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The myogen fraction of eight different muscles of four bovine animals was studied. Of the muscles considered, three were clearly differentiable from the others on the basis of water-soluble protein and aldolase content and "ultimate" pH. The relationship between "ultimate" pH and soluble protein, muscle buffering capacity, and inherent anaerobic glycolytic rate was discussed.

Electrophoretic patterns of aqueous extracts of bovine muscle were found to be comparable to those reported previously for rabbit muscle. Considerable variation in relative amounts of components was observed from muscle to muscle and from animal to animal, without any apparent regularity. Ultracentrifuge patterns of these same extracts were qualitatively similar, i.e., the soluble proteins fell into one of three groups: 2.3 *S*, 4.7 *S*, and 6.8 *S*. No systematic variation in relative amounts of components was observed.

Extracts of *longissimus dorsi* were fractionated by ammonium sulfate precipitation and the fractions further characterized. Tentative identification of myogen fraction components was made by comparison of solubilities, electrophoretic mobilities, and sedimentation constants for rabbit muscle proteins with those observed for bovine muscle extracts and fractions.

INTRODUCTION

The intracellular proteins of muscle can be conveniently divided on the basis of solubility into two groups having rather different properties and functions, i.e., the salt-soluble fraction, composed almost entirely of the contractile proteins actin and myosin, and the water-soluble group, consisting almost entirely of proteins with purely enzymic character.

The latter group, the so-called myogen fraction, has been studied from two viewpoints. For example, Dubuisson (1) and others at the Laboratory of General Biology, University of Liege, have been especially concerned with the properties and fractionation of the entire myogen fraction. Cori and Cori (2-5), and others, on the other hand, have been particularly active in the isolation of specific enzymic activities from this frac-

tion and with the study of the properties of these purified enzymes. Both approaches have tended to complement each other.

Recently a program was initiated in this laboratory to elucidate the post-mortem changes occurring in bovine skeletal muscle. Since many of these, without doubt, are enzymic in nature, our attention has been focused on the myogen fraction. Aldolase was of particular interest since it is one of the enzymes of anaerobic glycolysis, a process which is of special importance with regard to post-mortem changes in tissue pH. Furthermore, it was to be expected from the earlier work on rabbit muscle proteins that aldolase might lend itself to future isolation and characterization. This communication deals with the electrophoretic, ultracentrifugal, and enzymic properties of the myogen fraction of fresh bovine skeletal muscle. Subsequent papers will deal with the changes occurring in aged and frozen tissue, as well as properties of purified proteins.

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TABLE I
Bovine Skeletal Muscles Studied and Their Anatomical Location

Muscle No.	Muscle name	Location	Plate No. (6)
1	Longissimus dorsi	8th, 9th, 10th rib	XXIV
2	Psoas major	4th, 5th lumbar vertebrae	XVII
3	Semimembranosus	Proximal extremity of femur	IX
4	Serratus ventralis	8th, 9th, 10th rib	XXIV
5	Rectus abdominus		XXI
6	Semitendinosus	Proximal extremity of femur	IX
7	Latissimus dorsi	8th, 9th, 10th rib	XXIV
8	Trapezius	8th, 9th, 10th rib	XXIV

EXPERIMENTAL

MATERIALS

Reagent-grade chemicals were used with the exception of tris(hydroxymethyl)aminomethane (Tris). The latter was obtained from Commercial Solvents Corporation² and crystallized twice from water-acetone mixtures before use. Fructose 1,6-diphosphate was obtained from Schwarz Laboratories as the barium salt. It was converted to the sodium form immediately before use by the addition of a stoichiometric amount of sodium sulfate. All solutions were prepared with deionized water.

PREPARATION OF MUSCLE EXTRACTS

Animals were stunned and killed by the usual slaughterhouse procedure. Within 20 min. post-mortem, the desired muscles were dissected out of the left-hand side of the animal, placed in polyethylene bags, and cooled in crushed ice for transportation to the laboratory. The right-hand side of the animal was stored at 5°C. for 7 days for use in studies of post-mortem changes.

All operations were carried out at 2-4°C. One hundred and fifty to 300 g. of the chilled muscle were trimmed of external fat and connective tissue and put once through a chilled electric meat grinder having plate openings of 4 mm. One hundred grams of the ground tissue was stirred for one-half hour with 200 ml. of deionized water and squeezed by hand through one thickness of cheesecloth. The pulp was re-extracted with 100 ml. of deionized water. The combined extracts were then centrifuged in the Spinco model L centrifuge at $42,000 \times g$ for 60-90 min.

Animals Nos. 7, 8, and 9 were 18-month-old

² The mention of specific commercial products in this communication does not imply that they are endorsed or recommended by the Department of Agriculture over others of similar nature not mentioned.

steers of "choice" grade while animal No. 6 was a 5-year-old cow.

Shown in the first two columns of Table I are the muscles studied. In the case of animal No. 6, an average sample of the given muscle was taken over much of its length. In the case of animals 7, 8, and 9, sections were made according to diagrams given in "A Cross Sectional Muscle Nomenclature of the Beef Carcass" (6). Individual muscles were then removed from the proper section. Shown in Table I are the locations at which sections were obtained, as well as the illustration plate number in the previously cited reference (6).

ANALYTICAL METHODS

Protein concentrations were determined using the modified biuret procedure described by Beisenherz *et al.* (7).

