it better to use the central end of the tibial artery, for retrograde injections through the popliteal. The popliteal artery was prepared as for perfusion, but in this case a sufficient length of the tibial artery was similarly prepared by carefully finding and tying all side branches, so that fluid injected centrally into it must pass entirely into the popliteal artery and, when the latter was clamped, entirely into the vessels of the gastrocnemius. A cannula made from a syringe needle was then tied into the central end of the clamped tibial artery, and the butt of this cannula was fixed firmly in a small clamp. When an injection was to be made, this cannula, including even the bore of the needle, was filled with the solution to be injected by use of a finer hypodermic needle, so that there was no dead space outside the arteries themselves, and the syringe holding the measured volume was then firmly attached. The popliteal artery was then clamped near to the origin of its gastrocnemius branches, the blood in the muscle being free to escape by the veins. The clip on the tibial artery was then opened, and the contents of the syringe were quickly discharged into the vessels of the gastrocnemius. We found this to be the most effective of the methods used, and, when once the dissection and ligature of small arteries had been completed, the simplest to apply and the most easily controlled. In a few cases we found it convenient, after ligaturing and cutting all its side branches, to draw the divided tibial artery between the two heads of the gastrocnemius, so that the whole procedure of clamping and injection could be carried out proximally to the muscle.

Nerve stimulation. In those experiments in which the irrigating saline jet was used, the sciatic nerve was bared and laid on silver wire electrodes in such a way that it was kept warm and moist by the irrigation. When the jet was not used, the nerve was left in position in the thigh muscles and stimulated through a shielded electrode. The stimuli were slightly supramaximal break induction shocks, delivered at a known time interval, usually 10 sec., by a Lewis rotating contact breaker. In the experiments in which optical records were made, the stimuli were similar break induction shocks delivered by a Lucas pendulum.

Results

(i) *Action of acetylcholine on the normal muscle*

Effects with different methods of injection. Our earlier experiments had the object of extending Simonart's observations, with a sharper restriction of the injected dose to the muscle from which records were taken, and with the circulation stopped while the injection was made, so as to ensure a smaller and more constant mixture of the injection with blood. When a dose was injected through the opposite iliac artery, into the lower end of the clamped aorta, it could be seen momentarily to fill and distend the whole of the stretch of iliac, femoral and popliteal arteries, the side branches of which had been tied right down to the muscle. Part of the injection passed into the muscle as the artery collapsed again, and the remainder was swept in by the renewed blood stream, when the aorta was released. Under such conditions we sometimes obtained obvious responses of the muscle to the injection of doses as small as 5γ, all made in 1 c.c. of saline solution. The responses had the form of sharp, apparently simple contractions. With larger doses, of from 10 to 25γ, we obtained contractions with tensions as high as that of the maximal twitch excited through the sciatic nerve, and with doses of the order of 100γ, with tensions much higher. The results, however, were still irregular, and we found that for a constant dose there was an optimal volume for the injection. The reason of this was clear. If the volume was too small, practically none of the injection reached the muscle before the circulation was renewed; if it was too large, though much of the injection reached the muscle while its vessels were empty, it was in a correspondingly dilute solution; and the biggest response was accordingly obtained with solution in an intermediate volume, such that some of the injection passed into the still bloodless muscle, in a solution not too dilute to cause excitation, before the circulation was renewed. It was clear that, to obtain better and more regular results, we must further reduce the arterial dead space.

In our first attempts to do this, we were concerned at the same time to remove the last traces of blood from the vessels of the muscle, supposing that the esterase present in them might reduce the dose of acetylcholine reaching the muscle fibres. We accordingly adopted the technique of perfusion with warm, oxygenated Locke's solution above described, with the cannula inserted into the popliteal artery as near as possible to the muscle. In this way, giving the injections through the side tube of the cannula during stoppage of the perfusion, we obtained

much more regular results. At this period we were still giving the injections in a uniform volume of 1 c.c., and the smallest dose giving a definite contraction of the muscle was, in several experiments, 5γ. Later experience suggested that, even in the earliest stages of an artificial perfusion with Locke's solution, there was an incipient œdema, by which the weak solutions used for injection would be further diluted on the way to the muscle fibres. Under these conditions the effect increased very steeply with increase of the dose. Thus, with a resting tension of

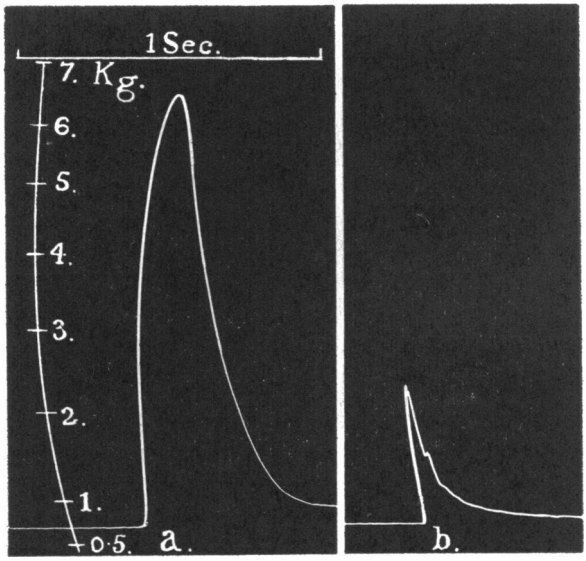

Fig. 1. Spinal cat. Tension record from gastrocnemius, perfused with warm Locke's solution. *a*, contraction in response to injection by arterial cannula of 20γ ACh.; *b*, maximal motor nerve twitch.

0·5 kg., when a maximal twitch excited through the nerve produced an additional tension of 2 kg., an injection of 5γ ACh. might cause a contraction of 0·5 kg., 10γ one with a tension equal to or greater than the maximal twitch, and 20γ one with the remarkable tension of 6 kg. in excess of the resting tension, as illustrated in Fig. 1. It will be seen that this contraction, recorded on a fast drum, though definitely slower than the twitch, is still very quick. The curved path of the lever point, which the calibration scale shows on the resting drum, somewhat exaggerates the apparent quickness of the earlier part of the rise; but it correspondingly diminishes the apparent speed of the latter part, and it can

be safely estimated that the whole rise to this high tension occupies not more than 200 msec. The relaxation is practically as quick, except for a weak contraction remainder, which delays the final return to the resting tension. We obtained a number of records of this kind with the perfusion technique, but we observed that, in order to obtain these large responses to small injections, it was necessary to make them very early in the perfusion. After perfusion had proceeded for 20–30 min., though the response of the muscle to stimulation through the nerve was undiminished, the effects of ACh. injections, though still striking, became smaller, and the threshold dose became greater. When this diminution of response had set in, it progressed steadily with continued perfusion, even when no intervening injections were given, and we observed after-effects of ACh. injections on the response to nerve stimulation, which will be considered later. We were led to associate these changes in response with the early beginning and rapid increase of an œdematous condition of the muscle perfused with the saline solution. Eventually, after long perfusion, when the muscle had become grossly œdematous, injections of acetylcholine which had initially evoked large, quick contractions, sometimes caused none at all, and only affected the subsequent response to nerve stimulation.

