THE COAGULATION OF MYOSIN IN MUSCLE

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The coagulation of myosin is one of the few changes in the proteins of muscle known to take place during contraction and rigor. Although under certain conditions as much as one-third of the total protein of muscle may become insoluble, the significance of this change for the shortening of muscle is not understood. And yet the recognition of a definite transformation in the substance of muscle should be of value in investigating the mechanism of contraction, especially when one recalls that nearly all of those abortive theories of contraction that have been formulated since the time of Descartes have been based on knowledge of systems supposed to be analogous to muscle, rather than on a knowledge of the properties of living muscle itself. As a step towards an understanding of the chemical properties of the "living machinery" of muscle (as distinguished from the metabolic transformations in muscle) I have, accordingly, investigated the coagulation of myosin. I have already shown how the coagulation of myosin in muscle is related to the denaturation and coagulation of isolated myosin (Mirsky, 1935–36 and 1936–37). By measurements of protein sulfhydryl groups it was found that the coagulation of myosin in muscle differs from the coagulation of myosin and other proteins brought about by the usual denaturing agents (such as heat and acid) but resembles the coagulation of myosin caused by dehydration. At this point it is important to recall that when myosin is said to coagulate in muscle it is not supposed that myosin actually precipitates from solution. In muscle probably only a very small part of the myosin present is dissolved (Smith, 1934). That there is a change in myosin in muscle is inferred from the fact that at one time the protein can be dissolved in certain media in which at another time it

cannot be dissolved; the myosin is said to have coagulated. Apparently myosin in muscle can pass from one gel state to another. How these states differ will be considered in this paper.

Powdered Muscle.—The first need is to find a method by which coagulation can be investigated under conditions more subject to control than those obtaining in intact muscle. It was formerly supposed that a rapid and spontaneous coagulation of myosin occurs in the juice expressed from minced muscle (Kühne, 1864). Recently, however, Smith (1930) showed that the myosin precipitated under these conditions re-dissolves on adding salt and maintaining the solution faintly alkaline. When, however, myosin coagulates in muscle it cannot be dissolved in a faintly alkaline salt solution. Since coagulation resembling that which occurs in muscle does not spontaneously occur in juice expressed from muscle, I decided to begin with a preparation much closer to intact muscle. Muscle in the form of a dry powder was made in the following way; the hind legs of a frog were cooled to 0°; the muscles were then removed, finely minced, placed in a vacuum desiccator at about −8° and dried *in vacuo* while frozen; the dried material was ground to a powder in a mortar. This dry powder has some of the properties of live muscle. The myosin in it is still soluble, as in intact muscle, and, more important still, under certain conditions the myosin can be caused to suddenly coagulate. Furthermore, the myosin in this preparation is stable; it can be kept for several months, at least, without undergoing more than slight changes. It is therefore convenient to prepare large quantities of the material, a uniform powder being obtained from many different frog legs.

If a quantity of water equal to that previously removed is added to the dry muscle powder, the myosin in it instantly coagulates. Deuticke (1930) has observed that myosin loses its solubility after a normal contraction as well as after rigor. The speed with which coagulation occurs in my preparation indicates that probably a similar change takes place in it as in the contraction of intact muscle. Further evidence that the change in myosin which takes place on wetting the powder is substantially the same as that which occurs in contraction is provided by an observation made by Hürthle twenty-seven years ago (1909) in his classical paper on the minute anatomy of muscle.