Aldolase activity was determined by measuring the amount of alkali-labile phosphorus developed in a given time in a reaction mixture having the composition:

	M
Fructose 1,6-diphosphate	8.3×10^{-3}
Iodoacetate	3.3×10^{-4}
Hydrazine	5×10^{-2}
Tris·HCl buffer, pH 7.4	0.25

After the reaction had proceeded for 15 min., it was stopped by addition of trichloroacetic acid. To decrease thermal inactivation of the enzyme, the reaction temperature was 25.5°C. rather than the value of 30°C. often employed.

The triose phosphate formed was hydrolyzed in 1.3 N NaOH, and the inorganic phosphorus was determined by the Fiske-SubbaRow procedure (8). The unit of aldolase activity is defined as a microgram of phosphorus produced during a 15-min. interval under the conditions of the assay. This unit corresponds to 3.8 μg . triose. Commercial 2 \times -crystallized rabbit muscle aldolase (Worthington) yielded a specific activity of 5.4 units/ μg .

TABLE II
Average Water-Soluble Protein and Aldolase
Content of Bovine Skeletal Muscle

Animal No.	Grams water-soluble protein/100 g. wet tissue	Units of aldolase/100 g. of wet tissue	Sp. activity
6	3.55 ± 0.35	2.0 ± 0.7 × 10 ⁶	0.56 ± 0.15
7	2.60 ± 0.44	2.9 ± 1.0 × 10 ⁶	1.1 ± 0.24
8	3.65 ± 0.32	2.8 ± 0.9 × 10 ⁶	0.77 ± 0.18
9	3.20 ± 0.42	2.8 ± 0.8 × 10 ⁶	0.84 ± 0.19
Avg.	3.25 ± 0.42	2.6 ± 0.3 × 10 ⁶	0.82 ± 0.15

ELECTROPHORETIC AND ULTRACENTRIFUGAL MEASUREMENTS

Electrophoretic measurements were made in the Perkin-Elmer apparatus (Model 38A) at 0°C. Twenty-milliliter portions of solutions were dialyzed in the cold room for a minimum of 24 hr. against at least 6 l. of pH 8.17, 0.05 ionic strength Tris-HCl buffer. These same solutions were used for ultracentrifuge measurements.

Sedimentation measurements were made in the Spinco model E ultracentrifuge equipped with RTIC* and phase plate at a speed of 59,780 r.p.m. In order to minimize thermal denaturation of protein, experiments were carried out at 10°C. or lower. The variation in temperature during the course of a run was generally less than 0.2°C.

Computations were made from projected images of the schlieren diagrams. Relative amounts of components were estimated from "peak heights," i.e., the height of the schlieren pattern above the base line, at a position corresponding to an electrophoretic or sedimentation component. In the case of ultracentrifuge experiments, separate solvent base-line runs were employed.

RESULTS AND DISCUSSION

PROTEIN AND ALDOLASE CONTENT

Summarized in Tables II and III are the water-soluble protein and the aldolase content of eight muscles from each of four animals. The averages shown in Table II are those for eight muscles from a given animal. The average of 3.25 g./100 g. muscle tissue, obtained from all 32 samples, is 25-30% lower than values reported for rabbit muscle (5, 7). Whether this is truly representative of bovine skeletal muscle cannot be stated with certainty, since relatively few animals were studied.

* Rotar temperature indicator and control.

The aldolase contents shown in Table II are comparable to those reported previously for rabbit muscle. If we assume that the value of 5.4 obtained for the specific activity of rabbit muscle aldolase (see *Experimental*) is applicable to pure beef muscle aldolase, then the observed specific activities indicate that 5-19% of the myogen fraction is aldolase (average value 15%). These are to be compared with the values of 5% (9) and 10% (7) previously obtained for the rabbit muscle enzyme.

Variations in water-soluble protein and aldolase content from muscle to muscle can be seen more readily if one makes allowance for variation observed from animal to animal. This was done by using relative quantities, e.g., relative protein content is defined as:

$$\frac{(\text{g. protein}/100 \text{ g. tissue}) \text{ muscle a, animal b}}{(\text{g. protein}/100 \text{ g. tissue}) \text{ avg., animal b}}$$

Examination of the relative protein and aldolase contents as shown in Table III indicates that muscles 1, 3, and 6 have high protein and aldolase contents while muscles 2, 4, 5, and 8 have low values. Muscle 7 appears to have intermediate values. It is of interest to note that the relative specific activities also fall into the same two groups. This indicates that the actual percentage of aldolase in the myogen fraction differs in the two groups of muscles. If we calculate the protein content due to aldolase and subtract this from the total soluble-protein content, we find that not only is the differentiation of muscles maintained, but in fact is even made more extreme. Thus, we conclude that differences in soluble protein content are not due solely to differences in aldolase content.

pH CHANGES

Shown in Table IV are pH values of the muscles studied as well as those of extracts of these muscles. As previously, in order to eliminate variations from animal to animal, these were calculated as the relative value, i.e.,

$$\frac{(\text{pH}) \text{ muscle a, animal b}}{(\text{pH}) \text{ avg., animal b}}$$

The averages shown in Table IV are those for animals 7, 8, and 9 with the exception of

