4. The activity of ultrasonically treated microsomes could not be restored by polyuridylic acid.

5. The application of ultrasonic vibrations on microsomes caused a several-fold increase in phosphodiesterase activity as measured by the breakdown of [³H]polyuridylic acid. As a breakdown product, an increased amount of 5'-UMP was obtained. There was no significant effect on the breakdown by ultrasonically treated ribonucleoprotein particles.

6. Electron microscopy showed that on treatment by ultrasonic vibrations the microsomal fraction retained membranes and particles. No apparent change in the structure of isolated ribonucleoprotein particles was observed. The membrane-bound enzyme glucose 6-phosphatase was not destroyed by treatment with ultrasonic vibrations for 1 min.

We thank Professor F. Dickens, F.R.S., for his interest and advice, Dr Marian Hicks of the Bland Sutton Institute of Pathology for the electron microscopy, Dr T. Hultin for the generous gift of [³H]polyuridylic acid, Mr B. Waynforth for performing the partial hepatectomies, and Miss Eileen Walters and Miss Elizabeth Lowe for their skilled technical assistance. The work was supported by a grant to the Medical School from the British Empire Cancer Campaign. The experiments with [³H]polyuridylic acid were carried out at the Wenner-Gren Institute and were made possible by a grant from the Swedish Cancer Society. A. v. d. D. thanks the Medical School for hospitality.

REFERENCES

- Allen, E. H., Glassman, E. & Schweet, R. S. (1960). *J. biol. Chem.* **235**, 1061.
- Campbell, P. N. & Cooper, C. (1963). *Biochem. J.* **89**, 94P.
- Campbell, P. N. & Kernot, B. A. (1962). *Biochem. J.* **82**, 262.
- Decken, A. von der & Campbell, P. N. (1961). *Abstr. 5th int. Congr. Biochem., Moscow*, p. 181.
- Decken, A. von der & Campbell, P. N. (1962). *Biochem. J.* **84**, 449.
- de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
- Fessenden, J. M., Cairncross, J. & Moldave, K. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 82.
- Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.
- Kalf, G. F. & Simpson, M. V. (1959). *J. biol. Chem.* **234**, 2943.
- King, E. J., Abul-Fadl, M. A. N. & Walker, P. G. (1951). *J. clin. Path.* **4**, 85.
- Kroon, A. M. (1963). *Biochim. biophys. Acta*, **72**, 391.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Martin, E. M., Malec, J., Coote, J. L. & Work, T. S. (1961). *Biochem. J.* **80**, 606.
- Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
- Ogata, K., Watanabe, I., Morita, T. & Sugano, H. (1962). *Biochim. biophys. Acta*, **55**, 264.
- Spahr, P. F. & Schlessinger, D. (1963). *J. biol. Chem.* **238**, PC2251.
- Sugano, H., Ogata, K., Mirota, T., Tominaga, H. & Watanabe, I. (1962). *Biochim. biophys. Acta*, **61**, 638.

Biochem. J. (1964) **91**, 201

The Influence of Post-Mortem Conditions on the Solubilities of Muscle Proteins

By R. K. SCOPES

Low Temperature Research Station, Downing Street, Cambridge

(Received 22 August 1963)

Of all animal tissues, muscle undergoes possibly the most marked biochemical and physicochemical changes after death. For this reason it is often essential to prepare homogenates or extracts as soon as is feasible after killing the animal, in order that neither the process of rigor mortis nor the lowering of muscle pH by conversion of glycogen into lactic acid adversely affects the factors being studied. Both the rigor-mortis process (Bate-Smith & Bendall, 1947) and the rate of post-mortem pH fall (Bendall, 1960; Marsh, 1954) have been subjects of considerable study both in this Laboratory and others; the present investigation sets out

to determine the biochemical changes that occur as a result of these processes, in particular the solubility characteristics of myofibrillar and sarcoplasmic proteins in various induced and simulated post-mortem circumstances.

