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A STUDY OF FOETAL AND NEW-BORN RAT MUSCLE FIBRES

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Normal adult, skeletal muscle fibres are sensitive to acetylcholine (ACh) only in the region of the neuromuscular junction and its immediate surroundings (Miledi, 1960*a*); but after dividing the motor nerve, the chemosensitive area spreads from the end-plate and eventually covers the whole fibre surface (Ginetzinsky & Shamarina, 1942; Axelsson & Thesleff, 1959; Miledi, 1960*b*).

Conversely, if the motor nerve fibres are allowed to regenerate into a denervated muscle whose fibres are sensitive on their entire length, the chemo-sensitive region then shrinks progressively and becomes again restricted to the junctional region (Miledi, 1960*c*). It is natural to wonder if a similar picture would obtain during ontogenetic development. Namely, are freshly innervated foetal muscle fibres sensitive to ACh over their entire length, and does the ACh-sensitive region then shrink in the same way as it does in reinnervated adult fibres?

In the summer of 1959 experiments were made to test these possibilities. It was found that even in 17-day rat foetuses most of the fibres already showed evidence of being innervated; and were sensitive to ACh over their whole length. A brief account of these results was published (Diamond & Miledi, 1959); and several months later a relevant paper was brought to our attention. We had it translated and found that Ginetzinsky & Shamarina, working in Leningrad about 20 years ago, had clearly shown the spread of ACh sensitivity which occurs after denervation. Furthermore, they also conducted some experiments on new-born rabbits and found that 2–5 days after birth some muscle fibres were sensitive to ACh along their entire length (see also p. 405). In view of this we hesitated to publish a more detailed account of our experiments. We do so now, to confirm the relatively unknown work of Ginetzinsky & Shamarina (1942), many copies of which, we are led to believe, lie in a sunken ship.

METHODS

Pregnant albino rats (local strain of Glaxo origin) were anaesthetized with ether and their foetuses removed. After anaesthetizing the foetuses their complete diaphragm muscle was isolated and mounted for intracellular recording as described by Krnjević & Miledi (1958*a*).

In the case of the younger foetuses the following procedure simplified the isolation and mounting of the delicate muscle. The abdomen was emptied through a longitudinal mid-line incision, and a slightly domed, cylindrical Perspex rod was pushed into the abdomen pressing lightly with the dome against the diaphragm. A thread was passed under the skin around the abdominal muscles and tied firmly against the rod. Everything else was then removed, leaving the diaphragm mounted (thoracic side upwards) on the rod which was transferred to the muscle chamber. The polished Perspex rod served, also, to trans-illuminate the muscle.

The muscles were kept at room temperature. The bathing solution was that described by Liley (1956) and was oxygenated by bubbling it directly with 95 % O_2 5 % CO_2 gas mixture. ACh was applied iontophoretically through a micro-pipette (del Castillo & Katz, 1955; Miledi, 1960*b*).

Gestation period. Vaginal smears were taken and if the rat was found to be in oestrus the male was allowed in the cage for about 12 hr. Counting from the end of this period, the rats were born 21–22 days later, usually 22 days. There was considerable variation of size and development of individual foetuses in any particular litter; but no attempt was made to correlate these variations with the state of the neuromuscular system.

RESULTS

Although our main objective was to see if foetal fibres were sensitive to ACh, some other points emerged during the course of the experiments. These will be considered first because they provide pertinent information on the state of development of the neuromuscular apparatus. It should be mentioned at the onset that we used muscles from 17- to 22-day-old foetuses, and that in these preparations stimulation of the phrenic nerve resulted in muscle action potentials and contractions.

Spontaneous electrical activity

While recording intracellularly from foetal fibres it was noticed that they exhibited spontaneous subthreshold depolarizations. These potentials resemble in many respects those which are continuously being produced in adult muscle by ACh, released randomly from normal nerve terminals (see review by Katz, 1958). For instance, they also appear at random and are abolished by curare; so presumably they are similarly produced by ACh. In view of these and other analogies which will emerge later, they shall be referred to as miniature end-plate potentials (m.e.p.p.s) as in the adult.

