ON MUSCLE-PLASMA[1]. By W. D. HALLIBURTON, M.D., B.Sc., *Assistant Professor of Physiology, University College, London.*

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Our knowledge of the properties of muscle-plasma depends almost entirely on Kühne's[2] researches on the muscle-plasma of frogs. In this investigation I have endeavoured to extend his observations to the warm-blooded animals, and to discover if possible the precursors of myosin in the muscle-plasma, as well as to ascertain more accurately the cause of the formation of myosin. Incidentally the research has included an investigation of the proteids of muscle-plasma, and of muscle-serum.

The subject may conveniently be discussed under the following heads:—

I. The influence of cold on the coagulation of the muscle-plasma of warm-blooded animals.

II. The influence of neutral salts on the coagulation of muscle-plasma.

III. The properties of saline extracts of muscles which have undergone *rigor mortis*.

IV. The properties of the muscle-clot.

V. The development of acid during coagulation.

VI. The preparation and properties of myosin-ferment.

VII. The proteids of muscle-plasma and of muscle-serum.

I. The Influence of Cold on the Coagulation of Muscle-Plasma of Warm-blooded Animals.

Kühne found that the muscle-plasma which he obtained from the frog could be prevented from coagulating by a temperature below 0° C.; about this temperature it clots slowly, and at a temperature of 40° C.

[1] The expenses in connection with this research have been defrayed out of a grant from the Scientific Grants Committee of the British Medical Association.

[2] Kühne, *Protoplasma*, Leipsig, 1864, and *Lehrbuch der physiologische Chemie*.

almost instantaneously; the muscle-plasma being a liquid of syrupy consistency, of a faintly alkaline reaction, and separating at a suitable temperature into a solid clot composed of the proteid substance called myosin, and a liquid residue which is squeezed out by the contraction of the clot and which has received the name of muscle-serum. Kühne was not able to employ the muscles of warm-blooded animals, as he found that they do not preserve their vitality sufficiently long to permit of the plasma being removed before coagulation occurred.

The first point therefore which it was necessary to settle before proceeding to the further questions involved in an investigation of the properties of mammalian muscle, was whether the facts above mentioned as true for the muscle-plasma of the frog are also true for the muscle-plasma of warm-blooded animals. These experiments together with several of the earlier ones which will be detailed under the next heading were performed in conjunction with Professor Schäfer.

Several unsuccessful attempts were made to obtain muscle-plasma from the rabbit, but it is unnecessary to enter into a description of these; the causes of failure will be mentioned in the following description of the method which was found to be wholly successful:—The animal was killed by bleeding from the carotids; the abdomen was quickly opened, and a cannula inserted into the abdominal aorta; the blood was then washed out from the lower limbs by means of a stream of cold salt solution (0·6 sodium chloride solution); it was found unadvisable to cool the salt solution to 0° C. as so cold a fluid caused such powerful contraction of the small vessels as to hinder the free flow of the fluid. It was found that a temperature of 5° C. was sufficiently low, and this was produced in a very simple way by putting lumps of ice into the salt solution; as they melted some dilution of the solution was produced, but this was immaterial. When the lower limbs were well swollen with the saline solution the *vena cava inferior* was opened, and the mixture of blood and salt solution allowed to flow out; in about five minutes the fluid came through perfectly clear. The lower limbs were then quickly skinned, and pieces of the muscles which were still excitable were cut off and plunged into a freezing mixture of ice and salt which had a temperature of about $-12°$ C. These pieces if small become almost instantly solid hard lumps: the outside of each lump is necessarily frozen first, this forms a protective case to the internal part of the piece of muscle and effectively prevents any but the smallest admixture of the strong saline solution with the muscular substance. It is necessary to put the muscles in this way directly into the freezing

mixture; our ill success in the earlier experiments was due to the fact that the lower limbs still covered by fur were immersed bodily in the mixture; for the fur being a bad conductor of heat, the muscles underwent *rigor mortis* and lost their irritability before the cold had time to take effect on them. The pieces of frozen muscle were removed from the freezing mixture, wiped dry quickly with blotting-paper, placed on a plate kept cold by ice and salt mixture; they still remained perfectly hard; they were then finely sliced; care being taken to cut as much as possible across the direction of the muscular fibres. The frozen slices were then wrapped in linen, and subjected to pressure. Some difficulty was experienced in finding a suitable press, but ultimately, the expedient of using a lemon squeezer of enamelled iron was hit upon. The instruments must all be cooled by immersing them before use in the freezing mixture, and an advantage which the iron lemon squeezer has in addition to its strength, over the ordinary wooden instrument, is the facility with which it may thus be cooled.

The muscle-plasma which is obtained in this manner can be collected in a test tube, or in watch-glasses. It is a yellowish, somewhat viscid fluid of a faintly alkaline reaction. Allowed to remain at the temperature of the air in winter time it is found to set into a solid jelly in the course of from one to two hours: at the temperature of 40° C. coagulation takes considerably less time, viz.—twenty to thirty minutes; simultaneously an acid reaction is developed. The naked eye phenomena of coagulation are the same in both cases; it begins by a thin film forming over the surface exposed to the atmosphere, and a similar film in those parts in contact with the glass; the process of clotting then spreads throughout the liquid until ultimately a firm translucent clot is obtained; in the course of some hours this contracts to a slight extent, and squeezes out a few drops of serum. That this clot is composed of myosin and not of fibrin formed from the lymph which might be mixed with the muscle-plasma can be shown by the fact that it does not swell in 0·2 per cent. hydrochloric acid, but immediately, rapidly, and completely dissolves both in this reagent and in a 10 per cent. solution of sodium chloride.

As a control experiment, the attempt was made to obtain a similar coagulable fluid from muscles which had undergone *rigor mortis*. The pieces of finely divided muscle were allowed to thaw, and in about half an hour's time were subjected as before to the pressure of the lemon squeezer; another specimen was not frozen, but allowed to undergo *rigor mortis*, and some twelve hours after death was chopped up finely

and squeezed; but in neither case did the expressed fluid undergo clotting, either at the temperature of the air or in the incubator at the temperature of 40° C. In both cases the expressed fluid was acid. In one experiment similarly performed to that just described an apparent exception to this statement occurred. Twelve hours after death, the muscles of the lower limbs of a rabbit freed from blood by a stream of salt solution injected by the abdominal aorta, were chopped up finely and wrapped in linen: this was squeezed and a strongly acid fluid expressed. This did not undergo clotting at the temperature of the atmosphere, but when kept in the incubator at 40° C. and examined half-an-hour afterwards it was found to have become thick and opaque. This was produced by a fine flocculent precipitate which pervaded the liquid, which was removable by filtration, and which resembled in appearance a heat coagulum much more than a clot of myosin formed spontaneously. That it was a heat coagulum, was proved by the following experiments; (1) The precipitate was collected on a filter and washed with water, and was found to be insoluble except in strong mineral acids. (2) This precipitation began immediately the temperature of the fluid reached 40° C., and gradually increased in amount. (3) If the temperature of the incubator was lowered to 35° C. this precipitation did not occur; while the coagulation of muscle-plasma occurs as readily or almost as readily at 35° as at 40° C. In connection with this explanation the question will naturally arise, why should a heat coagulum have occurred at so low a temperature as 40° C.? This is however to be accounted for by the acidity of the liquid, which was extreme as compared with that usually met with in fluids obtained from coagulated muscle, and the reddening of litmus paper of a decidedly blue shade was very marked. As will be seen later on, the lowest point at which any proteid occurring in muscle coagulates is 47° C., but the acidity in this case was presumably sufficiently great to account for the lowering by 7° of the temperature of coagulation.

The general conclusions that can be drawn from the foregoing experiments are as follows:—

1. That with a few slight modifications the facts previously discovered by Kühne in relation to the preparation of muscle-plasma from frog's muscle, are true also with regard to mammalian muscle.

2. That the preparation of muscle-plasma from mammalian muscle is by no means so difficult a process as has hitherto been supposed. Indeed, by following the simple method we have described, it is an experiment which can easily be demonstrated to a class.

3. That the coagulation of mammalian muscle-plasma, after it has been subjected to a low temperature, is a process of longer duration than that described by Kühne in the case of frog's muscle-plasma.

II. The Influence of Neutral Salts on the Coagulation of Muscle-Plasma.

The separation of muscle-plasma into a clot and muscle-serum is very similar to what occurs in the blood-plasma after its removal from the body, and the resemblance is borne out by the fact that a low temperature prevents or hinders the separation into clot and serum in both cases. In the case of blood-plasma cold is not the only agent which prevents the occurrence of coagulation, but the same result is brought about by mixing with the plasma certain proportions of neutral salts, such as magnesium sulphate, sodium chloride, or sodium sulphate. An important point then to determine is, whether the analogy between muscle-plasma and blood-plasma holds in this particular also; that is, whether it is possible to prevent the coagulation of muscle-plasma by mixing it with solutions of such salts as those just enumerated.

In order to elucidate this point, the following method of procedure was adopted. A rabbit was killed by bleeding, a cannula inserted into the abdominal aorta, and the blood washed out from the lower limbs by a stream of cold salt solution in the manner already described. The lower limbs were then quickly skinned; the muscles of one limb (*A*) were placed in the freezing mixture, the muscles of the other limb (*B*) not. When frozen the muscles of the limb *A*, were cut into fine slices, by means of a cooled knife[1]; the pieces being kept frozen during this proceeding by placing them on a cooled plate. The finely divided frozen muscle was then divided into three parts; one portion was put into 10 % sodium chloride solution, the second into 5 % magnesium sulphate solution, and the third into a half-saturated solution of sodium sulphate; in all cases these fluids were kept at the temperature of 0° C. or a few degrees below. By means of a cooled pestle and mortar, the pieces of muscle were thoroughly crushed, and mixed with the fluids in question; it was found that a very considerable amount of proteid went into solution; the fluids were then rapidly filtered, by using a large number of small filters which were also kept cold as far as possible by means of cooled glass funnels. The filtrates were in all cases alkaline in reaction,

[1] In some cases a freezing microtome was employed.

and had a temperature of 2°—3° C. They were kept at this temperature for thirty to forty minutes, and no coagulation occurred in the fluids, which we may speak of as salted muscle-plasma: they were then allowed to reach the temperature of the air (15° C.), and still no coagulation occurred.

