QUANTITATIVE STUDIES ON THE STRUCTURE OF CROSS-STRIATED MYOFIBRILS

II. INVESTIGATIONS BY BIOCHEMICAL TECHNIQUES

JEAN HANSON

Medical Research Council Biophysics Research Unit, Wheatstone Laboratory, King's College, London (England)

and

H. E. HUXLEY*

Medical Research Council, Department of Biophysics, University College, London (England)

INTRODUCTION

The contractile proteins of a muscle are situated in myofibrils packed together in the muscle fibres. The manner in which the molecules of these proteins are arranged in the fibrils is of great interest, for it must control the behaviour of the living muscle. Recent studies[1,2,3] on the cross-striated myofibrils of the skeletal muscles of rabbits have suggested that one of these proteins, myosin, is confined to the anisotropic (*A*) bands, which are denser than the rest of the fibril. The material responsible for their high density—the *A* substance—can be completely removed from fibrils under the microscope[1,2] by solutions which extract myosin completely and selectively from fresh muscle fibres[4] or from isolated fibrils in *rigor*[3]. The rest of the fibril appears to be unaffected by the extraction process. From this and other evidence (reviewed in [2]) it was concluded that myosin and the *A* substance are probably identical. This conclusion would be greatly strengthened if it could be shown that the quantities of *A* substance and myosin are the same, and that the myosin-extracting procedure removes only the *A* substance and no other materials from the fibrils. Since these fibrils consist almost entirely of protein[6] it might be possible to determine by interference microscopy the percentage of *A* substance in the total dry mass of the fibril, and then to compare this quantity with the percentage of myosin in the total myofibrillar protein estimated by biochemical methods; similar parallel determinations by the two techniques could also be made of the total amount of material taken out of the fibrils by the myosin-extracting procedure.

Results obtained by interference microscopy are described in the preceding paper[5]. It has been found that the *A* substance accounts for 50–55% of the total dry mass of washed, glycerol-extracted rabbit psoas fibrils, and that the myosin-extracting procedure removes all of the *A* substance together with another 10% of

* Part of this work was carried out whilst H. E. HUXLEY was a member of the Medical Research Council Unit for Research on the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge.

References p. 260.

the total material; thus, in all, 60–65% is extracted. The results obtained by chemical analyses performed on comparable fibrils are the subject of the present paper.

Although HASSELBACH AND SCHNEIDER[4] and others have analysed whole fresh rabbit muscle, information is needed about the rather different material which necessarily was used for interference microscopy, namely isolated glycerol-extracted fibrils from the psoas muscle. SZENT-GYÖRGYI, MAZIA AND SZENT-GYÖRGYI[8] have published results obtained on such material, but these show a wide scatter, and there are several other disturbing features about them, particularly the extraordinarily large total quantity of soluble proteins in the whole psoas muscle, *i.e.* proteins soluble in hypotonic saline; this fraction accounted for at least 52% of all the protein in the muscle, whereas the corresponding fraction in the analyses of HASSELBACH AND SCHNEIDER contained only 28% of all the protein in the muscle. Furthermore, SZENT-GYÖRGYI *et al.* obtained a value for the quantity of myosin in whole muscle (25–30% of the total protein) which is much lower than the value given by HASSELBACH AND SCHNEIDER (38%). Although SZENT-GYÖRGYI *et al.* found that myosin represents about 50% of the protein in the fibrils, and is therefore quantitatively similar to the *A* substance (50–55% measured by interference microscopy), they also found that another 18% of the total protein of the fibrils is extracted when myosin is removed from them, whereas the interference microscope measurements showed that only about 10% of material other than *A* substance is extracted.

Because of these uncertainties, new determinations have been made of the quantities of myosin in washed, glycerol-extracted fibrils and in the whole psoas muscle, and of the total protein taken out of fibrils by myosin-extracting procedures.

