ELECTROPHORESIS OF THE NON-STRUCTURAL PROTEINS FROM NORMAL AND ATROPHIC MUSCLES OF THE RABBIT AND OF MAN*

by

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INTRODUCTION

The experiments described in this paper aimed at ascertaining the influence of atrophy on the muscle proteins (globulin X, myogen, myoalbumin, myoglobin), soluble in salt solutions of low ionic strength and pH about 7, in which myosin and actomyosin are said to be insoluble. The proteins studied will further be called "easily soluble proteins" of muscle. They were studied by quantitative electrophoresis.

According to the results of experiments, carried out in this laboratory by BOSCH[1], the easily soluble proteins can be extracted completely by grinding the muscle with sand and a buffer solution of pH 7.15 and ionic strength 0.13. This was confirmed in the course of the work presented here. The numerical evaluation of the rather complicated diagrams by fitting in Gauss curves did not give fully satisfactory results in the hands of BOSCH. He believes that the differences between results calculated from various diagrams that appear to be identical upon superimposed projection must be attributed to the difficulty in drawing a pencil line exactly through the middle of the projected curves obtained by the Svensson-Philpot technique and to the arbitrariness inherent in the choice of the Gauss curves. Therefore he advises always to make the electrophoresis diagrams under exactly identical conditions and to decide upon visual inspection of superimposed projections whether differences exist or not. This procedure has the great disadvantage that one is not able to express these differences quantitatively. We believe, however, that we have demonstrated that a quantitative analysis of the diagrams of extracts of skeletal muscles is possible. Our procedure will be described in detail below.

Experiments were carried out with rabbit muscles and with human muscles.

EXPERIMENTAL PART

Methods

In general the procedure of BOSCH[1] was followed. Therefore the same buffer solution was employed for extraction, dialysis and electrophoresis. BOSCH has tried several solutions. We have

* This work forms part of the investigations on the biochemistry of muscle diseases by H. G. K. WESTENBRINK and co-workers, supported by a grant from the Netherlands Organisation for Pure Research (Z.W.O.).

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used a solution he recommends, containing 0.05 *M* KCl, 0.023 *M* Na_2HPO_4 and 0.01 *M* KH_2PO_4, of pH 7.15 and ionic strength 0.13, which gives the most detailed diagrams of all solutions tested. Some minor alterations described below were introduced.

Extraction of the proteins

The rabbits were anaesthetized with Numal-Roche (diethylammonium allyl*iso*propylbarbiturate) and bled by the aorta. After excision the muscle was stored for 1 hour at 1° C. Immediately after grinding *a* g muscle with *a* g sand and *a* ml extraction fluid the muscle debris were spun down at 1° C by 10 minutes' centrifuging at 14,000 *g* (centrifuging may also be done after 1 or 2 h). Contrary to BOSCH's experience viscous extracts were never obtained. No explanation of this difference can be offered.

In the experiments on muscles, atrophied as a consequence of inanition or vitamin-E-free feeding, it was necessary to excise the muscle of one leg before subjecting the rabbit to fasting or avitaminosis. Therefore this muscle certainly contained more blood than a muscle excised after the animal had been bled. As the experiments, referred to in Table II, show, this difference of blood content did not affect the results.

The human muscles were also stored, as soon as possible after the operation, for 1 hour at 1° C before extracts were made. Normal human muscles were excised during lung operations.

Dialysis

The dialysis was carried out at 5° C for at least 12 h, usually 18 to 24 h. The precipitate formed during dialysis was spun down by centrifuging at 0° C at 14,000 *g*. For rabbit muscle extracts 30 minutes' centrifuging was sufficient; in the case of human muscle extracts the centrifuging had to be continued for at least 50 minutes in order to prevent flocculation of some protein (myosin) in the electrophoresis cell.

Determination of protein content of the extracts

This was done by means of an Abbe refractometer. In the case of normal muscles the protein content was a little higher than 3%. In preliminary experiments the results of the refractometric determinations were compared with the results of Kjeldahl determinations. The agreement was excellent.

Electrophoresis

The dialyzed protein solution was diluted with the buffer solution to about 2%. Electrophoresis was carried out in a Tiselius apparatus (Strübin, Basle) according to the Svensson-Philpot method. Ilford Pan F film, 35 mm, 25° S, was used. No difficulties were encountered with the extracts of white muscles of rabbits. In the case of extracts of red muscles of both human and rabbit, it appeared to be necessary to cover the uncoloured portion of the ascending boundary in the electrophoresis cell during part of the rather long exposure time, and to prevent the entrance of stray light from the room into the optical system.

Some myosin solutions were examined. As they were very opalescent very long exposure times were required. Diagrams of reasonable quality were obtained. Yet we believe that cells as used by DUBUISSON *et al.*[2] are to be preferred to our cells of the common type for the investigation of myosin solutions.