Such results emphasized the fact that the stimulant effects were dependent, not only on the dose of ACh. entering the muscle, but on the rapidity with which it reached the site of its action, presumably at the motor end-plates, in a stimulating concentration. For such purposes the artificial perfusion method was suitable only in the earliest stages of its application. We had recourse, accordingly, to the other method already described, in which, during temporary arrest of the normal circulation, the injections were made into the empty arteries of the muscle through the central stump of the tibial artery. This gave us the best and most regular results hitherto obtained. The threshold dose was further lowered in most experiments. Even with this method the responsiveness of the muscle appeared to differ from one experiment to another; probably we failed, on occasion, to secure every one of the fine branches from the tibial artery, between the point where the cannula was inserted and its origin, with the branches to the gastrocnemius, from the popliteal. In the majority of preparations, however, an injection of 5γ of ACh. in 0·5 c.c. caused a contraction to a much higher tension than the maximal twitch, and even $2{\cdot}5\gamma$ in the same volume produced a tension equal to that of the twitch, or somewhat higher, as in Fig. 2. It is safe to conclude that, in such a case, a slightly smaller dose, about 2γ, would have produced the same tension as the twitch. In such cases a small but definite

contraction, of 250–500 g. tension, was usually produced by injecting as little as 1γ. We made no systematic attempt, however, to determine the threshold dose with any precision, since it could have no more than a conventional meaning. Slight differences in the technique of injection, as we shall see, could produce a large difference in the effect. We had evidence with this method, again, that the dead space due to the volume of the arteries and their branches in the muscle itself, though reduced to the practicable minimum, still had some effect; so that, if the volume of the injection was reduced too much, what was gained in concentration

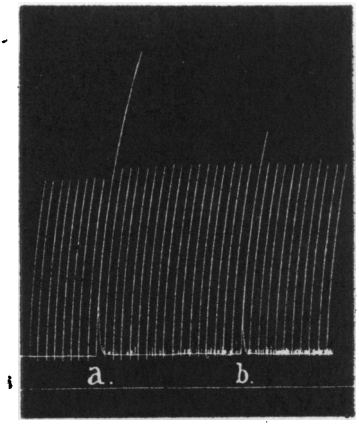

Fig. 2.

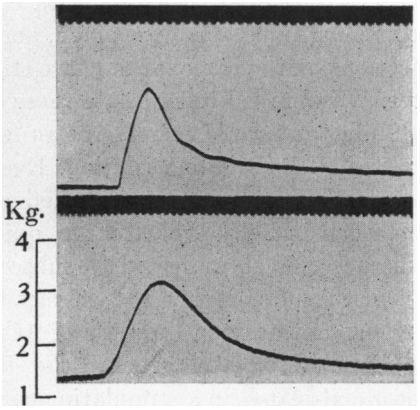

Fig. 3.

Fig. 2. Spinal cat. 3·3 kg. Record from gastrocnemius with natural circulation. Maximal twitches excited from nerve every 10 sec. At *a* 5γ, at *b* 2·5γ ACh., each in 0·5 c.c., by close arterial injection, during intermission of one shock to nerve.

Fig. 3. Spinal cat. Optical isometric myogram of gastrocnemius. Upper record—maximal motor nerve twitch; lower record—response to close arterial injection of 2·5γ ACh. in 0·25 c.c. Time 10 msec.

of ACh. in the fluid injected was more than offset by loss or delay in this dead space, and by dilution of what passed into the capillaries, and diffused out of them, by the interstitial fluids of the muscle. We found that reduction of the injection volume below 0·5 c.c. was not favourable to the effect, even of the smallest doses, though 0·25 c.c. was found, on occasion, to give as good an effect. One other point should be mentioned. The instability of ACh. in high dilutions, at the slightly alkaline reaction of Locke's solution or of the ordinary laboratory saline, led us, as a routine, to prepare our dilutions in saline made slightly acid (about *p*H 5) with HCl; and these dilutions were injected at the room temperature.

We repeatedly controlled the effects of similarly injecting 0·5–1 c.c. of such acidulated, cold saline. In almost all cases the result was completely negative, so that the definite response to the same volume containing 1γ of ACh. stood out in sharp contrast; and in one case we injected this dose in an immediately prepared dilution in ordinary saline, and found it similarly effective. In an exceptional case, however, we have observed a very small contraction as the result of injecting the acid saline alone into a very reactive muscle. In such a case we made no attempt to determine the threshold dose of ACh., but the effect was so small as not to complicate those of the strongly active doses.

Form of the contraction. With a method giving such regular responses to small injections, it seemed desirable to obtain a record more mechanically perfect of the course of the contraction. A few experiments were, therefore, made with the isometric torsion-lever, recording photographically. Fig. 3 shows such a record of a maximal twitch excited through the nerve, and the response to an injection of 2·5γ of ACh. in 0·25 c.c. The tensions produced are, in this case, identical. It will be seen that the curve recorded by the ACh. contraction is almost symmetrical about the maximum, and that the time taken to reach it is only about twice that taken by the twitch to reach the same tension. The response to ACh. has, in fact, the appearance of a rather slow and symmetrical twitch.

Effect of speed of injection. We had noticed that, even with the most direct method of injection into the blood vessels, the effect of a given dose was much reduced if some slight obstruction delayed its delivery from the syringe. We accordingly made a few injections in which the pace was deliberately altered. Fig. 4 illustrates the result. The injections were all made, during arrest of the natural circulation, by the tibial artery, and the dose in each case was 25γ of ACh. in 0·5 c.c., this being the dose required to elicit the maximum response to such injections, at this stage of this particular experiment. The records were taken on a slow drum. For the first injection, at *A*, the time was only roughly estimated, at less than 0·5 sec. The syringe was then carefully adjusted to allow the quickest injection possible, against the minimum natural resistance, and an injection, at *B*, was made in 0·2 sec., as nearly as could be measured by stopwatch. At *C* and *D* similarly timed injections were made in 1·5 and 2 sec. respectively, and at *E* and *F* two further injections were given at the maximum speed, the time being again recorded as 0·2 sec. in each case. Probably a more accurate record of the rates would have shown that these were, in fact, slightly slower than

that at *B*; but the results show that, in any case, the effect even of a relatively large dose can be greatly modified by the rate at which it enters the vessels of the muscle. Even an injection made at close range into the empty blood vessels will largely fail of its effect, if no special effort is made to give it quickly. One other observation, made in our most recent experiments, may be mentioned in the same connexion. We have obtained larger responses, to smaller doses, with the muscle vertically extended and pulling downwards on the lever, than in earlier experiments with horizontal extension and pull. We can only attribute the improvement to a readier and more complete drainage of the vessels

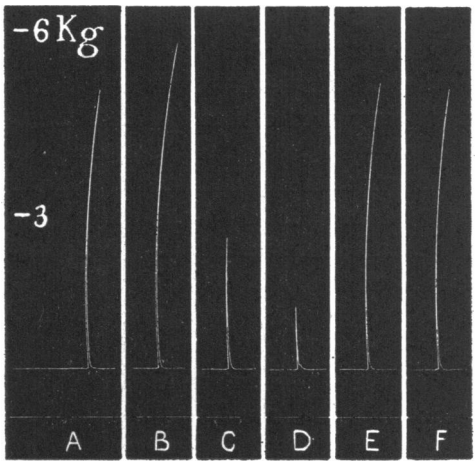

Fig. 4. Cat under ether. Response of gastrocnemius to 25γ ACh. in 0·5 c.c., injected at different speeds. See text.

by the vein, during the brief interval between closure of the popliteal artery and delivery of the injection; presumably this slightly accelerates the access of ACh. to the site of its action.