MATERIAL AND METHODS

The psoas muscles of domestic rabbits have been used. Thin bundles of muscle fibres from well-bled rabbits were tied at rest-length, or slightly stretched, to "Perspex" sticks and immersed at 4° C in a glycerol solution consisting of 50 volumes of glycerol, 40 volumes of water and 10 volumes of 0.067 *M* phosphate buffer, pH 7.0; in order to remove heavy metal impurities from the glycerol it was filtered, together with the water, through a column of ion-exchange resin (Amberlite IR 120 H); then the buffer was added and the pH adjusted to neutrality. After 24 hours at 4° C the fibres were immersed in fresh glycerol, and after another 24 hours at 4° C stored at —20° C for two weeks or longer before they were used.

All solutions were made from "Analar" grade reagents and water purified by "Pyrex" distillation or by ion-exchange resins. All procedures were carried out at 4° C.

Determination of protein extracted by glycerol

Protein extracted into the glycerol solution was precipitated by trichloracetic acid (final concentration 15%), washed with 15% trichloracetic acid to remove traces of glycerol, dissolved in sulphuric acid (micro-analytical grade) and estimated by the micro-Kjeldahl method.

Micro-Kjeldahl method

Nitrogen was estimated by a conventional micro-Kjeldahl procedure, and it was assumed that all the proteins contained 16% nitrogen. Control experiments showed that all the nitrogen in extracts from glycerol-extracted muscles was present in the fraction precipitated by trichloracetic acid (15%), and it was therefore taken to be entirely protein nitrogen.

Determination of soluble protein left after glycerol extraction

Bundles of glycerol-extracted fibres were equilibrated with about 50 volumes of a solution consisting of 0.04 *M* KCl, 0.0067 *M* phosphate buffer, 10^{-3} *M* $MgCl_2$, pH 7.0. This will be referred to as "hypotonic salt solution". Still in the same solution, they were divided into small pieces and blended in an M.S.E. homogeniser until broken up into fibrils. This procedure produced some foam which interfered with separation of the extract from the fibrils, so that at this stage the suspension was left overnight. Then it was centrifuged at 1600 *g* for 20 minutes and the fibrils

References p. 260.

resuspended in another 50 volumes of hypotonic salt solution and again centrifuged. This low centrifugal force was chosen because it was thought that subsequent extraction of the washed fibrils might be impaired if they were too tightly packed. Fibrils still remaining in the supernatant fluid were removed by centrifugation at 6500 *g*. The nitrogen of the combined extracts was estimated by the micro-Kjeldahl procedure.

Determination of total protein removed from washed fibrils by myosin-extracting procedures

Two different myosin-extracting solutions have been used. One is a modification of the pyrophosphate-KCl solution of HASSELBACH AND SCHNEIDER[4]; the total ionic strength was increased to about 0.73, and Mg^{++} was added, because it was observed that the original solution often failed to extract a little of the *A* substance. The composition of the modified solution is: 0.6 *M* KCl, 0.01 *M* sodium pyrophosphate, 0.1 *M* phosphate buffer, 10^{-3} *M* $MgCl_2$, pH 6.4. It will be referred to as "high μ H–S solution". The other myosin-extracting solution is 0.1 *M* pyrophosphate, 0.0067 *M* phosphate buffer, 10^{-3} *M* $MgCl_2$, pH 6.4. It will be called "pyrophosphate solution".

The sedimented fibrils left after washing away soluble proteins were suspended in a little isotonic salt solution (0.1 *M* KCl buffered at pH 7.0), and the myosin-extracting solution was slowly added. It had previously been noticed that the "ghost" fibrils left after extraction often appeared to be slightly damaged if they had been suspended immediately in the extracting solution instead of allowing the ionic strength to increase gradually. The solution of extracted protein was separated from the fibrils by centrifugation at 6500 *g*, and they were washed once more in myosin-extracting solution. The total time of extraction was about 90 minutes, and the quantity of extracting solution was either 100 volumes or 25 volumes. The nitrogen content of the combined extracts was determined.

