CHANGES IN RESTING AND CONTRACTILE PROPERTIES OF CHICKEN MUSCLES FOLLOWING DENERVATION

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ABSTRACT

The anterior latissimus dorsi muscle (ALD) of young chickens was examined for changes in contractile properties following denervation. Apart from an increase of wet weight the denervated ALD exhibited reduced slack length, a decrease of twitch and tetanic tension, and a marked increase of stiffness. The denervated posterior latissimus dorsi muscle (PLD) showed similar alterations, but its wet weight decreased. Optimum length for active tension was unchanged in the ALD, but reduced in the PLD. Ultrastructural signs of degeneration were obvious in the PLD, but almost absent in the ALD. K^+ - and caffeine contractures of the ALD were reduced to approximately 50% after denervation; the caffeine contracture was maintained, but the K^+ -contracture was not. The inability to maintain tension during K^+ -depolarization developed with time after denervation, and changes in the external Ca^{2+} concentration did not affect this type of contracture. Stretching to optimum length required much greater load in denervated than in normal ALD. The high resting tension decreased markedly in Ca^{2+} -free or Co^{2+} -containing solutions. The results suggest that the functional disorders of denervated ALD are due to changes in the Ca^{2+} permeability of the muscle fibre membrane. An increased resting Ca^{2+} influx seems to lead to a state of contracture. In contrast, the Ca^{2+} influx associated with the maintained phase of K^+ -contracture in normal ALD seems to be absent or markedly reduced after denervation.

The latissimus dorsi muscle of the chicken is divided into two major parts, anterior (ALD) and posterior (PLD), which have strikingly different morphological and physiological properties. The ALD has the characteristics of a slow tonic muscle and consists almost entirely of slow muscle fibres; in contrast, the PLD consists entirely of fast twitch fibres (1, 8, 10).

The ALD responds to denervation with a marked hypertrophy (6), but the tension developed in response to application of K^+ -rich solutions is reduced by about 50% (19). A reduction of contractile ability after denervation has also been described in mammalian and frog skeletal muscle fibres (2, 9, 14-16, 28). The reasons for this impairment of contractile force

are not yet well understood. Malvey *et al.* (18) have demonstrated that energy stores and contractile proteins are unchanged after denervation. Electronmicroscopical work has, however, revealed that denervation elicits a number of degenerative morphologic alterations such as disintegration of Z-lines, formation of vacuoles, disorganization of myofilaments, and swelling of the sarcoplasmic reticulum (11, 13). These observations suggest that a number of factors may contribute to the functional impairment of the contractile system. An important factor may be the inability of the internal membrane systems to handle Ca^{2+} properly (15, 25, 26, 29). It is also possible that changes in the Ca^{2+} permeability of the sarcolemma play an impor-

tant role. A reduction of the Ca^{2+} permeability would affect mainly the second (maintained) phase of the K^{+} -contracture of chicken ALD, which has been shown to depend strongly on the external Ca^{2+} concentration, whereas the initial phase is much less dependent on the Ca^{2+} of the medium (21). Experiments were therefore done to see whether denervation has a differential effect on the two phases of K^{+} -contracture. Caffeine contracture was also examined since it is known that caffeine elicits contractures without strongly depolarizing the sarcolemma (3, 12, 17). The results obtained suggest an increased resting influx of Ca^{2+} into the muscle fibres, whereas the depolarization-induced Ca^{2+} influx is reduced after denervation. Some of these results have been reported in the German Physiological Society (24).

MATERIALS AND METHODS

The experiments were performed from May to September 1981. Seven- to nine-day-old White Leghorn chickens were anesthetized with ether. An incision was made in the skin at the posterior junction between left wing and body. A segment of the main nerve trunk to the ALD and PLD (originating from the N. brachialis longus superior of the brachial plexus) was removed without impairing the blood supply to the muscles. The incision was closed by suturing the skin with surgical silk. The contralateral ALD and PLD were left untouched and served as controls. Three to fifteen days after the operation the chickens were decapitated, and the muscles to be examined were removed under continuous superfusion with Krebs-Henseleit solution bubbled with a mixture of 95% O_2 and 5% CO_2 . For measurement of contracture, control and denervated muscles were mounted horizontally in a common chamber. One tendon of each muscle was fixed at the bottom of the chamber, and the other tendons were attached to Devices transducers (type UF1) connected with a double channel recorder. For measurement of twitch and tetanic tension, only one muscle was mounted in a smaller chamber between two large Pt-plate electrodes (23). Square pulses of 2–4 msec and supramaximum intensity were used for transverse direct stimulation. The transducer was mounted on a separate stage which could be moved manually. The muscle was stretched in steps of 1 mm every 30 sec; 25 sec after stretching a twitch was elicited, and 5 sec later the next step of extension was applied. In order to avoid irreversible

