

EFFECT OF TEMPERATURE ON THE MAXIMUM RATE OF RISE OF TENSION IN DENERVATED RAT DIAPHRAGM MUSCLE

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ABSTRACT

Effects of chronic denervation and temperature were studied on the maximum rate of rise of tension in isometric twitch and tetanus of rat diaphragm muscle. The muscles were denervated for 15-20 days and the maximum rate of rise of tension was measured according to Hill (1951). The results showed that the maximum rate of rise of tension in twitch and tetanus was temperature dependent, exponentially increasing with increasing temperatures in both the normal and denervated muscles. A further observation was that the maximum rate of rise of tension in denervated muscle was always smaller than normal controls. It is suggested that probable changes in the series elasticity and duration of active state in the denervated muscle are responsible for the observed changes.

Introduction

The effect of temperature on the contraction properties of skeletal muscles is not well understood. Hill (1951) has shown that the twitch tension of frog sartorius and tortois illiotibialis muscles decreased following an increase in temperature. On the contrary, MacLagan and Zaimis (1957) have reported a decrease in twitch tension with a lowering of experimental temperature. Similarly, normal and denervated muscles have been shown to behave differently in many of their characteristics with ranges in experimental temperature (Padsha, 1968; Padsha and Winchester, 1968; Shaikh *et al.*, 1974). Later studies have confirmed that the denervated hypertrophied muscles develop less tension (Miledi & Slater, 1969) and that this tension development was temperature dependent in the fast and slow twitch muscles (Buller *et al.*, 1968a; Close and Hoh, 1968). Further, chronic denervation alone has also been demonstrated to produce changes in the contraction behaviour of mammalian skeletal muscles. The isometric twitch and tetanus become slower and prolonged while the twitch/tetanus ratio increased after denervation (Lewis, 1972; Shaikh *et al.*, 1979a). In addition it has also been shown that denervation period has a very profound effect on degree of these changes produced (Shaikh & Shaikh, 1987).

Our earlier studies on the contractile properties of skeletal muscles have further demonstrated that both denervation and temperature affected the isometric twitch and tetanic tensions (Shaikh *et al.*, 1979a) and duration of active state (Shaikh *et al.*, 1979a) in rat diaphragm. Further, the force velocity relationship of the denervated muscle has also

been shown to be independent at higher temperatures in rat diaphragm (Shaikh & Shaikh, 1985). It was therefore, concluded that while temperature affected the duration of active state, denervation must have altered the series elasticity, probably decreasing it in the muscles. A prolongation of contraction time is always followed by chronic denervation probably due to a decrease in Ca^{++} activated myosin ATPase activity (Gutmann & Hanzlikova, 1972) or probably due to an increase in the duration of active state and decreased series elasticity (Shaikh *et al*, 1979b; Shaikh & Shaikh, 1985).

The maximum rate of rise of tension is actually the time rate of change of tension and it is expected to have a direct effect on the total tension developed by a muscle. Since it is also expected to be influenced by factors such as temperature, chronic denervation, duration of active state and series elasticity etc., the present project was undertaken to investigate the effect of temperature and chronic denervation on the maximum rate of rise of tension in twitch and tetanus under isometric conditions.

Material and Methods

Adult white albino rats of both sexes, weighing from 170 to 250g were used and the diaphragm muscles were chronically denervated for 15-20 days. The experimental procedure for denervation, dissection, temperature regulation and the general experimental setup was the same as described earlier (Shaikh *et. al.*, 1979a). Since tension recordings were carried out under isometric conditions, where muscles were allowed to produce tension without shorting, an isometric force transducer (microdisplacement Myograph F-1000) was used. The buffer solution used for bathing the diaphragm strips was a slightly modified Krebs Henseleit solution (Winegrad and Shanes, 1962) which also contained 10mg/liter tubocurarine chloride to avoid spontaneous twitching. A mixture of 95% O_2 and 5% CO_2 was also bubbled through this solution. All the isometric tension measurements were carried out at temperatures ranging from 10°C to 35°C.

The measurements of rate of rise of tension were carried out directly from twitch and tetanus records, according to the method described earlier (Hill, 1951). For these measurements, initially a straight line was drawn superimposing the earliest rising phase of the twitch and tetanus and the area of superimposition, observed before the bifurcation of the twitch or tetanus from the drawn straight line showed the deepest slope (Fig. 1). This deepest slope represented the maximum rising of the tension. This maximum rise in tension was then divided by the time during which the superimposition of the drawn straight line and the rising phase of the twitch or tetanus continued. This gave a maximum rate of rise of tension in unit time.