Hürthle observed that in dried insect muscle the anisotropic layer is of the thickness characteristic of resting muscle, but that on wetting, this layer diminishes in thickness as it does during contraction. The properties of powdered muscle which I have described are sufficient to

TABLE I

Percentage of Soluble Protein in Various Preparations of Powdered Muscle

	Residual protein	Extracted protein	Percentage extracted
	gm.	*gm.*	
Preparation 3, dry powder extracted with KCl	0.30	0.67	69.0
Preparation 4, dry powder extracted with KCl	0.25	0.655	72.4
Preparation 6, dry powder extracted with KCl	0.252	0.597	70.3
Preparation 3, 5 cc. water added and the mixture allowed to stand at 20° for 30 min. Extract with KCl	0.47	0.37	44
Preparation 3, 5 cc. water added and the mixture allowed to stand at 20° for 30 min. Extract with 0.01 N HCl	0.45	0.402	47.3

TABLE II

Fractionation of Protein Extracted from Preparation 3 before and after Coagulation, Using Approximately 1 Gm. of Powder for Each Experiment

	Residual protein	Myosin	Myogen	Total extracted protein	Total protein	Protein extracted	Myosin	Myogen
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*
Before coagulation	0.237	0.305	0.163	0.468	0.705	66.4	43.2	23.1
After coagulation. Add 5 cc. water and stand at 20° for 30 min	0.482	0.096	0.203	0.299	0.781	38.3	12.3	26.0

show the usefulness of this preparation; its limitations will appear as it is used.

Wetting the powder causes an immediate change in myosin, but this change does not occur in the presence of much salt. The system remains stable, therefore, when wetted by a concentrated salt solution,

and on this depends the method of estimating the relative quantities of soluble and coagulated myosin. The procedure for estimating the quantity of soluble protein is to add a large volume of cold 1.2 M KCl, make the suspension slightly alkaline with a little K_2HPO_4 and stir in the cold for several hours. More protein is not extracted by more prolonged stirring. After centrifuging, the quantity of the protein in the supernatant is estimated. The soluble protein can also, of course, be fractionated and the several constituents estimated separately. In one experiment the quantity of soluble protein after coagulation was estimated by extraction with 0.01 N HCl,[1] the result being about the same as after extraction with KCl.

The fact that muscle can be dried without thereby coagulating the myosin in it is surprising if one recalls that isolated myosin is coagulated merely by drying (Mirsky, 1936–37). Apparently myosin in muscle differs in some way from isolated myosin, and the difference is important for an understanding of the coagulation of myosin in intact muscle. Fibres of dried isolated myosin have been made by Weber (1934) and after allowing them to swell in a physiological salt solution he considers them to be an exact model of the fine structure of the muscle fibre, or rather of its anisotropic band. The similarities between a muscle fibre and a myosin fibre observed by Weber are certainly noteworthy, but the difference discovered when dried, *live muscle* is compared with dried myosin is significant for this difference is not observed if *muscle* in *rigor* is dried and compared with dried myosin. The difference (which is detected when muscle is dried) between the states of myosin in living muscle and in a fibre made of isolated myosin is important because it is associated with the irritability of muscle.

Nature of Myosin Coagulation.—Various characteristics of the

[1] Dilute HCl was used because, according to Smith, it extracts more protein from minced resting muscle than does salt. Smith states that he has been unable to detect any coagulation of myosin as a consequence of rigor mortis. His results, which I have seen in abstract only (*Chem. Abstr.*, 1936, **30**, 496), contradict the results reported in this paper and elsewhere. He attributes the contradiction either to the strict maintainance of a low temperature during storage of the muscle or the absence of antiseptics in his experiments. In most of my experiments, however, the muscle was kept at 0° or below, and no antiseptics were used.

coagulation of myosin have been investigated, using preparations of powdered muscle.

1. The Kinetics of Coagulation.—This was studied by restoring to the muscle powder the water removed in drying and estimating at various intervals of time thereafter the quantities of soluble protein present. The speed of the process is striking; at 20° more than one-half of the myosin in muscle coagulates within a minute. At lower temperatures the change occurs more slowly. The temperature coefficient, approximately 2, is more characteristic of a chemical than

TABLE III

Coagulation Produced by Adding to 1 Gm. of Powdered Muscle 5 Cc. of Water, Allowing the Mixture to Stand for Various Lengths of Time and Subsequently Extracting with 1.2 M KCl

Time	Percentage of soluble protein			
	0°	10°	20°	0° adding 5 cc. of 0.02M $Na_2C_2O_4$ instead of water
Dry powder extracted immediately	70.0			
1 min.	70.0		52.8	
2½ min.	66.0	54.5	49.8	
5 min.	61.6	53.6	45.4	59.8
15 min.	56.1	51.0		
30 min.	54.3			54.9
60 min.	52.6			
120 min.	51.2		44.0	48.9
240 min.	47.4			
22 hrs.	44.8			

of a physical process. But when water is added to dry muscle many factors are involved, and it is unlikely that the resulting change can be described in terms of a single chemical reaction.