Numerical evaluation of the diagrams

The general type of diagram to be analyzed is shown in Fig. 1. In principle the analysis was carried out according to WIEDEMANN[3]. A Gauss curve was placed in peak VIII*. Then other Gauss curves were placed in the diagram to the left and to the right, taking great care each time that the area overlapped by two Gauss curves was as nearly equal as possible to the area of the diagram left uncovered between both curves. The tops of the Gauss curves need not coincide at all with the peaks which can be distinguished in the diagram. The part of the diagram between peaks II and III is too flat to be resolved into Gauss curves. The surface of

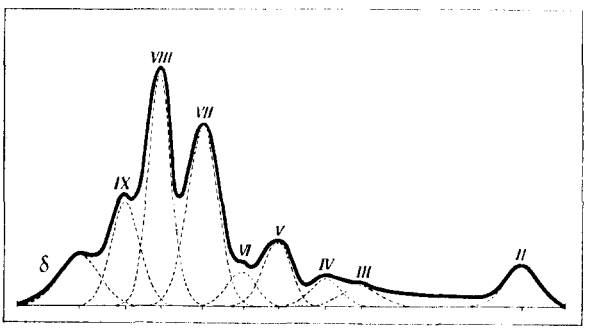

Fig. 1. Diagram of extract of fresh normal rabbit muscle with Gauss curves fitted in.

* The components are numbered from II to IX. A very small component I, moving very rapidly, was neglected.

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this part was measured with a planimeter. The sum of the surfaces of the Gauss curves and the unresolvable intermediate part between II and III should equal the total surface determined by planimeter and at every point of the abscissa the sum of the ordinates of points of the Gauss curves should equal the ordinate of the diagram at that point (see Fig. 1).

Rabbit muscles

The data assembled in Table I show that the preparation of the extracts as well as the numerical evaluation of the diagrams can be carried out in a highly reproducible way. In each of these experiments homologous muscles of the hind legs of a rabbit were compared. These muscles were excised immediately one after another.

The differences between homologous muscles were scarcely greater when the muscles were excised with an interval of 21 to 28 days (see Table II). This statement is important in view of later experiments, in which a muscle of one leg had to be excised before the rabbit was subjected to fasting or vitamin-E deficiency in order to provoke atrophy of the homologous muscle of the other leg.

Somewhat greater, though always still small, differences were found between various skeletal muscles of one rabbit (Table III), quite distinct differences, however, between the skeletal muscles of various rabbits (Table IV).

The mobilities at pH 7.15 of the eight components which can be derived from the diagrams were determined in 17 cases. The means and the standard deviations are given in Table V. To determine the iso-electric points of the components portions of an extract were dialyzed against solutions of ionic strength 0.13, which contained 0.05 *M* KCl and various ratios of primary and secondary phosphate, so that they had the following pH's: 8.00, 7.40, 7.00, 6.60, 6.20, 5.75, 5.30, 4.90*. With the protein solutions thus obtained pH-mobility curves of the various components were determined. The iso-electric points derived from these curves are given by Table VI. Similar determinations have been carried out by JACOB[4]. His results were: comp. VI: 5.7, comp. VII: 6.0, comp. VIII: 6.2, comp. IX: 6.8.

The iso-electric points of components II, III and IV are situated below 4.9. Component IX was split into 3 (ascending boundaries) or 2 (descending boundaries) components at pH values between 6.4 and 6.0 respectively. Each mobility determination was carried out in triplicate. All determinations were carried out as rapidly as possible one after another without interruption during the night in order to prevent undesired alterations in the protein solutions.

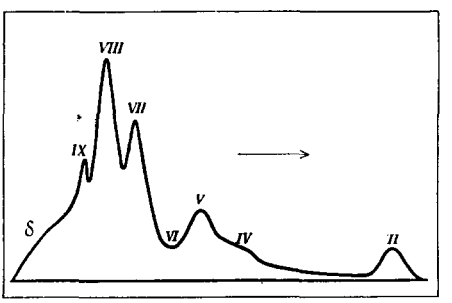

Fig. 2. Diagram of extract of normal rabbit muscle in rigor mortis.

Rigor mortis appeared to entail a quite distinct increase of the percentage of component V and possibly a slight increase of the intermediate part of the diagram between components II and III, no matter whether the rigor mortis had developed in the course of 8 h at 1° C in the isolated muscle or in the muscle *in situ* (see Table VII and Fig. 2).

Muscular atrophy was induced by various means, *viz.* severance of a motoric nerve,

* We did not prepare protein solutions of lower pH, as it is not possible to compose more acid phosphate buffer solutions, and buffers of other composition would give quite different diagrams (see BOSCH[1]).

