CHANGES IN CONTRACTILE MUSCLE PROTEINS OF VITAMIN-E-DEFICIENT RABBITS

II. OPTICAL PROPERTIES OF PROTEINS FROM NORMAL AND DYSTROPHIC MUSCLES

by

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In a previous paper[1] we have reported extraction experiments on muscle proteins from normal and vitamin-E-deficient rabbits. We stated there that, in muscular dystrophy produced by vitamin-E deficiency, there is a progressive decrease in the actomyosin content of the muscle extract. Myosin may completely disappear in the advanced states of dystrophy. In our opinion this is not merely due to a decrease in extractibility, but also to a real loss of such proteins. This opinion was supported by the study of the optical behaviour of both normal and dystrophic muscle fibres[2]. On the contrary, actin was found to have undergone no significant quantitative changes during the dystrophic process.

In the present paper we wish to report experiments on the qualitative changes of muscle proteins from dystrophic animals as shown by the study of the optical behaviour of protein solutions, namely their double refraction of flow (D.R.F.).

MATERIALS AND METHODS

Young rabbits (body weight 650–700 g) were divided into three groups: the first group was kept on a normal diet of bread and green vegetables; the second group was fed the vitamin-E-deficient diet of HOUCHIN AND MATTILL[11] slightly modified*, the third one was kept on the same diet, supplemented with vitamin E (20 mg given twice a week by mouth).

The progress of the dystrophic process was estimated from the rapid loss of weight of the animals. On the average, dystrophic rabbits died 35–45 days after the beginning of the experiment. Animals of the third group showed curves similar to those of rabbits fed the normal diet (group 1). Animals of the second group were killed 1. towards the end of the dystrophic process when the rabbits became unable to right themselves when pushed over, 2. at intermediate stages of dystrophy. Animals of the third group were killed after the same or longer experimental periods than animals of the second group.

Extraction of the different muscle proteins was carried out according to the technique of SZENT-GYÖRGYI[10] for myosin and actomyosin**, and of FEUER *et al.*[12] for actin. Myosin was purified

* 20% cellophane was substituted by 10% homogenized filter paper, 9% starch and 1% lard.

** Myosin is actin-free myosin and corresponds to L-myosin of SCHRAMM AND WEBER[14]. Actomyosin is the fraction of myosin precipitated by actin during the preparation of myosin (1.5% actomyosin according to SZENT-GYÖRGYI[10]).

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according to SZENT-GYÖRGYI[10] and actomyosin reprecipitated twice. The homogeneity of our protein fractions was also occasionally checked by electron microscopic examinations.

The composition of the solvents of the proteins will be found at the head of the tables summarizing our experiments.

The D.R.F. was measured using a Gerendás glass cell made according to the dimensions given by the author[13] *. This cell was mounted on a Leitz polarizing microscope. The velocity gradient (6,000) was calculated according to GERENDÁS. This is far beyond the range in which the D.R.F. is affected by temperature and viscosity[3-8]. Our measurements, however, were always carried out at 4° C for myosin and actomyosin, and at 18° C for actin.

Except in those experiments in which protein concentration had to be varied, we tried to keep it at the same level (estimated as N by the Kjeldahl method). With dystrophic rabbit muscles this was not always possible, especially for myosin.

The birefringence angle ψ of each protein solution as well as the retardation R measured with a 5 mm Bereck compensator, were determined. In order to make all measurements under comparable conditions R was estimated after having measured the angle ψ and having rotated the plane of polarization from the position corresponding to complete extinction to an angle of 45°.

RESULTS AND DISCUSSION

The values of ψ for *actomyosin* and *myosin solutions* corresponding to normal and dystrophic rabbits are reported in Tables I and III, Table II shows the values of ψ and R for actomyosin solutions at different concentrations of protein. It can easily be seen that the value of ψ for actomyosin are of the same order as those found by VON MURALT AND EDSALL[7] for the then called "myosin" extracted with Weber solution. The variations may be due to different methods of extraction which may yield actomyosin of different composition.