TABLE III
Composition of Individual Muscles

Muscle No.	Relative water-soluble protein content					Relative aldolase content					Relative specific activity				
	Animal					Animal					Animal				
	6	7	8	9	Avg.	6	7	8	9	Avg.	6	7	8	9	Avg.
1	1.16	1.14	0.95	1.23	1.2 ± 0.08	1.4	1.3	1.3	1.4	1.4 ± 0.05	1.2	1.2	1.3	1.1	1.2 ± 0.05
2	0.98	1.30	0.95	0.96	1.0 ± 0.1	0.60	0.66	0.75	0.87	0.72 ± 0.09	0.63	0.51	0.79	0.75	0.67 ± 0.10
3	1.03	1.23	1.22	1.39	1.2 ± 0.1	1.3	1.3	1.6	1.1	1.3 ± 0.10	1.3	1.1	1.3	0.85	1.1 ± 0.14
4	0.85	0.92	0.93	0.83	0.86 ± 0.04	0.35	1.2	0.39	0.65	0.65 ± 0.28	0.41	1.4	0.42	0.77	0.75 ± 0.33
5	0.86	0.63	0.97	0.99	0.86 ± 0.1	0.66	0.62	0.82	0.73	0.71 ± 0.07	0.77	1.0	0.84	0.72	0.83 ± 0.09
6	1.13	1.11	1.07	0.94	1.1 ± 0.03	1.4	1.6	1.1	1.5	1.4 ± 0.15	1.3	1.5	1.0	1.5	1.3 ± 0.18
7	1.10	0.80	1.01	0.95	0.96 ± 0.06	1.2	0.76	1.3	1.1	1.1 ± 0.16	1.1	1.0	1.3	1.1	1.1 ± 0.08
8	0.92	0.85	0.91	0.78	0.87 ± 0.07	0.88	0.62	0.82	0.66	0.75 ± 0.13	0.98	0.74	0.90	0.82	0.86 ± 0.08

the extract pH's which were for all four animals.

The tissue pH's were obtained with a hard-tipped glass electrode and the usual calomel reference electrode. The electrode pair was thrust, together with a thermometer, into a 2-cm.-deep slit cut into the muscle at the time of dissection. The observed pH values were corrected to 25°C. using the Beckman nomograph for these electrodes. Extract pH's were measured 1 hr. after the muscle had been extracted, which was approximately 3 hr. post-mortem. The "ultimate" pH values were those of extracts prepared from the right-hand portion of the animal 7 days post-mortem (see *Experimental*).

The relative "ultimate" pH's shown in Table IV are of particular interest in that muscles 4, 5, and 8 appear to differ from the others as was the case for soluble proteins and aldolase. This same pH differentiation has been recently observed by Swift and Berman (10) for the same eight muscles derived from five animals (steers, bulls, and cows). The actual pH's were of comparable magnitude to those reported here.

The post-mortem pH of a muscle will be

TABLE IV
Tissue and Extract pH's of Bovine Muscle

Muscle No.	Relative pH, avg.		
	Tissue ^a	Extract ^b	"Ultimate" ^a
1	1.03	1.040 ± 0.005	0.996 ± 0.010
2	0.94	0.940 ± 0.013	0.990 ± 0.010
3	0.99	1.017 ± 0.015	0.976 ± 0.008
4	1.02	1.029 ± 0.010	1.056 ± 0.020
5	1.02	1.007 ± 0.016	1.008 ± 0.003
6	0.98	0.978 ± 0.010	0.976 ± 0.004
7	0.98	0.988 ± 0.011	0.987 ± 0.005
8	1.03	0.997 ± 0.017	1.007 ± 0.003
Average Absolute pH	7.37 ± 0.24	6.39 ± 0.16	5.51 ± 0.10

^a Average for animals 7, 8, and 9.

^b Average for animals 6, 7, 8, and 9.

determined chiefly by the rate of lactic acid production, the buffering capacity of the muscle, and the rate of inactivation of the significant glycolytic enzymes. It is safe to assume that the latter factor is the same for all muscles. Thus, the fact that muscles such as Nos. 4, 5, and 8 yield relatively high pH values means either that their total buffering capacity is higher or they produce lactic acid through glycolysis at a slower rate. Although we do not have information on the relative buffering capacities of these muscles, we know that the total protein-buffering capacity of muscles 4, 5, and 8 is lower than the others (10). Furthermore, if the total phosphorus contents of such muscles is taken as a measure of phosphate content, the phosphate-buffering capacities of muscles 4, 5, and 8 are lower than or comparable to the others (10).

While it must be conceded that the relatively higher pH values of muscles 4, 5, and 8 could be a consequence of higher anserine and carnosine contents (34), we believe that they may be due to lower rates of lactic acid production. The fact that these three muscles have lower amounts of the water-extractable protein (enzyme-rich protein) suggests that the cellular concentration of rate-limiting glycolytic enzymes is lower in these muscles. Indeed, as we have shown in this communication, aldolase, one of the important enzymes of glycolysis, does make up a smaller percentage of the soluble proteins in these three muscles than in the others. The experiments of Andrews *et al.* (12), however, throw some doubt on the assumption that aldolase is a rate-limiting enzyme in glycolysis. Furthermore, gross variations in glycogen levels could also be responsible for differences in rates of lactic acid production. Small differences in glycogen level appear to have little effect on ultimate pH of beef muscle (11). Until more analytical data become available on the composition of these eight muscles, none of these factors can be excluded.