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TABLE I

HOMOLOGOUS MUSCLES OF THE HIND LEGS OF RABBITS

Muscles excised immediately one after another.
Area of the Gauss curves II to IX and of the intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient.

Rabbit No.	*Muscle*	*IX*	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*
					Ascending boundaries					
4	Vastus lateralis, left	14.7	30.0	24.3	3.7	11.8	4.8	2.5	2.5	5.8
	Vastus lateralis, right	14.6	29.9	24.1	3.7	11.6	4.8	2.4	2.7	5.8
10	Gastrocnemius, left	17.2	25.2	24.9	12.3	5.6	4.0	3.8	3.0	3.8
	Gastrocnemius, right	17.1	24.9	25.0	12.3	5.7	4.2	3.9	3.0	3.9
12	Serratus, left	15.3	30.0	25.3	6.1	6.7	3.8	4.1	3.4	4.1
	Serratus, right	15.7	30.6	25.3	6.3	6.8	3.8	4.1	3.2	4.1
					Descending boundaries					
4	Vastus lateralis, left	14.5	28.0	22.7	3.2	7.4	4.1	2.8	7.5	5.7
	Vastus lateralis, right	14.7	27.1	22.8	3.3	7.6	4.2	2.9	7.0	5.8
10	Gastrocnemius, left	17.2	19.7	29.3	10.3	7.3	6.7	1.8	5.2	3.6
	Gastrocnemius, right	17.7	20.0	29.1	10.1	7.3	6.6	1.7	5.5	3.5
12	Serratus, left	13.4	31.0	25.1	2.9	7.5	4.4	2.9	2.5	6.5
	Serratus, right	13.4	31.1	25.1	2.9	7.2	4.4	2.9	2.9	6.5

TABLE II

HOMOLOGOUS MUSCLES (VASTUS LATERALIS) OF THE HIND LEGS OF RABBITS, EXCISED WITH AN INTERVAL OF 3 AND 4 WEEKS

Area of the Gauss curves II to IX and of the intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient. Rabbit 3, muscle from right leg excised 21 days after left leg, rabbits 8 and 11, 28 days.

Rabbit No.	*Leg*	*IX*	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*
					Ascending boundaries					
3	left	9.6	28.9	26.4	6.0	10.0	4.2	4.6	3.0	4.8
	right	9.0	28.5	27.0	6.2	9.5	4.6	4.2	3.2	5.0
8	left	21.6	24.1	27.2	3.9	9.7	2.2	2.2	4.2	4.2
	right	20.4	24.3	26.8	3.1	9.2	3.0	2.6	4.0	4.0
11	left	14.6	32.1	22.2	6.7	10.2	7.2	3.7	1.5	3.1
	right	14.1	32.9	21.7	6.0	9.7	8.0	3.5	1.7	3.4
					Descending boundaries					
3	left	8.3	30.9	26.5	4.8	10.0	5.1	2.4	5.3	4.0
	right	8.8	31.2	25.8	4.6	9.7	5.5	2.4	5.5	4.2
8	left	11.7	32.5	25.6	6.8	8.3	2.8	2.9	4.5	4.8
	right	11.9	32.7	26.2	7.0	8.7	3.1	3.1	4.3	4.6
11	left	13.6	27.6	24.6	7.3	6.2	7.2	4.0	5.0	3.6
	right	13.2	27.0	25.2	7.6	6.5	6.6	4.0	5.3	3.8

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TABLE III

VARIOUS MUSCLES OF ONE RABBIT

Area of the Gauss curves II to IX and of the intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient.

Muscle	*IX*	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*
					Ascending boundaries				
Serratus	15.7	30.6	25.3	6.3	6.8	3.8	4.1	3.2	4.1
Gastrocnemius	15.4	30.1	25.0	5.8	6.9	4.6	3.9	2.6	4.5
Vastus lateralis	15.6	29.5	25.1	5.5	6.7	5.3	4.1	3.9	4.2
Vastus intermedius	15.6	29.4	25.0	5.6	6.8	3.9	3.5	4.1	4.3
					Descending boundaries				
Serratus	13.4	31.1	25.1	2.9	7.2	4.4	2.9	2.9	6.5
Gastrocnemius	13.2	31.2	25.0	2.9	7.2	4.5	2.9	3.4	6.5
Vastus lateralis	13.2	31.4	28.8	3.0	6.9	4.7	2.9	2.4	6.9
Vastus intermedius	12.5	31.7	26.1	2.9	6.9	4.5	2.9	3.6	6.5

TABLE IV

MUSCLES OF VARIOUS RABBITS

Area of the Gauss curves II to IX and of the intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient. Means and standard deviations.