Determinations of myosin

Determinations of myosin were all made on high μ H–S extracts of washed fibrils. In one series of experiments the extract was dialysed over one night or longer against about 30 volumes of hypotonic salt solution (μ 0.04), and the dialysis bag was gently agitated all the time. In another series of experiments the extract, whilst being stirred, was slowly diluted with water until the ionic strength had been reduced to 0.04; the precipitate was allowed to settle for 2–3 hours before it was collected.

The materials precipitated by these two methods were separated by centrifugation at 6500 *g* for 30 minutes, dissolved in sulphuric acid, and estimated by the micro-Kjeldahl method.

In a third series of experiments, the method of ROBINSON[7] for actomyosin determination was followed. Dilute ethanol (18%) was slowly added to the extract, which was stirred all the time, until the ionic strength had been reduced to 0.12 and the final ethanol concentration was 15% (v/v). The precipitate was immediately collected, dissolved in sulphuric acid and estimated.

Determination of final residue

After myosin had been extracted from washed fibrils the residue was dissolved in sulphuric acid and its nitrogen content determined.

RESULTS

Soluble protein content

Two soluble protein fractions have been estimated, firstly the protein that is extracted from fresh muscle by the neutral glycerol solution, and secondly the protein that is washed out of glycerol-extracted muscle by neutral hypotonic salt solution after it has been broken up into fibrils. Table I gives the results. In all experiments the fibril suspension was washed twice; in two control experiments (numbers 35 and 36) it was found that two further washes did not remove any more protein.

It is found that less than 10% of the total protein of the fresh muscle is extracted by the neutral glycerol solution. The average soluble protein content of glycerol-extracted muscle is 28% of the total protein, and is not obviously affected by the duration of glycerol extraction beyond three weeks. The average total soluble protein, expressed as a percentage of the total protein in fresh muscle, is 34%.

Total protein removed from washed fibrils by myosin-extracting solutions

Table II gives the results of determinations of the total protein removed from washed

References p. 260.

TABLE I

SOLUBLE PROTEIN CONTENT OF RABBIT PSOAS MUSCLE

1	2	3	4	5	6	7
Expt. No.	*Rabbit No.*	*Duration of glycerol extraction in weeks*	*Protein in glycerol as % of protein in fresh muscle*	*Protein in hypotonic saline as % of protein in glycerol-extracted fibres*	*Column 5 as % of protein in fresh muscle*	*Total of columns 4 and 6*
2	2	3		29		
3	2	5		25		
4	2	5		29		
5	2	6		28		
6	2	7		28		
7	2	8		30		
8	3	6		28		
9	3	7		24		
10	3	9		23		
11	3	12		30		
14	5	2	4	34	32	36
15	5	2	6	31	29	35
18	5	2	3	33	32	35
19	5	2	3	30	29	32
24	6	16	—	23	—	—
27	8	3	9	27	25	34
35	9	3	—	23	—	—
36	9	4	6	27	26	32
			Average	28	Average	34

TABLE II

TOTAL PROTEIN REMOVED FROM WASHED FIBRILS BY MYOSIN-EXTRACTING SOLUTIONS, HIGH μ H–S SOLUTION (H–S) OR PYROPHOSPHATE SOLUTION (PYRO)

Expt. No.	*Rabbit No.*	*Duration of glycerol extraction in weeks*	*Extracting solution*	*Protein extracted as % of total protein in washed fibrils*
12	3	13	H–S	61
14	5	2	H–S	69
18	5	2	H–S	69
24	6	16	H–S	69
30a	6	26	H–S	60
30b	6	26	pyro	65
32a	7	16	H–S	60
32b	7	16	pyro	67
33a	7	16	H–S	67
33b	7	16	pyro	65
34	6	30	H–S	56
35	9	3	H–S	58
37	9	5	H–S	52
38	9	6	H–S	56
			Average	62

glycerol-extracted fibrils by two different myosin-extracting solutions, high μ H–S solution and pyrophosphate solution. There is no significant difference between the results given by these two solutions.

The results are variable, the extreme values being 52% and 69%; the average quantity is 62%, expressed as a percentage of the total protein in the washed fibrils.