METHODS

Preparation of myofibrils. Muscle (100 g.) was homogenized in 300 ml. of 30 mM-sodium β -glycerophosphate (adjusted to pH 6.5 with N-HCl) in a high-speed blender. The homogenate was centrifuged at 1000g for 15 min., and the residue was resuspended in β -glycerophosphate buffer

and centrifuged at 600g for 3 min. to remove coarse material (Perry & Grey, 1956). These centrifugings were repeated, and then the myofibrils were washed twice more in the same buffer containing 30% (v/v) of glycerol by centrifuging at 2000g for 15 min. and finally resuspended in the buffer containing 35% (v/v) of glycerol and stored at -10° (Bendall, 1961). All operations before storage were carried out close to 0° .

Preparation of sarcoplasmic protein extract. Muscle was homogenized in 2.5 vol. of water and the homogenate centrifuged at 35000g for 45 min. The supernatant was passed through cotton wool to remove traces of fat, giving a clear protein solution at a concentration of 2–2.5%. Depending on the nature of the muscle sample and the purpose of investigation, the pH of the homogenate was sometimes adjusted upwards, before centrifuging, with *m*-tris.

Extraction of myofibrils. Samples of the glycerol-treated myofibrils were taken when required (storage for several months at -10° did not affect their extractability characteristics) and washed three times with cold 30 mM- β -glycerophosphate buffer. Finally they were resuspended in buffer and portions (2 ml.) of this suspension were pipetted into 6 ml. of suitable extraction mixtures contained in 25 ml. conical flasks. These were then sealed with Parafilm. The total concentration of myofibrillar protein during extraction was normally 0.2–0.3 mg./ml., but identical results were obtained in the range 0.1–0.8 mg./ml. The flasks were shaken periodically and extraction was allowed to proceed for 24 hr. The mixtures were then centrifuged at 20000g for 15 min. and the supernatants transferred to test tubes for protein estimation. All operations were carried out at $0-1^{\circ}$.

Protein estimation. All methods used were ultimately standardized against Kjeldahl nitrogen values, and a value of 16.7% of N has been used for converting nitrogen values into protein values, both for myofibrillar and sarcoplasmic proteins (Bailey, 1937). The supernatant solution after extraction and centrifugation contained only the extracting salts and protein. The latter could be estimated rapidly and accurately by measuring its absorption of ultraviolet light at suitable wavelengths, in particular at about 210 m μ (Tombs, Souter & MacLagen, 1959). The extinction of solutions was measured at 210, 220, 230 or 278 m μ , depending on the quantity of protein present, and the following extinction coefficients were employed: at 210 m μ , $E_{1\%}^{1\text{cm}}$ 203; at 220 m μ , $E_{1\%}^{1\text{cm}}$ 108; at 230 m μ , $E_{1\%}^{1\text{cm}}$ 55.2; at 278 m μ , $E_{1\%}^{1\text{cm}}$ 7.75.

Where the extractant contained ultraviolet-absorbing material, or when there was an unknown effect of denaturation changing the extinction coefficients at about 230 m μ (Glazer & Smith, 1961), the method of Lowry, Rosebrough, Farr & Randall (1951) was employed. This method was also used for total myofibrillar protein and for all estimations of sarcoplasmic protein.

Myoglobin was measured spectrophotometrically by the extinction of sarcoplasmic extracts at 555 m μ in the presence of sodium dithionite; an extinction coefficient of $E_{555}^{1\%}$ 7.6 was used (Bowen, 1949).

Precipitation of 'pH 5 protein' fraction. The pH of sarcoplasmic protein solutions was lowered by the addition of *N*-acetic acid, at 5° , until the desired value was reached as measured on a continuously recording pH-meter. The solution was then kept at 0° for 2 hr., centrifuged at

10000g for 5 min. and the precipitate washed with 50 mM-acetate or β -glycerophosphate buffer at the same pH. The precipitated protein was dissolved in 0.01 *N*-NaOH for estimation, or extracted with 0.1 *M*-tris-chloride buffer, pH 8.0, at 0° to obtain an undenatured solution.