The muscles of the second limb (*B*) were then taken about an hour after the death of the animal, they were chopped up finely, divided into three parts, which were pounded up in a mortar with the three saline solutions employed in making an extract of the limb *A*: namely a 10 per cent. sodium chloride solution, a 5 per cent. magnesium sulphate solution, and a half saturated solution of sodium sulphate. A large amount of proteid material went into solution; and the extracts of the dead muscle were in all cases acid, and underwent no spontaneous coagulation when exposed to the atmospheric temperature. So far then it would seem judging from the foregoing experiment which is described in detail as a sample of three similar experiments performed with the same result, that, granting the extracts obtained from the frozen muscle were really specimens of salted muscle-plasma, the salt solutions employed did prevent its coagulation; that this was the case was emphasized by the alkaline reaction of the extracts. But such an experiment as that just described cannot be regarded as conclusive, for it might be said that the fluidity of the saline solution is no proof that coagulation has been prevented, for the coagulum is composed of the proteid substance myosin which is itself soluble in the solutions used to prevent coagulation.

This would be a perfectly just criticism; my chief reason however for regarding it as probable that I had to deal with a muscle-plasma in which coagulation had been prevented, and not with a mere re-solution of myosin, was the reaction of the extracts. Subsequently, when coagulation was made to occur in them by diluting the saline liquid with water, the reaction became acid; and a conclusive experiment which confirmed the accuracy of this view was as follows:—a rabbit was killed as before, the blood removed from the lower limbs, and the muscles from those limbs frozen in the way already described; the muscles still frozen were finely minced, and squeezed in a cold lemon squeezer to extract the plasma; the plasma so squeezed out was collected in three vessels; in the first it was not mixed with anything, but in the course of half-an-hour underwent coagulation in the way already described; in the second, it was mixed with three or four times its volume of ten per cent. sodium chloride solution; in the third, it was mixed with three or four times its volume of

five per cent. magnesium sulphate solution. These two latter mixtures kept either at the temperature of the air or in an incubator of the temperature of 37° C. did not undergo coagulation. The sodium chloride plasma was alkaline in reaction, the magnesium sulphate plasma which was collected a few minutes later was neutral. Moreover these specimens of salted plasma were found to contain the same proteids by methods shortly to be described as the extracts obtained by pounding the cooled muscle with the salt solutions, and in fact exhibited precisely similar reactions, of which the most important was the fact immediately to be fully entered upon, that on dilution with water, coagulation occurred and an acid reaction was developed[1].

From this experiment I felt fully justified in concluding, that the extracts of cooled muscle were really specimens of salted plasma, and that in them, as in the case where the plasma itself was used, the salts employed had prevented coagulation. The former method, that of extracting frozen muscle with the salt solution, was the one employed in most experiments, particulars of which will follow; it was the less troublesome process of the two, and what was more important gave a larger yield of salted muscle-plasma.

The solutions employed for extracting the plasma from the frozen muscle, are those that have already been mentioned: a ten per cent. sodium chloride solution, or a five per cent. magnesium sulphate solution, or a half-saturated solution of sodium sulphate. A number of trials with solutions of these same salts of different strengths showed that the above mentioned are the best to use, since they extract a larger quantity of proteid than either weaker or stronger solutions. Saturated or strong solutions of sodium chloride, or magnesium sulphate, extract very little proteid, for, as will be shown later on, many of the proteids of muscle-plasma are precipitated by saturation with these salts: saturated solution of sodium sulphate has not this disadvantage to such an extent.

Solutions of the salts weaker than those mentioned do not with certainty prevent the formation of myosin, as will be seen by the dilution experiments next to be described. I have not employed ammonium chloride solutions in any of my experiments, although it has been largely used by others for dissolving myosin, for the reason, that in subsequent saturation experiments with magnesium sulphate or sodium chloride, the presence of a second salt would have complicated the reaction.

[1] The sodium chloride plasma mentioned above was in this case an exception; after clotting had occurred the reaction still remained very faintly alkaline, though whether it was less alkaline than previously I did not have sufficient material to investigate.

Following up the analogy between the coagulation of blood-plasma and muscle-plasma, the next point to investigate was one to which allusion has already been made; namely, whether dilution of the salted plasma with water produces coagulation in one case as in the other. Dilution of salted blood-plasma with water is supposed to produce coagulation by removing the inhibitory influence that a more concentrated salt solution has upon the formation of fibrin. Dilution of salted muscle-plasma with water does cause the formation of a clot of myosin, and acts presumably in a similar way by removing the inhibitory influence that a more concentrated salt solution has upon the formation of myosin.

The following are the notes of the observations made on the results of dilution of the three extracts described as having been made from the frozen muscle.

Extract 1; with 10 per cent. sodium chloride solution. Reaction alkaline; fluid slightly opalescent.

Time of observation	Amount of dilution	Result when allowed to stand at the temperature of the air (15° C.)	Result when placed in the warm chamber (temperature 37° C.)
10 a.m.	*a*. Diluted with an equal amount of water		
10 a.m. next day		No change	Slight increase of opalescence
10 a.m.	*b*. Diluted with three times its volume of water	Slight increase of opalescence	Slight increase of opalescence
10.30 a.m.		No change	Clot beginning to form
12.15 p.m.		No change	Clot extends throughout the liquid
12.30 p.m.		No change	Clot beginning to contract
2 p.m.		No change	Clot contracted and floats at the top a colourless clear fluid
10 a.m. next day		No change	No further change

Extract 2; with half-saturated solution of sodium sulphate. Solution opalescent, and reaction alkaline.

Time of observation	Amount of dilution	Kept at the temperature of the air (15° C.)	Placed in the warm chamber (temperature 37°C.)
10 a.m.	*a.* Diluted with an equal amount of water		
10 a.m. next day		No change	Small clot
10 a.m.	*b.* Diluted with three times its volume of water	Increase of opalescence	Increase of opalescence
10.30 a.m.		No change	Clot beginning to form
10 a.m. next day		No change	Abundant clot

Extract 3; with 5 per cent. magnesium sulphate solution. Solution opalescent; reaction neutral.

Time of observation	Amount of dilution	Kept at the temperature of the air (15° C.)	Placed in the warm chamber (temperature 37° C.)
10 a.m.	*a.* Diluted with an equal amount of water	No change	No change
5 p.m.		No change	Clotting commenced
10 a.m. next day		Small clot	Abundant clot
10 a.m.	*b.* Diluted with three times its volume of water	Increase of opalescence	Increase of opalescence
10.30 a.m.		No change	Clotting commenced
12.15 p.m.		No change	Clot contracting
4 p.m.		Clotting commenced	Clot contracted
10 a.m. next day		Abundant clot which had contracted	Abundant clot which had contracted

The clotting which is described as occurring at various times in the preceding tables was in appearance very like that which occurs when blood-plasma is similarly diluted; there is at first a jellying throughout the liquid; this very rapidly contracts, and floats in a clear fluid. The consistency of the clot is however not nearly so great as that of fibrin, and the jelly on shaking breaks up into fragments. In some instances, though not in the experiment just described, but when a very concentrated muscle-plasma was used, the jelly which formed was sufficiently firm to allow of the vessel in which it was contained, to be turned upside down without the contents being spilled. In all cases also the occurrence of the clot was accompanied by the change of reaction of the fluid from alkaline to acid. On redissolving the clot, it was found by its reactions to be in all cases myosin.

One point which is very important in the determination of the clotting is the temperature; the temperature of 37° C. is seen to be much more effectual in causing the coagulation of the myosin than the temperature of 15°, that of the atmosphere. The influence of temperature is more strikingly seen in the following experiment; specimens of salted muscle-plasma were obtained from a rabbit in the way described; it underwent coagulation more rapidly than in the experiment already detailed, and so comparative observations were more easily made. A preparation made with 10 per cent. sodium chloride was diluted with four times its bulk of water and divided into five portions, *a*, *b*, *c*, *d*, *e*.

a. Placed in a freezing mixture at a temperature of − 10° C. Thawed after three hours; no clot, clotting commenced 50—60 minutes after the temperature of the air 13° C. was reached.

b. Kept at the temperature of melting ice 0° C. No coagulation after four hours.

c. Kept at the temperature of 2° C. After 70 minutes a faint coagulation began to appear throughout the liquid.

d. Kept at the temperature of the air 13° C.: coagulation began in 25 minutes.

e. Kept at the temperature of 35° C.: coagulation began in 10 minutes.

With a specimen prepared by means of a five per cent. solution of magnesium sulphate, and then similarly treated, similar results were obtained.

a. b. c. corresponded exactly to *a*, *b*, and *c* above.

d. Coagulation began in 20 minutes.

e. Coagulation began in 8 minutes.

The specimens of salted muscle-plasma obtained by mixing the squeezed out plasma with salt solutions underwent precisely similar coagulation when diluted with water; at the temperature of the air the clot was very slowly formed (12—24 hours); at the temperature of 37° C. it appeared rapidly (10—15 minutes).

So far then the analogy between the occurrence of coagulation in salted muscle-plasma, and that in salted blood-plasma is very close, especially in the influence of temperature, a low temperature hindering, a temperature of 35°—40° C. hastening the formation of a clot. The development of acid however does not appear to take place in the separation of fibrin from blood-plasma as it does in that of myosin from muscle plasma. The close resemblance in other points however suggests a similar cause in the two cases; the formation of fibrin is believed to be due to the action of fibrin-ferment, which like other enzymes acts best at a temperature of 30°—40° C. and is inhibited by a low temperature: the question of a similar ferment action in the formation of myosin is one which will be entered into fully later on.