damage, the muscles were not stretched more than 90% over slack length. Slack length was determined as the length at which a small extension (<0.5 mm) elicited just measurable tension. Active tension was measured from the resting tension level, and represented the maximum tension developed in response to electrical or chemical stimulation. The solutions in the chambers could be exchanged within 2–3 sec; all solutions were bubbled with the O_2 - CO_2 mixture, and the temperature was kept at 22–26°C. The normal Krebs-Henseleit solution (solution A) had the following composition (mM): NaCl 150, KCl 5, CaCl_2 5, MgCl_2 2, NaHCO_3 20, glucose 11.1.

In order to elicit K^{+} -contractures, 5 mM Na- and 150 mM K-methylsulfate replaced NaCl and KCl of solution A. Caffeine contractures were evoked by adding 10 mM caffeine to solution A. Ca^{2+} -free Krebs-Henseleit was made by omitting CaCl_2 (no chelating agents added), and Co^{2+} was added as CoCl_2 to solution A.

Electronmicroscopy

The control and denervated ALD and PLD muscles were removed from 14-day-old chickens 7 days after denervation. Two small bundles of fibres were isolated from each muscle in Krebs-Henseleit solution under microscopic control. To avoid shortening during fixation, the bundles were pinned to a plastic plate. One bundle from both control and denervated ALD and PLD muscles was stretched slightly over slack length. The other bundles were stretched to 165% slack length in the ALD muscle, and to 140% slack length in the PLD muscle.

The bundles of muscle fibres were fixed in 2% glutaraldehyde in cacodylate buffer adjusted to pH 7.2. After 1 h, tissue segments of about 1 mm length were excised from the central parts of the bundles, and fixed for another hour in the same solution. The muscle pieces were rinsed in buffered solution, and postfixed for 1 h in a 1% OsO_4 solution buffered with cacodylate at pH 7.2, stained for 1 h with 1% uranyl acetate in 70% alcohol, and embedded in Epon. Ultrathin sections were cut with a Reichert OMU or Porter Blum II ultramicrotome, stained with uranyl acetate and lead citrate, and then examined with a Siemens Elmiskop 101 or JEM-100 B.

For the measurement of sarcomere lengths and other components of the myofibrils Epon sections 0.24 μm thick stained with lead citrate were examined in five muscle segments. Six to

ten electron micrographs were taken at low magnification and enlarged to more than 8,000 times.

Parallel lines at $0.5\ \mu\text{m}$ distances were drawn on micrographs along the alignment of myofibrils. The length of sarcomeres, A-bands, and the spaces extending from neighbouring Z-lines to both sides of the A-band of a sarcomere (Z-A distance) were estimated by measuring test points falling over these components.

RESULTS

Experiments on the ALD

Wet weight, slack length and length-tension diagram of resting muscle All denervated ALD muscles (d-ALD) were heavier by 15–110% (Fig. 1) than their controls (*cf.* reference 6). In addition, it was already noted during dissection that most denervated muscles were clearly shorter than their controls (c-ALD). The degree of shortening varied from 0 to 26%, and on the average it amounted to 9.3%, including 5 muscles which had control length (24 pairs). There was no obvious relation to the duration of denervation.

Resting length-tension diagrams of pairs of ALD muscles from normal chickens were identi-

cal. Up to three days following denervation no difference was noted between the d-ALD and their contralateral c-ALD. But from the 4th day all d-ALDs exhibited increased stiffness, and their length-tension diagrams became much steeper than those of their controls (Fig. 2b).

Relation between twitch tension and muscle

Fig. 1 Percentage changes in wet weight (circles) and slack length (crosses) of denervated ALD muscles; contralateral muscles served as controls. All denervated muscles were heavier than their controls, while all but five denervated ALD muscles had shorter slack lengths than their controls.

