The question to what degree the effects produced by the distortion of the microwave field in the cavity are significant requires separate treatment. It will be discussed elsewhere together with a more detailed description of the method. The results of preliminary tests, however, are encouraging and indicate that the 'magnet-rotation method', although comparatively simple, does not appear to be less exact than other techniques at present in use.

Riassunto. Si descrive un metodo che permette di misurare l'intensità di assorbimento ESR quando il campione standard e il campione su cui si esperimenta sono contemporamente presenti dentro la cavità risonante. Il campione standard viene situato in modo tale da rendere possibile l'eliminazione dell'assorbimento a cui esso da luogo, rotando il magnete attorno all'asse della cavità.

ST. LUKIEWICZ

Department of Experimental Zoology, Polish Academy of Sciences, Krakòw (Poland), March 11, 1963.

Bovine Muscle Proteins
IV. Preparation of Myosin and Actomyosin

For our early studies of bovine myosin[1], several classical procedures[2–4] for the preparation of rabbit myosin were adapted. However, because of the size of the animals used, and the slaughtering techniques involved, muscle samples generally are not available for extraction until as much as 1 h after slaughter. During this period the pH of the muscle decreases much more than occurs in the short period (5–15 min) before extraction of rabbit myosin is begun; this pH drop can be compensated for by extraction with solutions of higher initial pH. Any further delay results in greatly decreased yield, for myosin loses extractability as the onset of rigor approaches (2–4 h after slaughter for lean beef muscles).

For rabbit myosin, an extract containing the myosin from 1 kg of muscle must be diluted to 35 l in order to precipitate the protein by the usual procedure[2–4] of lowering the ionic strength. To precipitate bovine myosin, the ionic strength must be lowered more by diluting the extract to 70 l; even then the protein is partially soluble, and so the yield is poor. Further losses occur when adenosinetriphosphate (ATP) is used to precipitate actomyosin. Such preparations also are not always free of turbidity. Moreover, bovine preparations using the procedure of SZENT-GYÖRGYI[3] usually give a product which is unsatisfactory, showing polydispersibility in the ultracentrifuge.

For our recent studies on the properties of bovine myosin[5,6], we have developed a more satisfactory method of preparation, based in part on DUBUISSON's finding[7] that actomyosin is precipitated at a lower concentration of ammonium sulfate than is myosin.

We have found that ammonium sulfate solutions can be used to extract myosin and actomyosin directly from the muscle tissue. From the extract obtained, first actomyosin, and then myosin, can be precipitated selectively by increase in ammonium sulfate content. With this procedure, the maximum volume of liquid needed is about 6 l/kg of meat. Further, the yield of the final purified protein generally is between 15 and 20 g/kg for myosin.

Bovine myosin can then be prepared in water-clear solutions up to 3% concentration in 0.6 *M* KCl (pH 6.5). From analysis of sedimentation patterns such as that shown in the Figure, the intrinsic sedimentation coefficient ($s_{20,w}$) of bovine myosin has been calculated to be 5.78 Svedberg units. Both the customary ATPase[5] and 5'-adenylate deaminase activities are present in myosin and the derived meromyosin[6] products. These procedures have also been applied to the isolation of rabbit and lamb myosins with comparable yields in both cases.