The general conclusions which can be drawn so far with regard to the influence of neutral salts on the coagulation of muscle-plasma are as follows.

1. That admixture with solutions of neutral salts is able to prevent muscle-plasma from undergoing coagulation.

2. That dilution of the salted muscle-plasma brings about the coagulation prevented by the more concentrated salt solutions.

3. That the coagulation of diluted salted muscle-plasma occurs readily at temperatures between 30° and 40° C. more slowly at lower temperatures, and is wholly prevented by a temperature of 0° C.

4. That with the exception of the formation of acid which occurs simultaneously with the production of a clot of myosin, the phenomena regarding the formation of myosin are closely similar to those which are observed in the formation of fibrin from blood-plasma.

5. This similarity suggests that the formation of myosin may be due to a ferment, in the same way that the formation of fibrin from the fibrinogen of blood-plasma is due to the action of the fibrin-ferment.

III. The Properties of Saline Extracts of Muscle that have undergone Rigor Mortis.

It is now necessary to contrast and compare with the foregoing, the control experiments performed in a similar way with saline extracts of muscles in which the coagulation of myosin (which leads to what is called *rigor mortis*) had occurred. One must preface the account of these experiments by stating that the results obtained were very unexpected, and they may be briefly summarized by saying that they differ little from those obtained from salted muscle-plasma in the experiments just described; dilution of the extracts of dead muscle which contained a solution of myosin in addition to other proteids leading to a recoagulation of the myosin.

In the experiment described at the beginning of the last section, it was mentioned that three preparations (with different salts) were made from one lower limb of a rabbit: the other lower limb was not subjected to the freezing process, but after being allowed to remain an hour at the atmospheric temperature 15° C., the muscles were finely divided, and then pounded up with the same salt solutions as were employed to make salted muscle-plasma, viz.: ten per cent. sodium chloride, five per cent. magnesium sulphate, and half-saturated sodium sulphate solution, and finally filtered. On diluting these extracts, which were all distinctly acid, coagulation occurred in a manner indistinguishable in appearance from that already described; the coagulation also occurred more readily at the temperature of the body, than at a lower temperature.

The following are the details of the experiments performed with the magnesium sulphate extract.

Extract 3. With 5 per cent. magnesium sulphate solution. Extract opalescent, slightly acid.

Time of observation	Amount of dilution	Allowed to stand at the temperature of the air 15° C.	Placed in the warm chamber 37° C.
11 a.m.	*a.* Diluted with an equal volume of water	Increase of opalescence	Increase of opalescence
5 p.m.		No change	No change
10 a.m. next day		No change	Small filmy clot

Time of observation	Amount of dilution	Allowed to stand at the temperature of the air 15° C.	Placed in the warm chamber 37° C.
11 a.m.	*b.* Diluted with three times its volume of water	Marked increase of opalescence	Marked increase of opalescence
12 noon		No change	Jellying through liquid
12.30 p.m.		No change	Clot beginning to contract
5 p.m.		Jellying beginning	Clot contracted and abundant
10 a.m. next day		Small clot which had contracted	ditto.

Similar tables might be given of the behaviour of the sodium chloride, and sodium sulphate extracts, but the above will sufficiently indicate the results obtained. The coagulation was in appearance not a simple precipitation, but consisted first in a jellying throughout the liquid which subsequently contracted, squeezing out a colourless fluid, and this occurred more readily at the temperature of the body than at the temperature of the air.

This experiment was the first performed with apparently dead muscle, and it was at first thought that the result might be explained on the supposition that the muscle in question was not really dead, or at least had not undergone *rigor mortis*; since it was removed from the body of the animal only one hour after death. Observations were then made in a similar way on extracts of muscles removed from the body, four, ten, and twenty-four hours after death; in the first two cases those removed four and ten hours after death the muscles were markedly rigid; in the third case, that removed twenty-four hours after death, the stiffness had passed off; in a fourth case, the limbs were kept in an incubator at the temperature of 40° C. for twenty-four hours, a condition which would favour the coagulation of myosin in the muscles to the fullest extent; in this case the odour indicated that putrefaction had also commenced. But in all four cases, the result obtained was precisely the same; *i.e.* the myosin after being redissolved by the salt solution underwent a re-coagulation when that salt solution was diluted. In a further experiment it was found that this re-coagulation was entirely

prevented by a temperature of 0° C., took place slowly a few degrees above that temperature, and occurred readily at the temperature of the body.

I have been careful to speak of it as a recoagulation and not as a simple precipitation; the former term implying that the coagulum is of a similar nature and formation to the clot obtained from muscle-plasma or blood-plasma; whereas the term precipitation would simply imply that myosin, being a proteid of the globulin class, would naturally be precipitated by dilution, as all globulins are insoluble in very dilute solutions of salt. This latter statement is in fact that which one finds in the text books; the dropping of a solution of myosin into water is said to produce a precipitation of the myosin. This statement is perfectly true, and if one employs a large excess of water, as is implied in the above-mentioned statement, the clot-like character of the coagulum is lost. If one dilutes the muscle extract with ten or twenty times its bulk of water, a precipitation of the myosin occurs usually at the atmospheric winter temperature in less than an hour; and the precipitate is a flocculent one, which soon settles to the bottom of the vessel; but in this case also the precipitation is more rapid at the temperature of the body. A myosin clot under any circumstances is never such a coherent coagulum as one of fibrin, and when the dilution of a muscle extract is so great as that described above, the stage of jellying is so transitory, (the clot being immediately broken up into flocculi when it contracts in various directions throughout a large volume of liquid,) that it is apt to be, and in fact generally is overlooked. Still by very careful and continuous observation I have been able to perceive it even in such cases as these. But it can be perfectly well observed in cases in which the dilution is slight, as in the first experiment detailed at the commencement of this section. The clot of myosin which is formed has exactly the same characteristics as the clot of myosin formed when salted muscle-plasma is first made to coagulate.

The rabbit is not the only animal on which observations have been made by me; but in addition to some thirteen rabbits, I have observed an exactly similar phenomenon in the muscles of two cats, and three pigeons.

The appearance of the clot is however not the only ground on which I regard it as due to process of coagulation as distinguished from that of a simple precipitation. The other grounds on which this assertion is based are the following:—

1. *The influence of temperature.* It is prevented entirely by a temperature of 0° C. and occurs most readily at the temperature of the body.

These points have been already discussed. The remaining points have still to be described in detail, but it will be convenient here to summarize them also.

2. *The chemical characters of the clot* of myosin are the same when obtained from extracts of dead or living (frozen) muscle.

3. *The development of acid.* The already acid extract of dead muscle becomes still more acid after it has been diluted and coagulation of the myosin in it has taken place.

4. *The influence of a ferment.* The coagulation is hastened by the addition to the diluted muscle extract of a ferment which can be prepared from muscle, in the same way as Schmidt's fibrin ferment is prepared from blood.

These four points taken together show that the coagulation or rather the re-coagulation of myosin is something different from the simple precipitation of a globulin, as the result of dilution. The last three points have still to be supported by evidence, and as they open out into wider questions than the mere support of this statement with which we are at present concerned, it will be well to make the discussion of each, a separate section.

The next three sections will therefore be

IV. The properties of the muscle-clot.

V. The development of acid during coagulation.

VI. The preparation and properties of myosin-ferment.

Supposing that I make good in the three next sections the general statements which have just been made, it may here prematurely be laid down that the general conclusion to be drawn from the experiments described in this section is as follows:—

The saline extracts of muscle which has undergone *rigor mortis* resemble the salted muscle-plasma previously described in that after dilution a coagulation of the redissolved myosin occurs, which process resembles in all particulars the coagulation of muscle-plasma which in the first instance leads to the formation of myosin.

IV. The Properties of the Muscle-Clot.

1. *Solubility and re-coagulation.* I desired in the first place to determine whether myosin, the proteid substance of which the muscle-clot is composed, undergoes a similar re-coagulation on dilution of its saline solutions. Myosin was prepared either from specimens of salted muscle-plasma, or from saline extracts of muscle which had undergone rigor in the following way. The saline solution was diluted with about twenty times its volume of distilled water. This dilution caused a precipitation of the myosin, which settled into a flocculent deposit at the bottom of the vessel in which the operation was performed; it was then washed by decantation with distilled water three or four times until the supernatant fluid gave only a faint indication of the presence of proteid. (It was found impracticable to wash it more than this, as after prolonged washing, the precipitate of myosin becomes quite insoluble in saline solutions[1].) The myosin was then dissolved by adding solid sodium chloride, or magnesium sulphate, as the case might be, until the strength of the salt solution reached ten or five per cent. respectively. The solutions so obtained were neutral in reaction, and after dilution with two or three times their volume of water underwent re-coagulation; this as in the previous cases was first a jellying through the liquid, the coagulum subsequently contracting and squeezing out a clear fluid; this occurred more readily at the temperature of the body than at lower temperatures; it was accompanied by the development of an acid reaction in the liquid, and lastly, as will be fully stated later on, the addition of myosin-ferment hastened the process. The myosin thus obtained was again washed and redissolved in salt solutions, which were again diluted, and once more underwent a precisely similar coagulation. I have repeated this process in two cases four times, and in one case five times[2]. The clear liquid which is squeezed out by the contraction of the clot of myosin obtained from such solutions, contains only the faintest trace of proteid, such as might arise from the fact that the myosin was not for the reason stated washed perfectly free from other proteids. There seems therefore to be no other proteid formed during coagulation beyond the precipitate of myosin. Here is a difference between the formation of myosin and the formation of fibrin; for Schmidt stated that formation of the latter from fibrinogen under the influence of the fibrin ferment is accompanied by the simul-

[1] See below, p. 153.