Rather unexpectedly, m.e.p.p.s could be recorded from anywhere in foetal muscles. Even three days after birth, when the length of the fibres was 4–5 mm, m.e.p.p.s. could still be detected by a micro-electrode

inserted near either end. This finding raised the possibility that the potentials were being produced all along the fibres. Although in the adult diaphragm muscle the end-plates are situated near the middle of the fibres, it could be that originally the muscle fibres were innervated at several places and that later in development all but one of the junctions disappeared.

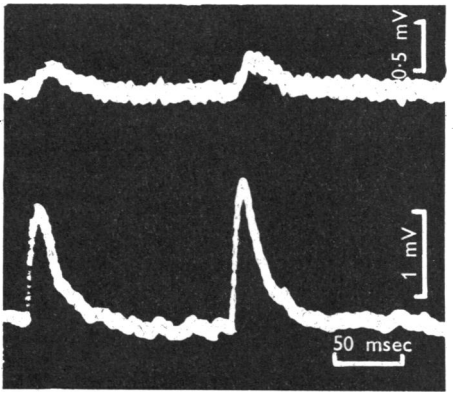

Fig. 1. Simultaneous intracellular record of spontaneous potentials at two points in the same muscle fibre. Top trace at 0·4 mm and lower trace at 1·6 mm from the central tendon end of the fibre. Total length of fibre *ca.* 4 mm. Sucrose (10%) added to raise tonicity of the fluid and increase frequency of m.e.p.p.s (see text). Rat diaphragm 1 day after birth.

To determine the site of origin of m.e.p.p.s, simultaneous records were made with two intracellular micro-electrodes in the same fibre, one in the middle of the fibre and the other near its end. In both foetal and new-born muscles the potentials were always larger, and faster in rise time, near the middle of the fibre (Fig. 1). This would be expected if that were the site where m.e.p.p.s are produced, the potentials recorded near the tendon being merely due to electrotonic spread from that site. Furthermore, on several occasions m.e.p.p.s were recorded with an extracellular micro-electrode placed near the middle of foetal fibres (Fig. 2) but not elsewhere on the fibre, and it has been shown by del Castillo & Katz (1956) that extracellular m.e.p.p.s are only detected very near their site of origin. Prodding this region with the micro-electrode sometimes increased the frequency of the potentials, presumably by damage to the nerve terminal.

Frequency of m.e.p.p.s

The most striking differences between 'adult' and 'foetal' m.e.p.p.s is their rate of occurrence, the frequency in foetal fibres being much slower.

This is illustrated in Fig. 3 which is a simultaneous record of potentials in an 'end-plate' from a foetus and in an end-plate from its mother.

M.e.p.p.s were seen in the majority of fibres of all foetal muscles examined; but some fibres were continuously observed for up to 10 min without detecting one m.e.p.p. We are unable to say whether these were merely cases of frequencies lower than 1 in 10 min or whether m.e.p.p.s did

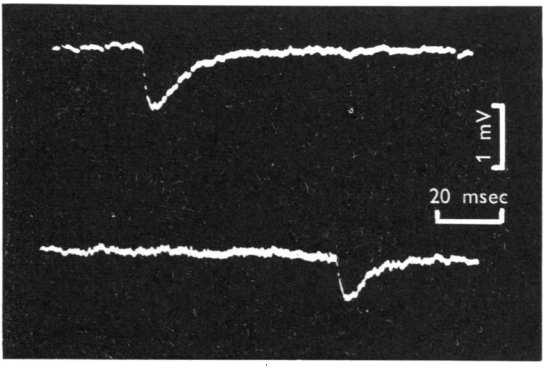

Fig. 2. Extracellular m.e.p.p.s recorded near the middle of a foetal muscle fibre (20 days). Length of fibre *ca.* 4 mm. In presence of sucrose (15%).

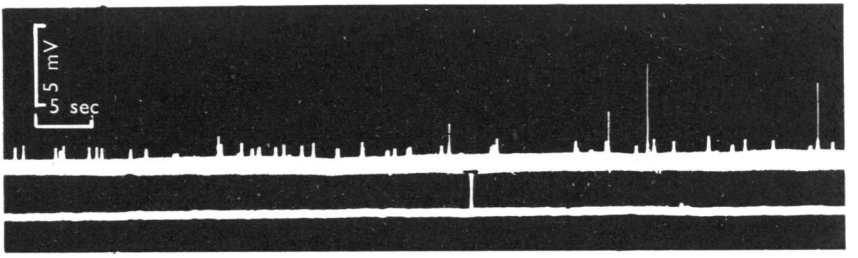

Fig. 3. Simultaneous recording of m.e.p.p.s from a muscle fibre of a 21-day foetus (bottom trace) and from an end-plate in the diaphragm of its mother (top trace); both muscles mounted in the same bath. Notice the difference in frequency. No sucrose added.

