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Effect of denervation on the resting membrane potential of healthy and dystrophic muscle

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Relatively few observations have been made of resting membrane potential of denervated muscle cells and these have given conflicting results. Ware, Bennett, and McIntyre (1954) reported that in mice denervation was followed by a progressive fall in membrane potential and Lüllmann and Pracht (1957) reported a similar fall in potential in the muscle fibres of the rat diaphragm. Nicholls (1956), however, found no change in the membrane potential of frog muscle fibres following denervation and Li, Shy, and Wells (1957) similarly found no alteration in the membrane potential of denervated skeletal muscle fibres in the rat.

In 1963 we reported that the resting membrane potential of dystrophic muscle in the mouse was lower than in healthy muscle, confirming earlier observations of Kleeman, Partridge, and Glaser (1961). Further confirmation of this observation has been reported by McComas and Mossawy (1964). In this study we have made further measurements of the resting membrane potential in healthy and dystrophic muscle and studied the changes which occur after denervation.

METHOD

The experiments were carried out on eight dystrophic mice of the Bar Harbor 129 inbred strain, and on 12 healthy mice. The age of the dystrophic animals varied between 63 and 118 days. The healthy animals were young adult mice and came from a stock unrelated to the dystrophic strain. Measurements of resting cell membrane potential were carried out *in vivo* in normally innervated muscle and also seven days after division of one sciatic nerve. In the case of three of the healthy animals measurements were also carried out 14 days after denervation. The animals were anaesthetized with pentobarbitone and after exposure of one gastrocnemius the hindquarters were placed in a bath of tyrode solution at 30 to 35°C. through which were bubbled Co_2 and O_2. Glass microelectrodes, drawn by an instrument similar to that described by Alexander and Nastuk (1953), and filled with 3Molar KCl, were connected through a cathode follower and a DC amplifier to a cathode ray tube. Suitable electrodes had a resistance of greater than 5 megohms and a tip potential not exceeding 5 mvolts. The active electrode was controlled by a micromanipulator. The muscle was observed through a dissecting microscope and an impalement was considered satisfactory if an abrupt fall in potential occurred as the electrode advanced. Only superficial fibres were studied. The membrane potential was measured by applying an equal voltage in opposite sense by means of a calibrator.

TABLE I

MEAN VALUES FOR RESTING MEMBRANE POTENTIAL IN DIFFERENT GROUPS OF ANIMALS

Animal	*No. of Insertions*	*Mean Resting Potential*	*S.D.*	*S.E.*
Healthy mouse	114	77·8	6·12	0·57
Dystrophic mouse	87	72·6	11·8	1·27
Denervated healthy mouse (7 days)	100	68·1	8·3	0·83
Denervated healthy mouse (14 days)	47	67·0	5·24	0·76
Denervated dystrophic mouse	74	63·6	9·50	1·10

TABLE II

MEAN VALUES FOR RESTING POTENTIAL OBTAINED IN EACH EXPERIMENT

Experiment No.	*Animal*	*No. of Observations*	*Mean Resting Potential*
1	Healthy mouse	21	75·3
2		5	74·0
3		26	76·9
4		17	82·1
5		27	79·4
6		18	77·3
7	Healthy mouse	28	70·9
8	7 days after	26	61·2
9	sciatic nerve	12	65·8
10	section	34	71·9
11	Healthy mouse	31	68·0
12	14 days after	10	66·1
13	sciatic nerve section	6	64·1
14	Dystrophic	21	74·5
15	mouse	19	70·0
16		8	78·3
17		8	72·5
18		7	67·9
19		3	72·7
20		21	71·3
21	Dystrophic	41	65·8
22	mouse 7 days	14	66·4
23	after sciatic nerve section	19	56·5