Size of dose. As already indicated, with successful injection under the best conditions obtainable, into a fresh, active muscle, the maximum response was obtained with an injection of about 20γ. Under the same conditions an increase of the dose up to 100γ produced no significantly greater effect, though after such larger doses there was more tendency to a contraction remainder—the appearance of a weak contracture delaying the latter part of the relaxation. This effect was sometimes obvious even with smaller doses. It is particularly so in the responses to 5 and 2·5γ seen in Fig. 2; and in this case it is also to be seen that the muscle

continued, for some time after the direct responses to the injections were finished, to give weak, partial twitches, between the maximal twitches excited through the nerve. Such weak twitches were often seen, however, in the muscle as exposed and arranged for our purpose, without any injection of acetylcholine; and the apparent accentuation of this tendency after the injections, seen in Fig. 2, was not a regular occurrence, and may have been accidental in this case.

All this experience with injection, by different methods and under varying conditions, gave a cumulative emphasis to the importance of speed of access to the site of action on the muscle fibre, presumably the motor end-plate, in the production of quick contractions of normal mammalian muscle by small doses of ACh. This was probably the chief cause of the failure of earlier workers to obtain any effects with such small doses, and of the irregularity of the effects which they observed with larger doses, when injections were made into the general circulation, apart from the destruction of ACh. by the blood esterase on its way to the muscle.

Effect of anæsthetics and other conditions. According to the Simonarts [1935 *a*] the effects which they described, as the result of intravenous injections of large doses of ACh., were not obtainable in animals under ether. Most of our experiments were made on spinal animals, after removal of the preliminary ether, but we made a few for comparison on cats kept continuously under ether. With our method of close-range arterial injection, ether certainly did not suppress the response of the muscle to ACh. The contractions illustrated in Fig. 4, for example, were obtained in a cat which was deeply under ether during the whole experiment. The threshold dose in this experiment was 2·5γ, rather higher than in most similar experiments on spinal animals, but not so much so as to suggest a severe depression. It must be remembered, however, that we made the injections in saline solution, directly into the vessels drained of blood. We note later the influence of ether on the effect of eserine. We think it probable, therefore, that the effect of anæsthetics has played some part in obscuring the stimulant action of ACh. on normal mammalian muscles, as in the recent observations of Rosenblueth *et al.* [1936], who injected large doses intravenously into cats under dial, without observing any directly stimulant effects. The ease with which the conditions requisite for the action of injected ACh. may be disturbed, is shown by another experience of our own. Wishing to keep the muscle longer under perfusion without producing the unfavourable œdema, we perfused, in one experiment, with the solution of hæmolysed corpuscles

described by Amberson & Höber [1932]. This gave us an admirable perfusion, and the muscle remained for hours free from significant œdema and fully responsive to stimulation of its nerve. Injections of ACh., however, were ineffective in doses less than those which had otherwise given maximal contractions, and even these produced only weak responses. In some way the use of this perfusion fluid apparently delayed the passage of ACh. from the capillaries to the muscle fibres, and thereby largely suppressed its stimulant effect.

After-effects of injection. When ACh. is injected into the vessels of the muscle during interruption of a normal circulation, or of an artificial saline perfusion in its earliest stages, the twitch given by the muscle in response to stimulation of the nerve appears to be completely normal, as soon as a contraction caused by ACh. is over and the circulation restored. This is the case, whether the dose injected produces a response of smaller or of greater tension than the twitch. Simonart [1935 *b*] records that the response of the muscle to nerve stimulation is potentiated as an after-effect of injecting 0·5 mg. of ACh. into the aorta, and depressed as an after-effect of 5 mg. The records which we illustrate in Figs. 2 and 7 might suggest a slightly increased response to the maximal shocks given regularly to the nerve, following the injections of 5 and 2·5γ in Fig. 2, and 5γ in Fig. 7. We suspect, however, that these effects, which are in any case small and inconstant, may be due to the vaso-dilator effects of the ACh. injections, which would persist long after the contractile response, and improve the nutrition and warming of the muscle. In Simonart's experiments it seems possible that such a dose as 0·5 mg., carried by the blood directly to the leg under experiment, might there cause some vaso-dilatation, and consequently improve the response of the muscle to nerve impulses, in spite of the presence of sufficient atropine to prevent a depressor action by that remainder of the ACh. which reached the general circulation. We have not used doses large enough to cause a significant depression of the nerve twitch in the fresh and normal muscle. On the other hand, we have regularly observed that when, after long perfusion with Locke's solution, the muscle showed much œdema, an injection of ACh., even in a dose too small to cause a contractile response, was followed by a definite weakening of the twitches evoked by maximal shocks to the nerve. The effect to be expected of these conditions would be an abnormal persistence of ACh. in the interstitial fluids, in a concentration too weak to excite the end-plates; and apparently a dose which has never produced a sufficiently rapid rise to a stimulant concentration, can produce this depression if it thus persists

in the muscle. We shall return to the point in considering the action of eserine.

On one matter of some interest we have as yet made no experiments, namely, the response of the muscle to a nerve volley *during* the contraction produced by an injection of ACh. The proper exploration of changes in such response, at different phases of the rapid ACh. contraction, will obviously require special arrangements for timing the nerve stimulation in relation to the injection, and we hope to undertake it in due course.

(ii) *Action on the denervated muscle*

The regularity with which powerful contractions are obtained from the denervated mammalian muscle, in response to the injection of even small doses of ACh. into the general circulation or the arterial stream of the limb, has been recorded by several observers [cf. Frank *et al.*, 1922; Dale & Gasser, 1926; Dale & Gaddum, 1930]. Such results made it clear, not only that the denervated muscle was much more sensitive to ACh. than the normal muscle, but also that its response was much less dependent on rapidity of access. We have confirmed these points with our new methods of injection in a few experiments on the gastrocnemius of the cat, denervated by aseptic section of the sciatic nerve under ether from 10 to 18 days previously. With injections made into the tibial artery during temporary arrest of the circulation, such muscles gave definite contractions in response to as little as $0{\cdot}001\gamma$, or 1 c.c. of a 1 in 10^9 dilution. It was necessary to make these low dilutions immediately, in ordinary saline, since the acid saline caused distinct contractions by itself. In several cases it was apparent that the smaller doses, when rapidly injected, produced contractions which, though slower than those of the normal muscle, especially in relaxation, were of the type of contractions with sharp summits, and not of contractures. With somewhat larger doses, such as 1γ, we observed in several cases a double effect, consisting of an initial, sharply topped contraction, followed by a secondary, slow contracture. Fig. 5 shows an example of this. Reference to earlier publications on the reaction of the denervated muscle shows that this double effect has often been figured, but without comment, as the result of injections into the general arterial stream [cf., for example, Dale & Dudley, 1929, Fig. 3]. With increasing doses the tension of the contracture rises above that of the initial contraction, and with still larger doses the two become fused. In responses such as those reproduced in Fig. 6, the contracture appears to be represented only by a somewhat irregular delay of the relaxation.