Starch-gel electrophoresis. A vertical apparatus as described by Smithies (1959a) was employed in the present study, and buffer systems were varied to obtain the best results with the proteins under observation. The two most useful buffer systems were:

(A) A slight modification of the method of Scopes (1963) in which the outer gel was made up in 60 mM-tris-100 mM-boric acid (pH 8.6 at 5°), and the inner gel in 12 mM-tris-2 mM-diethylenetriaminepenta-acetic acid. The latter buffer was made by desalting a 33% solution of the sodium salt of diethylenetriaminepenta-acetic acid (L. Light and Co. Ltd., Colnbrook, Bucks.) with Dowex 50 resin, and titrating the acid with *m*-tris to pH 8.25 at 18° . The final concentration of anions is approximately one-sixth that of the total tris present. The use of this anion has two slight advantages over the use of the citrate ion, namely the slightly enhanced 'discontinuity effect', due to the extra negative charge ($pK_a \sim 8.8$), and the stabilizing effect of a powerful complex-forming agent on some of the muscle proteins. The sodium borate in the buffer trays contained a little NaCl to keep the current from falling, and to raise the anion transport number, so that the discontinuity passed through the inner gel in 5 hr.

(B) Better separation of the protein components moving towards the cathode was obtained by using a tris-chloride buffer system with no discontinuity. The whole gel was made up in 4 mM-tris-2 mM-HCl (pH 8.6 at 5°), and the buffer trays contained tris-chloride ten times as concentrated as the gel buffer. The gel pH dropped by about 1 unit during the running of this system.

Electrophoresis was carried out at 300 v (approx. 11 v/cm.) for 5–6 hr., 15–20 mA, in a cold room kept at 1° . After being sliced, the gels were stained with a solution of 1% Naphthalene Black 10B plus 2% Nigrosine (water-soluble) in methanol-acetic acid-water (5:1:4, by vol.) and photographed (with an orange filter). The complementary slice was used for specialized reactions, principally the benzidine reaction for catalase activity (Smithies, 1959b). Samples for electrophoresis were dialysed against 0.50 *M*-sucrose in 10 mM-tris-1 mM-citric acid at 0° before being applied to the gel; a water extract was thus concentrated about twofold, resulting in sarcoplasmic solutions of about 4% protein content, which has been found to be the optimum concentration for a 0.5 mm. slot in the gel.

RESULTS

(a) *Extraction of ox muscle myofibrils.* Myofibrillar proteins are soluble only at high ionic strength, at extremes of pH, or in the presence of specific extractants. Several workers have devised solutions that are capable of solubilizing myofibrils to a high extent: e.g., Weber & Meyer (1933) used potassium chloride in a carbonate buffer; Bate-Smith (1937) found that lithium chloride was more efficient than potassium chloride; potassium iodide (Helander, 1957; Perry, 1953) and pyrophosphate

(Hasselbach & Schneider, 1951) have also been used for specific purposes. However, in the study of the general behaviour of myofibrils, the uncertainties of specific ionic effects, in particular those of phosphate buffers (Deuticke, 1932), can be avoided by using potassium chloride in an inactive buffer such as cacodylate or β -glycerophosphate, which have suitable pK values.

Extractions of myofibrils at an ionic strength of 1.0 were made over a range of pH values in 30 mM- β -glycerophosphate buffer. A similar set of extractions, in which the potassium chloride was omitted, was extended to extreme pH values to obtain a complete picture of the behaviour of myofibrils at low ionic strength.

The muscle sample employed had passed into rigor mortis at 20°, and had an ultimate pH of 5.55. The effect of pH on extractability of its myofibrils is shown in Figs. 1 and 2. Maximum extraction at ionic strength of 1.0 was found at pH 6.0, dropping sharply at lower pH owing to the insolubility of actomyosin under these conditions, and also dropping a little between pH 6.0 and 6.5. At low ionic strength (0.08) the myofibrillar structure is disrupted at pH values below 4.4 and above 10.1, rendering most of the protein soluble. A small residue (5 %) remains insoluble at pH 2, and rather less (3 %) at pH 12. Three myofibrillar preparations from the same muscle gave identical results throughout both ranges.