Muscle 1, and possibly Nos. 3 and 8, have relatively high pH's soon after the death of the animal, as can be seen from the tissue and extract pH's in Table IV. These differences between tissue and extract and "ul-

timate" pH values are probably due to processes other than lactic acid production. These processes likewise may differ from muscle to muscle. Howard and Lawrie (11) have shown, for example, that for periods up to 400 min. post-mortem, the rate of creatine phosphate and ATP splitting differ significantly from muscle to muscle.

ELECTROPHORETIC ANALYSIS

Shown in Fig. 1 are electrophoretic patterns of three typical muscle extracts.³ If one takes into consideration the great complexity of the system, the enantiography of the ascending and descending patterns is quite satisfactory. The patterns are quite similar in form to those reported by Jacob (13) and others for rabbit muscle. The letters refer to components identified by Jacob (13) and have been placed on the patterns at positions corresponding to the mobilities he observed at pH 8.2.

The patterns shown in Fig. 1 illustrate the great variation in relative amounts of components from muscle to muscle and animal to animal. No systematic variation was observed, i.e., muscles previously found to be differentiable from others on the basis of soluble protein and aldolase content exhibited no such regularity in electrophoretic pattern.

Summarized in Table V are the electrophoretic results for eight muscles from each of three animals. Relative amounts were estimated from the ratio of the height of a given peak to the total area of the pattern. This procedure was followed to allow for differences in protein concentrations of extracts. As is equally true for rabbit muscle (13), components m + n make up a large portion of the myogen fraction.

ULTRACENTRIFUGAL ANALYSIS

Shown in Fig. 2 are three representative ultracentrifuge patterns obtained for beef muscle extracts. As was the case for the electrophoretic patterns, no systematic variation of the relative amounts of each of the components was observed from muscle to muscle, although qualitatively the patterns were quite similar.

³ All the mobilities have the units cm./v./sec.

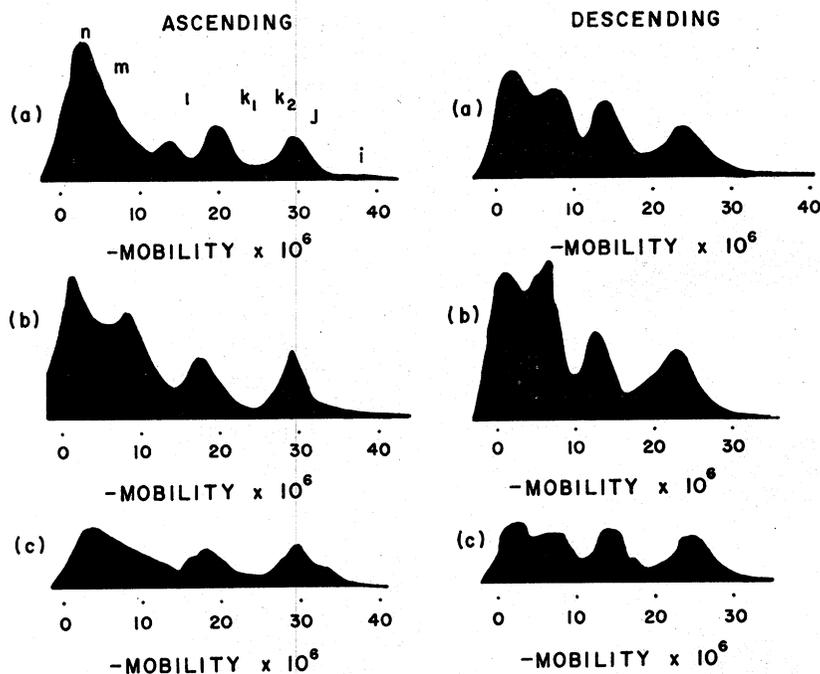


FIG. 1. Electrophoretic patterns of bovine skeletal muscle extracts. Field strength, 14.3 v./cm.; time, 6000 sec.; solvent, pH 8.17; Tris buffer, $\Gamma/2 = 0.05$.

- a. Animal No. 8, Muscle No. 1, concn. = 1.3 g./100 ml.
 b. Animal No. 9, Muscle No. 3, concn. = 1.8 g./100 ml.
 c. Animal No. 9, Muscle No. 4, concn. = 1.0 g./100 ml.

TABLE V
 Summary of Electrophoretic Results

Animal No.	Average mobility $\times 10^6$				Relative amount ^a			
	n	m	l	$k_1 + k_2 + J$	n	m	l	$k_1 + k_2 + J$
7	-3.0 ± 0.2	-8.9 ± 1.1	-15 ± 2	-25 ± 3	1.6 ± 0.2	1 ± 0.1	1.3 ± 0.3	1.3 ± 0.2
8	-3.4 ± 0.5	-12 ± 2	-19 ± 2	-31 ± 2	2.2 ± 0.3	1 ± 0.3	1.3 ± 0.2	1.1 ± 0.2
9	-2.9 ± 0.9	-11 ± 2	-19 ± 2	-31 ± 2	1.8 ± 0.2	1 ± 0.3	0.83 ± 0.2	0.92 ± 0.3
Avg.	-3.1	-11	-18	-29	1.9	1	1.1	1.1

^a Relative amounts were calculated taking component m as unity.