(iii) *Action of curarine*

Gasser & Dale [1926] found that the response of the denervated mammalian muscle to ACh. was still obtained after a dose of curare, given by the general circulation, which abolished the indirect excitability of the normal muscle. Simonart [1935 *b*] confirmed this resistance to curare of the action of ACh. on the denervated muscle, and we have confirmed it again, using the more accurate comparison made possible by our method of close-range injection. Fig. 6 shows the effects of injecting successive doses of ACh. into a denervated gastrocnemius by the

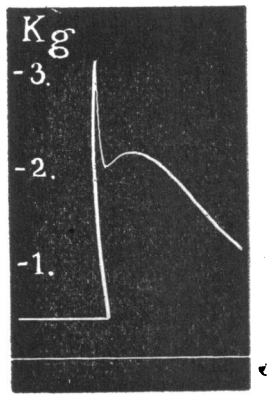

Fig. 5.

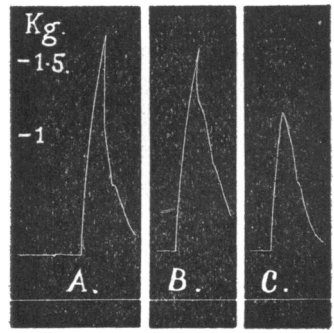

Fig. 6.

Fig. 5. Spinal cat. Record from denervated gastrocnemius, 14 days after nerve section. Perfusion with Locke's solution. Effect of 1γ of ACh. by close arterial injection.

Fig. 6. Spinal cat. 3·3 kg. Record from denervated gastrocnemius, 21 days after nerve section. Close arterial injections. *A*, 0·25γ ACh. Between *A* and *B* 1·4 mg. curarine intravenously. *B*, 1γ ACh. Between *B* and *C* a further 1·4 mg. curarine intravenously. *C*, 1γ ACh.

tibial artery, during arrest of normal circulation. Between *A* and *B*, 1·4 mg. of curarine hydrochloride had been given intravenously. This almost, but not quite, abolished the reaction of normal muscles to nerve stimulation; and it will be seen that 1γ of ACh. was now required, to produce an effect on the denervated muscle similar to that which was produced by 0·25γ before curarine. A second dose of 1·4 mg. of curarine, making a total of 2·8 mg., then completed the nerve paralysis of the normal muscles; but it only further reduced the response to ACh. of the denervated muscle, from that shown at *B* to that shown at *C*. It should be added that the denervated muscle, in any case, shows some

reduction of its response to successive, equal doses of ACh., though not of the extent here seen with curarine. The results show that, while the effect of ACh. on the denervated muscle is not completely resistant to curarine, it is, in conformity with previous evidence, much more resistant than the effect of nerve impulses on the normal muscle.

Simonart [1935 *b*] found, on the other hand, that the responses of the normal cat's muscle to ACh., which he observed with arterial injections, were suppressed by curare about as readily as the response to indirect stimulation. By comparing the effects on a regular series of maximal twitches excited through the nerve, and on the reproducible effects of small, close-range injections of ACh., we are able to state that

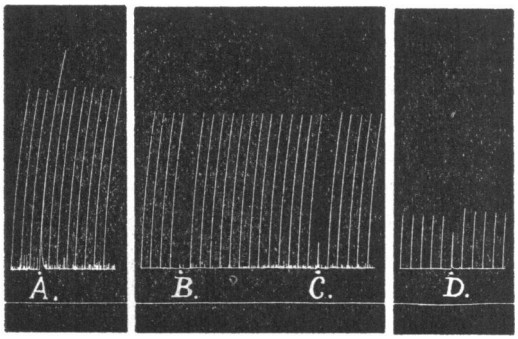

Fig. 7. As in Fig. 2. *A*, 5γ ACh., during intermission of one nerve shock. Between *A* and *B* 0·7 mg. curarine intravenously. *B*, 5γ ACh. *C*, 10γ ACh. Between *C* and *D* a further 0·7 mg. curarine intravenously. *D*, 25γ ACh.

these effects of ACh. on the normal muscle are even more sensitive to curarine than are those of nerve volleys. We have made such comparisons by the method of artificial perfusion, and by that used with natural circulation, the curarine in the latter case being given intravenously. Fig. 7 illustrates an experiment by the latter method. It will be seen that, before curarine, 5γ of ACh. produces a contraction stronger than the maximal twitches. After an initial dose of 0·7 mg. of curarine the twitch responses to maximal nerve volleys show only a small decline, while the formerly stronger effect of 5γ of ACh. is completely suppressed. After a second dose of 0·7 mg. of curarine the nerve twitch is greatly reduced, but even 25γ of ACh. now produces an even weaker contraction. It will be seen that, following each such dose of ACh. there is a slight recovery of the strength of the following nerve twitch; Rosenblueth *et al.* [1936] have recently described a partial recovery from a curare

paralysis following the injection of a large dose of ACh. (1 mg.) into the general circulation. We should add that, with a muscle rendered œdematous by perfusion with Locke's solution, so that injected ACh. tends to linger in its tissue spaces, the after-effect of an ACh. injection may be to intensify, instead of weakening, an incomplete paralysis by curarine.

(iv) *Actions of eserine*

We expected that eserine, by protecting the ACh. from the action of esterase, which it might meet on the way to the site of its action, would intensify the action of submaximally effective injections. The effect was clearly seen with long-range injections through the aorta and iliac artery; in one such case an injection of 10γ of ACh. produced no effect on the gastrocnemius before eserine, but, after intravenous administration of 0·3 mg. of eserine per kg., caused a contraction nearly as strong as a maximal nerve twitch. With the close-range injections this potentiating effect was perceptible, but was less pronounced. The tension produced by a submaximal injection might be increased by 30 or 50 p.c., and the threshold dose was lowered; but the potentiation was less than we had expected. The most striking effect of eserine on the effects of such injections was, indeed, not on the contractile response, but on the depression which followed it. We have seen that, with natural circulation, the nerve twitch following a response to an injection of ACh. was not depressed, but might show a fractional increase; under the action of eserine the tension of a maximal nerve twitch was always diminished, as an after-effect of an injection of ACh., to an extent and over a period increasing with the size of the dose injected. It appeared that ACh., after its stimulant effect at the end-plate was over, was enabled by eserine to linger in the spaces of the muscle, and to reduce its response to the nerve impulse. During this depressant after-action of one injection of ACh., another similar injection also had a greatly reduced stimulant effect. We have seen that œdema of the perfused muscle might produce a similar effect, and the phenomenon was seen in its most striking form, when a muscle had been perfused until œdematous with Locke's solution containing eserine in a high dilution, such as 1 in 10^6. In such a muscle, an injection of ACh. which failed to produce a contraction would sometimes completely abolish, for a time, the response to maximal nerve stimuli, which would slowly recover. Rosenblueth *et al.* [1936] have described such a curare-like action of acetylcholine injected into the general circulation under eserine. They found that, when thus paralysed to nerve stimulation, the muscle would still respond when stimulated

directly. Nevertheless, our evidence of the adjuvant effect of saline œdema strongly suggests that this depressant action is produced, not by ACh. which reaches the end-plates in stimulating concentration, but by that which lingers, especially when protected by eserine, in contact with the muscle fibres as a whole.