[2] In prolonged experiments such as these, decomposition was prevented by the addition to the liquid of a few crystals of thymol.

taneous formation of a globulin corresponding in its character to serum globulin.

We may call the hypothetical precursor of the myosin in muscle, myosinogen; this after death is converted into myosin; the passing off of *rigor mortis* seems to be due to the reconversion of myosin back to the former condition of myosinogen. This process occurs not only in the muscles after death, but something very similar can be made to occur in artificial solutions of myosin, the clotting and unclotting being brought about by alternate dilution and concentration of the salt solution used to dissolve it. We have been tracing hitherto the analogy between the coagulation of blood and that of muscle; we have noted already many resemblances; we have also come across certain differences of which the most important hitherto mentioned are the two following:—

1. The formation of myosin from myosinogen is accompanied by the development of acid, whereas that of fibrin from fibrinogen is not so far as we know.

2. The formation of myosin from myosinogen is not accompanied by the formation of another globulin, whereas that of fibrin from fibrinogen is.

We have now the question before us, have we a third difference to deal with in the readiness with which myosin is reconverted into myosinogen, which once again with suitable treatment will become myosin? or is it possible by similar means once more to reconvert fibrin into fibrinogen which in its turn will again become fibrin? Such an experiment was performed in the following manner.

Fibrin obtained by whipping from ox blood was first well washed under a tap with running water, and then with 10 per cent. sodium chloride solution to remove any adherent globulin. An extract of the fibrin was then made with 10 per cent. sodium chloride, and another with five per cent. magnesium sulphate solution. Thymol was added to each to prevent decomposition. After standing for two days in contact with these solutions, a considerable amount of the fibrin was dissolved. These solutions of fibrin were opalescent and gave a copious precipitate on heating; the heat coagulation temperature of saline solutions of fibrin is stated[1] to be between 60° and 63°. I tried the heat coagulation of the solutions mentioned above as well as in two others similarly prepared, the solution being in all cases made faintly acid by weak acetic acid. The results of these determinations may be stated in the following table.

[1] Gamgee, *Physiological Chemistry*, p. 36.

		Opalescence increased	Flocculent precipitate
Fibrin 1.	Solution in 10% sodium chloride	57° C.	64° C.
	Solution in 5% magnesium sulphate	66°	75°
Fibrin 2.	Solution in 10% sodium chloride	57°	65°
	Solution in 5% magnesium sulphate	66°	75°
Fibrin 3.	Solution in 10% sodium chloride	59°	69°
	Solution in 5% magnesium sulphate	68°	75°

The precipitate obtained was in all cases a finely flocculent one, and not at all like the sticky heat coagulum of fibrinogen.

The precipitation at the temperature given in the second column of the above table was a complete one; that is, after filtering off the heat coagulum, only sufficient proteid was present in the filtrate to give a faint xanthoproteic reaction.

The coagulation temperature of dissolved fibrin differs according to whether it is dissolved in a magnesium sulphate or a sodium chloride solution. It was of course possible that these two salts might dissolve different proteids from fibrin, and thus the difference of coagulation temperature accounted for; but a more probable explanation seemed to me to be that it was the presence of a large amount of sodium chloride in solution that produced a lowering of the coagulation point; that sodium chloride will do so, I knew from previous experiences with this salt: whereas the presence of magnesium sulphate never has any influence on coagulation temperatures. Accordingly I subjected both solutions to dialysis; after three days' dialysis the dissolved proteid was beginning to be precipitated; I added a trace of the salt to each to redissolve it, and then tried the heat coagulation temperature, and found that it was 73° C. in the sodium chloride extract, and 75° C. in the magnesium sulphate extract. These two temperatures are sufficiently near to indicate that I was dealing with the same proteid in both cases, especially as its other characters were identical in both extracts. It is a proteid of the globulin class, which is precipitated by heat at 73°—5° C., whereas fibrinogen is precipitated at 56° C. It is not precipitated by half saturation with sodium chloride, as fibrinogen is. It is completely precipitated by saturation both with sodium chloride and magnesium sulphate. It is thus a globulin, but it is not fibrinogen.

The effects of dilution upon the solutions of fibrin were then tried. Different specimens of the 10 per cent. sodium chloride extract were diluted to two, four, and six times their volume respectively; each was divided into two parts, one of which was kept at the temperature of the air (15° C.), the other at that of the body (36° C.). In those kept at the temperature of the air no change occurred after the lapse of forty-eight hours: in those kept at the temperature of the body there was no change after thirty-six hours: but after forty-eight hours a slight precipitate formed. This precipitate consisted of exceedingly fine flocculi, and a stage in which there was a jellying was neither in this nor in similar experiments ever observed: the precipitate represented a very small fraction of the total proteid dissolved, and there was no change in the neutral reaction of the liquid. A similar experiment was performed with another sodium chloride extract, with two specimens of magnesium sulphate (five per cent.) extract, with one specimen of an eight per cent. potassium nitrate extract, and with one made with half-saturated solution of sodium sulphate. But in all a similar result was obtained to that already stated. The different solutions differed somewhat in the length of time after dilution at which precipitation of a small amount of dissolved proteid occurred; but it was in all cases over twenty hours in the cold, and over twelve hours at the temperature of 37° C. In some of the above-mentioned solutions dilution was performed with water in some cases, and to an equal extent with an aqueous extract of fibrin ferment in others. But it was never found that the presence of the fibrin ferment had any influence in hastening the precipitation.

I should not be inclined to regard it as a coagulation on the following grounds:—

1. It is never, so far as could be ascertained, a jellying throughout the liquid.

2. It is not hastened by the addition of an aqueous solution of fibrin ferment.

It is however a curious fact, and one worth noting, that this precipitation occurred rather more rapidly at the temperature of the body than at the temperature of the air; and in this fact it resembles a ferment coagulation.

The coagulation in muscle thus differs from that in blood in a third particular which must be added to the two differences already stated p. 149.

3. Myosin, the solid proteid formed when muscle coagulates, is

readily soluble in solutions of salt; on dilution of these a recoagulation of the myosin occurs. Fibrin, the solid proteid formed when blood coagulates, is not so readily soluble in salt solutions; on dilution of these a small amount of reprecipitation of the dissolved fibrin occurs, but this does not appear to be a ferment coagulation.

2. *Heat coagulation.* The first property of the muscle-clot that we have hitherto considered is that of recoagulation.

The next point we have to take up, and it is closely connected with the first question, is the phenomenon of heat coagulation.

By applying the process of fractional heat coagulation, which I have fully described elsewhere[1], to salted muscle-plasma obtained by mixing the plasma squeezed out from frozen muscle with five per cent. magnesium sulphate solution, or to that obtained by extracting frozen muscle with the same solution, or to that obtained by extracting muscle which had undergone rigor with the same solution, I in all cases obtained the same series of precipitates. I also obtained the proteids in a state of comparative purity by saturating the muscle-plasma, or the extract of dead muscle with ammonium sulphate; this precipitates all the proteids in the solution. The precipitate was collected on a filter, washed with saturated solution of ammonium sulphate, and then dissolved again by the addition of water; in this solution the process was repeated, and to the solution finally obtained the process of fractional heat coagulation was applied; the result was precisely the same as that obtained from the muscle extract.

The series of precipitates obtained in all these cases was

47° C. A flocculent somewhat sticky precipitate.
56° C. A more abundant, and very sticky precipitate.
63° C. A finely flocculent precipitate: not sticky.
73° C. A finely flocculent precipitate: not sticky.

After filtering off the precipitate at 73°, a small amount of proteid remained in solution which had the characters of an albumose, but which will be fully described later on.

The point to which it is necessary here to call marked attention, is the fact that the series of precipitates in a muscle-plasma in which coagulation has not occurred, is precisely the same as in an extract of muscle in which *rigor mortis* has occurred.

The heat coagulation of the fluid left after the separation of myosin was brought about by dilution and filtration was found to be

[1] This *Journal*, Vol. v. p. 157.

63° C. a finely flocculent precipitate,
73° C. a finely flocculent precipitate.

The precipitates which formerly occurred at 47° and 56° had disappeared.

The clot formed by dilution was then washed with water and dissolved in magnesium sulphate solution, and by fractional heat coagulation gave precipitates at 47° and 56° C.

If, on the pattern of the word fibrinogen, we call the precursor of myosin, myosinogen, it is seen from the foregoing experiments that the myosinogen in muscle-plasma evidently consists of two proteids which coagulate at 47° and 56° C. respectively. But on redissolving myosin in a suitable saline solution, we obtain it once more reconverted into myosinogen, or rather into two proteids which resemble myosinogen in two particulars:—

1. They have the same heat coagulation temperatures.
2. They are convertible into myosin by dilution of their saline solvents; and this is again a ferment coagulation.

We have now to consider the remaining properties of the muscle clot.

3. *Insolubility after washing with water.* This has already been alluded to in speaking of the preparation of solutions of the clot. If the myosin is washed only three or four times with a large excess of water, it is then readily soluble in 5 or 10 per cent. sodium chloride, ammonium chloride, magnesium sulphate, sodium sulphate, or potassium nitrate solution. But if it be washed ten or twenty times in the same way, decomposition in all cases being prevented by thymol, it is then insoluble in any of the solutions named; the longer it is washed, the more insoluble does it become. This fact has been previously noted, at any rate with regard to ammonium chloride, by A. Danilewsky[1], and he finds it to be due to the removal of the salts, especially of the calcium salts which in the ash are exceedingly scanty as compared with the quantity in less thoroughly washed myosin. Addition of small quantities of calcium salts renders the myosin again soluble.