Another way of bringing about coagulation is to add the powder to a large volume of dilute saline. 1 gm. of powder may be stirred into 500 cc. of solution. If no salt is present coagulation does not occur under these conditions (the myosin remains in the muscle but it can be subsequently extracted) and if too much salt is present, myosin does not coagulate because it is extracted. Within these two limits there

is room for considerable variations in salt concentration. In 0.006 M NaCl not much coagulation occurs; in 0.02 M NaCl more occurs; and in 0.1 M about the same amount occurs. Other salts can be used. In a 0.02 M phosphate buffer practically all the myosin coagulates. Varying the pH from 6.15 to 7.35 made no apparent difference in the amount of coagulation. At 0° under these conditions the change is

TABLE IV

Myosin Coagulated by Adding 1 Gm. of Powdered Muscle to 500 Cc. of Dilute Salt Solution and Subsequently Extracting with 1.2 M KCl

	Time	First extraction	Extracted subsequently with 1.2 M KCl	Residual protein	Percentage of total protein extracted	Percentage of protein extracted by 1.2 M KCl (first protein extracted not reckoned in total)
	min.		*gm.*	*gm.*		
Water	60	0.247	0.282	0.296	64.2	48.8
0.006 M NaCl	5	0.133	0.325	0.341	57.3	48.8
0.02 M NaCl	5	0.135	0.263	0.414	49.0	38.9
0.02 M KCl	5	0.344		0.345	49.9	
0.1 M NaCl	5	0.322		0.305	51.4	
0.1 M KCl	5	0.343		0.339	50.4	
0.02 M $KHPO_4$ buffer pH 6.15	5	0.119	0.232	0.423	45.4	35.4
pH 6.15	30	0.142	0.238	0.438	46.5	35.4
pH 6.55	5	0.121	0.216	0.420	44.7	34.0
pH 6.55	30	0.160	0.190	0.430	44.9	30.7
pH 7.05	15	0.172	0.206	0.451	45.6	31.4
pH 7.35	5	0.141	0.207	0.431	44.7	32.4
First add 20 cc. of 1.5 M KCl and stand for 40 min. Then add 480 cc. H_2O. Final concentration 0.06 M KCl	5	0.215	0.302	0.234	68.9	56.5

practically complete in less than 5 minutes. This is much more rapid than when the powder at 0° is moistened with a quantity of water equal to that removed in drying.

2. *The Effect of Oxalate.*—This was investigated because the coagulation of myosin suggests comparison with the clotting of fibrinogen. In the formation of fibrin the rôle of calcium is concerned with the activation of thrombin. It has frequently been supposed that calcium

plays a similar rôle in the coagulation of myosin. Experiment shows, however, that myosin readily coagulates in the absence of free calcium. When a dilute oxalate solution is added to powdered muscle, coagulation occurs just as it does when water is added (see Table III).