Fig. 2 Twitch tension at different resting lengths (a) and length-tension diagrams (b) of control (circles) and denervated (crosses) ALD muscles. Abscissa, lengths of muscles in mm; ordinates, twitch (a) and resting (b) tension 25 sec after stretching the muscles in steps of 1 mm every 30 sec. Muscles were examined 8 (A) and 11 (B) days following denervation. Slack lengths were 19 and 14 (A), and 17.5 and 14.5 mm (B) for control and denervated muscles (see arrows near abscissa). Both denervated muscles exhibit much steeper length-tension diagrams than their controls. Optimum length of d-ALD is 2 mm shorter in A than that of its control, but in B both muscles have the same optimum length.

Fig. 3 Twitch (a) and tetanic (b) tension of control (A) and 9 days denervated (B) ALD muscles recorded at corresponding optimum lengths. Note slower time course of twitch contraction and relaxation as well as fatiguing during tetanus in B.

length Directly evoked twitches of the d-ALD were similar in amplitude to those of the c-ALD up to about 9 days following denervation (Figs. 2A and 3); 5 muscles denervated for 8–15 days showed much smaller twitches (Fig. 2B). On the average, twitch amplitudes of the d-ALD were 67% of those of the c-ALD (Table 2), and their time course was generally slower (Fig. 3a). Tetanic tension of the d-ALD decreased to 47% of control values (Table 2), and there was marked fatigue as compared with the c-ALD (Fig. 3Bb).

Fig. 2 also demonstrates that control muscles produced maximum twitch tension at resting lengths 5–8 mm longer than their slack lengths; the contralateral d-ALD had to be stretched 2–3 mm more to obtain the optimum length for twitch. On the average, this additional extension compensated for the reduced slack length of the d-ALD. Hence, the c-ALD and the d-

ALD produced maximum twitches at the same length (Table 1). At this length, however, the resting tension was 6.4 times as large in the d-ALD as it was in the c-ALD (because of the much steeper length-tension diagram).

K⁺-contracture The K⁺-contracture of a normal ALD typically consists of a rapid increase of tension leading to an early peak which is followed by a second phase of maintained tension (21). This time course of K⁺-contracture is illustrated in Fig. 4 (record c). When d-ALDs were stretched by the same amount (2 mm in the experiment of Fig. 4) they developed only a fraction of the tension produced by the control muscles (record d). After stretching both muscles by another 5 mm the peak contracture of the c-ALD increased by 25%, but that of the d-ALD was 4-fold. The time course of the contracture of both the c-ALD and the d-ALD was similar at smaller and larger extensions, but under both conditions the d-ALD was unable to maintain tension. Similar observations were made in many more experiments in which the muscles were put to different resting lengths. Table 2 shows the results of those experiments in which the resting length was set close to the optimum length for twitch. In these experiments peak tension was reduced on the average to 55%, whereas the second phase of contracture was only 34% of control values. Fig. 5 shows that there was a progressive decrease of the maintained phase of contracture with increasing time after denervation.

During relaxation following the K⁺-contracture, tension fell transiently well below the level prior to K⁺ application. This transient relaxation was independent of the Ca²⁺ concentration in the medium (Figs. 7 and 8).

Caffeine contracture Caffeine only moderately depolarizes the muscle fibre membrane (3, 12, 17). Therefore, it was of interest to examine the caffeine contracture of the d-ALD and its dependence on resting length. Fig. 6 and Table 2 demonstrate that the effect of resting length

Table 1 Slack Length (*L*), Optimum Length for Twitch (*L*₀), and Resting Tension at Optimum Length (*RT*₀) of Control (c) and 6–15 Days Denervated (d) ALD Muscles

c-ALD				d-ALD			
<i>L</i>	<i>L</i> ₀	<i>L</i> ₀ – <i>L</i>	<i>RT</i> ₀	<i>L</i>	<i>L</i> ₀	<i>L</i> ₀ – <i>L</i>	<i>RT</i> ₀
(mm)	(mm)	(mm)	(g)	% of control		(mm)	% of control
17.3±2.2	23.8±1.9	6.5±1.0	0.39±0.37	86.8±6.7	99.0±7.1	8.5±1.6	636.0±330.0

L of the d-ALD is shorter than that of the c-ALD, but *L*₀ of the d-ALD is on the average 99% of that of the c-ALD. Mean values ± SD of 8 experiments

Fig. 4 Effect of resting tension on K^+ -contracture of control (c) and 14 days denervated (d) ALD. Contractures were elicited by 150 mM K -methylsulfate solution as indicated. At small resting tension (both muscles extended by 2 mm) the K^+ -contracture of the d-ALD is much less than that of the control. Additional extension by 5 mm had a small effect on the contracture of the c-ALD, and a large effect on the d-ALD. Note that the d-ALD is unable to maintain tension under both conditions.