Gauss curve	*Number of determ.*	*Ascending boundaries*	*Descending boundaries*
IX	33	16.4 ± 4.8	15.5 ± 3.5
VIII	33	33.4 ± 6.7	28.7 ± 3.6
VII	33	24.2 ± 2.7	23.9 ± 3.2
VI	33	6.0 ± 1.7	5.7 ± 2.0
V	33	8.3 ± 1.6	7.9 ± 1.7
IV	33	5.0 ± 1.7	4.7 ± 1.6
III	27	3.3 ± 1.0	2.8 ± 1.0
Interm. part	27	4.7 ± 2.2	4.7 ± 1.9
II	33	4.0 ± 1.3	4.5 ± 1.1

TABLE V

MUSCLES OF VARIOUS RABBITS

Mobilities of components II to IX expressed in 10^{-5} cm^2 volt^{-1} sec^{-1}. Means and standard deviations of 17 determinations.

Component	*Descending boundaries*	*Ascending boundaries*
IX	0.8 ± 0.02	0.7 ± 0.02
VIII	1.3 ± 0.05	1.2 ± 0.03
VII	1.7 ± 0.02	1.6 ± 0.01
VI	2.2 ± 0.03	1.9 ± 0.03
V	2.7 ± 0.03	2.6 ± 0.02
IV	3.3 ± 0.05	3.2 ± 0.06
III	4.0 ± 0.05	3.8 ± 0.06
II	6.4 ± 0.06	6.2 ± 0.06

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immobilization of a leg in a plaster cast, prolonged fasting and vitamin-E deficiency.

Severance of a motoric nerve. Part of the nervus ischiadicus of one leg was excised and the skin sutured. The operation, which was terminated in 5 minutes, was carried out under Numal-Roche narcosis. Apart from the paralysis of the leg the animals were in perfect health. Homologous muscles of the hind legs, one normal and one atrophied, were excised after 2 to 5 weeks, in one case after 9 weeks.

Immobility in a plaster cast. In order to prevent damage by gnawing it appeared to be necessary to place a wooden collar around the neck of the rabbit. After three weeks the vastus lateralis muscles were removed from both hind legs and examined.

Inanition. Two rabbits were used for this experiment. The vastus lateralis was removed from one leg before the fasting period. (The results in Table II show that no change occurs in normal muscle in the course of some weeks.) The animals died after fasting for 6 and 9 days respectively. In this time the body weight had decreased by about 40%. As both animals died during the night the muscles were in rigor when the extracts were made. As will appear from Table VIII this did not affect the results.

Vitamin-E deficiency. A rabbit was fed on the GOETTSCH-PAPPENHEIMER diet[5]. The vastus lateralis of one leg had been removed before administration of the vitamin-E-free diet. The excretion of creatine and creatinine was determined every day. After about 18 days the creatine excretion increased from about 10 mg to 20 to 40 mg per 24 h. The 29th day showed a sharp rise of the creatine excretion to about 260 mg. On the next days it was somewhat lower again. The rabbit died on the 33rd day.

All atrophied muscles, which have been examined, with the exception of the muscle excized 9 weeks after severance of the nerve, had lost about 50% of their original weight. No matter which method was used to cause the atrophy, a 50% decrease in weight was accompanied by a 2- to 3-fold increase of the percentage of component II. This can be seen in Table VIII and Fig. 3. (The increase of the percentage of this component has already been observed by CRÉPAX[6], though only in the case of denervated muscles).

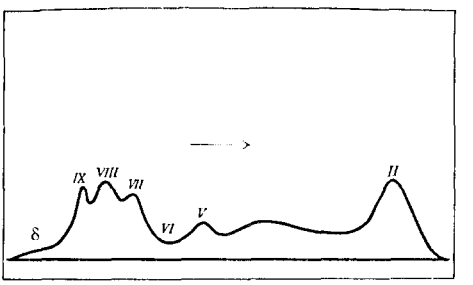

Fig. 3. Diagram of extract of atrophied rabbit muscle (2½ to 5 weeks after severance of nerve or immobilisation in plaster cast; or after death by inanition or vitamin-E deficiency).

The diagram obtained from the muscle which had atrophied during 9 weeks after severance of the nervus ischiadicus is shown in Fig. 4. All peaks had disappeared with the exception of component II, which accounted for about 50% of the total area.

As can be seen from Fig. 3 the unresolvable part of the diagram between components II and III also increases considerably, but this increase cannot be expressed numerically, as component III—and sometimes component IV—cannot be distinguished anymore in the diagrams of atrophied muscles, so that the flat unresolvable part extends from component II to component IV or V (see Table VIII).