Measurement of the maximum rate of rise of tension in a typical twitch

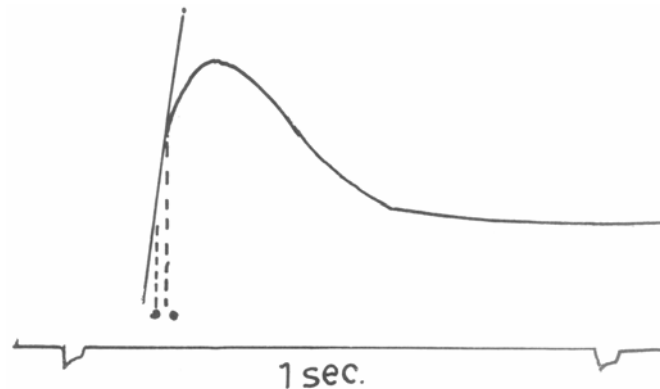


Fig. 1. The slope of the solid drawn line represents the maximum rate of rise of tension in the twitch record. The time is measured between the dotted lines a & b.

Results

1. Rate of Rise of Tension in Isometric Twitch:

The effect of temperature on the maximum rate of rise of tension was studied in both the twitch and tetanus obtained from the normal and 15-20 days denervated hemidiaphragms. The results showed that the rate of rise of tension was directly related to the experimental temperatures for the normal muscles, with the lowest value of this parameter being obtained at 10°C. When the temperature was increased upto 35°C, the rate of rise of tension also increased gradually and a maximum value was obtained at 35°C which was approximately 8 times the value obtained at 10°C (Table-1). In the case of denervated muscles, the fastest rate of rise of tension was again noted at 35°C. A major difference observed in the denervated muscles was that the maximum rate of rise of tension observed at 35°C was about 50 times greater than the same value obtained at 10°C. The rate of rise of tension and the experimental temperatures were again found to be related to each other for the denervated muscles (Fig. 2). It was further normal and denervated muscles showed that the average values of the maximum rate of rise of tension in denervated muscles were always significantly lesser than those of the normal muscles at all the temperatures studied ($P < 0.0005$ or $P < 0.001$). At 10°C, the rate of rise of tension, obtained from the normal muscles, were approximately 10 times greater than those of denervated muscles. However, at 35°C, this difference disappeared almost completely, with there being no significant difference remaining between the two muscles ($P > 0.05$).

Table-1: Effect of temperature on the maximum rate of rise of tension in isometric twitches recorded from the right normal and left 15-20 days denervated diaphragm strips

S.No.	Temperatures	Maximum Rate of Rise of Tension in Isometric Twitches (Kg/cm/sec)				P
		Mean±S.E.				
		Normal		Denervated		
1.	10°C	1.46±0.20	(13)	0.15±0.04	(9)	(P<0.0005)
2.	15°C	3.39±0.46	(13)	0.51±0.14	(9)	(P<0.0005)
3.	20°C	6.27±0.85	(13)	1.12±0.21	(9)	(P<0.0005)
4.	25°C	8.59±1.20	(13)	1.99±0.48	(9)	(P<0.0005)
5.	30°C	9.82±1.39	(13)	4.34±0.99	(9)	(P<0.001)
6.	35°C	11.14±1.68	(13)	7.88±1.65	(9)	(P<0.05)