4. *Conversion into syntonin.* The readiness with which myosin is converted into acid-albumin or syntonin is one of its best marked characteristics, and forms an easy test in distinguishing between fibrin and myosin. Danilewsky finds that this syntonin, or HCl-myosin as he terms it, seems to depend for its formation on the presence of

[1] *Zeit. physiol. Chem.* Bd. 5, 158—184.

calcium, for when the myosin is washed thoroughly in the manner first described it becomes insoluble in 0·1 per cent. hydrochloric acid. In my experiments which were performed previously to reading Danilewsky's paper, I found that the myosin was much less soluble in ·1 per cent. hydrochloric acid than before, but I always obtained a certain amount in solution. Doubtless my preparations, though sufficiently well washed to be insoluble in saline solutions, were not sufficiently calcium free to be quite insoluble in dilute hydrochloric acid.

The description of the properties of myosinogen, the precursor of myosin, and also the substance formed when myosin is redissolved, will come more conveniently in the section which treats of the proteids of muscle-plasma, and of muscle-serum, than in this place.

In concluding this section, the chief facts stated in it may be summarized as follows.

1. The muscle clot consists of myosin, and is formed from a substance (myosinogen) in the muscle-plasma which coagulates by heat at 47° and 56° C., showing that probably it consists of two proteids.

2. The more myosin is freed from salts by washing, the more insoluble does it become both in saline solutions and weak hydrochloric acid.

3. If myosin is not so thoroughly washed as this, it is readily soluble in 5 or 10 per cent. solutions of sodium chloride, magnesium sulphate, and other neutral salts.

4. The solution of myosin is formed by reconverting it into myosinogen; that this is the case is shown by the heat coagulation temperature and also by the fact that if the solution be diluted with water (or better still, as will be shown later on, with an aqueous solution of myosin-ferment) a clot of myosin is once more formed.

5. The conversion of myosinogen into myosin differs in the above particular from that of fibrinogen into fibrin: other differences being, the formation of acid in the former and not in the latter, and the formation of another globulin in the latter and not in the former case.

V. The Formation of Acid during Coagulation.

It has already been mentioned, that the conversion of myosinogen into myosin is accompanied by the formation of acid. This is seen from the alkaline reaction of salted muscle-plasma becoming acid simul-

taneously with the development of a clot; by the neutral reaction of a solution of myosinogen becoming acid when converted into myosin, and by the increase of acidity which occurs in an already acid extract of muscle which has undergone *rigor mortis*. It is this last statement that requires to be supported by corroborative experiments. The method adopted was as follows:—A two per cent. solution of sodium hydroxide was prepared, and placed in a burette, twenty-one drops from which were equal to one c.c. A magnesium sulphate (5 per cent.) extract made from muscle which had been removed from the animal three hours after death was prepared. The extract was diluted five times, i.e. 500 c.c. of water were added to 100 c.c. of the extract; 50 c.c. of the diluted mixture were taken immediately after dilution, and the acidity estimated by counting the number of drops of the soda solution it took to neutralise exactly. The remainder was then allowed to coagulate, and the clot filtered off; 50 c.c. of the filtrate were then taken, and the number of drops of the soda solution which it then took to neutralise were counted. The result showed in all cases that the acidity had increased after coagulation. Any putrefaction which might have given rise to acid was in all cases prevented by adding crystals of thymol to the liquid. The following table represents the results obtained from three different extracts thus quantitatively investigated.

Solution employed	Acidity as represented by the number of drops of a two per cent. soda solution required to neutralise 50 c.c. of extract.	
	Before coagulation	After coagulation
5% $MgSO_4$ extract of muscle, rabbit	7—8	11—12
5% $MgSO_4$ extract of muscle, pigeon	6	9
5% $MgSO_4$ extract of muscle, rabbit	5	8—9

The acidity of muscle which is produced both by the activity of muscle, and on the death of muscle, is now known from numerous researches to be lactic acid. In the earliest stage it is probable that the acid reaction may be due to an acid potassium phosphate produced from the alkaline phosphate by the development of new phosphoric

anhydride from the lecithin. Th. Weyl and H. Seitler[1] have shown that tetanized muscle contains less lecithin than resting muscle, but the amount is not lessened in amount corresponding to the increase of phosphoric anhydride; it is therefore supposed by them that the rest comes from some other phosphorized substance in muscle, probably nuclein. Numerous observers, including such names as those of Berzelius[2], Du Bois-Reymond[3], Kühne[4], and Heidenhain[5], have worked at the subject of the acidity of dead and tetanized muscle, and all coincide in opinion as to the presence of lactic acid.

I have not myself definitely set to work at this point, but accept the conclusions arrived at by these observers. I have however in a few cases demonstrated the presence of free acid[6] by adding a few drops of the muscle extract, or the acid filtrate after separation of a clot of myosin from a solution of myosinogen to a test liquid consisting of dilute ferric perchloride and carbolic acid (10 c.c. of a 4 per cent. solution of carbolic acid, 20 c.c. of water, and 1 drop of the liquor ferri perchloridi of the British Pharmacopeia). The violet solution instantly becomes of a faint yellowish colour. I have also performed the test as follows: the extract was boiled, filtered, and extracted with an equal volume of ether; the ethereal extract was evaporated to dryness, dissolved in water, and the test tried successfully with this aqueous solution. This test is especially delicate for lactic acid being given by a solution of it consisting of one part in ten thousand of water.

Observers differ however as to the origin of lactic acid. O. Nasse[7] believes that it comes from the glycogen present in muscle. Most observers however seem to regard the proteids as its source; this seems to me to be very conclusively shown by the experiments of R. Böhm[8]. Böhm found that the amount of glycogen in putrefaction and in rigor remains unaltered from that in fresh muscle; glycogen therefore cannot be the

[1] *Zeit. physiol. Chem.* Bd. 6, 557.

[2] *Lehrbuch der Chemie*, Vol. IX. p. 569.

[3] *Gesammelte Abhandlungen zur allgemeinen Muskel und Nervenphysik.* Leipsig 1877, Vol. II. p. 3.

[4] *Loc. cit.*

[5] *Mechanische Leistung*, p. 143.

[6] The description of this test for lactic acid will be found in a paper by J. Uffelmann, *Zeit. f. Klin. Med.* Bd. VIII. p. 392. I find however that in a brief note (*Deutsch. Archiv. f. Klin. Med.* Bd. XXXIX. p. 242), Caher and Mering cast doubt on the trustworthiness of the test.

[7] *Zur Anat. und Physiol. der quergestreiften Muskelsubstanz.* Leipsig, 1882.

[8] *Pflüger's Archiv.* Vol. 23, p. 44.

source of the acid. I quote one of Böhm's tables which brings out this point clearly.

	Fresh muscle. Percentage of		Muscle after rigor. Percentage of	
Experiment	Glycogen	Lactic acid	Glycogen	Lactic acid
1	0·71	0·22	0·71	0·57
2	0·28	0·16	0·28	0·44
3	0·036	0·35	0·041	0·56

This view of Böhm's is endorsed by Hoppe-Seyler[1].

The close relationship in point of time which my experiments show between the change in the condition of the proteids, and the development of an acid reaction, certainly support the view that lactic acid arises in some way from the proteids. How this precisely takes place will not be known until we are acquainted with the rational formulæ of proteids. It is here however worth noting that Dr Latham[2], who has a very distinct theory as to the composition of proteids, is also by his theory able to denote by a formula the way in which lactic acid arises after the death or during the contraction of a muscle. The theory is advanced that albumin is a compound of cyan-alcohols united to a benzene nucleus. The following are Dr Latham's words concerning lactic acid.

"The lactic acid developed when a muscle contracts or dies is a mixture of two kinds of lactic acid, the more abundant being paralactic acid or ethidene lactic acid $CH_3\ CH \begin{cases} OH \\ COOH \end{cases}$, the other, ethene lactic acid $CH_2\ CH_2 \begin{cases} OH \\ COOH \end{cases}$. Now by treating ethidene cyan-alcohol with acids or alkalies paralactic acid is obtained

$$CH_3\ CH \begin{matrix} OH \\ CN \end{matrix} + 2H_2O = NH_3 + CH_3\ CH \begin{cases} OH \\ COOH \end{cases}$$

[ethidene cyan-alcohol] [paralactic acid]

By treating ethene cyan-alcohol in the same way, ethene lactic acid is obtained

[1] *Physiol. Chemie*, pp. 666—7.

[2] *British Medical Journal*, Vol. I. 1886, p. 630.

$$CH_2\,CH_2\begin{cases}OH\\CN\end{cases} + 2H_2O = NH_3 + CH_2\,CH_2\begin{cases}OH\\COOH\end{cases}$$

[ethene cyan-alcohol] [ethene lactic acid]

Later on[1] Dr Latham says :—

"There is another way in which the molecules may split up, which is interesting as it appears to explain the formation of lactic acid and carbonic anhydride when a muscle contracts or dies.

If the molecule $CH_2\begin{cases}OH\\CNOH\end{cases}$ $CH_2\begin{cases}\\NC\end{cases}$ were hydrated forming $CH_2\begin{cases}OH\\CNOH\end{cases}$ $CH_2\begin{cases}\\COOH,\end{cases}$

and the portion CNOH then detached, the latter would be at once decomposed into CO_2 and NH_3 and we should have lactic acid left. This is shown in the following formula

$$CH_2\begin{cases}OH\\CNOH\end{cases} + 2H_2O = \begin{matrix}CH_2-OH\\|\\CH_2-COOH\end{matrix} + CO_2 + NH_3$$

[Two molecules of the lowest term of the cyan-alcohol series] [lactic acid]

The ammonia (NH_3) would not be set free, but would unite with some other cyan-alcohol higher in the series to form a cyanamide."