Shown in Table VI is a summary of the sedimentation data for eight muscles from animals 7, 8, and 9, respectively.⁴ Relative

⁴ Sedimentation data for muscle extracts obtained from animal No. 6 were in qualitative agreement with those obtained from animals 7, 8, and 9. These experiments, however, were carried out with an ultracentrifuge unequipped with either temperature control or phase plate. Consequently, the quality of the patterns obtained did not warrant their inclusion here.

amounts of each of the components were calculated from the ratio of peak height to total pattern area. Bovine muscle extracts all appear to yield three "components" characterized by average sedimentation constants of 2.3, 4.7, and 6.8, respectively, and here designated α , β , and γ . The broadness of each of these peaks, as can be seen from Fig. 2, for example, suggests that each of these so-called components consists of more than one protein.

Calculation of the total protein concentration of muscle extracts from the total area of ultracentrifuge patterns yielded values which were almost always lower than that obtained from biuret analysis. The former value was

obtained by comparing the area of the pattern with that of a solution of a pure protein of known concentration at the same bar angle. The concentration is given by:

$$\text{Unknown concn.} = \frac{\text{unknown area}}{\text{standard area}}$$

× standard concn.

Such calculations, using β -lactoglobulin as a standard, gave concentrations which were 25% (average for all muscles) lower than those obtained from the biuret analysis. This 25% apparently corresponds to the very heavy material which is seen to sediment to the bottom of the cell in the early phases of the run.

FRACTIONATION OF MUSCLE EXTRACTS

In order to obtain more information as to the composition of the myogen fraction, sub-fractions prepared by ammonium sulfate precipitation were studied. Solid dry ammonium sulfate was added to extracts of *longissimus dorsi* (muscle 1) at 3–5°C. until the desired concentration was reached. These concentrations of ammonium sulfate correspond to those used by Beisenherz *et al.* (7) in their simultaneous preparation of five enzymes. The ammonium sulfate solutions were allowed to stand for a minimum of 12 hr. and then were centrifuged in the Spinco model L at 42,000 × *g* for 1 hr. The precipitate was then taken up in the Tris buffer used for electrophoresis and ultracentrifuge experiments, dialyzed against a minimum of three changes of this buffer for at least 36 hr., and then centrifuged at 60,000 × *g* to remove insoluble protein.

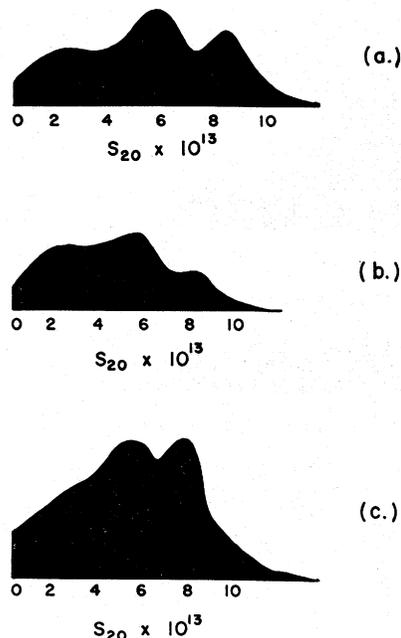


FIG. 2. Ultracentrifuge patterns of bovine skeletal muscle extracts. Speed 59,780 r.p.m.; bar angle, 50°; solvent, pH 8.17, Tris buffer, $\Gamma/2 = 0.05$; time, 80 min.

a. Animal No. 9, Muscle No. 2, 10°C., concn. = 1.1 g./100 ml.

b. Animal No. 8, Muscle No. 4, 6°C., concn. = 1.3 g./100 ml.

c. Animal No. 8, Muscle No. 3, 6°C., concn. = 1.7 g./100 ml.

TABLE VI

Summary of Ultracentrifugal Results

Animal No.	Average $S_{20} \times 10^{13}$			Relative amount ^a		
	α	β	γ	α	β	γ
7	2.4 ± 0.1	4.9 ± 0.2	6.9 ± 0.2	1 ± 0.1	2.2 ± 0.3	1.7 ± 0.2
8	2.2 ± 0.2	4.7 ± 0.2	6.8 ± 0.2	1 ± 0.2	1.9 ± 0.2	1.5 ± 0.2
9	2.4 ± 0.2	4.6 ± 0.2	6.6 ± 0.2	1 ± 0.1	1.8 ± 0.2	1.7 ± 0.3
Avg.	2.3	4.7	6.8	1	2	1.6

^a Relative amounts were calculated taking component α as unity.

TABLE VII
Water-Soluble Protein Distribution in *Longissimus Dorsi*

Fraction No.	$(\text{NH}_4)_2\text{SO}_4$ concentrations <i>M</i>	Grams protein/100 g. wet tissue				Per cent of original protein content			
		Animal No.				Animal No.			
		7	8	9	Avg.	7	8	9	Avg.
FI	0-1.75	0.16	0.19	0.19	0.18 ± 0.01	5.4	5.5	4.8	5.2 ± 0.3
FII	1.75-2.40	0.25	0.12	0.085	0.15 ± 0.06	8.5	3.5	2.2	4.7 ± 1.8
FIII	2.40-2.65	0.30	0.17	0.35	0.27 ± 0.07	10.1	4.9	8.9	8.0 ± 2.0
FIV	2.65-3.35	0.76	1.04	1.17	0.99 ± 0.15	25.7	30.0	29.7	28.5 ± 1.8
SIV	>3.35	0.53	0.41	0.16	0.36 ± 0.15	17.9	11.8	4.1	11.3 ± 4.8
—	Unrecovered protein	0.96	1.54	1.97	1.49 ± 0.48	32.4	44.3	50.3	42.3 ± 6.7
—	Total	2.96	3.47	3.93	3.45 ± 0.33	100	100	100	100

Shown in Table VII is a summary of the protein distribution of the myogen fraction for muscle 1. The largest single fraction (FIV) appears to be that precipitating between 2.65 and 3.35 *M* ammonium sulfate. What appears to be relatively poor reproducibility from animal to animal may be a consequence of variations in composition from animal to animal. The difficulties involved in carrying out a fractional precipitation in a reproducible manner, however, may be responsible for the observed variation.