TEMPERATURE EFFECT: RAT HEMIDIAPHRAGM
MAXIMUM RATE OF RISE: ISOMETRIC TWITCH

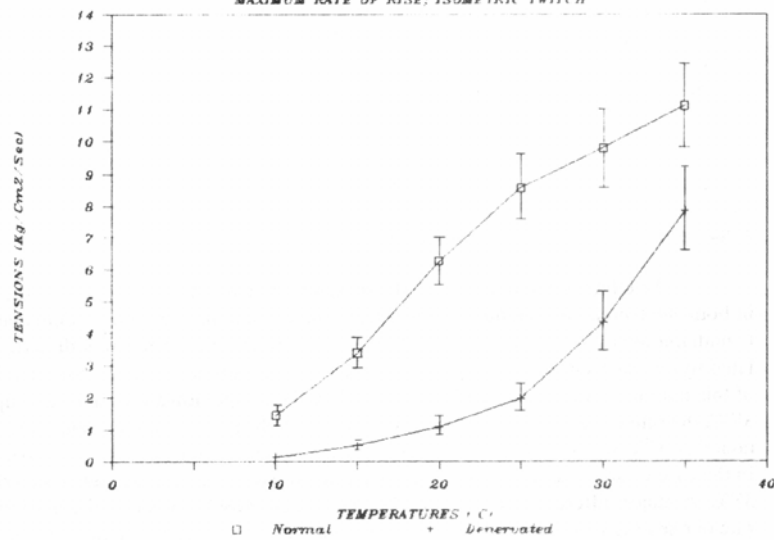


Fig.2

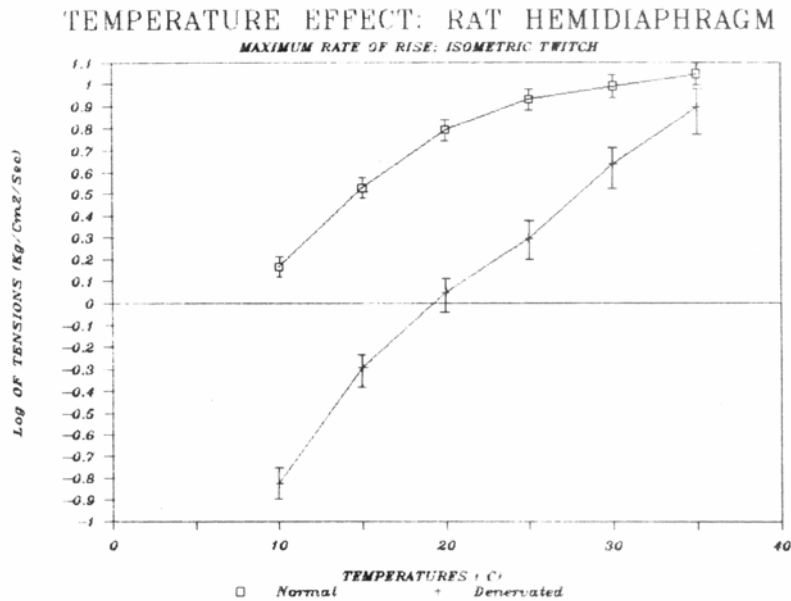


Fig.3.

2. Rate of Rise of Tension in Isometric Tetanus:

These results again demonstrated that the maximum rate of tension development in tetanus of the normal muscles was directly proportional to the experimental temperatures. The lowest values of this parameter were obtained at 10°C. When the temperature was increased for upto 35°C, there was a gradual increase in the rate of rise of tension which attained a maximum value at 35°C (Table-2). This value was approximately 12 times greater than that obtained at 10°C. Similar results were also noted for the denervated muscles. Here again, the rate of rise of tension in tetanus was directly proportional to the experimental temperatures. The lowest values of the maximum rate of rise of tension in tetanus were obtained at 10°C, which increased gradually with increasing temperatures till the highest values were attained at 35°C (Fig.4). When the log of these results were plotted, the relationship between the maximum rate of rise of tension in tetanus of the denervated muscles and the experimental temperatures was almost linear, suggesting an exponential relationship between the two (Fig.5). The Q to calculated for these results was again found to be 4. It was further noted that the difference in the maximum rate of rise of tension development in tetanus obtained from the denervated muscles at 10°C and 35°C were approximately 60 times. Another important observation was that for all the temperatures studied, the maximum rate of rise of tension in tetanus of the normal muscles were always greater than the same values obtained from the denervated muscles. This decrease in the maximum rate of rise of tension in tetanus of the denervated muscles was always extremely significant ($P < 0.0005$).

Table-2: Effect of temperature on the maximum rate of rise of tension in isometric tetanus recorded from the right normal and left 15-20 days denervated diaphragm strips. For tetanus recordings, the muscles were stimulated supramaximally with 100 V pulses and of 5mS duration at a frequency of 40/sec.