(b) *Yield of 'pH 5 protein' fraction from sarcoplasmic protein extracts.* One consequence of the falling pH *post mortem* is the insolubilization of the 'pH 5 enzymes', a preparation much used from other tissues in the study of the biosynthesis of proteins (Hoagland, Keller & Zamecnik, 1956; Keller & Zamecnik, 1956). It is known that many of these enzymes are unstable at the pH values necessary to precipitate them isoelectrically, and so this fraction was studied quantitatively to determine the effects of pH and temperature on them.

Sarcoplasmic protein was prepared from rigor ox longissimus dorsi muscle (pH 6.3 when obtained), and the quantity of protein that became insoluble either through isoelectric precipitation (at low temperatures) or through denaturation (at higher temperatures) was estimated after incubation at various temperatures and pH values. The ionic strength was 0.12, and the time of incubation 4 hr. The sarcoplasmic protein was first dialysed overnight at 0° against 100 mM-imidazole-50 mM-acetic acid buffer, pH 7.5. A very small precipitate, probably containing actomyosin extracted as a result of the ATP present in the muscle (Connell, 1958), was removed.

Samples (1 ml.) of the protein solution were added to centrifuge tubes containing 4 ml. of 100 mM-

imidazole-115 mM-acetic acid-30 mM-sodium β -glycerophosphate mixture, and appropriate quantities of 200 mM-acetic acid or imidazole, with 50 mM-potassium chloride to make the total volume up to 9 ml. The resulting mixtures were buffered over the pH range 4.4-7.5 with a constant ionic strength of 0.12. The final protein concentration was 3 mg./ml. Eight different pH values and eight temperatures were investigated, resulting

Fig. 1. Extractability of ox muscle myofibrils: variation of pH at constant ionic strength of 1.0. The conditions of extraction were: solutions contained 30 mM-sodium β -glycerophosphate-0.92 M-KCl, adjusted to the pH indicated with HCl. Extractions were for 24 hr. at 0°. The total number of experimental points was 64.

Fig. 2. Extractability of ox muscle myofibrils: variation of pH at low ionic strength (0.08). The conditions of extraction were: solutions contained the following: pH 1.0-3.6, HCl + KCl to give ionic strength 0.08; pH 3.6-5.3, 40 mM-acetate + KCl to give ionic strength 0.08; pH 5.0-7.2, 30 mM-sodium β -glycerophosphate adjusted with HCl; pH 7.2-9.7, 80 mM-tris-HCl + KOH; pH 10.0-11.0, 30 mM- Na_2CO_3 - NaHCO_3 ; pH 11.0-13.0, KOH + KCl to give ionic strength 0.08. Extractions were for 24 hr. at 0°. The total number of experimental points was 140.

Table 1. *Percentage of sarcoplasmic protein precipitated in 4 hr. at ionic strength 0.12 in imidazole- β -glycerophosphate-acetate-potassium chloride buffer at the pH and temperature indicated*

Temperature ... pH (at 18°)	Percentage of sarcoplasmic protein precipitated							
	0°	10°	15°	20°	25°	30°	37°	45°
4.45	4.4	3.8	4.2	4.0	4.4	4.7	5.1	8.1
4.79	6.6	5.7	4.8	6.4	6.6	7.4	5.4	15.0
5.24	5.0	4.9	5.5	6.2	7.8	10.5	15.8	35.0
5.67	3.1	2.9	3.2	3.1	4.2	6.2	12.2	34.0
6.00	2.1	1.9	2.2	2.5	3.2	6.3	8.5	29.0
6.53	0.6	0.8	0.7	2.1	2.8	3.4	6.6	24.5
7.09	0.35	0.35	0.6	0.5	1.2	2.1	5.2	24.0
7.52	0.25	0.4	0.7	0.8	1.1	2.3	7.6	22.0

in 64 separate experiments; the centrifuge tubes were incubated at the appropriate temperature for 4 hr. before the precipitate was centrifuged off, washed and estimated.