It is of interest to note that on the average $42 \pm 7\%$ of the protein is lost during the course of the fractionation. A small part of this may very well represent material unavoidably lost on transfers and in other operations during the fractionation. The fact that relatively large amounts of insoluble protein had to be removed during the course of the fractionation leads us to believe that the bulk of the 42% lost represents denatured protein. Qualitatively, it would appear that the bulk of the insoluble protein is precipitated with fraction FI. It is worth while to compare this value of 42% with the value of 34% of "missing" protein found for this particular muscle in ultracentrifuge experiments (see previous section). It seems probable that the heavy material which piles up on the bottom of the ultracentrifuge cell when muscle extracts are centrifuged is denatured protein having characteristically low solubility.

ELECTROPHORESIS AND ULTRACENTRIFUGATION OF AMMONIUM SULFATE FRACTIONS

Electrophoretic and sedimentation experiments were carried out under conditions comparable to those used for the extracts. Shown in Figs. 3 and 4 are tracings of the patterns obtained from fractions derived from animal No. 9.⁵ These are representative of patterns obtained from animals 7, 8, and 9. As one would expect, the patterns obtained from the fractions are sharper and more characteristic of paucidisperse systems than were those of the extracts. Fraction FI is especially interesting in that it shows concentration of ultracentrifugally heavy and electrophoretically fast protein as compared with the original extract. Fractions FIII and FIV do not appear to differ greatly from each other.

IDENTIFICATION OF COMPONENTS

Identification of electrophoretic and ultracentrifugal components from literature values of mobilities and sedimentation constants is complicated by the fact that, in addition to native protein, extracts may contain degradation products and components

⁵ The electrophoretic patterns of fraction SIV have not been included since the concentrations were too low to obtain satisfactory boundaries. Concentration of these solutions by pervaporation concentrates the myoglobin whose red color interferes with photographic registration.

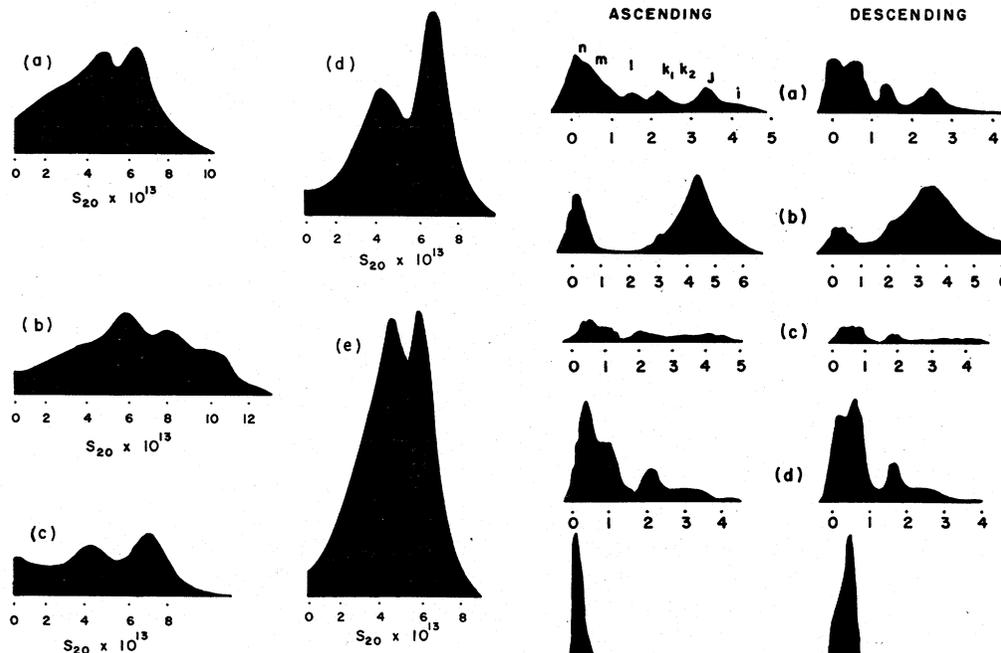


FIG. 3. Ultracentrifuge patterns of muscle extract fractions. Speed, 59,780 r.p.m.; temp. 10°C.; Animal No. 9, Muscle No. 1; solvent, pH 8.17, Tris buffer, $\Gamma/2 = 0.05$; time, 64 min.

- Original extract, bar angle, 50°, concn. = 1.3 g./100 ml.
- Fraction FI, bar angle, 40°, concn. = 1.5 g./100 ml.
- Fraction FII, bar angle, 40°, concn. = 0.95 g./100 ml.
- Fraction FIII, bar angle, 50°, concn. = 1.3 g./100 ml.
- Fraction FIV, bar angle, 55°, concn. = 1.7 g./100 ml.

arising from the mutual interaction of native proteins. The latter can be minimized, although not avoided entirely, by carrying out experiments, as we have done, in a pH region where the protein molecules have the same charge sign.