not occur at all in these fibres. There was considerable dispersion in the m.e.p.p. frequencies in different fibres of the same muscle; so comparison between muscles from different individuals is difficult—especially since one is dealing with transient random events of low frequency. Nevertheless, the frequencies measured at different ages are pooled in Fig. 4 with the cautionary note that in some cases the period of observation was only 2–3 min and in consequence only one or two m.e.p.p.s were observed. In spite of these shortcomings it appears that the frequency increases

slowly after birth; and that there is about a hundredfold difference in the frequency of foetal and adult m.e.p.p.s.

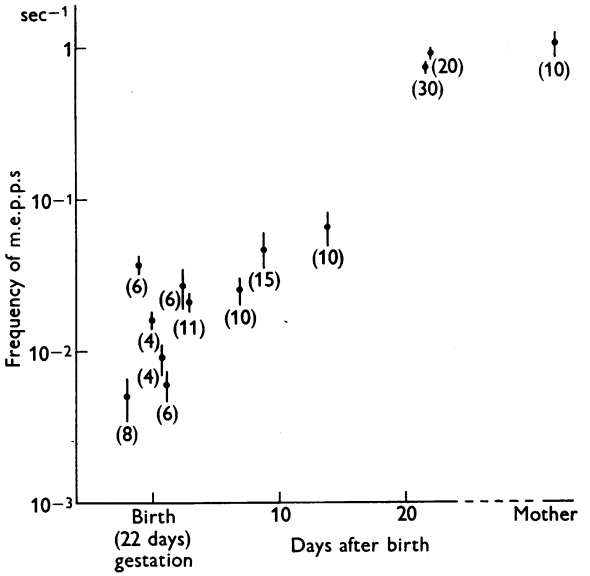

Fig. 4. Frequency of m.e.p.p.s in muscle fibres of rats at different ages. Mean ± S.E. of mean. In brackets, number of fibres examined. Two rats from the same litter were used at 22 days.

Effect of hypertonic solution on m.e.p.p. frequency

It has been shown by Katz & Miledi (1959) that low-frequency m.e.p.p.s can be detected in frog muscle after the motor nerve fibres have degenerated. In this case the ACh packages responsible for the m.e.p.p.s appear to be released from Schwann cells; and hypertonic solutions, which greatly increase the rate of release from nerve endings, have no effect or may even reduce the frequency of m.e.p.p.s in denervated muscle (Birks, Katz & Miledi, 1960). It was therefore desirable to find out if the effect of hypertonic solutions on m.e.p.p.s in foetal muscle conformed to one of these patterns.

Doubling or trebling, with sucrose, the tonicity of the solution bathing foetal (and new-born) muscles consistently increased the frequency of the m.e.p.p.s 100–1000 times. This was a useful finding, because it simplified the study of the site of origin, time course, etc., of the m.e.p.p.s.

Time course of miniature potentials

Foetal m.e.p.p.s had a slower time course than their adult counterpart: both rise-time (2–10 msec) and decay were longer (Fig. 5). The slower time course of foetal m.e.p.p.s was also evident when they were recorded

externally. For instance, the mean rise-time of nine potentials in the fibre of Fig. 2 was $3{\cdot}2 \pm 0{\cdot}1$ msec while in the adult the rising phase of m.e.p.p.s recorded extracellularly lasts only about 1 msec.

It is likely that the slower time course of miniature potentials in foetal fibres is mainly due to a protracted action of ACh, since it has been reported that the concentration of cholinesterase at the neuromuscular junction does not attain its adult level until some weeks after birth (Leibson, 1939; Kupfer & Koelle, 1951; Beckett & Bourne, 1958), but different electrical constants of the mucle fibre membrane could also be a contributing factor.