Fig. 8 shows, for comparison, the effects of 1, 5 and 25γ of ACh. injected by the perfusion cannula into two gastrocnemius muscles of

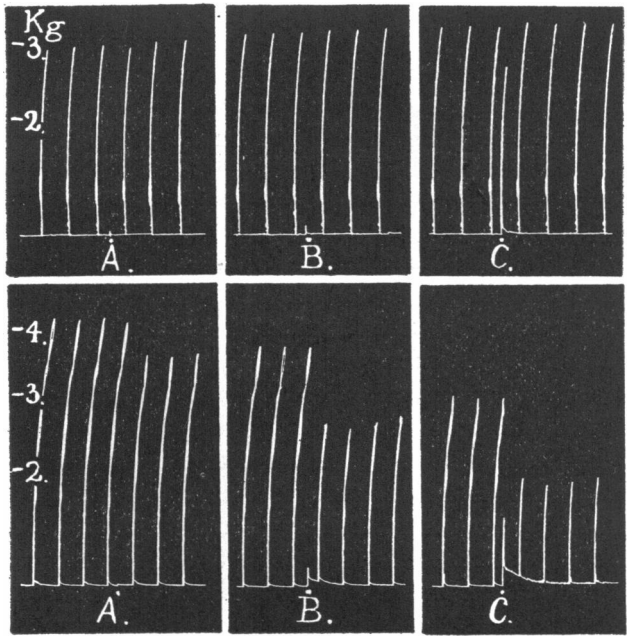

Fig. 8. Spinal cat. Both gastrocnemii perfused with Locke's solution, upper record without eserine, lower record with eserine 1 in 10^6. In each case, at *A* 1γ, at *B* 5γ and at *C* 25γ of ACh. by arterial cannula.

the same cat. The upper record is from one muscle perfused with Locke's solution only, the lower record from the other muscle, perfused with Locke's solution containing eserine 1 in 10^6. Both records were taken at corresponding, fairly early stages of perfusion, before any obvious œdema had appeared. It will be seen that the ACh. injections into the muscle perfused without eserine have no depressant effect on the succeeding nerve stimuli; whereas, in the case of the muscle perfused with eserine, even 1γ of ACh., producing no perceptible contraction of the muscle, is followed by a pronounced and long lasting depression of the response to nerve stimulation.

The most striking effect of eserine which we have observed, however, was not on the response of the muscle to injections of acetylcholine, but on the twitches excited by stimulating the nerve. The effect is well seen under the following conditions. In a spinal cat, freed from preliminary ether by artificial respiration, a tension record is made of the twitches of a muscle, such as the gastrocnemius or tibialis anticus, in response to a regular series of maximal break shocks applied to the nerve at 10 sec. intervals. When initial irregularities and staircase effects are at an end, and the muscle is recording a perfectly regular series of maximal twitches, an injection of 0·2–0·3 mg. of eserine per kg. is given into a vein, after a preliminary dose of 1 mg. of atropine to prevent circulatory depression by the eserine. After a minute or so, a twitch is recorded which is distinctly stronger than its predecessors in the regular series, and the potentiation then rapidly and steadily increases with successive twitches, until, after a further 4 or 5 min., each twitch reaches a tension which is often twice as great as that of the normal twitch before eserine. The greatest increase which we have recorded was to 2·3 times the normal. The effect remains at the maximum for some minutes, and then very gradually subsides. The suprarenal glands play no part in the effect, which is produced unchanged after their removal. After an hour or more, the twitches are usually slightly weaker than before eserine. The effect can then be repeated by a further similar injection of eserine; the second period of potentiation usually begins with a shorter delay, but, though sufficiently impressive, it is never so great as that following the first injection of eserine. We have repeated this experiment a number of times, and with uniform results under the conditions named. Some of these are illustrated in Figs. 9 and 10. In Fig. 10, interposed between the nerve twitches, omitting one stimulus to the nerve in each case, the effects of injections of ACh. are shown, made before and during the action of eserine. It will be seen that the first ACh. contraction under eserine, though somewhat potentiated, is definitely less so than the nerve twitches. The depressant effect of an ACh. injection after eserine, on the following responses to nerve stimulation, is also well seen in Fig. 10.

The potentiating effect of eserine on the maximal nerve twitch is so obvious, and so regular in its occurrence, that it is natural to enquire why it has, apparently, not been previously described. As long ago as 1876 Harnack & Witkowski, in a very early account of the action of eserine, stated that it lowered the threshold of response of the frog's muscle to stimulation through its nerve. Neither they, nor any subsequent observers, however, have recorded a potentiated response of the

frog's muscle to maximal nerve volleys, such as we have observed in the mammal. Kruta [1935] observed no such effect with immersion of the frog's muscle in Ringer's solution containing eserine; and, in a few

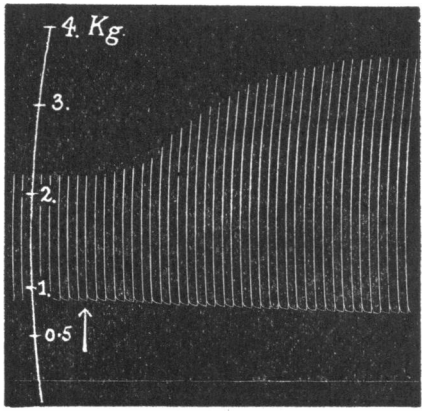

Fig. 9. Spinal cat. Eviscerated. Suprarenals removed. Gastrocnemius record. Maximal break shock to sciatic nerve every 10 sec. At ↑ 0·2 mg. eserine per kg. intravenously

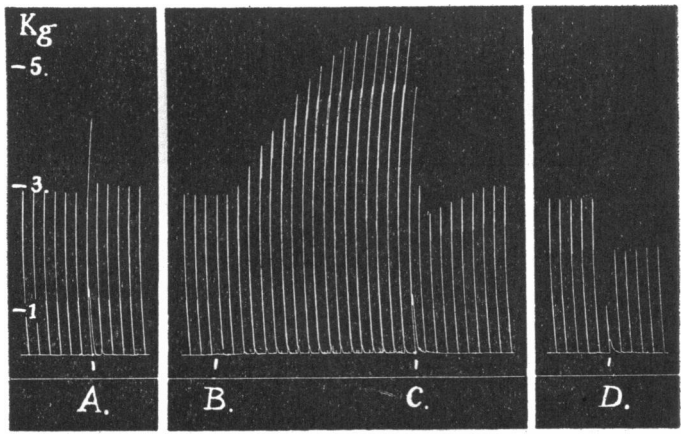

Fig. 10. Spinal cat. 2·6 kg. Gastrocnemius record. Maximal break shocks to sciatic nerve every 10 sec. At *A*, *C* and *D*, 25γ ACh. in 0·5 c.c. by close arterial injection, during intermission of one shock to nerve. At *B*, 0·8 mg. eserine intravenously.

preliminary experiments made in this laboratory, Z. M. Bacq has found that perfusion of frog's muscle with solutions of eserine, up to a strength of 1 in 10^5, causes no potentiation of its response to maximal break shocks applied to the nerve. We may conclude then that, for some reason, the

frog's muscle does not show the particular effect of eserine which we have observed so regularly in that of the mammal.