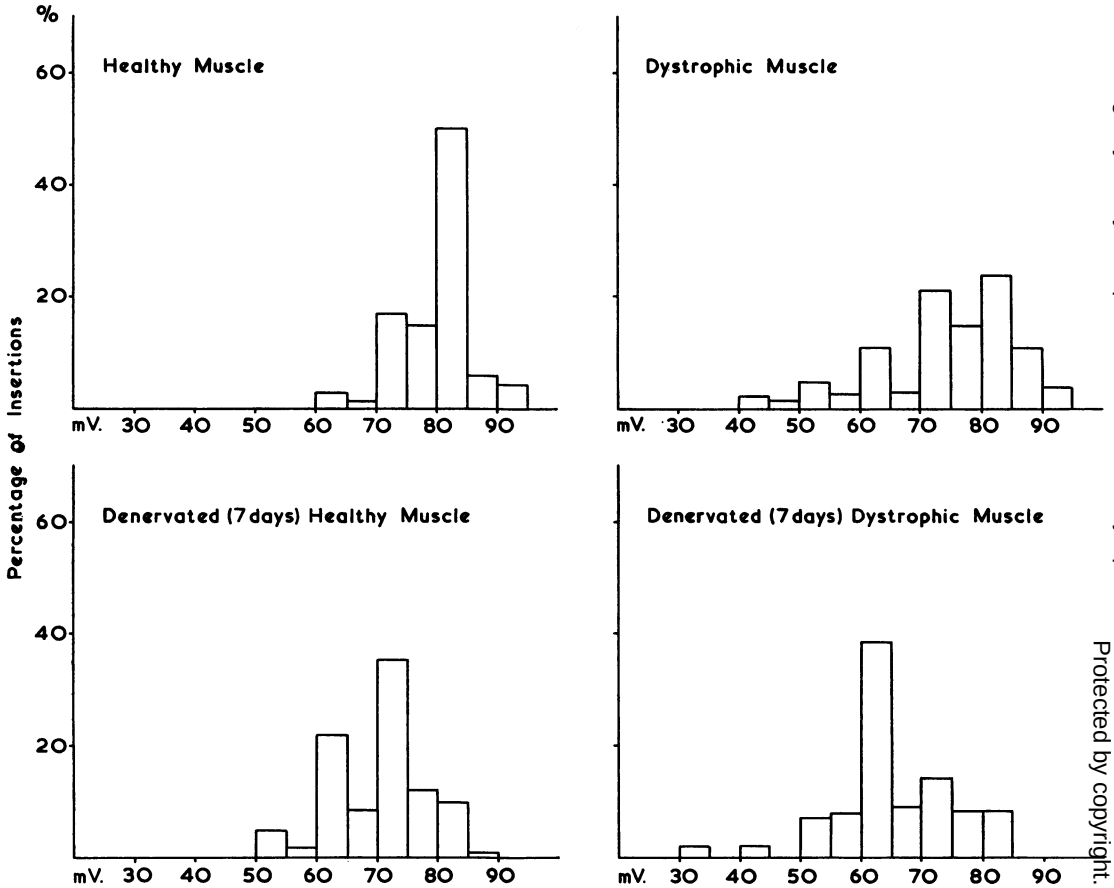

FIG. 1. *Distribution of potentials in different experimental groups.*

RESULTS

The results are shown in Table I. The difference in mean resting potential between healthy and dystrophic muscles is significant ($t = 4{\cdot}3$, $P < 0{\cdot}001$). So also is the difference between healthy muscle before and seven days after denervation ($t = 9{\cdot}0$, $P < 0{\cdot}001$) and between dystrophic muscle before and after denervation ($t = 5{\cdot}27$, $P < 0{\cdot}001$). The findings are consistent throughout the 23 experiments as is evident from Table II. It is clear from Fig. 1 that the scatter of values is greater in dystrophic muscle and is related to a wider spread of the distribution with an excess of low values; in denervated muscle, on the other hand, there is little increase in scatter but the histogram shows a shift to the left.

No systematic attempt was made to study action potentials but in denervated dystrophic muscle these were quite frequently seen occurring spontaneously (Fig. 2) at a frequency of about 5 to 10 c.p.s.

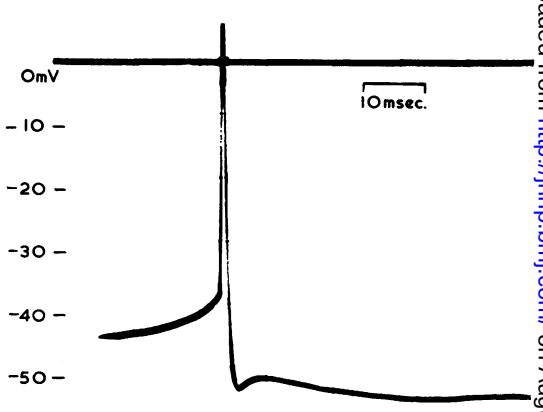

FIG. 2. *Intracellular recording of spontaneous action potential in denervated dystrophic muscle. Action potential is preceded by prepotential.*