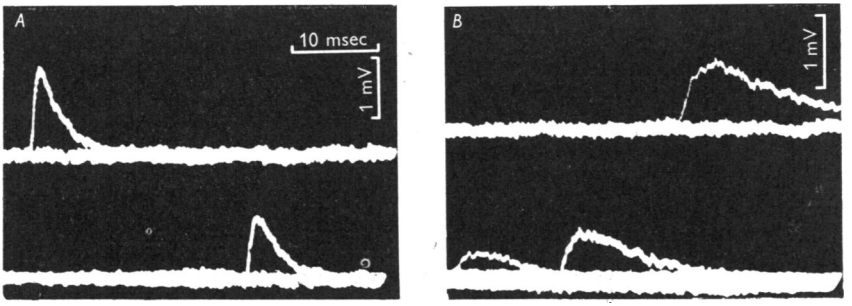

Fig. 5. Time course of m.e.p.p.s. *A*, from a fibre 22 days after birth. *B*, from a foetal muscle fibre (21 days); 10% sucrose was added to the fluid bathing the foetal muscle.

Size of m.e.p.p.s

Examples of the amplitude distribution of m.e.p.p.s in four fibres from muscles at different stages of development are shown in Fig. 6. There was a considerable variation in the mean size of potentials in different fibres, partly due to difficulties in obtaining 'clean' insertions with steady resting potentials. But m.e.p.p.s were usually larger in muscle fibres from foetuses and newly born rats than in the adult. Katz & Thesleff (1957) have shown that the amplitude of the m.e.p.p.s is inversely related to the diameter of the muscle fibre ($d^{\frac{1}{2}}$). It is therefore not surprising to find potentials of larger amplitude in foetal fibres, since these have a diameter of only a few microns, whereas adult fibres have diameters of 25–80μ (Krnjević & Miledi, 1958*b*). Another factor which would contribute to the increased size of m.e.p.p.s in foetal muscle is the relative lack of cholinesterase mentioned in the preceding paragraph. These considerations are important, because they indicate that in general the size of the quanta of ACh released from developing nerve fibres is not grossly different from that of mature endings. That is to say, the mean number of ACh molecules in a package is much the same, and the potentials only appear larger because of post-synaptic effects.

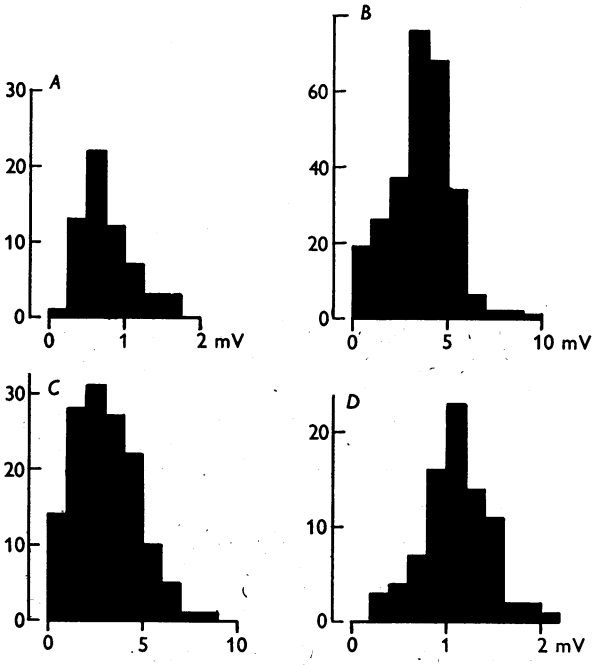

Fig. 6. Amplitude distribution of m.e.p.p.s in diaphragm muscle fibres of rats at different ages. *A*, from a 20-day foetus. *B*, *C* and *D* after birth; 1, 14 and 22 days, respectively. *B* was obtained in the presence of 10% sucrose.

Spontaneous mechanical activity

A considerable number of spontaneous contractions were observed in most foetal diaphragms of all ages examined; and the same was true of some muscles from animals up to about 1 week after birth. These contractions were accompanied by muscle fibre action potentials which had amplitudes of up to 80 mV and overshot the resting potential by 10–20 mV.

As seen through the microscope this activity consisted of a variety of events. Single fibres could be seen twitching regularly at frequencies of 1/sec or less for periods of many minutes, while other fibres in the same muscle were twitching irregularly. There were also contractions involving synchronously a group of fibres, which probably did not correspond to the same motor unit, for they lay close together in contrast to the widespread distribution of fibres belonging to a single motor unit (cf. Krnjević & Miledi, 1958*b*). Contractions of this type are commonly seen in tissue cultures of skeletal muscle, where at times all or some of the fibres in the explant appear to 'beat' in unison (Szepsenwol, 1946; Pogogeff & Murray, 1946). In other cases contractions were probably due to motor-unit activity, presumably arising from a nerve impulse of unknown origin.