The problem is further complicated by the fact that electrophoretic and ultracentrifugal experiments have been carried out in a variety of buffer systems. This is particularly critical in electrophoresis experiments where, even at a fixed pH, the mobility may vary markedly with the buffer concentration and type. Sedimentation constants, which are generally not as dependent upon buffer type,

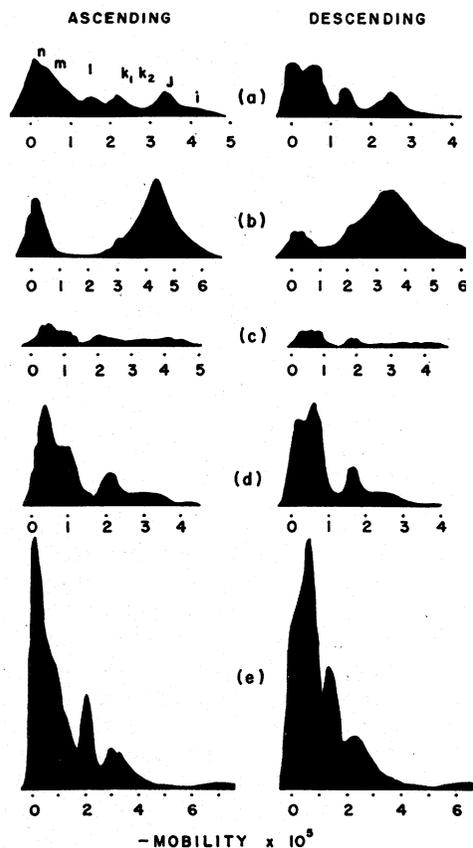


FIG. 4. Electrophoretic patterns of muscle extract fractions. Field strength, 14.3 v./cm.; solvent, pH 8.17, Tris buffer, $\Gamma/2 = 0.05$; Animal No. 9, Muscle No. 1.

- Original solution, concn. = 1.3 g./100 ml., time = 6000 sec.
- Fraction FI, concn. = 1.5 g./100 ml., time = 4300 sec.
- Fraction FII, concn. = 0.95 g./100 ml., time = 5000 sec.
- Fraction FIII, concn. = 1.3 g./100 ml., time = 5640 sec.
- Fraction FIV, concn. = 1.7 g./100 ml., time = 4052 sec.

concentration, and pH, are probably a more reliable means of identifying components.

Shown in Table VIII is a compilation of sedimentation constants and electrophoretic groups for a number of purified rabbit muscle proteins.⁶ With the exception of al-

⁶ Assignment of a protein to a group was based on Jacob's (13) data at pH 5-8, ionic strength 0.15. Since the values given in the litera-

TABLE VIII

Electrophoretic, Ultracentrifugal and Solubility Properties of Purified Rabbit Muscle Proteins

Protein	$S_{20} \times 10^{13}$	Electrophoretic group	Per cent of myogen fraction	Reference No.			
				Sol.	S_{20}	Electrophoresis	Percentage
		0-1.75 M ammonium sulfate (FI)					
Phosphorylase a	13.2	k_1, k_2	2	1	14	1	2
Phosphorylase b	8.2	k_1, k_2	—	1	14	1	—
(Actomyosin)	>20	j to k	—	1	15	1	—
(Myosin)	4.5-6.3	j to k	—	1	15	1	—
(Y protein)	5, (major), 8, 15	j to k	—	1	28	1	—
		1.75-2.40 M (FII)					
Glycerophosphate dehydrogenase	6.5	—	1	7	22	—	7
Lactic dehydrogenase	7.0	i, j	3	7	21	25	7
Phosphorylase b		See group FI					
Phosphofructokinase	6.9	—	10	1	29	—	30
		2.40-2.55 M (FIII)					
Phosphorylase b		See group FI					
Glyceraldehyde phosphate dehydrogenase	7.4 (avg.)	l, m, n	10-23	7	18, 20	1	3, 7
Aldolase	7.8 (avg.)	l, m, n	5-10	—	18, 19	1	5, 7
Phosphofructokinase		See group FII					
		2.65-3.35 M (FIV)					
ATP-creatine transphosphorylase	5.0	k	10-38	26	26	26	26, 27
Phosphoglyceric acid mutase	4.1 (avg.)	j	2	23	23, 24	24	23
Myokinase	2.0 (avg.)	l, m, n	2	1	16, 17	17	16
Aldolase		See group FIII					
		Above 3.35 M (SIV)					
Myoglobin	1.9 (avg.)	l	3	31	31, 32	31	32
Myokinase		See group FIV					
ATP-creatine transphosphorylase		See group FIV					

dolase, these have been grouped according to their reported solubility in ammonium sulfate solutions. Aldolase was placed in groups FIII and FIV on the basis of our own experimental work with bovine muscle. We have found that in a typical fractionation approximately 63% of the original aldolase activity can be recovered. Of this, 39% is

ture are for a variety of buffers and ionic strengths, the mobilities of the purified proteins for our purposes are only of qualitative interest.

found in FIII, 51% in FIV, and the remainder in FII and SIV.

Our experience with aldolase is an illustration of the limited reliability of protein solubility data; i.e., Beisenherz *et al.* (7) found that rabbit muscle aldolase is precipitated almost entirely in Fraction FII at a lower ammonium sulfate concentration than we employed. The assignment of proteins to more than one group in Table VIII is a reflection of this uncertainty. In spite of this, such data

when used with care can aid in the identification of components.