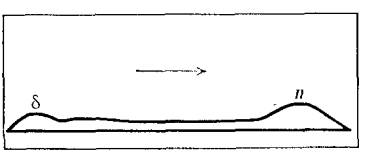

Fig. 4. Diagram of extract of atrophied rabbit muscle, 9 weeks after severance of nerve.

Human muscles

Fifteen normal muscles (m. serratus anterior) were examined. Fig. 5 shows the prototype of the diagrams. It bears a strong resemblance to the diagrams of normal rabbit muscles. The mobilities of the components very closely approached those of the components of the rabbit muscle diagrams. Therefore the same numbering could be used. Table IX shows the results

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TABLE VI

ISO-ELECTRIC POINTS

	V	*VI*	*VII*	*VIII*	*IX*
Ascending boundaries	4.9	5.0	5.3	5.8	6.2
Descending boundaries	4.9	5.0	5.3	6.0	6.4

TABLE VII

COMPARISON OF FRESH MUSCLES AND HOMOLOGOUS MUSCLES IN RIGOR

Area of the Gauss curves II to IX and of the intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient. Ascending boundaries. Rigor developed *in situ*. Each pair of homologous muscles taken from a different rabbit.

Muscle	*Rigor*	*IX*	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*
Serratus	—	15.7	30.6	25.3	6.3	6.8	3.8	4.1	3.2	4.1
	+	12.4	25.4	25.1	6.3	14.8	3.5	3.2	5.0	4.1
Gastrocnemius	—	15.4	30.1	25.0	5.8	6.9	4.6	4.0	2.6	4.5
	+	13.6	24.5	24.8	5.8	13.2	4.2	3.3	3.1	4.5
Vastus lateralis	—	15.6	29.5	25.1	5.5	6.7	5.3	4.1	3.9	4.2
	+	13.3	23.3	24.6	5.5	11.7	4.9	3.5	5.0	4.2
Vastus intermedius	—	15.6	29.4	25.0	5.6	6.9	3.9	3.6	4.1	4.3
	+	13.0	22.9	25.2	5.7	15.9	3.7	2.8	6.3	4.3

of the numerical evaluation of 13 diagrams. The most pronounced difference as compared to rabbit muscle is the comparatively high percentage of components II and VII, and the comparatively low percentage of component VIII. In most cases component IX was absent.

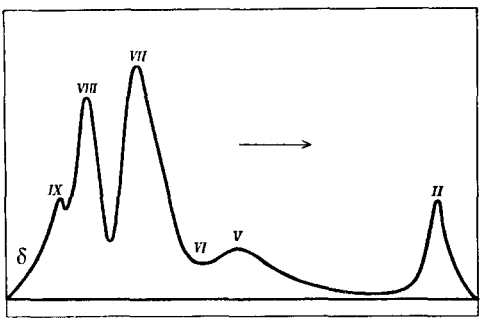

Fig. 5. Diagram of extract of normal human muscle.

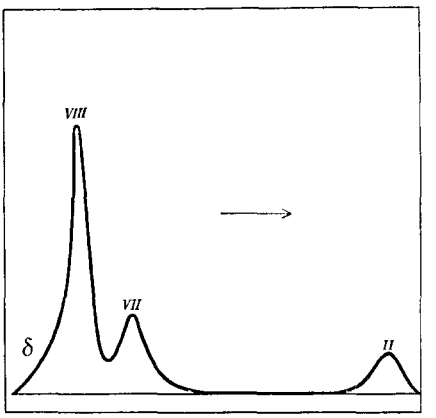

Fig. 6. Diagram of extract of human muscle atrophied since several decades.

Atrophic muscles were excised from patients suffering from prolonged muscular inactivity, often extending over several decades. Table X shows the results of the numerical evaluation of the diagrams obtained from 7 patients. As compared to normal muscles it appears that in general the percentages of components II and VIII have increased and the percentages of components VI and VII have diminished, while com-

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TABLE VIII

COMPARISON OF NORMAL AND ATROPHIED HOMOLOGOUS RABBIT MUSCLES

SN = atrophy by severance of nerve, P = atrophy by immobilization in plaster cast, I = atrophy by inanition, E = atrophy by vitamin-E deficiency.

If component III or components III and IV could not be distinguished in diagrams of atrophied muscles, one figure is given for the unresolvable part between components II and IV or II and V.

Ascending boundaries.