The pH values were measured at 18°. It was assumed that the temperature effects on the pH values of the buffer ions should be comparable with those on the pK values of the proteins themselves, as the groups concerned in this pH range are mainly imidazole and carboxyl residues, and so no corrections for temperature were made. The results are shown in Table 1. At temperatures above 25°, sarcoplasmic protein begins to be precipitated to a greater extent at all pH values, and in particular there is a marked rise at pH values close to the ultimate value usually met with in ox longissimus dorsi muscle, namely pH ~ 5.5. Thus at both pH 5.24 and at 5.67 an increase of 8% is found between 25° and 37°, indicating a considerable extent of denaturation occurring even at these mild temperatures. Moreover, at 45°, up to one-third of the sarcoplasmic protein is denatured in 4 hr., almost independently of pH.

(c) *Experiment investigating myofibrillar extractability and sarcoplasmic protein yields after controlling post-mortem temperature.* Ox longissimus dorsi was obtained immediately after slaughter, and cooled to 0° on ice as soon as possible; 1 hr. after death the muscle pH was 6.83. It was divided into three parts, which were treated as follows: A, homogenized at once: this is referred to as the 'pre-rigor' sample; B, kept at 0° for 20 hr. before homogenization: this is the '0°-rigor' sample; C, kept at 37° for 4 hr., then cooled to 0° and kept at 0° for 16 hr. before homogenization: this is the '37°-rigor' sample.

After homogenization, the pH was adjusted to 7.0, and then centrifuging was carried out at 4000g for 5 min. The supernatants were decanted and further centrifuged at 35000g for 45 min. to provide the sarcoplasmic protein fraction. The residue from the 4000g centrifuging was resuspended in 30 mM- β -glycerophosphate buffer, pH

Table 2. *Extraction of myofibrils with 0.92 M-potassium chloride-30 mM- β -glycerophosphate buffer, pH 6.0 (ionic strength 1.0)*

Experimental details are given in the text.

Myofibril preparation	Percentage of protein extracted ($\pm$ s.e.m.)
A ('pre-rigor')	73.0 $\pm$ 1.0
B ('0°-rigor')	73.5 $\pm$ 1.0
C ('37°-rigor')	46.5 $\pm$ 0.5
Previous preparation [see Results section (a)]	74.0 $\pm$ 1.5

6.5, and myofibrils were prepared from the suspension as described above.

The myofibrils were extracted with 0.92 M-potassium chloride-30 mM- β -glycerophosphate buffer, pH 6.0. The results are shown in Table 2.

These results show that allowing rigor mortis to proceed at 0° does not affect the myofibrillar extractability as compared with a pre-rigor preparation, whereas the 37° treatment decreased the extractability by nearly one-half.

The 'pH 5 proteins' were prepared as described above by using final pH values for precipitation of 4.8 and 5.6 (Table 1 indicates a maximum yield near pH 4.8 at low temperatures). The 'pre-rigor' extract contained an unknown quantity of actomyosin (Connell, 1958), and so the results are not shown; they were in fact very close to those of the '0°-rigor' sample. In Table 3 are given the yields of 'pH 5 proteins', together with the total sarcoplasmic protein and the myoglobin concentration. The percentages of sarcoplasmic protein are based on a maximum of 33% of the total muscle protein being soluble in the extraction conditions described.

From Table 3 it is clear that the 37° treatment has not only denatured, and so made insoluble in the extraction conditions of pH 7.0, a large part of the 'pH 5 proteins', but also some other protein or proteins amounting in all to about 20% of the total; the myoglobin content was unchanged.