In connection with the presence of lactic acid in muscle after death, Catherine Schipiloff[2] has formed an interesting theory of *rigor mortis*. She finds that myosin can be precipitated from its saline solutions by weak acids without change, and is soluble in excess; the same occurs in the body; by injecting small quantities of lactic or hydrochloric acids (0·1 to 0·25 per cent.) into the vessels of a recently killed frog, the muscles become rigid; this passes off by injecting stronger acid 0·3 to 0·5 per cent. If such stronger acids be injected in the first instance, no rigor, or rigor which is only momentary, occurs. After rigor, muscle contains more acid than either during rigor, or before it sets in. The conclusion is therefore drawn that rigor is due to the post-mortem development of acid, and its passing off is due to the development of more acid which redissolves the precipitated myosin.

If one however examines the reasoning which results in the above conclusion the premisses will be found not to bear it out. Because myosin can be precipitated and redissolved by acid when in solution, or

[1] *Loc. cit.* 634.

[2] *Centralblatt f. d. Med. Wissensch.* 1882, 291.

after death in the muscles, it does not necessarily follow that this is the cause of rigor in the normal course of things, even if the amount of lactic acid is increased after rigor; for the increase in lactic acid may not only be the cause of rigor, it may also be the result, or merely a concomitant of the coagulation of muscle.

According to the theory which I shall presently advance, I believe it to be a concomitant merely of the conversion of myosinogen into myosin, and that both are due to the same cause, a ferment action. Moreover the theory of C. Schipiloff seems to involve that lactic acid is formed from something other than proteids, presumably glycogen.

This section may be summed up by saying

That lactic acid is formed simultaneously with the occurrence of the coagulation or recoagulation of myosin, the agreement in point of time suggesting that the source of the acid is a proteid.

VI. The Preparation and Properties of Myosin-ferment.

We now turn to the full consideration of the ferment which brings about the coagulation of myosin, and to which allusion has been several times made in the foregoing pages.

I have prepared three specimens in all; and the method of preparation is almost precisely that adopted by Schmidt in the preparation of the fibrin-ferment from blood.

Muscle was first allowed to undergo rigor; it was then chopped up into small pieces and kept under absolute alcohol for a long time.

The first preparation made from cat's muscle was kept under alcohol for ten months; and two preparations from rabbit's muscle were kept under alcohol for three months.

The pieces of muscle after having been thus treated were then dried over sulphuric acid, and powdered. An aqueous extract of this powder contained the myosin-ferment, as shown by the fact that dilution of muscle-plasma, or a solution of myosin with it brought about coagulation much more quickly than dilution with distilled water.

The chemical properties of the aqueous extract were as follows:

1. Alcohol gave a precipitate soluble in water.
2. Boiling gave no precipitate.
3. The xantho-proteic reaction showed that a small amount of proteid was present.

4. Nitric acid gave a slight precipitate in the cold; this disappeared on boiling, and reappeared on cooling.

5. On adding to the liquid after concentration a trace of copper sulphate and excess of caustic potash, a faint pink colour was produced (biuret reaction).

Thus myosin-ferment resembles fibrin-ferment in giving the proteid reactions faintly. The reactions show however that we have to deal, not with an ordinary proteid, but with one which has similar properties to those intermediate products of peptic digestion, to which Kühne has given the name of albumose; we may therefore call it muscle-albumose. The full details of the properties of this albumose will be given in the description of the proteids of muscle-plasma (section VII.).

The question then is, what have we in the aqueous extract of dried muscle? Have we to deal with an albumose, and a ferment; or is the albumose identical with the ferment? I hope to be able to show that the latter is the more correct view to take. I have not found it possible to separate the albumose from the ferment; so that if the albumose and ferment are not identical, they are at any rate in very close union with one another.

The ferment and albumose are precipitable by means of ammonium sulphate, when that salt is added to saturation; the filtrate was freed of salt by dialysis, and then found to have no ferment-action; that is, it did not hasten the coagulation of muscle-plasma, as the following experiments show that the aqueous extract of the dried alcoholic precipitate of muscle does. On the other hand the precipitate produced by these salts, on being redissolved by the addition of water and freed from excess of salt by dialysis, had the properties of the ferment as well as those of the albumose.

The first experiment performed with the ferment was done roughly as follows—not with muscle-plasma but with extracts of already rigid rabbit's muscle. The following are the details of the experiment:

Rabbit killed by bleeding 10.15 a.m., and the muscles freed from blood by a stream of salt solution—the cannula being placed as in previous experiments in the abdominal aorta. When the limbs were examined at eleven o'clock they were found to be stiff; the muscles were then removed, chopped up finely, and pounded up in a mortar, with a saline solution, and filtered; two extracts were made:—

I. Extract made with 10% sodium chloride solution.

II. Extract made with 5% magnesium sulphate solution.

With these extracts dilution experiments were performed as follows, the diluted specimens not being kept in an incubator, but at the temperature of the air, which never rose higher during the experiment than 10° C.

I. *Sodium chloride extract.*

Dilution. 5 p.m.	Result. 10 a.m. next day
a. Diluted with an equal amount of water	No clot
a'. Diluted with an equal amount of aqueous extract of dried alcoholic precipitate of muscle	No clot
b. Diluted with three times as much water	No clot
b'. Diluted with three times as much aqueous extract of dried alcoholic precipitate of muscle	Good clot
c. Diluted with five times as much water	No clot[1]
c'. Diluted with five times as much aqueous extract of dried alcoholic precipitate of muscle	Good clot

II. *Magnesium sulphate extract.*

Dilution. 5.30 p.m.	Result. 10 a.m. next day
The dilution in each case was the same as performed with extract I.	
a	No clot
a'	No clot
b	Slight film
b'	Good clot
c	Slight film
c'	Good clot

The experiment it is thus seen was a tentative one, and was not carefully watched throughout; it shows that in the course of a very cold

[1] There was no general clotting throughout the liquid, but a particle of the dried alcoholic precipitate of muscle had fallen accidentally into the tube, and adhered to the glass this formed the centre of a tiny clot.

night coagulation did not occur when the dilution of the saline extract was performed with water simply, but if instead of water an aqueous extract of the dried alcoholic precipitate of cat's muscle was used, the result was the formation of a well-marked clot of myosin.

This experiment was however sufficiently conclusive to warrant further investigations, and so far as it went supported the idea I had formed, that coagulation of myosin was a ferment; the occurrence of coagulation after dilution of a salted muscle-plasma, or of a saline extract of dead muscle, being due to the ferment present in such solutions but prevented from acting previously by the presence of excess of salt. As in the foregoing experiment, coagulation is considerably hastened by the addition of an aqueous solution of dried alcoholic precipitate, that is of a solution of myosin-ferment as it may now be called.

The next experiment performed with the solution of myosin-ferment was as follows:

A 5% magnesium sulphate extract of rabbit's muscle prepared as before.

1. Diluted four times.
   a. With water.
   b. With ferment solution. } Kept at 35° C.

   In three quarters of an hour, both had clotted; and five minutes later the clot had contracted to a considerable extent.

2. Diluted four times; all specimens kept at temperature of air 11° C.
   a. With water. Coagulation had not begun in 24 hours.
   b. With ferment solution. Coagulation began in one hour: and an hour later the clot had contracted.
   c. With water; some particles of the dried precipitate being also added. Four hours later each particle was the centre of a small clot, which later extended into the liquid.

This shows very markedly the acceleration of the process of clotting produced by adding the ferment solution.

The coagulation at the temperature of 35° C. advances so rapidly as a rule, that little or no difference in the rate of clotting can be made out between specimens diluted with water or with ferment solution. In some cases however by very careful watching it is possible to do so. This is illustrated in the following experiment which was performed with a 10% sodium chloride extract of muscle prepared from the same animal.

10% sodium chloride extract of rabbit's muscle: diluted four times.

a. With water.

b. With ferment solution.

Both were kept at the temperature of 35° C. The experiment was begun at 12.30 p.m. Addition of water to the muscle extract produced, as it always does, increase of cloudiness.

	a.	*b.*
12.35 p.m.	Cloudy liquid	Cloudy liquid
12.40	,,	,,
12.42	,,	Bubbles beginning to be entangled
12.54	Slight entanglement of bubbles	Clot well marked
1.5	Clot well marked	Clot contracted

Similar tables might be quoted at length to illustrate still further the activity of the several specimens of ferment prepared. They however all illustrate the same thing, that the addition of the ferment solution hastens, often very considerably, the formation of the clot of myosin. There was however one exceptional case which I am totally unable to explain: the particulars of this experiment are as follows:—

5% magnesium sulphate extract of rigid muscle (from a rabbit). Each specimen was diluted to three times its volume; all were kept at the temperature of the air.

1. Diluted with water at 3.15 p.m.
   Clotting occurred at 4.55 p.m., that is in 1 hour and 40 minutes.
2. Diluted at 3.16 p.m. with ferment solution prepared from cat's muscle.
3. Diluted at 3.25 p.m. with ferment solution prepared from rabbit's muscle.
4. Diluted at 3.45 p.m. with ferment solution prepared from another specimen of rabbit's muscle.
   Clotting had not occurred in these last three preparations by ten o'clock the following morning.

This experiment in its results is so opposed to the six other experiments I have performed similarly, that I am forced to put down its failure either to idiosyncrasy, or morbid condition of the animal employed, or to some mistake or accident in manipulation.

The next experiments were performed with salted muscle-plasma:

that is the extracts were made when the muscle was in a frozen condition before it had undergone rigor; the method has already been fully described. These experiments fully corroborate those just detailed and so bear out the ferment theory of myosin formation. I will quote two of these experiments, as they not only illustrate the hastening of the coagulation by the ferment, but were also designed to elucidate the question as to whether, as in the case of fibrin-ferment, exposure to a high temperature destroyed the activity of the ferment. The aqueous solution of the ferment was exposed to various high temperatures, and cooled; then it was added, always to the same extent, to specimens of salted muscle-plasma.