Rabbit No.	*Cause of atrophy*	*Time of development of atrophy*	*mg total protein extracted from whole muscle*	*Percentage of total area*									*mg comp. II extracted from whole muscle*
				IX	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*	
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
9	—	—	310	18.0	30.0	21.2	6.7	9.5	6.8	2.2	3.0	4.9	15
	SN	2 weeks	154	12.8	19.2	18.9	5.9	9.5	7.5		15.8	10.4	16
13	—	—	750	17.4	29.8	21.4	7.2	9.7	6.6	2.2	3.6	4.7	35
	SN	2½ weeks	300	12.5	19.8	19.2	5.7	7.9	6.2		19.6	12.5	36
14	—	—	400	14.4	27.4	30.4	5.6	6.1	4.0	4.5	9.5	4.0	16
	SN	3 weeks	137	12.7	17.1	15.1	5.7	7.0	5.0		20.0	12.1	17
15	—	—	460	12.5	26.8	25.2	7.1	6.9	6.0	4.2	4.5	6.4	29
	SN	4 weeks	190	7.7	27.2	18.0	6.4	12.0			14.2	15.2	29
16	—	—	290	9.5	25.1	20.2	8.7	7.7	6.2		14.2	5.2	15
	SN	4 weeks	120	8.2	17.3	12.2	4.8	7.8	4.9		25.6	13.2	16
19	—	—	338	11.7	27.4	25.0	6.8	7.3	6.4	4.0	4.1	6.8	23
	SN	4 weeks	140	12.2	17.0	15.3	5.7	7.0	5.1		20.2	17.1	24
33	—	—	290	12.3	24.8	20.9	9.1	7.9	6.0	8.1	5.6	5.3	15
	SN	5 weeks	120	7.7	10.8	9.9	8.1	5.7	5.6		24.2	13.6	16
20	—	—	550	13.0	29.6	24.4	7.9	7.8	5.2	3.2	5.6	4.2	23
	P	3 weeks	230	9.2	18.4	17.6	7.1	6.8			30.1	10.0	23
18	—	—	570	26.8	21.2	21.0	4.5	8.2	4.1	2.4	5.0	6.1	35
	I	9 days	134	12.5	12.5	17.6	7.6	11.4			26.8	26.0	35
24	—	—	480	16.9	23.4	29.8	6.3	10.7	6.3	4.6	8.2	6.7	32
	I	6 days	162	9.6	18.0	14.5	5.8	18.2			14.8	23.3	37
22	—	—	112	18.6	30.1	26.6	5.4	6.3	4.2	4.0	5.0	4.1	4.6
	E	33 days	39	8.4	18.2	16.5	5.7	6.2			32.8	11.1	4.3

TABLE IX

NORMAL HUMAN MUSCLES

Area of Gauss curves II to VIII and of intermediate part between II and III expressed in percentage of total area minus δ- (resp. ε-) gradient. Means and standard deviations.

Gauss curve	*Ascending boundaries*		*Descending boundaries*	
	Number of determinations	*Percentage*	*Number of determinations*	*Percentage*
VIII	13	27.3 ± 6.8	12	26.0 ± 5.7
VII	13	33.7 ± 6.4	12	36.2 ± 6.0
VI	13	7.7 ± 2.1	12	7.3 ± 2.0
V	13	7.7 ± 1.9	12	6.4 ± 2.0
IV	10	5.5 ± 1.5	12	6.7 ± 1.4
III	4	4.1 ± 1.6	4	5.3 ± 1.3
Interm. part	4	4.3 ± 1.3	4	5.3 ± 2.3
II	13	6.6 ± 1.2	12	7.2 ± 1.4

TABLE X

ATROPHIED HUMAN MUSCLES

Area of Gauss curves II to VIII and of intermediate part expressed in percentage of total area minus δ- (resp. ε-) gradient.

Age of atrophy in years	*Fatty degenerations*	*VIII*	*VII*	*VI*	*V*	*IV*	*III*	*Interm. part*	*II*
				Ascending boundaries					
2	—	44.0	28.8	3.2	0	0	0	17.4	9.0
3	—	48.8	24.3		0	0	0	15.0	11.7
4	—	41.3	28.8	4.3	0	0	0	14.4	11.3
10	+	56.4	20.0		0	0	0	12.1	11.5
20	+	55.2	27.2		0	0	0	0	16.9
23	+	50.6	30.6		0	0	0	0	18.6
46	+	49.2	32.8		0	0	0	0	18.0
				Descending boundaries					
2	—	40.0	24.4	5.2	0	0	0	18.8	11.6
3	—	46.2	28.8		0	0	0	13.1	12.8
4	—	36.2	29.4	9.0	0	0	0	26.0	8.3
10	+	52.2	26.6		0	0	0	9.1	11.4
20	+	51.8	35.8		0	0	0	0	13.2
23	+	52.4	33.2		0	0	0	0	14.2
46	+	50.2	31.6		0	0	0	0	18.2