Table 3. *Quantitative values for sarcoplasmic proteins prepared from '0°-rigor' ox longissimus dorsi and '37°-rigor' longissimus dorsi from the same animal*

Experimental details are given in the text. Values are expressed as percentages of the original amount of sarcoplasmic protein *in vivo*, assumed to be 33 % of the total muscle protein.

	B (‘0°-rigor’ preparation)	C (‘37°-rigor’ preparation)	Fall from B to C
Percentage of sarcoplasmic protein extracted	100	78	22
Percentage of myoglobin	10.9	10.8	0.1
Quantity of precipitate to pH 5.6 (3 expts.)	6.7±0.10	2.1±0.05	4.6
Quantity of precipitate to pH 4.8 (3 expts.)	8.3±0.15	5.7±0.10	2.6

(d) *Starch-gel electrophoresis of sarcoplasmic protein.* A preliminary note on the present work has been published (Scopes & Lawrie, 1963) with a diagram representing the pattern obtained from ox muscles in a tris-borate buffer system, pH 8.6. Some components migrate different distances on varying the buffer system, and so the diagram shown in Fig. 3 does not correspond exactly with the previous publication. For the convenience of the present discussion, the bands obtained in the discontinuous buffer system (A) have been enumerated by a letter representing an area on the starch gel between one major component and the next, followed by a serial number within this area; a suffix letter has been used for further minor components that do not always show. These numbers apply only to ox sarcoplasmic protein. In Fig. 3 a few examples relevant to the text demonstrate the nomenclature.

Plate 1 (a) shows a stained starch gel that was run in buffer system (A), and compares sarcoplasmic protein extracts from muscles that had passed into rigor mortis at room temperature or lower with samples of the same muscle that passed into rigor mortis at 37°. Three species, ox, rabbit and pig, all show the same effects, in particular a large diminution of band D1, creatine kinase (ATP-creatine phosphotransferase, EC 2.7.3.2). This identification was made by comparing the mobility of a partially purified preparation of creatine kinase (up to the magnesium acetate-extraction stage of Noda, Kuby & Lardy, 1955), which is shown beside the ox and pig samples. Another component, F3, which is diminished by ‘37°-rigor’ treatment, is shown more clearly in Plate 1 (b), a gel run in buffer system (B) in which the cation-migrating bands are better separated; in each case one minor component is removed at high temperatures. Although it is not clear on the photographs, other minor bands, belonging to the ‘pH 5 protein’ fraction, are diminished or completely removed in the ‘37°-rigor’ samples. In particular, band A2 is decreased even in the ‘0°-rigor’ samples, as compared with the ‘pre-rigor’ extract; this is the only consistent difference

between ‘pre-rigor’ and ‘0°-rigor’ extracts. The creatine kinase of rabbit sarcoplasmic protein is the band 9 tabulated by Hartshorne & Perry (1962); the other cation-migrating band that is susceptible to ‘37°-rigor’ conditions is possibly their band 1. There are several very fast bands in pre-rigor extracts all running ahead of the myoalbumin (band B1), and at least 30 bands altogether can regularly be observed in sarcoplasmic extracts from ox, rabbit or pig by using either buffer system (A) or (B).

Other bands on the electrophoretogram have been identified by comparing the mobilities of purified preparations of particular proteins (myoalbumin, pyruvate kinase, phosphoglyceraldehyde dehydrogenase), and myoglobin and metmyoglobin by their catalase activity.

DISCUSSION

It has been suggested by Bendall & Wismer-Pedersen (1962) that the substantial decrease in myofibrillar extractability met with in muscles from pigs which suffer from ‘white muscle disease’, in which the pH drops rapidly while the muscle temperature is still about 37°, may be associated with denaturation of sarcoplasmic proteins. Indeed, dark bands of what is presumed to be denatured protein have been observed histologically in such muscles. Although this disease is unknown in ox, similar effects are obtained by inducing conditions of high temperature and low pH in the muscle. Thus by holding the muscle at 37° for 4 hr., causing rapid glycolysis and a fast fall in pH, some 20 % of the sarcoplasmic protein is denatured. Included in this fraction is the majority of the creatine kinase of the muscle (approx. 10 % of the sarcoplasmic protein), and much of the ‘pH 5 protein’ fraction, which includes amino acid-activating enzymes as well as many uncharacterized proteins. At the same time, the myofibrillar extractability is greatly decreased, suggesting that the denatured sarcoplasmic protein may act to inhibit the solubilizing activity of high concentrations of potassium