Rabbit. 10% sodium chloride muscle-plasma, in each case diluted to four times its bulk, the diluted liquids being kept at the temperature of the air, 10° C.

a. Diluted with water; coagulation in 14 hours.

b. With ferment solution (cat); coagulation in 3—4 hours.

c. With ferment solution previously heated to 50° C.; coagulation in 3½ hours.

d. With ferment solution previously heated to 60° C.; coagulation in 3 hours.

e. With ferment solution previously heated to 80° C.; coagulation in 4 hours.

The foregoing experiment though incomplete illustrates the fact that a temperature of 80° C. will not destroy the activity of the myosin-ferment. A temperature of 80° C. does destroy the activity of the fibrin-ferment. A temperature of 100° C. will however destroy the activity of the myosin-ferment. An experiment illustrative of this is as follows:—

5% magnesium sulphate muscle-plasma from rabbit. Each specimen was diluted to three times its bulk, and kept at the temperature of the air, 15° C.

a. Diluted with water. It began to coagulate in 90 minutes.

b. Diluted with aqueous solution of myosin-ferment (rabbit). It began to coagulate in 25—30 minutes.

c. Diluted with the same solution previously boiled. It began to coagulate in 70—80 minutes.

d. Diluted with aqueous solution of myosin-ferment prepared from another rabbit. Coagulation began in 20 minutes.

e. Similarly diluted; but a few particles of the dried alcoholic precipitate of muscle were added also. Coagulation began in 20

minutes; the specimen illustrated very well how these particles become foci of coagulation.

f. Diluted as in *d*; but the solution was previously boiled. Coagulation began in 90—95 minutes.

This experiment was performed one afternoon: they were left standing over night; next morning there was a clot in all; but that in the specimens *a*, *b*, *d* and *e* was very abundant as compared with that in *c* and *f*.

Lastly we come to experiments performed with the muscle-plasma itself, as squeezed out from the muscle. The only way of testing the activity of the ferment on such, is to use particles of the solid precipitate; the addition of an aqueous solution would introduce the additional complication of dilution. The addition of these solid particles did not however produce any hastening of the coagulation; probably because the viscous muscle-plasma is not capable of dissolving out the ferment from the dried precipitate before it coagulates spontaneously. In one case however I obtained very distinct evidence that the dried precipitate really does contain myosin-ferment. It was as follows:—

Muscle-plasma was prepared by means of the pressure exerted by a lemon-squeezer in the way already described. Towards the end of the squeezing process, when the muscle was getting out of its frozen state, the plasma was collected in a separate vessel, and filtered from a few minute particles of muscular tissue which had got mixed with it. The muscle-plasma collected at the beginning of the experiment underwent coagulation on reaching the temperature of the air: that collected at the end of the experiment and filtered did not; for it must have undergone at any rate partial coagulation. It was kept at the temperature of 40° C. for some hours and even then did not coagulate: to a similar specimen kept at the same temperature a few particles of the dried alcoholic precipitate were added: after the lapse of about an hour, there was the formation of fine films through the liquid; it did not however become a jelly throughout. This seemed to me to show that, although coagulation had probably in great measure occurred, it required the stimulus of the ferment to complete the transformation of myosinogen into myosin.

From the general similarity of the coagulation of blood-plasma and muscle-plasma, it might no doubt have been inferred that the cause of one is similar to that of the other, and that both are the result of ferment actions. The most striking difference, as it seems to me, between the two is the way in which the myosin can be so easily reconverted into

myosinogen which can be then made to recoagulate with the formation of myosin once more; while fibrin and fibrinogen are not so interchangeable. One suggestion that might arise is this: if myosin can be made to clot and unclot so easily out of the body, is it possible that a similar condition exists in the body? Are these experiments in fact any confirmation of Hermann's views of the contraction of muscle being the partial death of the muscle? I do not myself think that they are. Myosin certainly will clot and unclot readily out of the body, but we have no proof of the formation of a coagulum during contraction; it is true that lactic acid is formed both during contraction and during rigor mortis; but this is the only known point of chemical resemblance between the two processes. On the other hand one physical change in particular shows there is a great distinction between the two processes, and that is the change in the extensibility of the muscle, which after rigor is much diminished and during contraction is increased; that is to say, rigor makes the muscle less extensible because it becomes more solid, due to the formation of the myosin clot; but as during the contraction of a muscle, it becomes more extensible, we may consider that in a sense it becomes more liquid, which is certainly against the theory of the formation of a solid clot during contraction.

The next point in connection with the ferment theory of the coagulation of myosin is this; is there any rise of temperature during the conversion of myosinogen into myosin? I have tried the experiment several times, using exceedingly delicate thermometers, but have never been able to detect any rise of temperature which might be produced by the occurrence of coagulation.

I quote only one of these experiments as an illustration.

Extract of rigid muscle (rabbit) was made with 5% magnesium sulphate solution in the usual manner. 100 c.c. of this extract were taken and diluted with 400 c.c. of water; the temperature of the air was 15·6° C. The temperature of the diluted extract was noted every few minutes, as was also the temperature of 500 c.c. of water placed by the side of it, and which served as a control experiment.

Time	Temperature	
	Of diluted muscle extract	Of the water in the control experiment
12.45 p.m.	14·7° C.	
12.50	14·7°	
12.55	14·75°	14·25° C.
1.0	14·75°	14·25°
1.5	14·8°	14·3°
A. 1.10	14·8°	14·4°
1.15	14·825°	14·5°
B. 1.20	14·85°	14·55°
1.25	14·9°	14·6°
1.30	14·92°	14·65°
1.35	14·95°	14·7°
1.50	15·0°	14·8°

A. 1.10. Clotting commenced.
B. 1.20. ,, distinct.

It might be suggested that 500 c.c. of liquid is a large quantity to be raised in temperature by the production of more heat, if any such production does take place. No doubt this is the reason which prevents the increase of temperature being sufficiently great to show itself on the thermometer. Experiments tried with smaller quantities gave however equally negative results; in these doubtless the amount of myosin formed was too small to give rise to sufficient heat to raise the temperature of the fluid. I have not made any thermoelectric observations.

It was stated at the commencement of this section that it had not been found possible to separate the ferment from muscle-albumose. Ammonium sulphate was employed to precipitate the albumose present in the aqueous extract of the dried alcoholic precipitate of muscle. The

precipitate was collected on a filter; the filtrate was free from proteid of any kind. It was freed from ammonium sulphate by dialysis, and was then found to have no power of hastening the formation of myosin. This is illustrated by the following experiment.

Cat's muscle. 5% magnesium sulphate extract prepared in the usual way. Two specimens were diluted to three times their volume with water, and two others with the filtrate mentioned above. Coagulation occurred in all at the temperature of the air (17° C.) in from 8—9 hours, but the clot was a small one.

The precipitate produced by saturating the aqueous extract of the dried alcoholic precipitate of muscle with ammonium sulphate was collected on a filter, washed with saturated solution of ammonium sulphate, and redissolved by the addition of distilled water.

This solution of the albumose was freed from ammonium sulphate by dialysis, and it was found to possess the power of hastening the formation of myosin. This is illustrated by the following experiment, which was performed with the same extract, as in that just related.

Two specimens were diluted to three times their volume with the solution of the albumose just mentioned. Coagulation occurred in each in from 2—3 hours, and the clot formed was a large one.

The last point which has still to be considered is the very important one, whether the fibrin-ferment, and the myosin-ferment, are or are not identical. The term ferment is at present a conventional one; we use it to denote an agent of unknown but probably unstable chemical nature which by its presence induces certain changes in the materials with which it comes into contact. These enzymes however agree in certain points with regard to their manner of action, the chief being the influence of temperature upon them: they act most favourably at the temperature of the body, are prevented from acting by a low temperature, and are destroyed by a high temperature.

The fibrin-ferment has not so far as I am aware been obtained so free from proteid, that it does not give the proteid reactions faintly. Dr Gamgee's[1] method of preparing fibrin-ferment also gives a solution which contains a proteid, which seems to have the properties of a globulin. The myosin-ferment is also, as I have shown, either a proteid (of the albumose group), or is so closely associated with a proteid, that I have not been able to dissociate them. So far then, there are certain points of resemblance between the fibrin-ferment and myosin-ferment.

[1] This *Journal*, Vol. II. p. 145. 1879.

We have already seen one point of difference; viz.—that the activity of the fibrin-ferment is destroyed by a temperature of 80° C.: that of the myosin-ferment is not destroyed till the temperature of 100° C. is reached.

But with our imperfect knowledge of the constitution of ferments, and even of proteids, the burden of proof or disproof of the identity of the ferments will rest not so much upon these properties as upon the activity of the ferments. In other words, we have to answer these two questions:

1. Will myosin-ferment hasten the coagulation of blood-plasma?
2. Will fibrin-ferment hasten the coagulation of muscle-plasma?

If the answer to both these questions is in the affirmative, we may conclude that fibrin-ferment and myosin-ferment are identical; if in the negative, we may conclude that they are not identical.

Before considering the experiments which I have performed to elucidate this point, it is first necessary to allude to certain papers which appeared from the Dorpat laboratory in the year 1883.

Edgar Grubert[1] remarks that there is great analogy between fibrin formation, and the rigor mortis of muscle; hence there is great probability that the latter is due like the former to a ferment action. Frogs were the animals employed in Grubert's experiments; the blood was removed by an injection of salt solution; the muscles were removed, cut up finely, subjected to pressure, and to the juice was added twelve times its volume of absolute alcohol: the precipitate was collected, dried and extracted with water. This extract had great power in causing the coagulation of salted blood-plasma; the ferment seems to be formed at the moment of rigor mortis. Injection of a concentrated solution of fibrin-ferment into the vascular system of a frog from which the blood had been removed by a stream of salt solution, produced however no hastening in the onset of rigor. The identity of the ferment obtained from muscle and from blood was also shown by the fact that the watery extracts of muscle free from blood contained almost no fibrin-ferment, but produced rapid coagulation of filtered horse-plasma, which can only be explained by supposing that they contain the same substance from which the ferment is formed by the blood-plasma.