The mechanism initiating these contractions is not fully understood. Occasionally a large m.e.p.p. triggered an action potential, especially in hypertonic solutions when the frequency of m.e.p.p.s was increased. But it is obvious that the infrequent, random m.e.p.p.s cannot, alone, account for the regular contractions described above. D-tubocurarine (10^{-6} to 10^{-5} g/ml.) was tried in twelve different foetal muscles (ages 18–22 days) and in all cases the spontaneous mechanical activity was greatly reduced or abolished (cf. Fig. 7) which indicates that ACh is at least partly responsible for it.

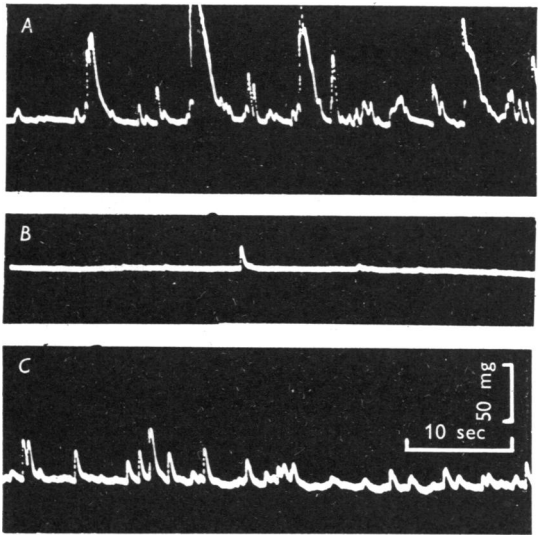

Fig. 7. Effect of curare on spontaneous contractions of a foetal rat diaphragm (21 days). *A*, control. *B*, 5 min after adding curarine to bath solution (1×10^{-6} g/ml.). *C*, 1 min after washing out curare.

Sensitivity to ACh

Before birth. Iontophoretic application of ACh to any surface spot on foetal muscle fibres produced a depolarization (Fig. 8). It was merely necessary to lower the pipette until it just touched the muscle fibre, when, on passing a pulse of current through the pipette, an ACh response was invariably obtained: there was no need to search for the end-plate region, as is the case with normal adult fibres.

General ACh sensitivity of the muscle was observed in foetuses which ranged in age from 17 to 22 days. The length of the muscle fibres in the diaphragm varied from about 2 to 3 mm in young ones to 5 mm in those more mature; and one had, therefore, to be certain that the whole muscle fibre surface was sensitive to ACh and that responses were not simply produced by ACh diffusing to a single sensitive region. This was done in

two ways. First, ACh was released at points several hundred microns apart along individual muscle fibres, and at each position a similar dose was ejected at a distance some 50–100 μ sideways from the fibre in question. A depolarization was recorded only when the ACh was applied to the surface of the fibre being examined. A second and more decisive test is the one used to demonstrate the presence of ACh receptors just outside the junctional region of normal adult muscle fibres (cf. Miledi, 1960*a*). Potentials were recorded simultaneously from two points in the same muscle fibre,

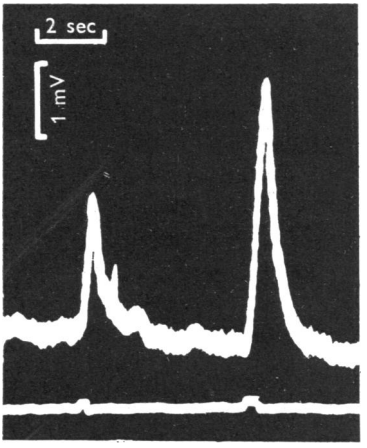

Fig. 8. Potentials produced by ACh applied iontophoretically to a foetal muscle fibre (21 days) at a spot 0·25 mm from the central tendon. The voltage calibration represents $1{\cdot}5 \times 10^{-8}$ A for the lower trace, which monitors the current through the ACh pipette.

one near the central tendon, and one near the middle of the fibre. ACh was then applied to different spots along the fibre and the ensuing depolarization appeared greater on the nearest electrode. If the ACh, wherever released, were acting by diffusion, for instance, to the primitive neuromuscular junction near the middle of the fibre, then the ACh potential should have always appeared greater on the electrode inserted near the middle of the fibre. It may then be concluded, that the ACh was acting mainly at the spots where it was delivered and that the entire muscle fibre membrane was sensitive to ACh.