Table 2 Isometric Tension Developed upon Electrical Stimulation or Application of Caffeine or 150 mM K^+ Solution

	ALD		PLD	
	Control (g)	Denervated (%)	Control (g)	Denervated (%)
Twitch tension (n=8)	2.7 (1.9–4.1)	67.4 (7.0–125.5)	12.3 (7.0–18.2)	29.2 (14.1–54.3)
Tetanic tension (n=8)	11.5 (9.6–13.6)	46.8 (7.8–65.1)	29.5 (28.1–31.2)	36.2 (30.0–46.5)
K^+ -contracture (n=8)				
Initial peak	8.6 (6.0–10.9)	55.2 (14.5–105.8)	—	—
After 6 min	8.9 (7.0–10.6)	33.8 (5.8–83.7)	—	—
Caffeine contracture (n=6)	6.3 (4.6–8.4)	64.8 (22.9–112.9)	—	—

Twitch and tetanic (40/sec) tensions recorded at optimum length. Tensions developed by denervated ALD are given in % of control values. Mean values with ranges in parentheses. Duration of denervation was 6–15 days (ALD) and 7–11 days (PLD).

was essentially the same as it was for the K^+ -contracture. There was, however, an important difference; during application of caffeine (with 1.8 or 5 mM Ca^{2+}) the tension was maintained on a high plateau level. In addition, the d-ALD relaxed more slowly than the c-ALD, especially at high resting tension.

Effect of Ca^{2+} and Co^{2+} on K^+ -contracture
According to Page (21), the maintained phase of K^+ -contracture is dependent on the external Ca^{2+} concentration. This observation was confirmed in a number of experiments on the c-ALD (Fig.

7). In contrast, the K^+ -contracture of the d-ALD was only slightly affected by variation in the Ca^{2+} concentration. In Ca^{2+} -free solution the peak tension was somewhat reduced, and tension declined somewhat faster during the second phase of the contracture (Fig. 7); on the other hand, maintenance of tension could not be improved to any great extent by increasing the Ca^{2+} concentration from 5 to 20 mM (Fig. 8). An effect similar to that of Ca^{2+} -free solution was observed upon addition of Co^{2+} (10 mM) to the 150 mM K^+ solution containing

Fig. 5 Effect of denervation on ability of the ALD to maintain tension in response to 150 mM K-methylsulfate solution. Circle on the ordinate indicates the average amplitude of isometric tension 6 min after application of the K⁺-rich solution (15 control muscles); the initial peak of the contracture (see Fig. 4) was taken as 100%. Note that with increasing duration of denervation (abscissa) the maintained phase of contracture decreases.

normal Ca²⁺ concentration. In the c-ALD the early peak tension was somewhat smaller, but the maintained phase of contracture was strongly reduced (Fig. 9). This effect was similar to that observed by Huerta and Stefani (12) upon application of other blockers of Ca²⁺ channels. In the d-ALD there was, however, no substantial effect except a slight reduction of peak tension and a somewhat faster relaxation.

Effect of Ca²⁺-free solution and Co²⁺ on resting tension Control ALDs were usually set to a resting length corresponding to optimum length for twitch. This required stretching by 5–8 mm

and a resting tension of approximately 0.39 g (Table 1). Variation of external Ca²⁺ concentration between 0 and 5 mM did not affect this tension.

In contrast, the resting tension of the d-ALD could be markedly reduced by removal of Ca²⁺ or by addition of Co²⁺. In six experiments the resting tension was set to values from 1.9–4.9 g (mean 3.6 g) in Krebs-Henseleit containing 5 mM Ca²⁺. After removal of Ca²⁺ the tension dropped by 29–70% (mean 43.4%). An even stronger effect was observed after adding Co²⁺ (10 mM) to normal Krebs-Henseleit (Fig. 10). In four experiments the resting tension dropped from 3.2 g (2.7–3.6 g) to 0.82 g (0.5–1.2 g), which corresponds to an average reduction of 74.5%. This effect was slowly reversible. Fig. 10Ba also illustrates that the spontaneous contractile activity sometimes observed in the control ALD following a K⁺-contracture was quickly and reversibly blocked by the Co²⁺.