3. Removal of Water-Soluble Constituents.—It is possible to thoroughly extract powdered muscle with water without thereby coagulating more than a fraction of the myosin. Although restoring to the powder the quantity of water removed in drying results in rapid coagulation of myosin, if the powder is stirred into a large volume of cold water, most of the myosin remains within the muscle undissolved but still soluble. When the residue, after extraction with water, is extracted with 1.2 M KCl more than half of the myosin dissolves. Extraction with water can be prolonged, lasting for several hours, and the extracted material can be kept for at least 8 hours without any further change taking place in the myosin than occurred when the powder was first stirred into water. Quantitatively the most important constituent of muscle extracted by water is the protein myogen, and all the myogen is extracted (along with a small amount of myosin). Myogen extracted in this manner constitutes 24 per cent of the total protein in muscle. If, using 1.2 M KCl, myogen is extracted along with myosin from powdered muscle, and if the two proteins are then separated by dialysis, the same quantity of myogen is obtained as by simple extraction with water (see Table II). If, after extraction with water, the muscle is suspended in a dilute phosphate solution for a moment, quickly centrifuged, and then extracted with 1.2 M KCl, myosin is found to be coagulated.

4. The Significance of Structure for Coagulation.—Myosin must be in its proper place in the structure of the muscle fibre if the rapid coagulation described above is to occur, as the following experiment shows. If to some muscle powder a small volume of relatively concentrated salt solution is added (to 1 gm. of powder 20 cc. of 1.5 M KCl) no coagulation occurs, for the salt is concentrated enough to dissolve myosin. The mixture can be kept at 0° for at least an hour without any change in the quantity of soluble myosin. The salt is now diluted by addition of a large volume of cold water (480 cc. so that the final concentration of KCl is 0.06 M). If muscle powder is added directly to this volume of such a dilute salt solution coagulation occurs

immediately. In the diluted suspension of muscle, however, myosin does not coagulate; nor is it dissolved in the dilute saline; it is simply precipitated and can be dissolved by adding salt. Once myosin has been removed from its place in the muscle fibre, it does not coagulate as it previously did even if it is mixed with the rest of the muscle from which it was displaced (see Table IV).

5. The Rôle of Water in Coagulation.—When muscle powder is treated with a volume of water, so large that no coagulation occurs,

TABLE V

1 gm. of powdered muscle extracted with 500 cc. of water and subsequently with 1.2 M KCl. In one experiment the water-extracted material was coagulated by freezing and in another experiment it was coagulated by mixing with a dilute phosphate buffer.

	Protein extracted with water	Protein extracted with KCl	Residual protein	Total protein extracted	Protein extracted with KCl (not reckoning water extracted protein)
	gm.	*gm.*	*gm.*	*per cent*	*per cent*
Experiment 1	0.247	0.282	0.296	64.2	48.8
Experiment 2	0.235	0.320	0.257	68.4	55.4
Experiment 3	0.240	0.288	0.330	61.7	46.6
Experiment 4	0.203	0.225	0.259	62.2	46.5
Freezing at −8° after extraction with water	0.340		0.295	53.6	
Mixing the water-extracted material with 0.02 M pH 6.55 phosphate buffer for 5 min. at 0°	0.203	0.174	0.312	54.8	35.8

the muscle swells to a considerable volume. When a little salt solution is added coagulation occurs and there is at the same time a striking shrinkage in volume. 1 gm. of muscle powder swells to about 25 cc. and then shrinks to about 7 cc. The correlation between loss of water and coagulation of myosin may be explained by supposing that as long as the myosin particle is enveloped by a shell of water as in swollen muscle it can be dissolved, but when that shell is removed the groups of one particle unite with those of neighboring particles to form an insoluble coagulum. It has already been shown that simply dehydrating isolated myosin by drying or freezing causes it to become

insoluble. On the other hand, myosin in intact muscle is not coagulated by freezing and drying. Experiments on swollen muscle show that when this is frozen at $-8°$, the myosin in it is coagulated (see Table V). In this respect, then, myosin in swollen muscle behaves like isolated myosin and unlike the myosin of intact muscle. But myosin in swollen muscle can be coagulated simply by adding a little neutral salt, and this extreme instability recalls the myosin of intact muscle rather than isolated myosin.

DISCUSSION

On the basis of the new information presented in this paper and of other knowledge of myosin in muscle, it is perhaps possible to conceive of how the particles of myosin are arranged in muscle and what change occurs during coagulation.