The potentiating effect of eserine on the response of the mammalian muscle, however, is completely suppressed by ether. We have not yet studied the effect of other anæsthetics upon it, but it seems likely that their use accounts for the failure of earlier workers on mammalian muscle to observe so clear an action. Rosenblueth *et al.* [1936], using cats anæsthetized with dial, state that the effects of injecting eserine alone,

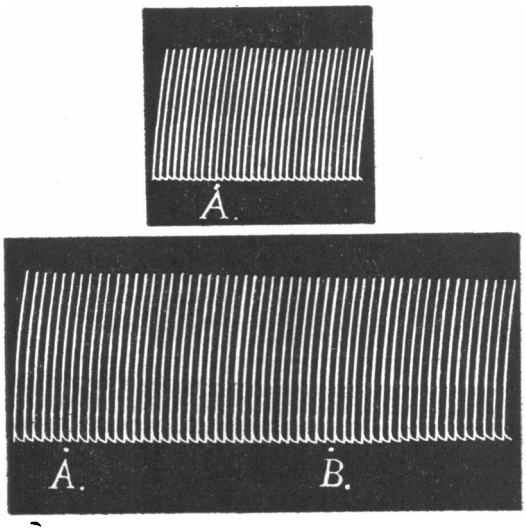

Fig. 11. Upper tracing: record from gastrocnemius of cat, 2·6 kg., under ether. Maxima break shock to nerve every 10 sec. At *A*, 0·52 mg. eserine intravenously. Lower tracing: record from denervated gastrocnemius (11 days) of spinal cat, 3 kg. Maximal break shock directly to muscle, every 10 sec. At *A* 0·6 mg., at *B* 0·9 mg. eserine intravenously.

on the response of the muscle to nerve stimulation, were "weak and irregular." In Fig. 11 the upper record is from one of our experiments in which the cat was throughout under the influence of ether. It will be seen that an injection of eserine has here no effect of the kind which it regularly produces in the spinal animal; the effect was regularly absent in the experiments which we made under ether. In the muscle denervated by degeneration, eserine produces no potentiation of the twitches caused by direct stimulation with maximal induction shocks, as shown in the lower record of Fig. 11; and in the normal muscle which has been rendered completely insensitive to excitation through the nerve, by the action of

a large dose of curarine, there is similarly no trace of potentiation by eserine of the twitches caused by direct stimulation. The potentiation by eserine, therefore, represents an abnormal enhancement of the response of the muscle fibre to the excitatory effect of a nerve impulse,

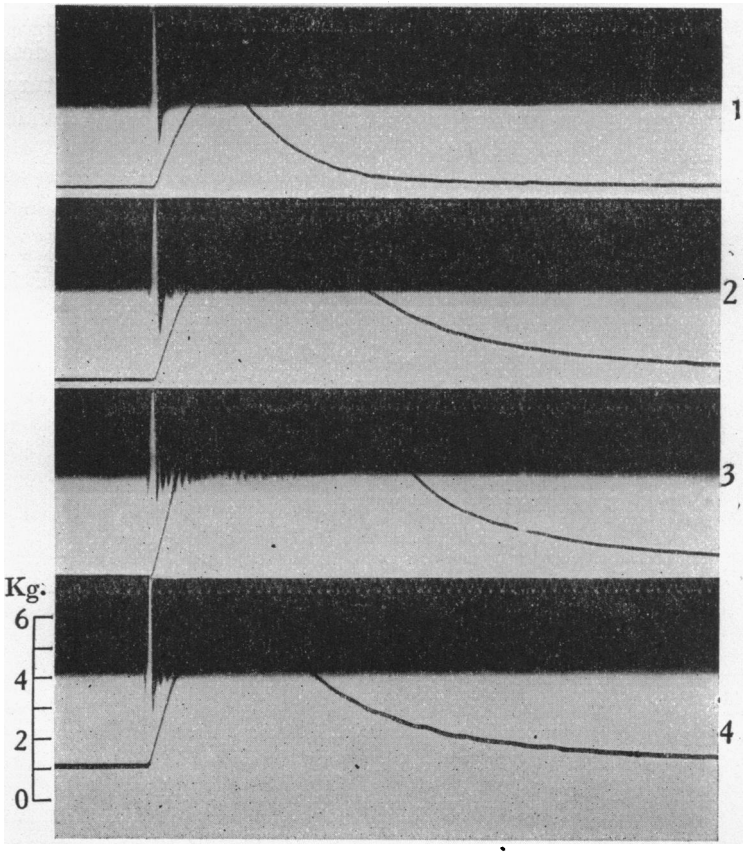

Fig. 12. Optical myogram and action potential of gastrocnemius of spinal cat, 2·6 kg. (1) before eserine; (2) 3 min. after 0·8 mg. eserine intravenously; (3) 10 min. later; (4) 2 hours later.

and to no other kind of stimulation of those we have tried, except that produced by injection of acetylcholine.

The potentiated nerve "twitch" under the influence of eserine is longer in duration, as well as higher in tension, than the normal twitch. If the all-or-none principle holds good, it must represent the effect of a repetitive response, and presumably of a more or less synchronous

series of responses of all the fibres. Such preliminary records as we have made, of the electrical variations accompanying such a response, entirely confirm this view of its nature; in place of the single wave of negativity seen with the normal twitch, the response under eserine shows a series of waves in rapid succession, the second following the first at an interval of 5 msec. or less, and the others following at increasing intervals and with diminishing amplitude. The effect merits a more exact and detailed study than we have yet been able to give it, but its general nature can be seen in Figs. 12 and 13. The potentiated effects under eserine evidently

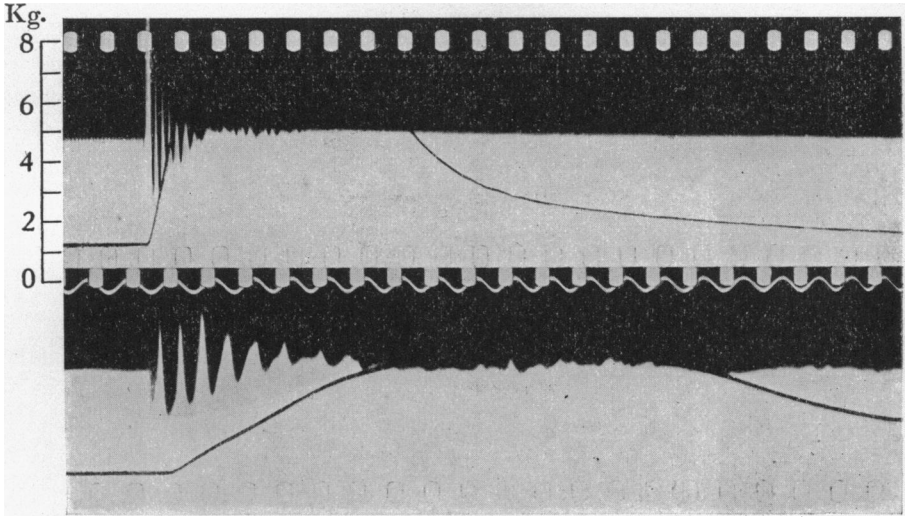

Fig. 13. Same experiment as Fig. 12. Upper record: as in Fig. 12, 14 min. after eserine, with higher amplification of action potential. Lower record: higher film speed; action potential with concentric electrode; 2 hours after eserine.

have the nature of a brief tetanus, composed of a series of diminishing responses. It is, in fact, the kind of effect which might be expected as the result of a continuous but rapidly diminishing stimulus to the muscle, causing it to give a series of responses, each at a later stage of the refractory period which follows its predecessor, until the stimulus falls below the threshold. We have seen that this effect is only produced on the response of the muscle to nerve stimulation, and there seem to be only two ways in which it could be produced. On the theory that the wave of physico-chemical disturbance constituting the nerve impulse is directly transmitted to the muscle fibre at its end-plate, the effect must be due to a repetitive response of the nerve, under the influence of eserine,

to a stimulus normally causing a single volley. So far, our electrical records from the nerve, during the potentiation of the muscle response by eserine, have given us no evidence of any change in its single reaction to a single shock. On the theory that the excitation is not transmitted from the nerve to the muscle as a continuous impulse, but by the stimulation of the muscle end-plate by acetylcholine, released by arrival of the impulse at the nerve ending, the effect of eserine appears to find a natural explanation, in terms of its known function as an inhibitor of the action of cholinesterase. It may, perhaps, be suggested that eserine causes a volley, single during its conduction in the part of the nerve accessible to electrical recording, to change into a series of diminishing volleys in the intramuscular part of the nerve. If the impulse were, indeed, repetitive on reaching the nerve ending, the response of the muscle could be accounted for on either theory. But such a supposition would have no relation to any known action of eserine, or to any known manner of conduction in nerve. The chemical transmission theory seems to be the only one which, in this case, can be related to facts already known.