Fig. 3. Schematic representation of bands obtained in starch-gel electrophoresis of ox-muscle sarcoplasmic protein, by using the diethylenetriaminepenta-acetic acid discontinuous buffer system, pH 9.0 at 5°. The nomenclature of the bands is indicated by bracketed zones and individual examples. Components with catalase action are indicated by arrows. Identified proteins are as follows: C1, myoglobin; C4, metmyoglobin; D1, creatine kinase; B1, myoalbumin; E1, phosphoglyceraldehyde dehydrogenase; F1, pyruvate kinase.

chloride by binding together the myofilaments, or even by forming chemical bonds with them. It is difficult to verify this hypothesis by incubating isolated myofibrils with and without sarcoplasmic protein at relevant pH values, as isolated myofibrils are very much swollen by water-binding, compared with their state *in situ*, and behave quite differently. The results of such an experiment *in vitro* were inconsistent and inconclusive.

The 'pH 5 proteins' have been found to be rather unstable, and especially so at temperatures above 25°. Thus, in Table 3, about 20 % of the denaturing sarcoplasmic protein is part of the 'pH 5 protein' fraction which at 0° is insoluble at pH 5.6. Moreover, even the method of preparation of this fraction at 0° causes denaturation of about half of it, as only half is re-soluble on raising the pH to 7.0. Such a solution, after centrifugation at 35000g, retains an opalescence, and its ultraviolet-absorption spectrum indicates the presence of nucleotide or nucleic acid, as the extinction between 250 and 270 m μ is almost constant. Undoubtedly the solution contains amino acid-activating enzymes with bound RNA, as well as many other minor proteins, possibly lipoproteins of mitochondrial origin. On subjecting this preparation to starch-gel electrophoresis, some 20 bands are seen, mostly minor components in the B-C area that are not concentrated enough to show in a whole sarcoplasmic protein sample, and also the fast-moving band A2.

The 'pH 5 protein' fraction described above was always prepared at an ionic strength of about 0.1, comparable with the value in muscle *in situ*. Very much more can be precipitated by lowering the pH at a very low ionic strength. In this case the protein includes 'Globulin X' (Weber & Meyer, 1933), which corresponds on the starch-gel electrophoretogram to several of the myogen proteins in the E-F area. At higher ionic strength, e.g. in 1.0 M-ammonium sulphate, both creatine kinase and metmyoglobin are precipitated and denatured readily at pH 5.0.

EXPLANATION OF PLATE 1

Plate 1. (a) Proteins of muscle sarcoplasm separated on starch gel by using the diethylenetriaminepenta-acetic acid discontinuous buffer system (A). (i) Partially purified ox creatine kinase; (ii) ox '0°-rigor' muscle; (iii) ox '37°-rigor' muscle; (iv) rabbit '0°-rigor' muscle; (v) rabbit '37°-rigor' muscle; (vi) pig '0°-rigor' muscle; (vii) pig '37°-rigor' muscle; (viii) partially purified pig creatine kinase. (b) Cationic proteins of muscle sarcoplasm, separated on starch gel by using tris-HCl buffer (buffer system B). (i) Ox '0°-rigor' muscle; (ii) ox '37°-rigor' muscle; (iii) rabbit '0°-rigor' muscle; (iv) rabbit '37°-rigor' muscle; (v) pig '0°-rigor' muscle; (vi) pig '37°-rigor' muscle.