The coagulation of myosin in muscle bears a certain resemblance to coagulation of myosin caused by dehydration. In neither is there that change in sulfhydryl groups characteristic of coagulation due to the familiar denaturing agents, such as heat, acid, urea, etc. In neither is the coagulum soluble in dilute HCl, in which, however, myosin denatured by acid readily dissolves. And in swollen muscle prepared by extracting powdered muscle with a large amount of water, in which myosin appears to be still placed somewhat as it is in intact muscle, myosin coagulates when dehydrated. When myosin coagulates due to dehydration the profound change in internal configuration caused by the usual denaturing agents does not occur. The groups of one myosin particle combine with those of its neighbors to form a coagulum without causing more than a relatively slight alteration in internal configuration.

The way in which myosin is imbedded in the structure of muscle is important for coagulation. If myosin is dislodged from this position (as it is by addition of concentrated salt solution), that rapid coagulation characteristic of muscle no longer occurs. The significance of structure is also indicated by the fact that myosin in intact muscle does not coagulate on dehydration.

A fairly satisfactory picture of the arrangement of myosin particles in muscle can be formed. One of the layers of cross-striated muscle has long been known to be doubly refractive. On the basis of Wiener's

theory and the conceptions of Ambronn and Frey (1926), Stübel (1923) has shown that the anisotropic layer contains a multitude of minute rod-shaped particles (small compared with the wave-length of light) oriented with their long axes parallel to the axis of the muscle fibre. The anisotropic properties of myosin discovered by von Muralt and Edsall (1930) and by Weber (1934), make it probable that rod-shaped particles in muscle consist, in part at least, of myosin. The linear arrangement of myosin particles in muscle is also indicated by the observations of Rubner (1922) and Hürthle (1931) on the changes in dimensions of muscle on drying. When a muscle, such as the frog's sartorius, is dried there is a pronounced shrinkage in volume but its length diminishes only slightly, the great decrease occurring transversely to the axis of the fibres. The great mass of water in muscle appears to be located between longitudinally arranged strips of protein.

If this picture of the fine structure of the anisotropic layer is accepted and if coagulation in muscle resembles the coagulation of myosin caused by dehydration, then it may be supposed that when myosin coagulates in muscle, the myosin particles join to each other end to end. In resting muscle the rod-shaped myosin particles are loosely arranged in a line end to end, and when coagulation occurs they string together to form a thread of myosin. This can be accomplished with speed because although there is much water separating the particles laterally, the ends of the particles are close to each other. If the particles are dislodged from their longitudinal arrangement, they are enveloped on all sides by water so that rapid coagulation can no longer occur.

It is now necessary to consider why the myosin in intact muscle can be dried without coagulating. Possibly this myosin has a different configuration from myosin prepared by the present methods, however carefully they are carried out. Gorter (1936) has recently shown how the properties of myosin are markedly changed by a slight enzymatic hydrolysis. Some such change, it may be imagined, takes place during the extraction of myosin, and even in the moment when powdered myosin is merely moistened with water; and this modified myosin then coagulates. Another explanation would postulate a change not in the configuration of myosin but in the substance lying

between the ends of myosin particles. In intact muscle and in frozen and dried muscle the substance in this region prevents the ends of the particles from joining, but when powdered muscle is moistened this substance is changed, so that it no longer acts as a barrier between the ends of myosin particles. This substance must be in the form of a thin film, and the freezing of muscle, preceding drying, would probably change its structure so that subsequent wetting might destroy it. Whatever the explanation of the stability of soluble myosin in muscle may be, it seems likely that a similar change occurs both in intact muscle during contraction and in muscle powder on wetting, a change which causes myosin to coagulate.