DISCUSSION

The present experiments on innervated muscle in healthy and dystrophic mice confirm the preliminary observation (Lenman, 1963) that the mean resting membrane potential is lower in dystrophic than in healthy muscle. The mean values for each group are higher than those recorded previously and agree closely with those obtained by McComas and Mossawy (1964) for the thigh muscles of healthy and dystrophic mice. The results reported by Kleeman *et al.* (1961) are similar although the technique of their experiments differed in important respects and their observations were not confined to superficial fibres. Observations *in vitro* on the other hand (Conrad and Glaser, 1961; McLennan, 1961) have shown smaller differences in membrane potential between healthy and dystrophic muscle.

The present experiments have shown a reduction in membrane potential following denervation in both healthy and dystrophic muscle. This effect of denervation on the resting membrane potential is in agreement with the observations of Ware *et al.* (1954) and Lüllmann and Pracht (1957). The similar findings of Ware *et al.* are of interest in that the technique employed differed in important respects from that of the present study. They confined their observations to fibres deep within the muscle substance because they considered that superficial fibres were more likely to give low values as a result of injury during dissection. They excluded all values below a certain level as being due to faulty impalement. In our experience insertion of the electrode deeply into the muscle is liable to give rise to tip potentials, a possible source of error (Adrian, 1956), and we have therefore confined our observations to superficial fibres and included all measurements where the criterion of an abrupt fall in potential has been fulfilled. Lüllmann and Pracht (1957), in addition to finding a fall in membrane potential in the fibres of the rat diaphragm after denervation, found that the potential reverted to normal as reinnervation took place.

Histological studies of muscular dystrophy in the mouse have shown that there is greater variation in the size of muscle fibres in dystrophic than in healthy muscle and that in either case denervation is followed by a decrease in mean fibre size. Moreover both dystrophic and denervated muscle have a larger amount of fibrous tissue than healthy muscle (Banker and Denny-Brown, 1959). These are two possible sources of error which must be considered since faulty penetration of small cells is more likely to give low values due to leakage of electrolyte from the cell and excess of fibrous tissue may cause electrode damage. In this study observations of denervated muscle have been restricted to measurements made at intervals of not greater than 14 days after nerve section when changes in fibre size are relatively small and little fibrous tissue has formed.

Nicholls (1956) reported that denervation of frog muscle was followed by an increase to double in the membrane resistance. Although this could be related to a lowering of the potassium conductance of the membrane, which would tend to lower the resting potential, he found no alteration in the membrane potential in the denervated muscles. Species differences may be significant here, since the membrane characteristics of amphibian muscle differ considerably from those of mammalian muscle (Boyd and Martin, 1959), and fibrillation potentials occur later in the frog after denervation than in mammalian muscle.

Li *et al.* (1957) did not find any difference in the mean values for resting potential in the fibres of rat skeletal muscle before and after denervation but they noted considerable fluctuations in membrane potential and observed that fibrillation potentials were frequently preceded by a slow fall in the resting potential towards the depolarization threshold. Lüllmann reported similar excursions in resting potential in the rat diaphragm and regards these as a significant factor in initiating the fibrillation potential (Lüllmann, 1960). In our limited number of observations of spontaneously occurring action potentials in denervated dystrophic muscle prepotentials have been a conspicuous finding (Fig. 2).

McLennan (1961), in a study *in vitro* of dystrophic muscle, found that the resting membrane potential conformed closely to the potassium equilibration potential. Horvath and Proctor (1960) have suggested that the potassium concentration within dystrophic muscle cells is less than in healthy muscle cells and this could explain in part the reduced resting potential recorded in experiments in muscular dystrophy *in vivo*. A very substantial change in intracellular potassium concentration would be necessary to account for the changes in membrane potential following denervation reported here. Moreover it is doubtful whether any significant change in intracellular potassium concentration occurs following denervation (Drahota, 1962). It is more likely, therefore, that the changes are to be explained in terms of alterations in membrane conductance, and Lüllmann (1960) has reported a lowering of the potassium permeability of the cells of the rat diaphragn after denervation.

SUMMARY

Measurements *in vivo* of resting membrane potential in healthy and dystrophic muscle confirm that the

resting potential is significantly reduced in dystrophic muscle. In both healthy and dystrophic muscle denervation is followed by reduction in the mean resting membrane potential.

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