S.No.	Temperatures	Maximum Rate of Rise of Tension in Isometric Twitches (Kg/cm/sec)				P
		Mean + S.E.				
		Normal		Denervated		
1.	10°C	1.56±0.23	(13)	0.15±0.04	(9)	(P < 0 . 0005)
2.	15°C	3.40±0.45	(13)	0.51±0.14	(9)	(P < 0 . 0005)
3.	20°C	6.61±0.90	(13)	1.12±0.2	(9)	(P < 0 . 0005)
4.	25°C	10.58±1.29	(13)	2.04±0.48	(9)	(P < 0 . 0005)
5.	30°C	15.14±1.92	(13)	4.57±0.95	(9)	(P < 0 . 0005)
6.	35°C	18.65±0.46	(13)	8.92±1.7	(9)	(P < 0 . 0005)

3. Ratio of the Maximum Rate of Rise of Tension in Isometric Twitch and Tetanus:

The results showed that these ratios in the normal muscles, were more or less constant at temperatures ranging from 10°C to 20°C. However, these ratios gradually decreased with an increase in temperature and a minimum value of 0.60 was obtained at 35°C (Table-3). These results demonstrated clearly that although the maximum rates of rise of tension were the same for both the twitch and tetanus at low temperature, the rate of rise of tension in twitch was about 60% of that of tetanus at 35°C. These results further demonstrated that in the denervated muscles, this ratio remained more or less constant through out all the temperatures studied from 10°C to 35°C (Fig.6). A further observation was that the ratios of the maximum rates of rise of tension were more or less the same in the normal and denervated muscles for temperatures upto 25°C with there being no significant difference observed between the two sets of data ($P > 0.05$).

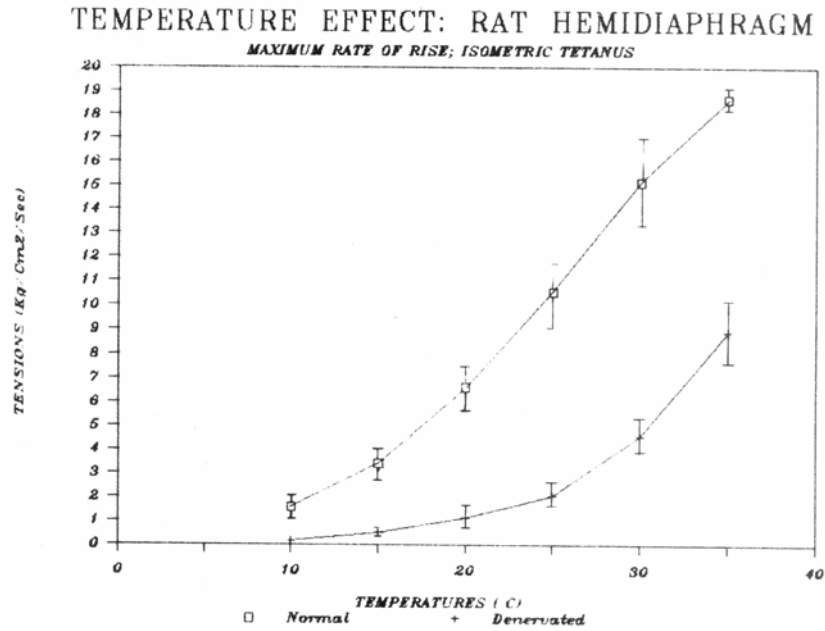


Fig.4.

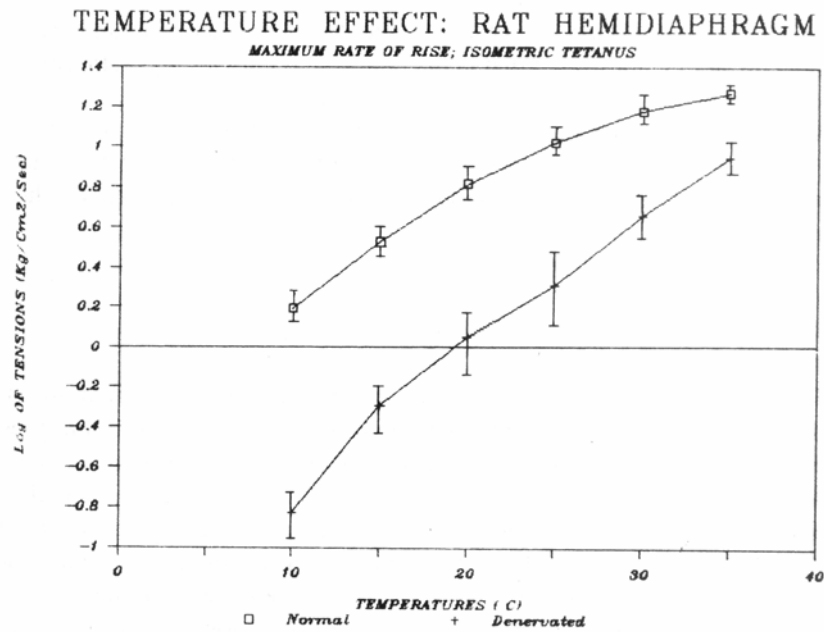


Fig.5.