TABLE I

DOUBLE REFRACTION OF FLOW OF ACTOMYOSIN FROM NORMAL AND VITAMIN-E-DEFICIENT RABBITS

Values of ψ ($\pm$ 1°) of a solution containing 1,100–1,200 mg N/l.
Temp. 4° C; velocity gradient 6,000.

Rabbits on normal diet		*Rabbits on H. and M. diet*		*Rabbits on H. and M. diet + vitamin E*	
No.	ψ	*No.*	ψ	*No.*	ψ
1	72°	1	72°	1	72°
2	72°	2	70°	2	69°
3	68°	3	72°	5	70°
4	71°	4	71°	6	72°
5	69°	5	68°	7	68°
6	71°	6	67°		
7	70°	9	67°		
		11	69°		
		12	69°		
		13	70°		
		36	72°		

Actomyosin extracted from dystrophic rabbits, although considerably reduced in quantity[1], has the same optical properties as that from normal rabbits or from rabbits of group 3.

When ψ and R were measured on *actomyosin solutions of different concentration of*

* The cell was perfectly made by Mr. E. NISTRI, Via La Spezia 28, Rome.

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protein (Table II), it was found that, parallel to the obvious fall in R with decreasing concentration, there is also a fall in the angle ψ. This is evidently due to anomalies in the law of birefringence of flow shown by several organic substances (PETERLIN AND STUART[9]).

TABLE II

DOUBLE REFRACTION OF FLOW OF ACTOMYOSIN FROM NORMAL RABBITS

Effect of various concentrations of protein in 0.6 M KCl on ψ ($\pm$ 1°) and R ($\pm$ 10). Temp. 4° C; velocity gradient 6,000.

Rabbit no. 7-on normal diet			*Rabbit no. 7 – on H. and M. diet + vit. E*	
Concentration of Actomyosin mgN/l	ψ	R $\mu\mu$	*Concentration of Actomyosin mg N/l*	ψ
1,236	70°	175	1,138	68°
988	68°	145	910	68°
865	67°	98	569	64°
741	66°	74	455	62°
494	65°	67	284	59°
370	63°	54		
312	59°	43		

When myosin was available from vitamin-E-deficient rabbits, it showed the same ψ as that from normal rabbits (Table III). It may be necessary to recall that the dystrophic disease in the rabbit is an almost acute process and that when it is possible to extract a good amount of myosin from vitamin-E-deficient muscles this is likely to be due to the immaturity of the dystrophic process, the muscles not yet being severely damaged.

TABLE III

VALUES OF ψ ($\pm$ 1°) FOR AQUEOUS MYOSIN SOLUTION

N Concentration: 1,100–1,200 mg/l.
Temp. 4° C; velocity gradient 6,000.

Normal rabbits		*Rabbits – on H. and M. diet*		*Rabbits – on H. and M. diet + vit. E*	
No.	ψ	*No.*	ψ	*No.*	ψ
1	85°	1	85°	1	85°
2	85°	9	85°	2	83°
3	85°	40	85°	3	86°
4	85°	41 and 45	85°	5	84°
5	84°			6	84°
6	85°				
7	84°				

The optical behaviour of *myosin solutions at various concentration of KCl* both from normal and vitamin-E-deficient rabbits, is shown in Tables IV, V and VI.

The values of ψ for non-purified myosin show a marked fall when the concentration of KCl reaches 0.3–0.35 M (Table IV). With purified myosin the fall of ψ corresponds

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to a lower concentration of KCl (0.2–0.3 *M*) (Table IV). This effect has been analyzed by SZENT-GYÖRGYI[10] without any particular claim to precision. In his work (1945) he apparently followed a rudimentary technique and stated that at 0.4 *M* KCl myosin loses all birefringence of flow. Obviously this is not the case and while we find a rapid drop in ψ down to a value of 66° at the critical level, a birefringence of flow of the mycsin solution is still present beyond this limit and the value of ψ remains unchanged at whatever concentration of KCl.