Discussion

The evidence which we have presented shows that, when applied so as to obtain sufficiently rapid access to the muscle fibres, acetylcholine will regularly elicit a rapid contraction from normal mammalian muscle. Such a contraction is superficially not unlike the twitch elicited by a single volley of nerve impulses, and the dose required to produce it, with a tension similar to that of the maximal motor nerve twitch, has fallen, with improvement of our technique of injection, to the small dimensions of about 2γ. If clear evidence had been earlier available of this type of reaction of mammalian muscle to acetylcholine, we believe that the action of the latter as a chemical transmitter of excitation from nerve to muscle would have presented itself as a possibility, even before there was evidence of its release by nerve impulses at the motor nerve endings. It is still necessary, however, to consider whether the contractions now produced by its artificial application are yet sufficiently rapid, and whether the doses producing them are yet small enough, to entitle us to regard these effects as supporting the chemical transmission theory.

In the first place, it is true that the contractions obtained in response to arterial injections of acetylcholine are not so rapid as the twitch produced by a single nerve volley. As already indicated, however, they could not be expected to equal that rapidity. Assuming, as seems to be

proper, that such rapid contractions are due to excitation propagated from the motor end-plates, acetylcholine reaching these structures by diffusion from the blood vessels cannot excite them as synchronously as its sudden and simultaneous release, in direct contact with every end-plate, by a volley of nerve impulses. The best that can be expected, from a dose of acetylcholine artificially introduced, is an excitation of the end-plates in rapid succession. We shall have to consider later whether its effect, when so applied, would be to elicit a single or a repetitive response from each fibre. In any case, the fact that, with rises to the same tension, the contraction in response to an injection of acetylcholine takes about twice as long to reach that tension as does the motor nerve twitch, cannot be regarded as presenting any theoretical difficulty to the conception of chemical transmission. Superficially, the acetylcholine contraction has the appearance of a twitch, though a rather slow one. It is an entirely different phenomenon from the contracture elicited from the slow muscles of certain lower vertebrates, by immersion in solutions containing acetylcholine.

In considering the size of the dose necessary to elicit such contractions we are faced with an apparent discrepancy. The smallest injection with which, under the best conditions yet attained, we have produced a contraction with a tension comparable to that of a maximal nerve twitch, is about 2γ. On the other hand, the largest amount of acetylcholine collected by Dale *et al.* [1936] from a similar muscle, during stimulation of its nerve with maximal shocks, corresponded only to one-hundredth of that dose—viz. about $0{\cdot}02\gamma$ per shock. It must be borne in mind, however, that the whole of the acetylcholine liberated by the nerve impulses would impinge directly on the muscle end-plates, while a dose injected into the blood vessels would diffuse from them into the fluid of the interstitial spaces, so that only a small fraction of it would reach the end-plates. We have shown, again, that speed of injection, as well as dose and concentration, has a pronounced effect on the strength of the contractile response; it must be supposed, therefore, that the rate of increase of the concentration of acetylcholine up to threshold value, in contact with the end-plate, is one of the determinant factors in the stimulant effect. A much smaller dose will obviously be effective in producing a given rate of such increase, if it is liberated on the spot into a minute volume of fluid, than if it has to reach the site of action by diffusion through the tissue fluids. It must further be noted that the figures given above represent quantities actually injected and collected; and while, as already indicated, only a small fraction of the amount

injected can arrive with effective rapidity at the site of stimulation, it is also certain that less than the whole of the amount liberated by nerve impulses reaches the vein for collection, and probably much less. For these various reasons, we conclude that the apparent discrepancy, between the quantities of artificially injected and naturally liberated acetylcholine associated with the occurrence of equivalent motor responses, has no real significance, and creates no difficulty for the conception of acetylcholine as the chemical transmitter.

We have shown that somewhat larger injections of acetylcholine, such as 10–20γ, produce apparently single contractions with relatively very high tensions. Simonart [1935 *b*], using distant arterial injections with uninterrupted circulation, recorded contractions comparable in tension to short tetani. He was dealing, however, with relatively very large doses, reaching the muscle at unknown rates, and his records do not suggest a single contraction of the muscle. We are concerned with the rapid, strictly localized injection of doses only from 2 to 10 times that required to match the maximal nerve twitch in tension. Such an injection often produces a contraction with a much greater tension than the twitch, even three times as great, but still with the time-relations of a slow twitch, and not of a contracture or a prolonged tetanus. If the all-or-none principle is valid for such responses to the direct application of a chemical stimulant, we can only explain these high tensions by the summation of repetitive responses of the muscle fibres. According to Asmussen [1934] the all-or-none principle does not apply to a muscle fibre when directly stimulated, but only to its indirect stimulation through its motor end-plate, where the arrival of a nerve impulse produces a constant response, but not the greatest of which the fibre is capable. It does not seem to be certain whether on this evidence, interpreted in terms of chemical transmission, we should picture the nerve impulse as releasing a constant but submaximal dose of acetylcholine, and the muscle fibre as capable of giving a stronger single twitch in response to the application of a stronger dose to its end-plate; or whether the indirect twitch represents the maximum single response which the muscle can give to any stimulation of its end-plate. In any case, a larger response of the fibre to an injection of acetylcholine cannot properly be assumed to be single, without definite evidence that it is not, in fact, multiple. We are endeavouring to obtain evidence on the point by recording the accompanying electrical changes, though we realize that the lack of synchronism in the responses of individual fibres, even within the limits of a single motor unit, will create unusual difficulties. An

indirect suggestion of a probable multiplicity is afforded, however, by our evidence concerning the nature of the potentiated response to a maximal nerve volley under the influence of a small dose of eserine. In this case we have already definite evidence that the potentiation is due to a repetitive response of the muscle to the single volley, producing a contraction which must be of the nature of a very brief tetanus. If this effect, as we suggest, is due to the persistent presence of acetylcholine in supraliminal concentration at the end-plates, it seems natural to suppose that a similar persistence, produced by an artificial injection, would similarly cause a repetitive response of the muscle fibres, though with such a lack of synchronism as to make its nature more difficult to demonstrate. This seems the most likely interpretation of the high tension responses to injections of 10–20γ. The mechanical records of these responses, however, have the same appearance, resembling rather slow twitches, as the contractions evoked by smaller injections, with tensions similar to, or lower than, that of the single nerve twitch. There is no sign of any change in the type of the response, from the weakest to the strongest so obtained, but simply an increase in the tension produced, and a concomitant increase in the time taken to attain it and to relax from it. It seems very probable, therefore, that any conclusion as to the nature of the stronger acetylcholine contractions will apply also to the weaker, and that these latter also involve some degree of repetitive response by individual fibres. On this view, any contraction produced by injection of acetylcholine would differ in type from a single nerve twitch which it might superficially resemble, and would have the nature, rather, of a short, asynchronous tetanus[1]. As already pointed out, only a part of the dose injected will reach the motor end-plates rapidly, and much of it will remain for a brief period in the interstitial tissue fluid, until destroyed by esterase. During that interval it may be supposed that it will continue to reinforce the concentration at the end-plates, maintaining it at a supraliminal level, and thus causing repetitive responses of the muscle fibres.