(a)

(i) (ii) (iii) (iv) (v) (vi) (vii) (viii)

(b)

(i) (ii) (iii) (iv) (v) (vi)

The post-mortem pH fall can cause substantial changes to the muscle proteins if the temperature remains at its value (37°) *in vivo*. However, no significant quantitative change occurs to the proteins, either myofibrillar or sarcoplasmic, if the muscle is kept at 0° while the pH falls to its ultimate value. The temperature at which the transition between these two circumstances occurs depends largely on the actual pH values, but, from the results of Table 1, this would appear to be about 25° for pH 5.2 and nearer 30° for pH 5.7 as far as denaturation of sarcoplasmic protein is concerned. It is probable that the precautions taken in muscle biochemistry to prevent substantial pH falls are often unnecessary, provided that these critical conditions are not reached.

SUMMARY

1. Myofibrillar proteins from ox muscle are found to be most soluble in M-potassium chloride at pH 6.0. At low ionic strength the myofibrils disperse at pH values below 4.4 and above 10.1.

2. Sarcoplasmic proteins are denatured readily at pH values below 6.0 at 37°, and at higher temperatures more independently of pH.

3. Denaturation of sarcoplasmic protein *in situ* is associated with decreased myofibrillar solubility in M-potassium chloride.

4. Starch-gel electrophoresis indicates one major and several minor proteins that are specifically denatured in conditions of low pH and high temperature; the major one has been identified as ATP-creatine phosphotransferase.

This work was financed by the U.S. Department of Agriculture under the authority of Public Law 480. I thank Dr R. A. Lawrie and Mr J. R. Bendall for their suggestions and encouragement.

REFERENCES

- Bailey, K. (1937). *Biochem. J.* **31**, 1406.
 Bate-Smith, E. C. (1937). *Proc. Roy. Soc. B*, **124**, 136.
 Bate-Smith, E. C. & Bendall, J. R. (1947). *J. Physiol.* **106**, 177.
 Bendall, J. R. (1960). In *Structure and Function of Muscle*, vol. 3, p. 227. Ed. by Bourne, G. H. New York: Academic Press Inc.
 Bendall, J. R. (1961). *Biochem. J.* **81**, 520.
 Bendall, J. R. & Wismer-Pedersen, J. (1962). *J. Food Sci.* **27**, 144.
 Bowen, W. J. (1949). *J. biol. Chem.* **179**, 235.
 Connell, J. J. (1958). *Biochem. J.* **69**, 5.
 Deuticke, H. J. (1932). *Hoppe-Seyl. Z.* **210**, 97.
 Glazer, A. N. & Smith, E. L. (1961). *J. biol. Chem.* **236**, 2942.
 Hartshorne, D. J. & Perry, S. V. (1962). *Biochem. J.* **85**, 171.
 Hasselbach, W. & Schneider, G. (1951). *Biochem. Z.* **321**, 462.
 Helander, E. (1957). *Acta physiol. scand.* **41** (suppl.), 141.
 Hoagland, M. B., Keller, E. B. & Zamecnik, P. C. (1956). *J. biol. Chem.* **218**, 345.
 Keller, E. B. & Zamecnik, P. C. (1956). *J. biol. Chem.* **221**, 45.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Marsh, B. B. (1954). *J. Sci. Fd Agric.* **5**, 70.
 Noda, L., Kuby, S. & Lardy, H. (1955). In *Methods in Enzymology*, vol. 2, p. 605. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Perry, S. V. (1953). *Biochem. J.* **55**, 114.
 Perry, S. V. & Grey, T. C. (1956). *Biochem. J.* **64**, 184.
 Scoopes, R. K. (1963). *Nature, Lond.*, **197**, 1201.
 Scoopes, R. K. & Lawrie, R. A. (1963). *Nature, Lond.*, **197**, 1202.
 Smithies, O. (1959a). *Biochem. J.* **71**, 585.
 Smithies, O. (1959b). *Advanc. Protein Chem.* **14**, 65.
 Tombs, M. P., Souter, F. & MacLagen, N. F. (1959). *Biochem. J.* **73**, 167.
 Weber, H. H. & Meyer, K. (1933). *Biochem. Z.* **266**, 137.