TABLE IV

DOUBLE REFRACTION OF FLOW OF NON-PURIFIED MYOSIN FROM NORMAL RABBITS

Effect of various KCl concentrations on ψ ($\pm$ 1°). For each experiment the concentration of myosin was kept constant.

Temp. 4° C; velocity gradient 6,000.

Solvents	*Rabbits on normal diet*			*Rabbits No. 1 – on H. and M. diet + vit. E*
	No. 2 ψ	*No. 3* ψ	*No. 4* ψ	ψ
H_2O	85°	85°	85°	85°
0.1 *M* KCl	86°	86°	86°	86°
0.2 *M* KCl	86°	86°		86°
0.3 *M* KCl	86°	86°	86°	84°
0.35 *M* KCl	76°			73°
0.4 *M* KCl	68°	67°	70°	
0.5 *M* KCl	68°	69°		68°
0.6 *M* KCl		68°		
0.7 *M* KCl		68°		
0.8 *M* KCl			68°	
1 *M* KCl			68°	

TABLE V

DOUBLE REFRACTION OF FLOW OF PURIFIED MYOSIN FROM NORMAL RABBITS

Effect of various KCl concentrations on ψ ($\pm$ 1°) and *R* ($\pm$ 10). For each experiment the concentration of myosin was kept constant.

Temp. 4° C; velocity gradient 6,000.

Solvents	*Rabbits on normal diet*						*Rabbit No. 2 – on H. and M. diet + vit. E*	
	No. 5		*No. 6*		*No. 7*			
	ψ	$R\,\mu\,\mu$	ψ	$R\,\mu\,\mu$	ψ	$R\,\mu\,\mu$	ψ	$R\,\mu\,\mu$
H_2O	84°	84	85°	84	84°	43	83°	175
0.1 *M* KCl	85°	273	85°	254	84°	120	85°	235
0.2 *M* KCl	85°	240	85°	252	83°	127	85°	199
0.3 *M* KCl	75°	99	80°	140	70°	46	71°	98
0.35 *M* KCl	67°	85	66°	80	60°	33	66°	98
0.4 *M* KCl	66°	85	67°	80	61°	33	66°	98
0.5 *M* KCl	67°	85	68°	80	60°	33	65°	98
0.8 *M* KCl	66°	85	68°	80				
1.0 *M* KCl	66°	85	68°	80				
1.5 *M* KCl	66°	85	67°	80				

Passing from a solution of myosin in water to a solution in KCl (0.1–0.2 *M*) there is a large increase in *R* (intensity in birefringence of flow or birefringence power) which

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becomes three times higher. As the concentration of KCl increases and reaches the same concentration at which ψ declines to its lowest values, R falls to about the same values as shown by aqueous solutions or to lower values. Concentrations of KCl at which R shows its maximum values seem therefore to be the optimal ones for the fibrous aggregation of myosin. Myosin extracted from rabbits of group 3 shows an optical behaviour analogous to that of normal myosin.

TABLE VI

DOUBLE REFRACTION OF FLOW OF NON-PURIFIED MYOSIN FROM VITAMIN-E-DEFICIENT RABBITS

Effect of various KCl concentrations on ψ ($\pm$ 1°) and R ($\pm$ 10). For each experiment the myosin concentration was kept constant.

Temp. 4° C; velocity gradient 6,000.

Solvents	*Rabbits on H. and M. diet*								
	No. 9		*No. 12*	*No. 13*		*No. 40*		*No. 41 & 45*	
	ψ	$R\,\mu\,\mu$	ψ	ψ	$R\,\mu\,\mu$	ψ	$R\,\mu\,\mu$	ψ	$R\,\mu\,\mu$
H_2O	85°	33				85°	43	85°	98
0.1 *M* KCl	85°	33		83°	67	85°	82	84°	199
0.2 *M* KCl	86°	33	83°			84°	98	84°	210
0.3 *M* KCl	86°	25	81°			84°		84°	135
0.35 *M* KCl	68°	14							
0.4 *M* KCl	57°								
0.6 *M* KCl			64°						