This last suggestion implies a further and very important assumption, namely, that, without such reinforcement, the injected acetylcholine would disappear more rapidly from the neighbourhood of the nerve endings than from the general body of the muscle, presumably on account of a local concentration of the generally distributed cholinesterase. If evidence of this could be produced, it would not only confirm the

[1] *Note added in proof.* Electrical records, which one of us (G. L. B.) has now obtained, prove that this deduction is correct.

suggested mode of action of acetylcholine when artificially injected; it would also meet one of the chief difficulties which the theory of chemical transmission encounters, when applied to the excitation of ganglion cells and voluntary muscle fibres. This difficulty is due to the brief refractory periods of these quickly reacting cells. We have suggested that a persistent supraliminal concentration of acetylcholine at the end-plates will produce a repetitive response of the muscle. The fact that a single motor nerve impulse evokes only a single response of the muscle fibre would accordingly entail, on the chemical theory, a fall in the local concentration of acetylcholine to a subliminal value within the very brief limits of the refractory period. The only conception which would provide for such a rapid disappearance, on the basis of present knowledge, would be a local concentration at the nerve ending, on surfaces in relation to which acetylcholine is liberated by the nerve impulse, of the widely distributed cholinesterase, which even in weak solution destroys acetylcholine with such remarkable rapidity. If the mechanism required for the removal of acetylcholine during the refractory period were, indeed, of this nature, it would be expected that eserine, by weakening the action of the esterase and thus delaying the removal, would alter the effect of the nerve impulse, and probably cause a repetitive response. This assumption, of a repetitive response by the voluntary muscle fibre to a continuous stimulus applied to its end-plate, does not appear to be supported by any exact analogy from previous knowledge. It seems to be necessary, however, to account for the effects both of injected acetylcholine and of the nerve impulse under eserine. In earlier papers from this laboratory [Feldberg & Vartiainen, 1934; Dale *et al.*, 1936] it has been argued that failure to obtain decisive evidence of such an effect of eserine, either in the case of the ganglion or the voluntary muscle, could not be regarded as necessarily fatal to the conception of acetylcholine as the transmitter of excitation, as Eccles has suggested: it might signify no more than the failure of a particular form of experiment to demonstrate the existence of one possible mechanism, admittedly the most obvious one, for the rapid removal of acetylcholine from the site of action. The results which we have now obtained with small doses of eserine change the position completely as regards the mammalian voluntary muscle. We have there observed, with great regularity, exactly the effects to be expected, on the conception of acetylcholine as the transmitter and cholinesterase as the agent for its rapid removal. A single nerve volley, when eserine is present, causes not a single, but a repetitive response of the muscle, the successive responses declining in the manner

to be expected if the acetylcholine liberated by the impulse disappeared at a retarded rate. The details of the effect have yet to be critically examined, and considered in relation to the theory. At present it is already established that eserine, in the doses employed, has no similar effect on the response of the nerve to an induction shock, or on that of the denervated or curarized muscle to direct stimulation. The repetitive response to a single nerve impulse is therefore due to a modification, by eserine, of the transmission of the excitation from nerve ending to muscle fibre.

The readiness with which this action of eserine can be demonstrated in the case of the mammalian voluntary muscle, suggests that it may be worth while to examine yet further, by different methods, its action on the ganglion. In this case, however, there are two complicating actions which are, at least, less obvious in the case of the muscle—the depressant effect on ganglion cells of eserine itself in still weak concentrations, and the secondary depressant effect of acetylcholine, persisting in the ganglion after its stimulating action is ended. This latter effect is clearly perceptible in the muscle also, when acetylcholine is given by injection during the action of eserine. It appears to be due to the persistence of acetylcholine in the muscle outside the nerve endings; for when, under eserine, the muscle is responding regularly to successive nerve volleys by maximally potentiated contractions, an injection of acetylcholine, even in a dose causing a much smaller contraction, is followed by a depression of the response to the after-coming volley. The prominence and persistence of this depressant effect of injected acetylcholine in the perfused muscle, in which stagnant fluid easily accumulates in the interstitial tissue, and the fact that under these conditions it can be produced by an injection of acetylcholine even without eserine, are further in favour of the view that it is due to action on the muscle fibre as a whole, and not on its end-plate. Such facts, again, are in harmony with the conception of the cholinesterase as concentrated at the nerve endings, and as more weakly represented in the rest of the muscle, where eserine will more completely suppress its action, and where acetylcholine will more readily persist. Briscoe [1936] and Cowan [1936] have described phenomena which recall the Wedensky inhibition, when rapid rhythmic stimulation is applied, at a rate normally producing a maintained tetanus, to the nerve of a muscle under eserine; and it is tempting to speculate whether these effects may not be due to the escape of acetylcholine, accumulating at the nerve endings under those conditions, into the general tissue spaces of the muscle. The phenomena have certainly a suggestive similarity to those described in the ganglion by Brown &

Feldberg [1936]. There are, however, many of the phenomena of the excitation of muscle through its nerve, the interpretation of which will need to be reconsidered in the light of a theory of chemical transmission, if this should eventually win acceptance. In particular, the reactions of the voluntary muscles of different vertebrate types, with their different kinds of nerve ending and different forms of reaction to acetylcholine, may require separate consideration. We are here dealing only with the transmission of excitation from nerve to muscle in the mammal, and the observations presented in this paper, with those described in the foregoing paper by Dale *et al.* [1936], seem to us to be compatible with a form of chemical transmission, in which the direct stimulant of the muscle fibre at its end-plate, acetylcholine, is liberated by arrival of the nerve impulses at the nerve ending, and destroyed during the refractory period by a local concentration of cholinesterase. These facts are: (1) that acetylcholine, identified as such, is liberated by impulses at motor nerve endings; (2) that acetylcholine, when suitably injected into the muscle, produces the kind of contraction which the transmitter should produce; and (3) that a suitable dose of eserine causes the muscle to give a short, waning, tetanic response to a single, synchronous volley of nerve impulses.

Summary

1. Acetylcholine, when injected, with adequate rapidity, directly into the empty arteries of a normal mammalian muscle, causes contraction of the muscle at not less than half the speed of a maximal motor nerve twitch. An injection of 2γ will cause a contraction with a tension similar to that of such a twitch, and an injection of 10 or 20γ one of several times that tension.

2. This quick response of normal mammalian muscle, to injected acetylcholine, is abolished by curarine even more readily than its response to nerve impulses. It is not affected by atropine, in doses which annul the "muscarine" action of acetylcholine.

3. In the spinal cat, a moderate intravenous dose of eserine (0·2–0·3 mg. per kg.) causes the response to a maximal nerve volley to change from a simple twitch to a repetitive response of the nature of an evanescent tetanus, with a maximum tension twice that of the normal twitch, or even greater. Eserine has no effect on the twitches given by the denervated or curarized muscle, in response to directly applied induction shocks.

4. It is suggested that these observations are compatible with a particular method of chemical transmission of excitation, from nerve to voluntary muscle, in the mammal.

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