EXPERIMENTAL

1. Preparation of Muscle in the Form of a Dry Powder.—Muscles of the hind legs of frogs, *Rana catesbiana*, were used. The hind legs were severed by one cut with a heavy cleaver just above the pelvis, and kept at 0° for 5 hours. The muscles were then rapidly dissected and finely minced in a cold room at 0°. The minced muscle, spread in a thin layer on a large watch glass, was frozen at −8°, and the rest of the procedure was carried out at this temperature. The frozen muscle was placed in a vacuum desiccator, containing anhydrous calcium chloride, which was evacuated by a pump which ran continuously. After 24 hours the muscle was in the form of a fairly dry solid cake. This was removed from the watch glass, broken up, and then replaced in the desiccator. At the end of another 24 hour period the muscle was as dry as it can be made by this procedure. It was ground to a fine powder in a mortar and stored at −8°. This powder lost 3.6 per cent of its weight when dried in an oven at 110°.

The effect of the various steps in the procedure after mincing on the quantity of extractable protein was determined. The myosin in minced muscle is quite stable; the quantity of protein extracted from muscle immediately after mincing is the same as after the minced muscle has stood at 0° for 2 hours. Freezing of minced muscle causes a slight change; a few more per cent protein were extracted after freezing, probably because the frozen tissue was more completely disintegrated. Drying had no further effect. In eleven different preparations, all, however, made at the same time of the year,—in March, April, and May, the quantities of soluble protein varied from 69.0 to 72.8 per cent of the total protein. In the course of 3 months (from May 25, to September 4) the quantity of soluble protein in one preparation dropped from 70.0 to 68 per cent. How the other properties of powdered muscle change with time is not known, since most of the experiments described in this paper were performed on a large single preparation over a period of 3 months, and there was no opportunity at that time of making a fresh preparation. It is possible, therefore, that some of the properties of powdered muscle described

in this paper are due to the fact that the powder had been stored for some time, even though storage was at a low temperature.

It was at first thought that a preparation more like that of intact resting muscle would be made if the tissue were frozen before mincing and then minced while frozen. Muscles were accordingly frozen at $-8°$ and then finely minced without thawing. The minced frozen tissue was added to the extraction fluid which was rapidly stirred so that only a short time intervened between thawing of a particle and penetration of it by concentrated saline. Only 59 to 60 per cent of the total protein dissolved. It is well known that freezing a muscle serves as a stimulus to contraction; apparently the effect of freezing is much greater on intact than on minced muscle.

Extraction.—1 gm. of powder was extracted at 0° with 240 cc. of 1.2 M KCl and 4 cc. of 1 M K_2HPO_4 in a 250 cc. centrifuge flask. This solvent was used because Howe found that of the various salts he tried, potassium salts of this concentration were the most effective extractants. Extraction was aided by continual stirring and lasted 2 hours. No more protein was extracted in 6 hours. The pH of the mixture was about 8.0, just red to cresol red. After extraction under these conditions, stirring the residue for an hour in a 0.25 M pH 9.4 borate buffer failed to extract any more protein. The extracted and residual material were separated by centrifuging at high speed, a residue of less than 10 cc. and a perfectly clear supernatant being obtained.

Estimation of Protein.—The extracted protein was precipitated by adding 20 cc. of a 50 per cent trichloracetic acid solution (trichloracetic acid dissolved in an equal weight of water). The suspension was centrifuged and the protein precipitate was freed of salt by washing twice, each time with 250 cc. of a 5 per cent trichloracetic acid solution. The protein was dehydrated by stirring with 250 cc. acetone and the suspension was centrifuged after adding to it 1 cc. of concentrated HCl. Most of the acetone adhering to the precipitate was removed by suction, and lipoids remaining with the protein were extracted with 250 cc. of a mixture containing two parts of alcohol and one of ether. The precipitate was transferred to a tared crystallizing dish and dried to constant weight at 110°. Since it was found that the relative quantities of extracted and residual protein were the same if the protein were dried after washing with acetone, in most experiments the treatment with alcohol-ether was omitted. The proteins in the residue after extraction were washed in the same way and then dried and weighed.