References p. 260.

Lower values, some of them below 50%, were given in earlier experiments in which the material included fragments of fibres incompletely broken up into fibrils. Under the microscope, fibrils suspended in the myosin-extracting solutions were observed, before centrifugation, to have the same appearance as fibrils treated by the irrigation technique (see photographs in preceding paper); all of the *A* substance had been removed.

Quantities of myosin and X-*protein in the extract*

The myosin content of high μ H–S extracts of washed fibrils has been determined by measuring the quantity of protein that precipitates when the ionic strength is reduced to 0.04. The amount of *X*-protein, defined as the fraction that does not precipitate, has been estimated by difference. Since very little material precipitates from pyrophosphate extracts they have not been used in these investigations.

Dialysis experiments. Table III gives the results of experiments in which the extract was dialysed against about 30 volumes of hypotonic salt solution over one, two or three nights.

The results are very variable and it is clear that they are influenced by the concentration of protein in the dialysis bag (see experiment 31) and by the duration of dialysis. Amounts ranging from 57% to 87% of the total protein in the extract were precipitated. The average was 71%. The three results that are perhaps the most reliable (experiments 31a, 33 and 35), because the duration of dialysis was short and the protein concentration high, are 73%, 76% and 77%. It is interesting that the only amounts above 80% were obtained by using muscles that had been glycerol-extracted for only two weeks.

A few determinations were made of the total protein remaining within the dialysis membrane after one night, and it was found that 20% or more of the protein of the original extract had escaped through the membrane.

Dilution experiments. Table III also gives the results of estimations of myosin made by diluting the extract with water until its ionic strength was reduced to 0.04. This Table also includes the results of a few experiments in which the extract was mixed with dilute ethanol until its ionic strength was 0.12 and its ethanol concentration 15% (v/v).

The quantity of material precipitated when the ionic strength of the extract is reduced by diluting it with water is greater than the quantity usually precipitated when the ionic strength is reduced by dialysis (see, for example, experiment 31). The average quantity precipitated by the dilution method is 82% of the total protein in the extract. Ethanol precipitation gives a still greater yield, on the average 91%.

Quantity of X-*protein.* The quantity of *X*-protein, estimated by difference and expressed as the percentage of the total protein in the extract, is 29% (dialysis method) or 17% (dilution method).

A control experiment

In order to show whether or not extra-fibrillar components contribute to the *X*-protein content of the high μ H–S extracts, an experiment (number 36) was performed in which the fibrils were prepared as free as possible from other muscle elements. The suspension of homogenised fibrils in hypotonic salt solution was centrifuged lightly to remove any large fragments. It was then centrifuged at 600 *g*, bringing down a

References p. 260.

TABLE III

QUANTITIES OF PROTEIN PRECIPITATED FROM HIGH μ H–S EXTRACTS BY THREE DIFFERENT METHODS, DIALYSIS, DILUTION WITH WATER, AND TREATMENT WITH ETHANOL

Expt. No.	*Rabbit No.*	*Duration of glycerol extraction in weeks*	*Method**	*Protein conc. of extract mg/ml***	*Precipitated protein as % of protein in extract*
2	2	3	Dialysis (2)	1.53	73
6	2	7	Dialysis (2)	1.25	67
11	3	12	Dialysis (2)	0.98	71
12	3	13	Dialysis (2)	0.32	74
14	5	2	Dialysis (2)	1.55	87
18	5	2	Dialysis (2)	1.64	82
20	3	20	Dialysis (2)	0.66	67
24	6	16	Dialysis (3)	0.90	57
25	6	18	Dialysis (2)	0.30	68
31a	7	15	Dialysis (1)	1.77	73
31b	7	15	Dialysis (1)	0.88	67
31c	7	15	Dialysis (1)	0.09	58
31d	7	15	Dilution	0.15	82
31e	7	15	Ethanol	0.29	96
32	7	16	Dilution	0.10	77
33a	7	16	Dialysis (1)	1.68	76
33b	7	16	Dilution	0.14	90
34a	6	30	Dilution	0.10	84
34b	6	30	Ethanol	0.20	90
35a	9	3	Dialysis (1)	2.12	77
35b	9	3	Dilution	0.12	84
35c	9	3	Ethanol	0.35	83
36	9	4	Dilution	0.14	84
37	9	5	Dilution	0.15	78
40a	9	6	Dilution	0.10	82
40b	9	6	Ethanol	0.30	97
41a	9	6	Dilution	0.04	77
41b	9	6	Ethanol	0.12	88
				Average by dialysis	71
				Average by dilution	82
				Average by ethanol	91