Experiments on PLD

A few comparative experiments were performed on PLD muscles. It is known that these muscles undergo substantial atrophy following denervation (6). Wet weights were taken from five pairs of PLDs; the (9–14 days) denervated muscles were 32% lighter than their controls. Slack lengths of d-PLDs were also reduced, but the reduction was only 5% on the average (compared with 9.3% in the d-ALD; see above).

Resting length-tension diagrams of control PLD muscles differed from those of control ALD muscles. Although both types of muscles had

Fig. 6 Effect of resting tension on amplitude of caffeine contracture of control (above) and 6 days denervated ALD (below). Caffeine (10 mM) was added (as indicated) to normal Krebs-Henseleit solution at different resting tensions. The muscles were first stretched by 9 mm each, and after the first caffeine contracture their extension was reduced to 3 mm. At the single arrows recording speed was reduced to 1/10, and at the double arrow it was restored to the value indicated by the calibration line. Note stronger contracture of the d-ALD at higher resting tension, and maintenance of tension throughout the period of caffeine application.

Fig. 7 Effect of low external Ca^{2+} on K^{+} -contracture of control (above) and 9 days denervated (below) ALD. In the c-ALD the maintained phase of K^{+} -contracture is strongly reduced by removal of Ca^{2+} . In contrast, the time course of contracture of d-ALD is very much the same under both conditions. Note transient relaxations below level prior to K^{+} -contractures in the d-ALD. Upon readmission of Ca^{2+} resting tension of the c-ALD remains constant, but that of the d-ALD rises to a level slightly above that prior to first K^{+} -contracture. Following the first K^{+} -contracture the chamber was flushed twice with Ca^{2+} -free Krebs-Henseleit solution.

Fig. 8 K^{+} -contracture of control (above) and 9 days denervated (below) ALD at normal (5 mM) and high (20 mM) external Ca^{2+} concentration. Loss of tension of the d-ALD is not inhibited by the high Ca^{2+} solution. At the arrows recording speed was reduced to 1/6 of the value indicated by the calibration line. 0-Lines indicate zero resting tension.

similar slack lengths (about 18 mm), the resting tension of PLD started to rise steeply when they were stretched by more than 8 mm (Fig. 11); ALD muscles at similar lengths showed only a slight increase of resting tension (Fig. 2). Denervated PLDs exhibited a marked shift of the

steep branch of their length-tension diagram to a shorter extension range. In sharp contrast to the d-ALD muscles (Fig. 2), d-PLDs had to be stretched less than their controls in order to produce a maximum twitch (Fig. 11a). Since their slack length was also shorter by 5%, the

Fig. 9 Effect of Co^{2+} (10 mM) on K^+ -contracture of 15 days denervated (A) and control (B) ALD. 150 mM K-methylsulfate solution was applied as indicated by the horizontal lines below the recordings. Co^{2+} were applied 10 min prior to K^+ application in b. In both muscles Co^{2+} reduced slightly the amplitude of the initial phase of K^+ -contracture. The second (maintained) phase of the contracture was, however, strongly affected only in the control ALD (Bb). The resting tension of the denervated ALD was markedly reduced by Co^{2+} (Ab), but there was no effect in the control ALD (Bb). 0-Lines indicate zero resting tension; note difference in calibration.

optimum length of the d-PLDs was 12.5% shorter than that of the c-PLDs.

Twitch and tetanic tensions were measured in three pairs of PLDs (Table 2); twitch amplitude of denervated muscles was reduced to 29% (14–54%); amplitudes of 40/sec tetani were reduced to 36% (30–46%) of control values. In contrast to the ALDs, the time course of PLD twitch and tetanic tensions was virtually unchanged after denervation.

Electronmicroscopy

The ultrastructure of the ALD fibres is characterized by thick and wavy Z-lines, an indistinct M-line, and the absence of a regular pattern of sarcoplasmic reticulum associated with each sarcomere (Fig. 12a). The mean sarcomere length of c-ALDs was $2.28 \mu\text{m}$. Seven days after denervation the general appearance of ALD fibres did not differ appreciably from that of control muscles. The orderly banding of myofibrils was preserved; signs of degeneration were rare although disintegrated and irregularly oriented bundles of myofilaments were occasionally seen in the intermyofibrillar spaces. The length of sarcomeres had decreased on the average to $1.48 \mu\text{m}$ and the I-bands had shortened correspondingly (Fig. 12b).