When myosin was available from vitamin-E-deficient rabbits the above effects were also investigated (Table VI). Myosin could not be purified owing to its scanty amount. Owing to the difficulty of having available at the same time a sufficient number of animals showing an incomplete dystrophic condition, only in one case (rabbits 41 and 45, Table VI) was it possible to collect the muscles of more than one rabbit and measure the optical properties of myosin at a concentration of protein comparable with those used for myosin from normal rabbits. The values of R obtained in this case are obviously more reliable than the others shown in Table VI, since with myosin concentrations so low as to give initial values of R of 33 or 43 in aqueous solutions, the visibility is so reduced that measurements become rather uncertain. Taking into account the above considerations we nevertheless may conclude that in dystrophic rabbits the rise in birefringence of flow of a myosin solution in 0.1–0.2 *M* KCl is not abolished but is apparently reduced. It should be interesting to establish whether this behaviour represents a change in the ability of extracts from dystrophic rabbits to form K-myosinate. In this connection it is also worth noting that the gross appearance of myosin solutions from dystrophic rabbits is somewhat different from that from normal rabbits, the viscosity and solubility of the latter being far more evident.

The values of ψ for *actin* are shown in Table VII. As is already known, Table VII shows that when activation occurs, it is equally carried out by KCl, NaCl, LiCl and NaI.

Actin prepared from normal and vitamin-E-supplemented rabbits was always activated, whereas that from dystrophic rabbits was only very seldom activated. Activation failed always in those cases in which myosin was also absent from the extracts, *i.e.* when the dystrophic process was very much advanced.

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TABLE VII

DOUBLE REFRACTION OF FLOW OF ACTIN FROM NORMAL AND VITAMIN-E-DEFICIENT RABBITS

Values of M (± 1°) of aqueous and various saline solutions.
Concentration of actin 500–1,000 mg N/l.
Temp. 18° C; velocity gradient 6,000.

Rabbits on normal diet				
No.	*φ Actin in 0.1 M*			
	KCl	*NaCl*	*LiCl*	*NaI*
2	86°			
3	85°			
6	85°			
7	87°	86°	87°	83°

Rabbits – on H. and M. diet + vit. E				*Rabbits – on H. and M. diet**			
No.	*φ Actin in 0.1 M*			*No.*	*φ Actin in 0.1 M*		
	KCl	*NaCl*	*LiCl*		*KCl*	*NaCl*	*LiCl*
1	87°			9	86°		
2	86°	84°	85°	12	86°	86°	87°
3	87°		87°	13			85°
5	84°	83°	84°	36			86°
6	87°		87°	40	86°		86°
7	86°	86°	87°	41 and 45			86°

* On H. & M. diet no myosin could be extracted and the actin could not be activated from 10 additional rabbits.

Electron microscopic examinations of the actin solutions confirmed morphologically this optical behaviour[15]. We may conclude that the process of polymerization of actin and of fibre formation from solutions of this protein, is severely impaired in the muscular dystrophy of vitamin-E deficiency.

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SUMMARY

1. The optical behaviour (D.R.F.) of actomyosin, myosin and actin prepared both from normal and dystrophic rabbit muscles has been investigated. Quantitative estimations are reported.

2. In dystrophic muscle myosin and actomyosin, as far as these proteins could be extracted with the method employed, do not show marked changes in the optical behaviour of their solutions as compared with normal. The only notable change requiring further investigation is the reduced increase in birefringence shown by myosin solution in 0.1–0.2 *M* KCl. This phenomenon may be interpreted as a change in the ability of myosin from dystrophic muscles to form K-myosinates.

3. In advanced dystrophy, actin loses its capacity to be activated by different salts. This may be interpreted as an inability of actin to polymerize.

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RÉSUMÉ

1. Les auteurs ont étudié au point de vue quantitatif la biréfringence de flux de protéines musculaires (actomyosine, myosine, actine) provenant de lapins normaux et de lapins distrophiques par carence de vitamine E.