* Duration of dialysis, in nights, is given in parenthesis.
** Concentration in dialysis bag, or after dilution, or after ethanol treatment.

large number of fibrils but leaving many others, and presumably any lighter particles that were present, in the supernate; the sediment was resuspended in hypotonic salt solution and this procedure repeated three more times. The high μ H–S extract prepared from these washed fibrils yielded 84% of precipitated protein when it was diluted with water to μ 0.04. Thus the *X*-protein is derived from the fibrils.

Calculations of quantities of myosin in washed fibrils and in whole muscle

If the quantity of protein removed from washed fibrils by myosin-extracting solutions is 62% of their total protein (see Table II), and if myosin constitutes 82% of the total protein in the extracts (average of water-dilution determinations, Table III),

References p. 260.

then the extracted myosin represents 51% of the total protein in washed fibrils, and the *X*-protein 11%. If myosin is estimated from the results of the dialysis experiments (the precipitate being on the average 71% of the extract), then the amount of myosin is 44%, and the amount of *X*-protein 18%.

The percentages of myosin and *X*-protein in the total protein of the fresh psoas muscle can now be calculated. If the soluble protein content of the muscles is 34% (Table I), then the myosin content is 34% (by dilution) or 29% (by dialysis) and the *X*-protein content is 7% (by dilution) or 12% (by dialysis).

Synopsis of results

Table IV summarizes all the results.

TABLE IV

SYNOPSIS OF RESULTS

Protein fraction		*As % of total protein in fresh muscle*	*As % of total protein in glycerol-extracted muscle*	*As % of total protein in washed fibrils*
Glycerol-extracted		up to 9	—	—
Rest of soluble		—	28	—
Total soluble		34	—	—
Myosin + *X*-protein		41	45	62
Myosin	by dilution	34	37	51
X-protein	by dilution	7	8	11
Myosin	by dialysis	29	32	44
X-protein	by dialysis	12	13	18
Residue		25	27	38

DISCUSSION

In this paper we shall confine discussion to assessing the reliability and significance of the results and to comparing them with previous estimates made by HASSELBACH AND SCHNEIDER[4] and SZENT-GYÖRGYI, MAZIA AND SZENT-GYÖRGYI[8] (see Table V). The implications of all these findings, together with the interference microscope results, are more fully discussed in the preceding paper[5].

TABLE V

COMPARISON OF THE PRESENT RESULTS (H–H) WITH THOSE OF HASSELBACH AND SCHNEIDER[4] (H–S) AND SZENT-GYÖRGYI, MAZIA AND SZENT-GYÖRGYI[8] (S–G)

Figures in parentheses have been calculated by the present authors

Protein fraction	*As % of total protein in fresh muscle*			*As % of total protein in glycerol-extracted muscle*			*As % of total protein in washed fibrils*		
	H–S	*S–G*	*H–H*	*H–S*	*S–G*	*H–H*	*H–S*	*S–G*	*H–H*
Soluble	28	(47)	34	—	42	28	—	—	—
Myosin	38	(26)	34	—	29	37	(53)	50	51
X-protein	—	(9)	7	—	10	8	—	18	11
Myosin + *X*-protein	—	(35)	41	—	39	45	—	68	62
Actin + residue	34	(17)	25	—	19	27	(47)	32	38

References p. 260.