Although the whole muscle was sensitive to ACh, the ACh sensitivity along a fibre was not evenly distributed. It tended to be greatest near the middle of the fibre and least near its ends. This was particularly clear in some foetuses near term, where the terminal 50–100 μ of a few fibres appeared to be insensitive to iontophoretic pulses of ACh. These same fibres had m.e.p.p.s at frequencies of only 0·005–0·03/sec, while other fibres in

the same muscles were sensitive along their entire length and had m.e.p.p.s at rates up to 0·6/sec.

It would be interesting to determine the level of ACh sensitivity of muscle fibres at different times in foetal life, but it was difficult to compare sensitivities of fibres from different individuals. It was also difficult—especially in the younger foetuses—to ascertain the relative sensitivity of different spots along single muscle fibres. The main reasons for this were that foetal muscle fibres are only a few μ in diameter and difficult to impale with the micro-electrode, and that the muscles showed spontaneous contractions. These factors resulted in low, unsteady and declining resting potentials. A further complication was that ACh produced contractures quite readily, disturbing the recording electrode even more.

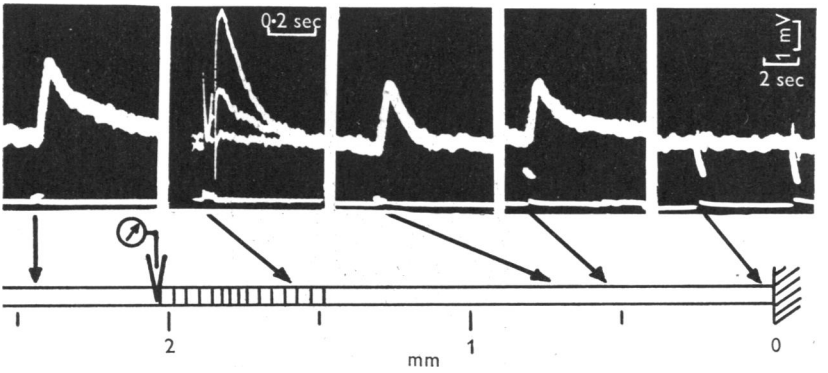

Fig. 9. Potentials produced by ACh along a fibre in the diaphragm of a rat, $2\frac{1}{2}$ days after birth. Lower diagram illustrates site of intracellular recording electrode and positions at which ACh was released. Distance is measured from the central tendon end of the fibre, shown at right. For comparison the ACh-sensitive length in an adult end-plate is represented by vertical stippling. Total length of muscle fibre *ca.* 4·5 mm. Frequency of m.e.p.p.s in this fibre was 0·005/sec.

After birth. During the first 3 days after birth, the ACh sensitivity near the ends of the fibres continued to fall, but the over-all picture is not very different from that of mature foetal fibres; i.e. some fibres are sensitive over their whole length, while in others ACh had no effect when applied within 0·2 mm from the tendon (see Fig. 9). The local distribution of ACh sensitivity expressed as the ratio of depolarization to the coulombs which released the ACh (Miledi, 1960*b*) is illustrated in Fig. 10, and shows a peak near the site of origin of m.e.p.p.s. This peaked spatial distribution of sensitivity resembles that described previously for normal adult end-plate regions, except that its gradients are less steep (cf. Miledi, 1960*a*, 1962).

One week after birth the total length (5–6 mm) of a few muscle fibres was still sensitive to ACh, but in most fibres the sensitivity had fallen to an undetectable level 0·2–0·3 mm from either tendon. As development progressed the chemo-sensitive properties of the membrane continued to recede from the ends towards the site of the neuromuscular junction. For instance, 9 days after birth, the sensitive length of eight fibres was about

1 mm, the length of the muscle fibres being 6–7 mm, while the frequency of m.e.p.p.s in these and other fibres in the muscle was still quite low (cf. Fig. 4).

About 2 weeks after birth the muscle fibres attain their adult chemosensitive length and its size does not alter much with further development although the total length of the fibres still trebles.