In several experiments extracted protein was fractionated by dialysis. The supernatant fluid, after centrifuging, was poured into a long cellophane tube, 1 inch in diameter, which was placed in a rocking dialyzer. Inside the tube was a marble ball which rolled from one end to the other and thus stirred the contents. The extract was dialyzed in the cold against a continually flowing 0.01 M pH 6.6 phosphate buffer, for 16 hours. The contents of the cellophane tube, now partly precipitated, were centrifuged. The precipitate, which readily dissolved in 1.2 M KCl at pH 8 was considered to be myosin. The supernatant, which remained clear on more prolonged dialysis, was considered to be myogen. A search was made for globulin X, a protein in mammalian muscle described by Weber as being

insoluble in absence of salt but requiring for solution much less salt than does myosin. None, however, was found.

Coagulation.—To 1 gm. of powdered muscle was added 5 cc. of water. The powder was readily wetted by the water, and by mixing with a glass rod, the muscle was in a few moments of the consistency of a dough. In experiments at definite temperatures both powder and muscle were cooled, the powder being kept in a stoppered tube to prevent condensation of moisture on it. At any given time coagulation was interrupted by adding 235 cc. of cold 1.2 M KCl.

In experiments on the effect of oxalate, to 1 gm. of powder was added 5 cc. of 0.02 M sodium oxalate at 0°. The calcium content of frog muscle is less than 0.0025 M.

When muscle was stirred into 500 cc. of a dilute salt solution, the solution was at 0°. The mixture was stirred for 2 minutes and then centrifuged, the total time taken from adding the powder to pouring off the supernatant fluid being 5 minutes. The residue was extracted with 1.2 M KCl in the usual manner. The protein in each of the two extracts (in dilute saline and 1.2 M KCl) was precipitated and estimated separately.

Extraction with Water.—1 gm. of powder was stirred into 500 cc. of water at 0°, and the stirring was continued for an hour. After centrifuging, the residue was observed to be greatly swollen, volume 25 to 35 cc. By stirring with another 500 cc. of water, no more protein was extracted. In some experiments the residue was extracted with 1.2 M KCl, in another it was frozen at −8° for 12 hours, and then extracted with 1.2 M KCl. And in others it was suspended in 225 cc. of a 0.02 M pH 6.55 phosphate buffer, stirred for 5 minutes so that the myosin present coagulated, centrifuged, and the residue extracted with 1.2 M KCl, all the extracted protein being precipitated and estimated.

An attempt was made to measure the speed with which coagulation occurs when dilute phosphate is added by suspending the washed muscle in 225 cc. of water, adding a little phosphate, and then immediately thereafter sufficient concentrated KCl to make the final concentration of KCl 1.2 M, and so extract soluble protein. It was found that, even without adding dilute phosphate, addition of concentrated KCl to the suspended muscle caused coagulation. Coagulation also occurred if the aqueous suspension of washed muscle was poured into concentrated KCl solution. In the experiments described above, for extraction 225 cc. of 1.2 M KCl is added to water-extracted muscle compressed to 25 cc. by centrifuging, and under these conditions much less coagulation occurs. This curious situation requires further investigation.

The quantities of myosin and myogen extracted from muscle with water were estimated after dialysis, as described above.

SUMMARY

1. Muscle can be prepared in the form of a dry powder in which myosin exists in a state similar to that in intact muscle. As in intact

muscle, myosin in powdered muscle is soluble and can be caused to rapidly coagulate.

2. Restoring to powdered muscle the quantity of water previously removed causes coagulation of myosin. The rate of coagulation is considerably slower at 0° than at 20°.

3. Adding the powder to a large volume of dilute salt solution also results in coagulation.

4. The water soluble constituents of muscle can be removed from the powder without thereby causing coagulation. Coagulation occurs in water extracted muscle when it is suspended in a dilute salt solution.

5. Coagulation of myosin in muscle resembles the coagulation of myosin caused by dehydration.

6. Myosin coagulates readily only when it is imbedded in the structure of muscle. The significance for coagulation of the arrangement of myosin particles in muscle has been indicated.

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