Table-3: Effect of temperature on the ratios of maximum rate of rise of tension in twitch and tetanus calculated from the tension data obtained from the right normal and left 15-20 days denervated diaphragm strips

S.No.	Temperatures	Ratios of Maximum Rate of Rise of Tension from Isometric Twitch and Tetanus (Kg/cm/sec) Mean±S.E.				P
		Normal		Denervated		
1.	10°C	0.93±0.05	(13)	1.00±0.01	(9)	(P > 0.05)
2.	15°C	1.00±0.05	(13)	1.00±0.03	(9)	(P > 0.05)
3.	20°C	0.94±0.05	(13)	1.00±0.04	(9)	(P > 0.05)
4.	25°C	0.81±0.04	(13)	0.97±0.09	(9)	(P > 0.05)
5.	30°C	0.64±0.03	(13)	0.95±0.08	(9)	(P < 0.01)
6.	35°C	0.60±0.05	(13)	0.88±0.075	(9)	(P < 0.01)

Discussion

The maximum rate of rise of tension, which is also denoted by dp/dt (Hill, 1951; Winchester, 1969), is actually the time rate of change of tension. In the present experiments, this parameter has been measured directly from twitch and tetanus records, according to the method used by Hill, (1951) although it can also be measured directly by a dp/dt transducer (Winchester, 1969). Our results of the effect of temperature on the maximum rate of rise of tension in isometric twitch and tetanus indicated that normal muscles developed isometric tensions at a greater maximum rate than the denervated muscles at all the temperatures studied. Thus at 35°C, the normal muscles had an average maximum rate of rise of tension in isometric tetanus which was approximately twice that of the denervated muscles. This could only have been due to changes in the series elasticity of the muscles since, active state duration changes were not a factor in tetanus (Shaikh *et. al.*, 1979b). Similarly, the results of our previous studies on the force velocity relationship of the denervated muscle showed no muscle alteration at 25°C to 30°C (Shaikh & Shaikh, 1985). Thus, a change in elasticity would have to be such that following denervation, the series elasticity was decreased.