Fig. 10 Effect of Co^{2+} (10 mM) on resting tension in control (above) and denervated (below) ALD muscles. Beginning of records shows restoration of resting tension in normal Krebs-Henseleit after previous treatment with Ca^{2+} -free Krebs-Henseleit (A) or 150 mM K-methylsulfate solution (B). Addition of Co^{2+} (horizontal lines) strongly reduced the (high) resting tension of the denervated ALD muscles and stopped spontaneous contractile activity of control muscle (B). Removal of Co^{2+} had an immediate effect on control muscle (Ba) but not on the denervated muscles. Duration of denervation was 10 (A) and 11 (B) days, respectively. Short lines labeled 0 indicate zero resting tension. At the arrows bath was flushed again with normal Krebs-Henseleit.

Fig. 11 Optimum length for twitch (a) and length-tension diagrams (b) of control (circles) and denervated (crosses) PLD muscles. Abscissa, muscle length in mm; twitch tension (a) and resting tension (b) 25 sec after extending the muscles in steps of 1 mm every 30 sec. Slack lengths were 19.5 and 19 (A) and 21 and 20 (B) mm for control and denervated muscles, respectively. Note steepness of length tension diagrams of denervated muscles at short extensions. Duration of denervation was 7 (A) and 11 (B) days.

Fig. 12 Electron micrographs of longitudinal sections through control (a, c) and 7 days denervated (b, d) ALD muscles at two different lengths; a and b at slack length, c and d stretched to 165% slack length. The length of sarcomeres (s), the distance from the Z-line to one side of the A-band (Z-A), and the corresponding distance (A-Z) in the other half of the same sarcomere were measured. Bar, 1.0 μ m. $\times 12,500$

Fig. 13 Distribution of lengths of sarcomeres (s) and Z-A distances (ZA) in control (empty columns) and 7 days denervated (stippled columns) ALD fibres. The measurements were made in fibres at slack length (a) and at 165% slack length (b).

When the c-ALD was stretched to 165% slack length, sarcomere length and waviness of Z-lines increased, but the A-bands kept their position in the central region of sarcomeres (Fig. 12c). In the d-ALD stretched to 165% slack length the actin-filaments were asymmetrically pulled out of the A-bands such that Z-A distances varied considerably (Fig. 12d). These changes are illustrated also in the histograms of Fig. 13. At slack length the Z-A distances were shorter in the d-ALD than in the c-ALD, but they varied similarly in both types of muscle (Fig. 13a). In fibres stretched to 165% slack length, the Z-A distances increased by equal amounts in c-ALD fibres, but in the d-ALD fibres the corresponding values were smaller and more widely distributed (Fig. 13b). There was no difference between the c-ALD and the d-ALD in the lengths of the A-bands; these varied between 1.19 and 1.4 μm , which is less than the 1.6 μm generally measured in fresh muscles. This difference is certainly due to shrinkage during the fixation procedure.

The PLD fibres were affected more severely by the denervation than the ALD fibres, showing extensive atrophy and ultrastructural changes, such as disintegration of Z-lines and filaments in a number of myofibrils. In contrast to the

ALD fibres, there was no significant difference in sarcomere length between control and denervated PLD fibres (2.11 and 2.10 μm , respectively). When the PLD was stretched to 140% slack length, the Z-A distances increased evenly on either side of the A-bands, which remained at constant length (1.25–1.30 μm).