Quantity of soluble protein

The total quantity of soluble protein found in the psoas muscles of well-bled rabbits is on the average 34% of the total protein, and no doubt nearly all of it is sarcoplasmic. About a quarter of this soluble protein is taken out by glycerol extraction. The average quantity of soluble protein washed out of the glycerol-extracted muscle after it has been broken up into fibrils is 28% of the total protein remaining in the muscle after glycerol extraction. Deviations from this average figure of 28% may reflect variations in the thickness of the fibre bundles put into glycerol, but even the highest result obtained (34%) is much lower than the figure quoted by SZENT-GYÖRGYI *et al.* —41.6%.

HASSELBACH AND SCHNEIDER found only 28% of soluble protein in the total protein content of fresh skeletal muscles. This estimate was made by extracting both myosin and the soluble proteins simultaneously from muscles broken up into fibre fragments, and then precipitating the myosin. If this type of extraction also removes *X*-protein, then the quantity of this protein will be included in HASSELBACH AND SCHNEIDER's figure of 28% for total soluble protein. Their estimate should therefore be compared with the *sum* of the amounts of soluble protein and *X*-protein found in the present analyses and in those of SZENT-GYÖRGYI *et al.* From the results of SZENT-GYÖRGYI *et al.* it can be estimated that this total quantity is 52% or more (41.6% soluble protein + 11% *X*-protein + a correction for protein extracted in glycerol); however, their figure of 41.6% soluble protein was the result of only a single experiment. In the present experiments, the *total* quantity was found to be 41% (34% soluble protein + 7% *X*-protein). Nevertheless, the discrepancy between 41% and 28% still seems too large to be explained on the basis of incomplete extraction of the ordinary soluble proteins from the fresh minced muscle used by HASSELBACH AND SCHNEIDER; and their method was designed to extract the myosin as completely as possible. It seems more likely that the method used in the present experiments removes a larger quantity of *X*-protein than the HASSELBACH-SCHNEIDER procedure; this possibility is considered in more detail in the preceding paper. Their figure of 28% would then be comparable to the present figure of 34% for soluble protein *not* including the *X*-protein.

Quantity of protein removed by myosin-extracting procedures

When the *A* substance is completely removed from washed glycerol-extracted fibrils by pyrophosphate solution or high μ H–S solution, an average of 62% (spread 52–69%) of their total protein is extracted (Table II). SZENT-GYÖRGYI *et al.*, who have also estimated the quantity of this extracted material, used two myosin-extracting solutions, Guba-Straub (buffered KCl, μ 0.6, pH 6.5) with adenosine triphosphate, and the original HASSELBACH-SCHNEIDER solution (KCl-pyrophosphate, buffered at pH 6.4 — μ 0.6) with magnesium. They found that the average quantity of protein extracted by the first solution was 72% (spread 45–88%) of the total protein of the washed fibrils, and by the second solution 63% (spread 40–72%). The average of the combined results from both methods was 68%. It is perhaps surprising that the average results are not lower than these, because when such extractions are observed under the microscope neither of these solutions takes out all of the *A* substance, and the duration of extraction in the experiments of SZENT-GYÖRGYI *et al.* was very short. However, the wide spread of the results probably renders any

References p. 260.

averaging rather unrealistic. The present results (average 62%, spread 52–69%) are very similar to those given by interference microscopy (average 61.5 ± 4%) which is considered to be the better of the two methods, because it gives values which are unaffected by the inclusion of any measurements made on incompletely-extracted fibrils, and because contact with the extracting solution is not unnecessarily prolonged; these considerations probably explain why the results obtained by chemical analysis are the more variable.

Quantities of myosin and X-protein

If myosin is defined as the material which precipitates when the ionic strength of the myosin-containing extract is reduced to 0.04, then its quantity is most probably about 82% of the total protein in the extract. This is the average of the results obtained by the dilution method (spread 77–90%). When other samples of the same extracts were dialysed to reduce their ionic strength, the quantity of precipitate obtained was nearly always lower, and the average result of all the dialysis experiments was only 71% (spread 57–87%); very similar results were obtained by SZENT-GYÖRGYI *et al.* (average 73%, spread 65–89%) by the dialysis method, and by a viscometric method whose accuracy we cannot assess.