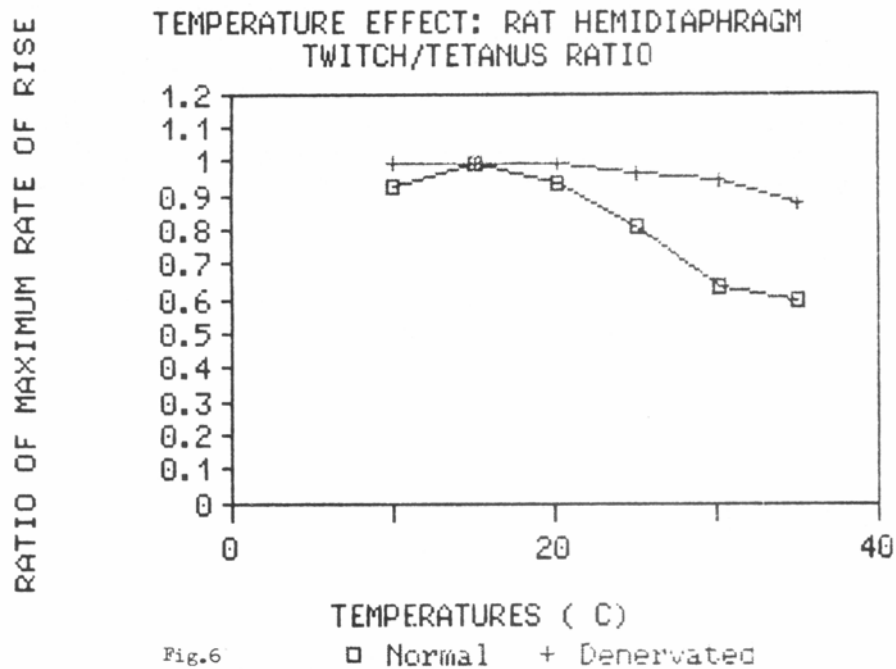


Fig.6

In a previous study (Shaikh *et al.*, 1979a), the twitch tension were seen to be altered by both denervation and temperatures. Similarly, the twitch/tetanus ratios essentially set the maximum intensity of the active state of the muscles to unity independent of temperature and nervous supply. The peak tension seen in twitch was thus easily compared with respect to temperature and with respect of muscles being denervated or normal, with variations in the maximum intensity of the active state eliminated from the comparison. The larger twitch/tetanus ratios of normal muscle at lower temperature, as compared to those of denervated muscles, suggested that either the normal muscles had stiffer series elasticity or that the active state time course for normal muscles fall more slowly than that of denervated muscles, or both. However, other results (Shaikh *et al.*, 1979b) indicated that the duration of the active state plateau in the normal muscles exceeded those of denervated muscles for all the temperatures studied below 25°C. If it is assumed that in this temperature region, the active state time course was also longer so that the active state intensity fell more slowly, then it is probably sufficient to say that the twitch/tetanus ratios of the normal muscles exceeded those of denervated muscles at temperatures below 25°C. Further, as indicated above, the series elasticity of the muscles is decreased by denervation. Thus, both the effects would appear to cause the reduced twitch/tetanus ratios seen following denervation, below 25°C. This statement has also been represented graphically in Fig.7. Further, it is to be noted that an increased series elasticity, coupled with a faster fall in time course of the active state, would only result in a decrease in the twitch/tetanus ratio, if the effect of the active state was greater than the effect of the series elasticity. This appears to be the case in our experiments at temperatures above 25°C. In addition, the duration of the active state plateau in the

normal muscles was seen to be less than that of the denervated muscles above 25°C (Shaikh *et. al.*, 1979b). Again, if it is assumed that the duration of the active state plateau indicated the nature of the time course of the active state in general, it would mean that above 25°C, the active state in denervated muscles falls more slowly than in the normal muscles. This would have the effect of "cutting off the twitch earlier in normal muscles than in the denervated ones. Thus, as a result, in spite of normal muscles having the stiffer elasticity, denervated muscles would develop more relative tension than their controls, in twitches above 25 C. This has also been represented graphically in Fig.8.

TWO HYPOTHETICAL TWITCHES REPRESENTING ALTERED SERIES ELASTICITY
AND ACTIVE STATE TIME COURSE

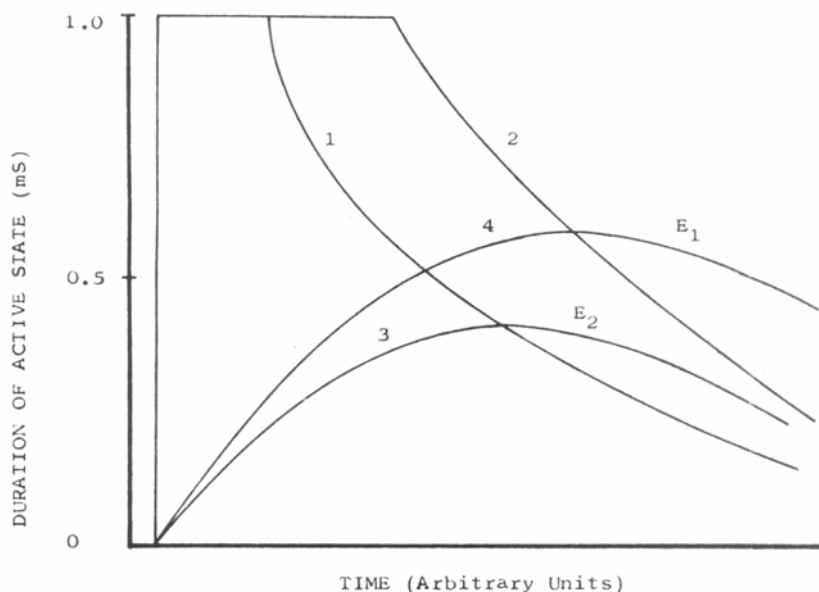


Fig. 7

Curve 1: Active state No.1

Curve 2: Active state No.2

Curve 3: Twitch for active state No.1, $E_2 < E_1$

Curve 4: Twitch for active state No.2, E_1

E_1 and E_2 are measures of series elasticity for the muscles.