TABLE XI

AMOUNTS OF PROTEIN EXTRACTED PER G MUSCLE

Muscles	*Easily soluble proteins*		*Myosin*	
	Number of determinations	*mg per g*	*Number of determinations*	*mg per g*
Normal rabbit muscles	44	24 ± 3.5	16	16 ± 2.5
Atrophic rabbit muscles	12	19 ± 3.1	8	4 ± 2.6
Rabbit muscles in rigor mortis	12	24 ± 4.2	11	3 ± 1.5
Normal human muscles	12	24 ± 4.1	12	17 ± 3.2

ponents III, IV and V have completely disappeared. In those cases in which the atrophy had only existed for a few years the unresolvable intermediate part was present in the diagrams. If the atrophy had been manifest for many years (*e.g.* 40 years) this intermediate part had completely disappeared. Fig. 6 shows the prototype of the diagrams of the latter muscles.

Myosin, extracted from muscle by salt solution of pH 7.15 and ionic strength 0.13

As described above, a precipitate forms upon dialysis of the muscle extracts against the KCl-phosphate solution of pH 7.15 and ionic strength 0.13. This precipitate was spun down, repeatedly washed with the same solution and then dissolved in Greenstein-Edsall solution (0.5 *M* KCl, 0.03 *M* $NaHCO_3$; pH 8.10; ionic strength 0.53). After dialysis against this solution the electrophoresis diagram obtained appeared to be practically identical with that of a myosin solution prepared from rabbit muscles according to STEYN-PARVÉ AND GERRITSEN[7]. Hence it appears that myosin may be extracted from the muscle by a practically neutral solution of low ionic strength.

Amounts of proteins extracted from normal and atrophied muscles

Table XI shows the means of the amounts of the easily soluble proteins and of myosin, extracted per g muscle by KCl-phosphate solution of pH 7.15 and ionic strength 0.13. Regarding the easily soluble proteins no difference exists between fresh normal rabbit muscles, fresh normal human muscles and rabbit muscles in rigor, but the amount extracted per g of atrophic rabbit muscles is significantly lower ($P < 0.001$). The amount of myosin extracted per g tissue from fresh normal rabbit muscles and from fresh normal human muscles did not differ, the amounts extracted per g tissue from the atrophied rabbit muscles and from normal rabbit muscles in rigor were very much lower, however.

Notwithstanding the fact that the total amount of easily extracted protein per g of atrophic rabbit muscle was lower than the amount extracted per g of normal muscle, and that the weight of the atrophic muscles was much less than the weight of the normal homologous muscles, the amount of the protein(s) giving rise to component II of the diagram, extracted from the *whole* atrophied muscle, had not diminished as compared with the amount of this protein extracted from the whole homologous normal muscle. This can be seen from column 14 of Table VIII, the figures of which have been calculated from the total amounts of easily soluble protein per whole muscle (column 4) and the percentage of component II (column 13).

Though the diagrams of extracts of atrophied muscles had also shown an increase of the flat unresolvable part between peaks II and III, observed in the case of normal muscles, a similar calculation could not be carried out here, for this part of the diagram now extended from component II to component IV or from component II to component V.

DISCUSSION

It should be realized that the high reproducibility of the numerical evaluation of the diagrams could only be attained if it was carried out by one and the same person, always following the same procedure. We have placed Gauss curves in the diagrams, starting with the rather arbitrarily chosen peak No. VIII (the highest one in the case of extracts of rabbit muscle) and then trying to fill up the whole diagram with the

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smallest possible number of Gauss curves. The figures obtained for the various Gauss areas as percentages of the total area minus δ- (resp. ε-) gradient may obviously only be regarded as characteristics of the muscle extracts serving for mutual comparison of these extracts. It will be necessary to employ other methods, *e.g.* salting out according to DERRIEN and enzyme determinations, in order to obtain a fuller understanding of what happens to the proteins in the course of the development of atrophy.

The most remarkable fact established in this work seems to be the practically identical changes that are observed irrespective of whether the atrophy is caused by immobilization (as a consequence of severance of the motoric nerve or of application of a plaster cast), by inanition or by vitamin-E deficiency. In all cases the proteins that give rise to component II (and probably also those composing the flat part of the diagram between components II and III) are maintained, while the other proteins disappear in the course of the development of the atrophy.

Special attention should furthermore be given to the difference between the diagrams of rabbit muscles, rendered atrophic by experiment and human atrophied muscles. GERRITSEN[8], working in this laboratory on the glucokinase content of normal and atrophied muscles, also found a difference between these two kinds of atrophy. The enzyme appeared to have been fully maintained in the atrophied rabbit muscles, but to have decreased considerably in the atrophied human muscles. In both cases we believe that the differences between rabbit and human muscles are caused by the different times during which the atrophies have existed. The intermediate unresolvable part of the electrophoresis diagrams of extracts of atrophied rabbit muscles and the glucokinase of these muscles would probably also have disappeared if it would have been possible to incite a very slowly developing muscular atrophy in rabbits.