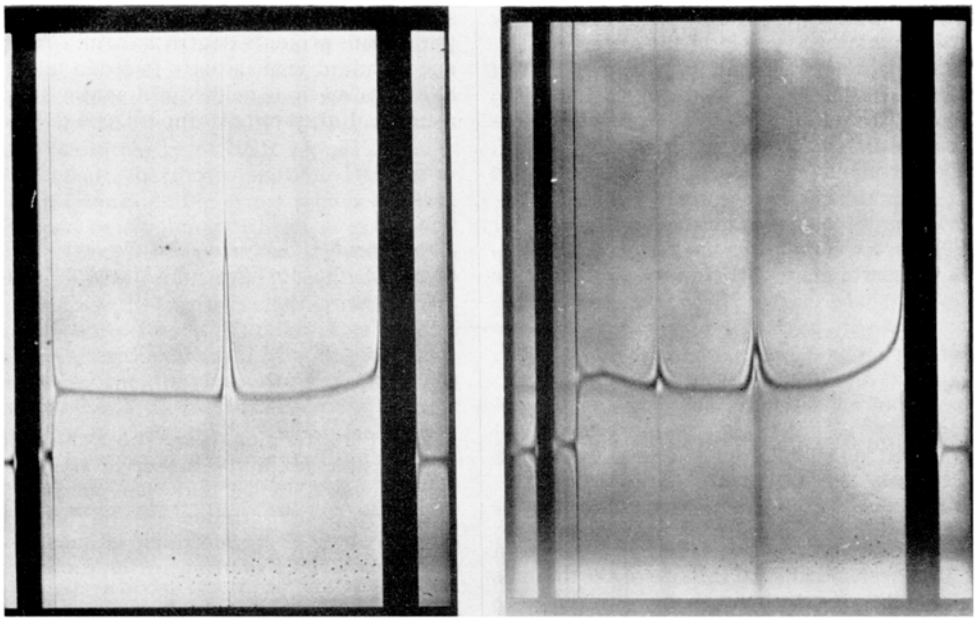
The procedure we have used is described below in detail as it has been applied to bovine muscle tissue obtained within 1 h after slaughter. The lean is separated quickly from visible fat and connective tissue, sliced into thin strips, chilled in ice and then ground in a pre-chilled Hamilton Beach[8] grinder.

Each 500 g of ground tissue is washed twice with 1 l of cold 0.05 *M* KCl solution; after each washing press out the liquid through cheese-cloth. Make a slurry of the meat residue with 1 l of cold 30%-saturated ammonium sulfate[9] solution (pre-adjusted with K_2HPO_4 to pH 6.8). After 10 min of gentle stirring, add another liter of the same solution, and squeeze quickly through cheesecloth. Adjust the pH between pH 6.5 and 7.0, and measure the volume of the extract. Because of the liquid in the original meat residue, the extract at this point contains about 25%-saturated ammonium sulfate. For each liter of extract, add 120 ml of 90%-saturated ammonium sulfate solution to raise the concentration to 32% of saturation. Using the GSA rotor with the Servall SS-3 centrifuge, separate out the actomyosin precipitate by centrifuging the mixture at 9000 × *g*, 10 min, at 4°C.

The myosin in the supernatant can be precipitated by carefully stirring in, for each liter of solution, 185 g of finely granulated ammonium sulfate, thereby bringing its concentration up to 60% of saturation. The preparation is again centrifuged as above; the sediment contains the myosin. For each 12 g (which is equivalent to 10 ml of 60%-saturated ammonium sulfate solution), add 10 ml of cold water, followed by an equal volume of cold 30%-saturated solution; adjust the pH between pH 6.5 and 7.0, if necessary.

Further purification is achieved by two or three reprecipitations, until the color of trace amounts of myoglobin is eliminated. After dissolving the myosin pellet with 5/6 vol of cold water, the solution at this point should

[1] R. J. GIBBS, A. J. FRYAR, C. LOCKETT, and C. E. SWIFT, Fed. Proc. *17*, 228 (1958).
[2] A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction* (New York 1951).
[3] A. G. SZENT-GYÖRGYI, J. biol. Chem. *192*, 361 (1951).
[4] V. KESSLER and S. S. SPICER, Biochim.biophys. Acta *8*, 474 (1952).
[5] W. L. SULZBACHER, R. J. GIBBS, C. E. SWIFT, and A. J. FRYAR, Proc. 12th Research Conference, American Meat Institute, 61 (1960).
[6] A. J. FRYAR and R. J. GIBBS, Arch. Biochem. Biophys. *88*, 177 (1960).
[7] M. DUBUISSON, Exper. *2*, 412 (1946).
[8] Trademarks are mentioned for identification, implying no endorsement.
[9] The concentrations of ammonium sulfate solutions are expressed in terms of its solubility in water at 25°C (S. P. COLOWICK and N. O. KAPLAN, *Methods in Enzymology* (New York 1955), vol. I, p. 76). The solutions were all stored and used at 4°C.