There seem to be two possible explanations for the difference between the results obtained by the dilution and the dialysis methods. On the one hand, the extract may contain some protein of low molecular weight which can escape through the dialysis membrane, but which also tends to be carried down by the myosin when the latter precipitates. Thus, during precipitation by dialysis, some of this protein would escape, and the amount of precipitate would be less than in a dilution experiment. Indeed, both SZENT-GYÖRGYI *et al.* and ourselves have found that some protein does escape through the dialysis membrane. Alternatively, a small part of the myosin itself may break down, during the 1–2 day dialysis, into components which remain in solution. A decision between these two explanations does not lie within the scope of this paper, where "myosin" will refer to the whole of the material precipitated by dilution; the possibility that actomyosin, which also would precipitate under these conditions, is present in the extract, does, however, seem to be excluded. The conventional test for the presence of actomyosin, namely measurement of the viscosity of the solution with and without adenosine triphosphate, has been applied by SZENT-GYÖRGYI *et al.*, who have found that the actomyosin content of these extracts is negligible. HASSELBACH[3], also using viscosity methods, determined that there was one part of actin to 19 parts of myosin in similar extracts made from fibrils isolated from muscles in *rigor mortis*. The present experiments do not exclude the possibility that the precipitate contains proteins of more than one molecular species, but, using the conventional definition of myosin given above, the results show that it represents 82% of the extracted material.

Thus, if the total extracted material is 62% of the protein in the washed fibrils, the extracted myosin represents 51% of the protein in these fibrils. Alternatively, using the value found by interference microscopy (64.5%) for the total extracted material, the quantity of myosin extracted from these fibrils is 53% of their total protein content. This result is in exact agreement with the value for myosin in the fibrils (53%) calculated from the findings of HASSELBACH AND SCHNEIDER that fresh muscle contains 38% myosin and 28% sarcoplasmic protein. SZENT-GYÖRGYI *et al.*

References p. 260.

obtained the result that the quantity of myosin extracted from washed glycerol-extracted fibrils is about 50% of their total protein, *i.e.* the average total extracted protein was 68%, and the average amount of myosin in the extracts was 73% of the total protein. Experiments of HASSELBACH AND SCHNEIDER[3,4] have shown that it is probably safe to assume that myosin is completely extracted by the procedures used in the present investigations.

The myosin content of the whole psoas muscle (34%) calculated from the present results is comparable to that found by HASSELBACH AND SCHNEIDER (38%). The low value, 25–30%, obtained by SZENT-GYÖRGYI *et al.*, is due to that fact, already commented on, that their single determination of soluble protein in the psoas muscle gave a very high figure.

The automatic corollary of the fact that the dilution method used here and the methods used by SZENT-GYÖRGYI *et al.* give different values for myosin, is that they also give different values for the *X*-protein content of the fibrils —11% of the total protein in the present experiments, and 18% in those of SZENT-GYÖRGYI *et al.*

In conclusion, then, it is very probable that myosin represents about 53% of the total protein of washed glycerol-extracted rabbit psoas fibrils, and that another 11% is also removed by the myosin-extracting procedure. These values are almost exactly the same as the estimates made by interference microscopy of the quantity of *A* substance (54.5%) in these fibrils and the quantity of other material (10%) extracted together with the *A* substance by the same myosin extracting solutions. The simplest hypothesis to account for all these findings is that myosin is confined to the *A* bands in these fibrils. However, the fact that myosin does not make up the whole of the extracted material, still leaves the possibility that a small part of the *A* substance consists of *X*-protein, and that a small fraction of the total myosin is elsewhere than in the *A* bands. In spite of this slight uncertainty, it can be concluded that at least four-fifths of the myosin is confined to the *A* bands in these muscles.