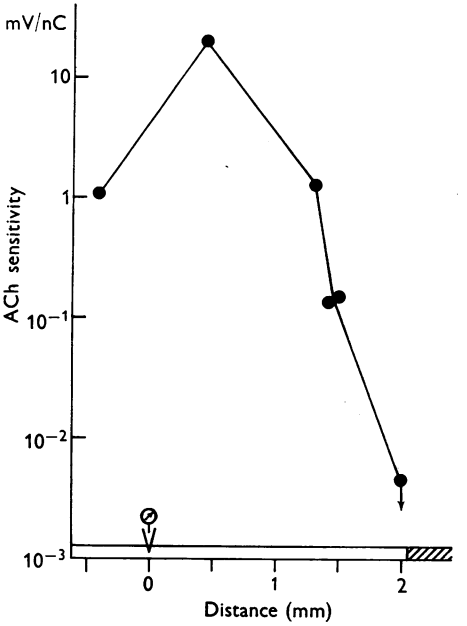

Fig. 10. Distribution of ACh sensitivity along the fibre shown in Fig. 9. Abscissae, distance from recording electrode. Ordinates, ACh sensitivity expressed as ratio of depolarization (10^{-3} V) to coulombs (10^{-9} C) which release the ACh.

DISCUSSION

Our experiments have given decisive electrophysiological evidence that effective nerve–muscle contact has occurred in most fibres as early as the 17th day of foetal life. However, there seems to be no agreement in the histological literature as to the time of development at which nerve fibres are first seen to make contact with muscle fibres. Cuajunco (1942) believes that contact is made at a time when muscle nuclei are still centrally placed. On the other hand Tello (1917) and Couteaux (1941, 1960), after a careful study of the development of neuromuscular connexions, conclude that the junction is formed somewhat later, when the nuclei have migrated to their peripheral position. In either case neuromuscular contact would be expected to have taken place in the diaphragm muscles which we

studied because nuclear migration presumably occurs earlier than the 17th day of gestation.

Surprisingly enough Sitaramayya (1951) could find no evidence of innervation in the diaphragm of rat foetuses up to 21 days; and Kupfer & Koelle (1951) state that in the forelimb of foetal rats 'up to and including the 20th day of intra-uterine life, no motor endings of any form were found within the muscle cell'. The latter authors were aware that movement of the limbs first appears in the 15- to 16-day-old foetus (East, 1931; Angulo y González, 1932), and that at this stage muscle contraction may be produced by stimulation of the nerves (Straus & Weddell, 1940). To explain this, Kupfer & Koelle suggest that ACh may be released by the nerve fibres from where it would diffuse to the muscle fibres causing them to contract.

As mentioned above, stimulation of the phrenic nerve in the foetuses elicited a contraction of the muscle. It might be thought that this is a consequence of a long-range action of ACh. But in foetal muscle fibres we found m.e.p.p.s which are essentially like those of adult end-plates; and which, like these, are presumably produced by ACh spontaneously released, at close quarters, from nerve fibres. Therefore, it seems very likely that there was *synaptic* contact between nerve and muscle in most of the foetal fibres examined. It is true that foetal m.e.p.p.s have a slower time course, but this is very probably related more to a low level of cholinesterase and other factors than to a greater distance between the source of ACh and the receptors. In view of these findings, it would be interesting to re-examine neuromuscular connexions in foetal muscle at both the light and electron-microscope level.

The frequency of m.e.p.p.s in foetal fibres is much lower than in the adult. It could be that synthesis, and storage, of ACh in foetal nerve fibres are limiting the release of ACh; but this is probably not the case because the release can be increased by the nerve impulse, by mechanical irritation of the ending and by hypertonic solutions. It is conceivable that the progressive increase in m.e.p.p. frequency during development of the neuromuscular junction is related to an increase in the area of nerve ending which is in contact with the muscle fibre.

In rat muscle, cholinesterase first becomes detectable in the 16–17th day of gestation (Kupfer & Koelle, 1951; Zelená & Szentágothai, 1957). Since our results indicate that nerve fibres are already in junctional contact with muscle fibres before the 17th day, it would appear that cholinesterase is formed mainly as a result of neural activity. This agrees with the work of Lewis & Hughes (1960), who find that motor nerves of tadpole muscles arrive either before or at the same time as the first evident signs of cholinesterase. Lewis & Hughes (1957) suggested that the enzyme is

synthesized in the perikaryon and reaches the neuromuscular junction by flow along the axon. Another, rather attractive, hypothesis would be that the ACh released by the nerve induces the formation of cholinesterase in the muscle cell through a mechanism analogous to that of adaptive enzyme formation in other systems (Monod & Cohn, 1952).