Ultracentrifuge patterns of bovine myosin (left) and actomyosin (right), in solutions at 0.6 *M* KCl, pH 6.8, 59,780 × *g*, 20°C. Myosin: 0.5%, 96 min, bar angle 70°; actomyosin: 0.3%, 16 min, bar angle 70°.

be almost completely clear. If not, it may be clarified by adjusting to 33% of saturation, and centrifuging at 30,000 × *g*.

To remove the ammonium sulfate, the supernatant (adjusted to pH 7.0) should be dialyzed at 4°C against 10 vol of 0.6*M* KCl. Change the dialysate every 3–4 h, until the ammonium sulfate has been removed. Since the pH of the myosin solution decreases steadily during dialysis, it may be necessary to open the tubing and adjust the pH during the dialysis. The contents of the tubes are then centrifuged at 30,000 × *g*, 15 min, to give the final bovine myosin preparation. If desired, a gelatinous precipitate of myosin could be obtained at this point by dialysis against water instead of the KCl solution. The purified protein (yield: 15–20 g/kg meat) is then available for further studies.

When an actomyosin preparation is desired, the extraction with 2 vol of 30%-saturated ammonium sulfate solution should be continued for 24 h. Immediately before separation through cheesecloth, 2 l of 20%-saturated ammonium sulfate should be added. The actomyosin can then be prepared by precipitation at 30–35% of saturation, followed by redissolving at 20% of saturation. After two reprecipitations, the ammonium sulfate can be removed by dialysis as above, giving a yield of 10–20 g/kg meat. An ultracentrifuge pattern of a typical preparation is shown in the Figure.

Zusammenfassung. Es wird eine Methode zur Extraktion und Reinigung von Myosin und Actomyosin aus Rindermuskelgewebe mittels Ammoniumsulfat beschrieben. Aus 1 kg Muskelfleisch wurden 15–20 g Myosin und 10–20 g Actomyosin gewonnen.

A. J. FRYAR and R. J. GIBBS

Meat Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, Beltsville (Maryland, U.S.A.), April 18, 1963.

EXPERIENTIA MAJORUM

100 Jahre tägliche Wetterkarte

Am 11. September 1863 wurde im «Bulletin international de l'Observatoire impérial» (Paris) die erste synoptische Wetterkarte veröffentlicht, die von MARIE-DAVY abgefasst worden war. Wir wollen dieses Jubiläum dazu benutzen, um einen Blick auf die Entwicklung zu werfen, die zu diesem für die Meteorologie so bedeutsamen Ereignis führte.

Seit jeher hat der Mensch dem Wetter ein starkes Interesse bekundet, denn hiervon hingen Gesundheit und Wohlstand ab. Es sei hier nur der vielen, teils jahrhundertealten Bauernregeln gedacht. Sie zeigen, dass der Mensch schon eh und je versuchte, sich eine gewisse Kenntnis über den Ablauf des Wetters zu verschaffen. Sobald ihm durch TORRICELLI ein Gerät zur Luftdruckmessung und durch den Grossherzog Ferdinand II. von Toskana ein Thermometer zur Temperaturmessung in die Hand gegeben war, ging er dazu über, erst mehr sporadisch, bald aber an mehreren Stellen systematisch, diese Wetterelemente zu messen und dazu die Bewölkungsmerkmale zu notieren.

So ist es kein Wunder, dass bereits seit vielen Jahren das Bestreben bestand, Vergleiche der Witterungserscheinungen über grössere Räume zu erzielen.

Zum ersten Male wurde diese Methode von BRANDES entwickelt. Er schrieb dazu am 1. Dezember 1816 aus