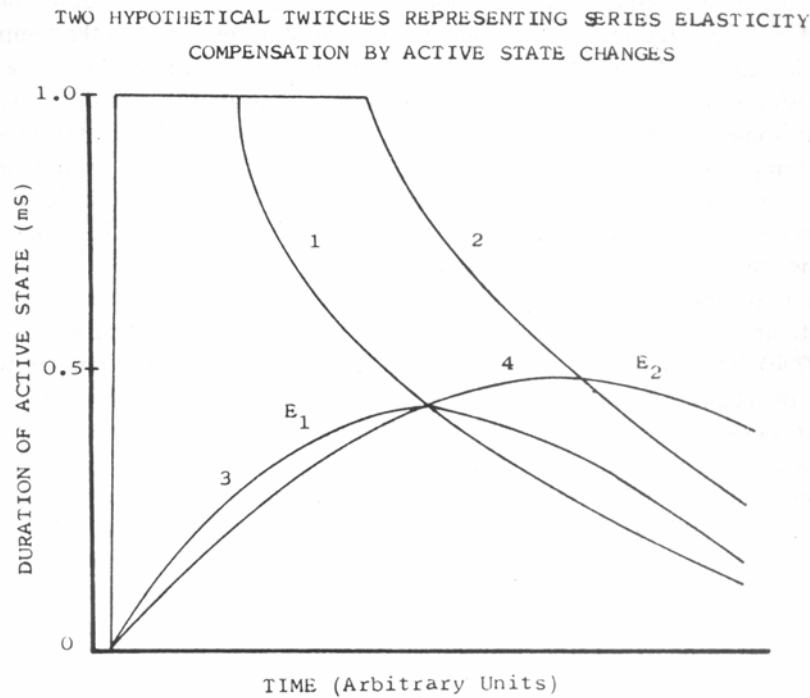


Fig. 8

Curve 1: Active state No.1

Curve 2: Active state No.2

Curve 3: Twitch for active state No.1, E_1 Curve 4: Twitch for active state No.2, $E_2 < E_1$ E_1 and E_2 are measures of series elasticity for the muscles.

The studies of Winchester (1969) have further indicated that the duration of time between the stimulus and the achievement of peak tension in twitch was increased by denervation. This was also found to be true in our experiments over the temperature range 10°C to 30°C, the difference increasing with increasing temperatures. These results suggested that time course of the active state for normal muscles falls more rapidly than that of denervated muscles at temperatures below 25°C, in spite of the duration of the active state plateau being longer in normal muscles at these temperatures (Shaikh *et al.*, 1979b). However, results presented in Table-3 provide evidence to support the hypothesis that the series elasticity is altered more by denervation at lower temperatures than at the higher ones. As can be seen from Table-2 and Fig.4, the maximum rate of rise of tension in tetanus in normal muscles at 35°C was twice that of denervated muscles, but at 10°C, the maximum rate of rise of tetanus in normal muscles was ten times that of denervated

muscles. Thus, it is clear that, in addition to being decreased by denervation, series elasticity was also differentially decreased in denervated muscles as compared to that of normal ones as the temperature was decreased. The results of the effect of temperature on the maximum rate of rise of tension in isometric twitch and tetanus further indicated that normal muscles developed isometric tensions at a greater maximum rate than the denervated muscles at all the temperatures studied. Thus, at 35°C, normal muscles had an average maximum rate of rise of tension in isometric tetanus which was approximately twice that of the denervated muscles. This could only have been due to changes in the series elasticity of the muscles, since active state duration changes were not a factor in tetanus.

The present results again indicate that denervation results in changes of the active state and series elasticity of the muscles as has been suggested earlier (Shaikh *et al.*, 19796). The changes in elasticity are smaller especially at lower temperatures. However, the changes observed earlier in active state (Shaikh *et al.*, 19796) are not so simple. At temperatures between 25°C and 35°C, the duration of active state plateau was increased by denervation alongwith a simultaneous alteration in the time course of active state, so that it fell more slowly than that observed in the normal muscles. At lower temperatures however, the duration of active state plateau was reduced by denervation but the rate of decay of the active state was less than That of the normal muscles. This hypothesis fits the twitch/tetanus ratio and maximum rate of change data obtained in our experiments.

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