DISCUSSION

The present series of experiments on 1–3-week-old chickens fully confirmed the earlier findings (6) that the ALD muscle responds to denervation with an increase in weight. In addition, we could show now that this muscle undergoes considerable shortening after removal from the animal although the drooping wing must have extended in *in situ*. The shortening is obviously due to a state of contracture for the following reasons. 1) A transient reduction of resting tension was regularly observed during relaxation following a K^+ -contracture. 2) After stretching the denervated ALD, application of Ca^{2+} -free solution or addition of Co^{2+} markedly reduced the resting tension. Co^{2+} are known to block Ca^{2+} channels (4, 7), and it is therefore likely that the denervated ALD fibres exhibit an increased Ca^{2+} permeability; this could lead to a rise of the Ca^{2+} activity in the cytoplasm and hence to shortening of sarcomeres below control length (or high resting tension if shortening is counteracted by stretching the muscle). Electron microscopy revealed that the denervated muscles exhibited a marked reduction of sarcomere lengths. Since the classical experiments of Ramsey and Street (22) it is well known that a muscle develops maximum tension only at an optimum sarcomere length. Therefore, in order to develop tension the d-ALD had to be stretched more than the c-ALD, and on the average optimum lengths of the d-ALD and the c-ALD were almost identical. Similar observations were made by Schottelius *et al.* (25) in experiments on rat gastrocnemius muscles subject to different procedures resulting in inactivity of the muscle. Although care was taken to measure active tension at or near optimum length, our experiments revealed a markedly reduced ability of the d-ALD to develop tension independent of the nature of the stimulus. The reduction in contractile force may have been overestimated to some extent because an unknown part of the resting tension in the denervated muscles was due to a state of contracture, as discussed above. The total tension attained during electrical or chemical stimulation was, however, in most

muscles appreciably smaller than the corresponding values of control muscles. Hence, there can be no doubt that the denervated muscles had lost contractile force. In contrast to the findings reported by Jirmanová and Zelená (13), we did not observe severe signs of degeneration such as disruption of Z-lines, disarrangement of myofibrils, or vacuolization. This difference in results is probably due to the shorter observation period and the young age of the chickens in our experiments. The asymmetric displacement of A-bands observed in the d-ALD after stretching, and the occasional observation of aberrant myofilaments in the space between myofibrils must, however, be taken as first signs of degeneration and as a reflection of the functional alteration.

Apart from the generally reduced ability to develop tension there was an important qualitative difference between normal and denervated ALD. The latter was unable to maintain tension in response to K^+ -rich solution. This finding is very interesting because it has already been shown many years ago (21) that the K^+ -contracture of normal ALD consists of two phases. The first, rapid one is not immediately affected if Ca^{2+} are removed from the medium; the second, maintained phase is, however, strongly and rapidly abolished under this condition. These observations were fully confirmed in the present experiments; the conclusions drawn by Page (21) are further supported by our finding that Co^{2+} drastically reduce the maintained phase of K^+ -contracture but do not substantially affect its early phase. In addition, it was noted that the ability to maintain tension became weaker with increasing duration of denervation. Malvey *et al.* (19) explicitly state that the contracture responses of the c-ALD and the d-ALD were similar, and that both were able to maintain tension for prolonged periods of exposure to high potassium concentrations. This finding does not disagree with our results, because Malvey *et al.* (19) examined the muscles only 7 days after denervation, when according to our Fig. 5 the maintained phase is still at about 70% of the peak value. Removal of Ca^{2+} or addition of Co^{2+} did not appreciably affect the maintained phase of K^+ -contracture, nor could this be restored by increasing the Ca^{2+} concentration to 20 mM. The following mechanisms could explain this finding. 1) The Ca^{2+} channels (which seem to open during exposure of normal ALD to high- K^+ solution) do not open or inactivate quickly in the d-ALD. 2) The sarcoplasmic reticulum (SR) of the d-

ALD binds more Ca^{2+} than normally, and thereby reduces the Ca^{2+} activity inside the fibres. 3) The contractile system is unable to maintain tension, although the intracellular Ca^{2+} activity may be high. The latter explanation has to be discarded because the caffeine contracture is maintained at a high level. There is also no evidence available that the SR of the d-ALD binds more Ca^{2+} than normal ALD. Contrasting results as regards the Ca^{2+} -binding capacity of mammalian muscles are reported in the literature (5, 20, 26, 29). Thus, the most likely explanation of the reduced ability of the d-ALD to maintain the K^+ -contracture is a reduction or a rapid inactivation of the Ca^{2+} influx induced by K^+ .

Comparison between Denervated ALD and PLD

Opposite weight changes of the ALD and the PLD after denervation (6) were confirmed. In contrast, the other types of responses to denervation were qualitatively similar in the two muscles. The d-PLD also had reduced slack length, but the shortening was less marked than in the ALD. Both muscles exhibited increased stiffness, but the response of the PLD was more accentuated than that of the ALD. These changes were correlated with structural alterations, the signs of degeneration being much more obvious in the d-PLD than in the d-ALD. Finally, there was also a difference in the effect of denervation on optimum length. In the d-ALD it remained at its control value, but in the d-PLD it was clearly shorter than in the control muscles. The reasons for this difference between the two types of muscles are unclear.

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