As regards the sensitivity to ACh in new-born muscle, our results confirm the earlier work of Ginetzinsky & Shamarina (1942). Using glass pipettes (25–50 μ tip diameter) filled with ACh, they applied small droplets to muscle fibres while through a microscope they looked for signs of contraction. In this way they demonstrated that denervation of the gracilis and semi-membranosus muscles of white mice leads to a progressive increase in the length of fibre sensitive to ACh; and that 2 weeks after section of the nerve the entire fibre reacted to ACh. They then proceeded to examine the semi-membranosus and gracilis muscles of 2- to 10-day-old rabbits and found that 2–5 days after birth the total length of some fibres reacted to ACh, and that the ACh-reactive zone diminished progressively with time after birth.

We have further shown that the entire surface of rat *foetal* muscle fibres is sensitive to ACh. Most, possibly even all, the fibres studied were already innervated; but we presume neuromuscular contact had been established not long before they were tested. In its chemo-sensitivity embryonic muscle fibres were in a state similar to that of reinnervated muscle recently described (Miledi, 1960*c*). In both cases it seems that the sensitivity of the muscle fibre becomes gradually restricted to the end-plate region, after neuromuscular contact has been established. The analogy is close, and strongly suggests that embryonic muscle fibres have general sensitivity to ACh before they receive their motor nerves, and that the effect of innervation is to produce a drastic reduction of sensitivity outside the neuromuscular junction.

What effect the nerve has on the sensitivity of the developing *junctional* muscle membrane we do not know. The maximal sensitivity observed in foetal fibres was smaller than that of the most sensitive spots at adult end-plates, and the values tended to be higher with increasing time after birth; but all this could merely be due to technical difficulties (cf. p. 402) which are reduced as the tissue grows.

Although the control of ACh sensitivity appears to be independent of nerve impulses (Miledi, 1960*c*), it might still depend on the spontaneous release of transmitter. For example, the action of ACh associated with the m.e.p.p.s in foetal muscle and in regenerating frog junctions might be responsible for the reduction of the chemo-sensitive area. This view is taken by Thesleff (1960) who finds that during botulinum intoxication the whole muscle fibre becomes sensitive to ACh when m.e.p.p.s are reduced to

frequencies lower than 0·1/sec. But this relation does not seem to hold generally. Thus, in some foetal fibres the ACh sensitivity had receded from the tendon ends, although the frequency of m.e.p.p.s was still below 0·1/sec. Furthermore, Birks *et al.* (1960) and Miledi (1960*b*) have shown that denervated frog muscle fibres have m.e.p.p.s—in some cases at frequencies which overlap the lower range in normal fibres—and yet they become super-sensitive to ACh. Finally, the sensitive length was found to return to normal in a few regenerating junctions before an increase in the frequency of m.e.p.p.s occurs (Miledi, 1960*c*). All this indicates that the spontaneous quantal release of ACh may not be responsible for the reduction in chemo-sensitivity.

SUMMARY

1. Muscle fibres in the diaphragm of foetal (17–22 day) and young rats up to several weeks old were examined with intracellular micro-electrodes. Their sensitivity to acetylcholine was tested with the method of iontophoretic drug application.

2. Spontaneous subthreshold potentials could be recorded anywhere in foetal muscle fibres, but their site of origin was in the middle of the fibres where the adult end-plates are ultimately formed. These potentials are due to acetylcholine and resemble in most respects the miniature end-plate potentials of adult fibres; but they occur at a much lower frequency and their time course is slower.

3. Most foetal muscles showed spontaneous contractions (and action potentials) which were greatly reduced or abolished by tubocurarine.

4. The entire length of foetal muscle fibres was sensitive to acetylcholine. At birth the tendon ends of some fibres do not react to the drug. With further development the chemo-sensitive properties of the muscle fibre membrane continue to recede from the tendon ends towards the middle of the fibre. The adult sensitive length is attained a few weeks after birth.

5. It is concluded, from these and earlier observations, that the establishment of neuromuscular connexions restricts the chemo-sensitivity of muscle fibres to the junctional region, and its immediate surroundings.

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