ACKNOWLEDGEMENTS

We are very much indebted to Miss CHRISTINE THURNELL for her efficient and enthusiastic assistance in all these experiments. We also wish to express our gratitude to Professor J. T. RANDALL for his encouragement of this research and for the facilities of the Wheatstone Laboratory, and to Dr. R. H. SMITH for reading and criticizing this paper.

SUMMARY

The quantities of various protein fractions in the psoas muscle of the rabbit have been determined by chemical analysis.

The soluble proteins removed by glycerol extraction and by the subsequent isolation and washing of the fibrils in neutral hypotonic salt solution, make up 34% of the total protein of the whole muscle.

62% of the total protein of the washed fibrils is taken out by myosin-extracting procedures.

82% of the extracted protein precipitates when the ionic strength is reduced to 0.04; this component is conventionally known as myosin. Thus the myosin extracted makes up 51% of the total protein of the washed fibrils, and the removal of the myosin is accompanied by the extraction of a further 11% of the total protein.

In the accompanying paper[5] it has been shown by interference microscopy that the quantity of *A* substance in similar fibrils is 50–55% of their total protein, and that the same myosin-extracting procedures remove the *A* substance and another 10% of the total protein, 60–65% in all.

References p. 260.

These comparative measurements by interference microscopy and chemical analysis prove that at least four-fifths of the myosin in these fibrils is present as the *A* substance, and the results are in excellent agreement with the hypothesis that *all* the myosin is concentrated in the *A* bands.

REFERENCES

1 J. HANSON AND H. E. HUXLEY, *Nature*, 172 (1953) 530.
2 J. HANSON AND H. E. HUXLEY, *Symposia Soc. Exptl. Biol.*, 9 (1955) 228.
3 W. HASSELBACH, *Z. Naturforsch.*, 8b (1953) 449.
4 W. HASSELBACH AND G. SCHNEIDER, *Biochem. Z.*, 321 (1951) 461.
5 H. E. HUXLEY AND J. HANSON, *Biochim. Biophys. Acta*, 23 (1957) 229.
6 S. V. PERRY, *Symposia Soc. Exptl. Biol.*, 9 (1955) 203.
7 D. S. ROBINSON, *Biochem. J.*, 52 (1952) 621.
8 A. G. SZENT-GYÖRGYI, D. MAZIA AND A. SZENT-GYÖRGYI, *Biochim. Biophys. Acta*, 16 (1955) 339.

Received July 5th, 1956

ELECTRON MICROSCOPY AND X-RAY DIFFRACTION OF BONE

H. FERNÁNDEZ-MORÁN

Ultrastructure Department, Instituto Venezolano de Neurologia e Investigaciones Cerebrales, Caracas (Venezuela)

and

A. ENGSTRÖM

Department of Medical Physics, Karolinska Institutet, Stockholm (Sweden)

Recently, the apatite-containing structures in the organism have attracted considerable attention because of their marked ionic exchange properties. The capacity of the apatites in biological systems to bind and release various types of ions has a special physiological significance. Special interest attaches to these phenomena in connection with infection with "bone-seeking" radioactive substances.

The adsorptive properties of the bone apatite depend upon the extremely small size of the individual hydroxyapatite particles, and hence the elucidation of the submicroscopic organization of bone tissue is of fundamental importance.

Polarized light studies[1] have revealed that the collagen fibres, constituting the bulk of the organic fraction of bone, are capable of "adsorbing" the apatite particles in a highly ordered way. The hydroxyapatite belongs to the hexagonal system and since in X-ray diagrams the *oo*l-reflections are less broadened than the *h*oo-lines it is clear that the crystallites are elongated with their *c*-axis parallel with the long axis of the crystallite. Moreover, the wide-angle X-ray diffraction diagrams of oriented bone specimens indicated that the *c*-axes of the crystallites are arranged parallel with the collagen fibres[2,3]. Bone tissues gives a diffuse low-angle X-ray scattering[4] most probably attributable to the apatite particles. The low-angle X-ray diagrams, also, indicated that the particles were elongated and aligned along the collagen fibre axis. From these studies the dimensions of the apatite particles were estimated to be 40–75 A

References p. 264.