SUMMARY

The proteins of normal and atrophied skeletal muscles of the rabbit and of man, soluble in salt solutions of low ionic strength and pH about 7, were studied by means of electrophoresis. Atrophy of rabbit leg muscles was caused by severance of the motoric nerve, by immobilisation of a leg in a plaster cast, by inanition and by vitamin-E deficiency. No matter which of these methods was used to induce muscular atrophy, the electrophoresis diagrams obtained always showed the same alterations as compared with the diagrams obtained for normal muscles. The most remarkable feature was the increase of the percentages of the more rapidly moving components and the decrease of the percentages of the slower components. While the total amount of protein, extractable by a salt solution of ionic strength 0.13 and pH 7.15 from the whole muscle, had considerably decreased, the amount of the most rapidly migrating component (II), distinguishable in the diagrams, had remained constant.

Normal human muscles differed little from normal rabbit muscles. The flat intermediate part of the diagrams, which had been maintained in the case of the atrophied rabbit muscles, had, however, completely disappeared in the case of chronic human muscular atrophy caused by immobilization, existing during several decades. The time of existence of the atrophy may be the cause of this difference, as the atrophy of the rabbit muscles had developed in the course of a few weeks.

RÉSUMÉ

Les protéines des muscles squelettiques normaux et atrophiés de l'homme et du lapin, solubles dans des solutions salines de force ionique faible et de pH environ 7, ont été étudiées par électrophorèse. L'atrophie des muscles de la patte du lapin est provoquée par lésion du nerf moteur, par immobilisation de la patte dans un moule de plâtre, par inanition ou par carence en vitamine E. Quelle que soit la méthode utilisée pour provoquer l'atrophie musculaire, les diagrammes d'électrophorèse obtenus présentent toujours les mêmes modifications par rapport aux diagrammes obtenus avec des muscles normaux. La plus remarquable de ces modifications est l'augmentation des pour-

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centages des constituants se déplaçant le plus rapidement, et la diminution des pourcentages des constituants les plus lents. Alors que le contenu total en protéines extractibles par une solution saline de force ionique 0.13 et de pH 7.15 à partir du muscle entier, diminue considérablement, la teneur du constituant qui possède la plus grande vitesse de migration (II), qu'on peut distinguer sur le diagramme, reste constante.

Les muscles humains normaux diffèrent peu des muscles de lapins normaux. La partie intermédiaire plate du diagramme, qui est maintenue dans le cas des muscles de lapin atrophiés, disparait cependant complètement dans le cas d'une atrophie musculaire humaine chronique provoquée par une immobilisation de plusieurs dizaines d'années. Cette différence est peut-être due à la durée de l'atrophie, car l'atrophie des muscles du lapin est obtenue en quelques semaines.

ZUSAMMENFASSUNG

Die Proteine normaler und atrophierter Skelettmuskeln von Kaninchen und Menschen, die in Salzlösungen mit niedriger Ionenstärke und einem pH von ungefähr 7 löslich sind, wurden mit Hilfe der Elektrophorese untersucht. Die Atrophie der Beinmuskeln von Kaninchen wurde hervorgerufen durch Trennung des motorischen Nervs, durch Immobilisation des Beines im Gipsverband, durch Entkräftigung und durch Vitamin E-Mangel. Verglich man die erhaltenen elektrophoretischen Diagramme mit denen normaler Muskeln, so zeigten sie immer die gleiche Veränderung, ganz gleichgültig welche dieser Methoden zur Herbeiführung der Muskelatrophie benutzt wurde. Das Ansteigen des Prozentgehalts der schneller beweglichen Komponenten und das Absinken des Prozentgehalts langsamer Komponenten war das meist bemerkenswerte Merkmal. Während der Gesamtanteil des mit Salzlösung der Ionenstärke 0.13 und pH 7.15 aus dem ganzen Muskel extrahierbaren Proteins beträchtlich abnahm, blieb der Anteil der am schnellsten wandernden Komponente (II) welche man in den Diagrammen unterscheidet, konstant.

Normale menschliche Muskeln unterschieden sich wenig von normalen Kaninchenmuskeln. Der flache Zwischenteil der Diagramme, der im Fall des atrophierten Kaninchenmuskels erhalten blieb, verschwand jedoch vollständig im Fall der über mehrere Jahrzehnte bestehenden, durch Immobilisation verursachten menschlichen chronischen Muskelatrophie. Die Ursache dieses Unterschiedes könnte in der Dauer des Bestehens der Atrophie liegen, da sich die Atrophie des Kaninchenmuskels im Laufe einiger Wochen entwickelte.

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