2. Chez les lapins distrophiques, tant que la myosine et l'actomyosine peuvent être extraites en quantités suffisantes, celles-ci ne présentent pas de différences remarquables, comparativement aux protéines obtenues de lapins normaux. Le seul fait à noter, mais qui d'ailleurs doit être étudié ultérieurement, est l'augmentation minime de la biréfringence de flux de la myosine lorsqu'elle est dissoute en 0.1–0.2 *M* KCl. Ce phénomène peut être interprété comme une diminution de la capacité de former des myosinates de potassium de la part de la myosine provenant de muscles distrophiques.

3. Dans les cas de distrophie avancée, l'actine cesse de s'activer. Ceci est dû à l'incapacité de la part de l'actine à se polymériser.

ZUSAMMENFASSUNG

1. Die Verfasser haben quantitative Bestimmungen der Strömungsdoppelbrechung der Muskelproteine (Aktomyosin, Myosin, Aktin) durchgeführt, die aus normalen Kaninchen und solchen in Dystrophie aus E-Avitaminose gewonnen wurden.

2. In dystrophischen Kaninchen weisen Aktomyosin und Myosin, soferne sie extrahierbar sind, keine merkbaren Veränderungen der Strömungsdoppelbrechung gegenüber den aus normalen Kaninchen gewonnen Proteinen auf. Das einzige bemerkenswerte Ergebnis, das jedoch noch weiterer Prüfung bedarf, besteht in der geringen Vermehrung der Strömungsdoppelbrechung der Myosin-Lösungen in 0.1–0.2 *M* KCl. Dieses Phänomen darf als eine verminderte Kapazität des Myosins im dystrophischen Muskel aufgefasst werden, K-Myosinate zu bilden.

3. In Fällen fortgeschrittener Dystrophie verliert das Aktin die Fähigkeit der Aktivierung, sobald es mit Salzen versetzt wird. Diese Tatsache darf als Unfähigkeit seitens des Aktins sich zu polymerisieren aufgefasst werden.

REFERENCES

1 E. BONETTI, M. ALOISI AND P. MERUCCI, *Experientia*, 8 (1952) 69.
2 M. ALOISI, A. ASCENZI AND E. BONETTI, *J. Path. Bact.*, 64 (1952) 321.
3 J. T. EDSALL, *J. Biol. Chem.*, 89 (1930) 389.
4 J. T. EDSALL, *Advances in Colloidal Sci.*, 1 (1942) 269.
5 J. T. EDSALL AND J. W. MEHL, *J. Biol. Chem.*, 133 (1940) 409.
6 J. P. GREENSTEIN AND J. T. EDSALL, *J. Biol. Chem.*, 133 (1940) 397.
7 A. L. VON MURALT AND J. T. EDSALL, *J. Biol. Chem.*, 89 (1930) 315.
8 A. L. VON MURALT AND J. T. EDSALL, *J. Biol. Chem.*, 89 (1930) 351.
9 A. PETERLIN UND H. A. STUART, *Doppelbrechung, insbesondere künstliche Doppelbrechung. Hand- und Jahrbuch der Chemischen Physik* Bd. 8. Eucken und Wolf, 1943.
10 A. SZENT-GYÖRGYI, *Studies on muscles, Acta Physiol. Scand.*, 9 (1945) suppl. 25.
11 O. B. HOUCHIN AND H. A. MATTIL, *J. Biol. Chem.*, 146 (1942) 301.
12 G. FEUER, F. MOLNÁR, E. PETTKÓ AND F. B. STRAUB, *Hung. Acta Physiol.*, 1 (1948) 150.
13 M. GERENDÁS, *Enzymologia*, 9 (1940) 123.
14 G. SCHRAMM AND H. H. WEBER, *Kolloid-Z.*, 100 (1942) 242.
15 M. ALOISI, A. ASCENZI AND E. BONETTI, *Experientia*, 8 (1952) 266.

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