Comparison of ultracentrifugal and electrophoretic results with the data compiled in Table VIII enables us to draw some conclusions as to the composition of each of the fractions. As we have already pointed out, and as Figs. 3 and 4 illustrate, fractions FIII and FIV appear to be rather similar in composition. Closer examination of these fractions, making use of aldolase assay data, indicates that the similarity is only an apparent one. The specific activities of 2.8 and 1.1 obtained for fractions FIII and FIV indicate that 51% of fraction FIII and 20% of FIV are aldolase.⁷ Thus, comparison of Figs. 3*d*, 3*e*, and Table VIII leads us to conclude that the bulk of the 7 *S* peak for fraction FIII is aldolase.⁸ In the case of FIV, less than half of the fast peak (6 *S*) consists of aldolase.⁹ Glyceraldehydephosphate dehydrogenase, having a somewhat lower sedimentation constant than aldolase, may make up the bulk of this fast peak. Furthermore, the fact that both aldolase and glyceraldehydephosphate dehydrogenase have mobilities placing them in the *l*, *m*, *n* group could explain in part the similarity in electrophoretic patterns of FIII and FIV.

Phosphoglyceric acid mutase and ATP-creatine transphosphorylase have sedimentation constants which would place it in the

⁷ These were calculated from the specific activity of purified rabbit muscle aldolase as discussed previously.

⁸ The sedimentation constants given in Table VIII were generally calculated for infinite dilution or low concentration of protein. Since the protein concentrations in our experiments were generally relatively high, we would expect the observed sedimentation constants to be somewhat lower than the literature values.

⁹ In general, the peak height of one component in a schlieren diagram is not independent of that of adjacent components. In the case of the diagram for FIV shown in Fig. 3*e*, the 5 *S* component without doubt enhances the height of the 6 *S* component and, furthermore, shifts the position to lower values of *S*. Resolution of these peaks would probably shift the slower peak somewhat closer to 4 *S* and the faster one somewhat closer to 7 *S*. The polydispersity of these fractions, however, does not warrant this detailed a process.

4–5 *S* group. The electrophoretic mobilities of these enzymes are likewise consistent with components found in the mobility range -2 to -3.5×10^{-5} (see Figs. 1*a*, 4*d*, and 4*e*).

Comparisons of the electrophoresis patterns (Figs. 4*a*, 4*b*) and the ultracentrifuge patterns (Figs. 3*a*, 3*b*) for the original extract and fraction FI shows that this fraction has become enriched in protein faster than 7 *S* and with mobilities in excess of -3×10^{-5} . Solubility data, sedimentation constant, and electrophoretic mobility all suggest that this fraction is rich in phosphorylase b. No 13 *S* component was seen in this or in earlier photographs, which would indicate that phosphorylase a is absent. This is consistent with the findings of Krebs and Fischer (33) that under the usual conditions of extraction, phosphorylase a is converted almost entirely to the b form. While as much as 40% of fraction FI might consist of phosphorylase, it is apparent from Figs. 3*b*, 4*b*, and Table VIII that other components are present. Actomyosin, myosin, and Y protein all have mobilities which would place them in the main peak in Fig. 4*b*. However, these proteins are usually extracted at relatively high ionic strength, i.e., above 0.15. Calculated from the total ions found in bovine skeletal muscle, the ionic strength of our extracts would be about 0.06.¹⁰ It is therefore unlikely that appreciable amounts of actomyosin, myosin, or Y protein are present in aqueous muscle extracts.

A relatively large amount of fraction FI consists of protein having mobilities in excess of -4×10^{-5} . None of the proteins assigned to group FI have the required mobility. Lactic dehydrogenase, assigned to group FII, appears to be the only protein having a sufficiently large mobility. The sedimentation of lactic dehydrogenase would probably account for much of the protein slower than 8 *S*.

The specific activity of aldolase in fraction FII corresponds to 27% of the fraction, probably accounting for the bulk of the 7 *S* peak

¹⁰ The ionic strength calculated for muscle extracts from their specific resistance is about 0.03 (as NaCl). The actual ionic strength probably lies somewhere between 0.03 and 0.06.

(Fig. 3c) and a large part of the *l*, *m*, *n* electrophoretic group (Fig. 4c). The sedimentation constants and solubility of glycerophosphate dehydrogenase, and phosphofructokinase would appear to place them in the 7 *S* group of fraction FII.

Although the ammonium sulfate fractions are certainly less heterogeneous than the extracts from which they were derived, they are obviously still quite polydisperse. Furthermore, virtually all of the 2.3 *S* group (Table VI, group α) remains unidentified. The only proteins given in Table VIII that have the required sedimentation constants are myokinase and myoglobin, which constitute only about 5% of the myogen fraction. This is insufficient to account for the amount of the 2.3 *S* group.

While the 11 proteins listed in Table VIII probably make up a large percentage of the total myogen fraction on a weight basis, they certainly represent only a portion of the proteins (mainly enzymes) known to be present in muscle tissue. Indeed, Dubuisson (1) has estimated that there are about 50 enzymes in the muscle cell! Therefore, for practical reasons, in a study of the post-mortem changes occurring in muscle tissue it is undesirable initially to resort to very specific measurements such as enzymic assays. We have found that a study of gross properties, such as reported in this communication, yields information useful in formulating more specific experiments. This approach will be considered at greater length in subsequent communications.

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