In connection with this last statement it is necessary to allude to a paper by F. Rauschenbach[2]. He states that the filtered blood-plasma

[1] *Ein Beitrag zur Physiologie des Muskels.* Inaug. Diss., Dorpat, 1883.

[2] *Ueber die Wechselwirkungen zwischen Protoplasma und Blutplasma.* Inaug. Diss., Dorpat, 1883.

of the horse contains but little ferment, and less paraglobulin than plasma to which white corpuscles are added; two products of the action of plasma on the cells being the ferment and paraglobulin. The ferment does not exist in the cells as such, but as something which is subsequently changed into it. He further supposes that leucocytes are of two varieties; α leucocytes which split up in the way just mentioned, and β leucocytes which do not, and which remain unaltered in the plasma.

Continuing now the summary of Grubert's paper, he goes on to say, that greater ferment action was obtained if instead of the muscle itself, the juice pressed out from the muscle was coagulated by alcohol. The separation of the muscle-juice from the muscular tissue seems to have therefore the same effect as the removal of the blood from the vessels.

J. Klemptner[1] has worked at another aspect of the same question. He says that the quantity of fibrin-ferment in living muscle, as well as in that which has undergone rigor, is very small and variable. Rigor was hastened by injecting water, or better a 2 per cent. solution of caffeine into the vessels of one leg of a frog from which the blood had been removed by a stream of salt solution; the other leg served for a control experiment. The quantity of ferment in the expressed juice, and also in the watery extract of the dried alcoholic precipitate, was tested by noting the time in which coagulation was produced in Schmidt's salted blood-plasma: the leg in which rigor had occurred contained more fibrin-ferment than the other. The ferment solutions obtained from muscle work much more slowly on salted plasma than those obtained in the usual way from blood; the amount of ferment obtained from frog's blood is however not so great as that obtained from the blood of warm-blooded animals.

The last of this series of dissertations is from the pen of Ernst Kügler[2], whose researches were on the muscle of mammals, and therefore bear more directly than the preceding papers on the present question. The animals used were dogs; the blood was washed out of the vessels by salt solution; the muscle was removed and divided into two parts: one half was cut up and kept in ice till the next day; in the case of the other half caffeine was injected and after rigor the comparison was made as in Klemptner's experiments. He found that muscle

[1] *Ueber die Wirkung des destillirten Wassers, und des Caffeins auf die Muskeln und über die Ursache der Muskelstarre.* Inaug. Diss., Dorpat, 1883.

[2] *Ueber die Starre des Säugethiermuskels.* Inaug. Diss., Dorpat, 1883.

always contains fibrin-ferment, that great differences exist as to its amount in frog's muscle, but that most occurred in that which had been made to undergo rigor by the action of caffeine. The muscle juice itself was much more active than the watery extracts of the alcoholic precipitate.

The conclusions that one can draw from the foregoing summaries of these papers are as follows:—

1. In answer to the question—Does fibrin-ferment hasten the formation of myosin? we must say—that so far, as these experiments give any answer at all to this question, the answer is in the negative.

2. The answer to the question—Does the ferment obtained from muscle hasten the formation of fibrin in blood-plasma? is an uncertain affirmative. The fresh aqueous extract of muscle certainly does produce a hastening of the formation of fibrin, but an aqueous extract of the dried alcoholic precipitate of muscle contains very little ferment, and that in variable amount, and sometimes has so little influence in producing the formation of fibrin, that recourse is had to the theory that it is the precursor of fibrin-ferment which is present in the fresh juice, not fibrin-ferment itself.

Passing now to the experiments which I have myself performed in attempting to answer the two questions already propounded: they may be easily arranged into two categories:—

1. Experiments designed to ascertain whether fibrin-ferment produces any hastening in the formation of myosin.

2. Experiments designed to ascertain whether myosin-ferment produces any hastening in the formation of fibrin.

These will be taken seriatim.

1. *Experiments designed to ascertain whether fibrin-ferment produces any hastening in the formation of myosin.* The results here obtained are entirely negative, and so confirm the experiments of Grubert, who found that injection of a concentrated solution of fibrin-ferment into the vascular system of a bloodless frog produced no hastening of *rigor mortis*. I have not however followed Grubert's method, but experimented on the same plan by which I have been enabled to show that the aqueous extract of the alcoholic precipitate of muscle contains a myosin-ferment. That is to say, saline extracts of muscle were taken and diluted with solution of fibrin-ferment[1], and the time at which the

[1] The fibrin-ferment employed in these and subsequent experiments was prepared from horse's serum by Schmidt's method.

coagulation of myosin occurred noted in comparison with specimens of the same muscle extract, similarly diluted with water, and also with solution of myosin-ferment. The result was that in those specimens which were diluted with the solution of myosin-ferment, coagulation occurred more rapidly than in those which were diluted either with water or with a solution of fibrin-ferment; and that coagulation occurred simultaneously in those diluted with water and those diluted with solution of fibrin-ferment.

I quote the following as an illustrative experiment.

Rabbit's muscle-plasma prepared by extracting the frozen muscles (freed from blood in the usual way), with 10% sodium chloride solution, and also with half-saturated sodium sulphate solution.

1. 10% sodium chloride muscle-plasma.

a. Diluted with an equal amount	of water	of solution of fibrin-ferment	of solution of myosin-ferment
24 hours afterwards, having been exposed to the temperature of the air 13°—14° C. for that time	No clot	No clot	Slight clot

b. Diluted with three times its volume of water	of solution of fibrin-ferment	of solution of myosin-ferment
The liquid became cloudy immediately	Became cloudy	Became cloudy
It was kept at the temperature of the air, and myosin coagulated in from 15—18 hours	Kept at the temperature of the air. Coagulation in 15—18 hours	Kept at the temperature of the air. Coagulation in 2—3 hours

2. Sodium sulphate (half-saturated) muscle-plasma.

a. Diluted with three times its volume of water	Diluted with three times its volume of solution of fibrin-ferment.

Coagulation did not occur after 24 hours in either specimen: the fluids being kept at the atmospheric temperature.

b. Diluted with six times its volume of water	Diluted with six times its volume of solution of fibrin-ferment.

Coagulation in both occurred at the temperature of the air in from 20—24 hours.

In other specimens particles of the dried alcoholic precipitate of serum were added to specimens of diluted muscle-plasma; but here again

there was no hastening of the process of coagulation, nor did the particles of precipitates become foci of coagulation as do the particles of the dried alcoholic precipitate of muscle.

2. *Experiments designed to ascertain whether myosin-ferment produces any hastening of the formation of fibrin.* In these experiments the results I have obtained are also entirely negative. The method of the experiments was as follows:—salted plasma, usually of the horse, was obtained by receiving the freshly drawn blood into an approximately equal volume of a saturated solution of sodium sulphate; the mixture was kept in a cool room till the corpuscles had subsided, and then the supernatant salted plasma was pipetted off. Specimens of this salted plasma were diluted to equal extents, usually to five or six times their volume, with water, with solution of myosin-ferment, and with solution of fibrin-ferment respectively; the time of dilution, and the time at which coagulation occurred at the atmospheric temperature, were carefully noted, and the results are embodied in the following tables.

Horse's sodium sulphate plasma. Diluted in each case six times.

	A. With water	B. With solution of fibrin-ferment	C. With solution of myosin-ferment from cat
Coagulation occurred in	230 minutes	81 minutes	226 minutes

The next experiment illustrates the same fact:—

Horse's sodium sulphate plasma. Diluted in each case six times.

	A. With water	B. With solution of fibrin-ferment	C. With solution of myosin-ferment (cat)	D. With solution of myosin-ferment (rabbit)
Coagulation occurred in	154 minutes	65 minutes	180 minutes	150 minutes

In other words, the coagulation of the plasma was invariably hastened, as illustrated in the two foregoing experiments, by the addition

of fibrin-ferment, while specimens to which a solution of myosin-ferment had been added coagulated at approximately the same time as those which had been diluted with water simply; sometimes a little before, sometimes a little after these.

These solutions of the ferment were prepared by extracting the dried alcoholic precipitate with cold water. It was found however in the case of fibrin-ferment, that if the water used to extract the ferment from the dried alcoholic precipitate of serum was not cold but warm water, the solution of fibrin-ferment obtained was more concentrated or at any rate more active; the warm extract was always allowed to cool to the temperature of the air before it was used. The following experiment illustrates this.

Horse's sodium sulphate plasma. Each specimen was diluted six times.

1.	Diluted with cold watery extract of fibrin-ferment	Coagulation occurred in		50	minutes
2.	ditto	,,	,,	65	,,
3.	ditto	,,	,,	54	,,
4.	Diluted with a solution of fibrin-ferment made with water at the temperature of 45° C. and subsequently cooled	,,	,,	18	,,
5.	ditto	,,	,,	19	,,
6.	ditto	,,	,,	15	,,

In this experiment it is necessary to call attention to another point; namely that the coagulation does not occur absolutely simultaneously in specimens apparently under the same conditions; the temperature of the air, the presence of draughts, and the shape of the containing vessel are probably the chief varying conditions which produce these slight differences. It need hardly be said however that care was always taken to have as far as possible all these conditions as much alike as possible.

Having performed this and one similar experiment on fibrin-ferment with a corroborative result, it was thought advisable to try a similar experiment with the myosin-ferment. If warm water be used with which to extract the dried alcoholic precipitate of muscle, not only would a stronger solution of the myosin-ferment be obtained, but also any fibrin-ferment which might be present and mixed with it, would run a better chance of getting into solution. The result is illustrated by the following table.

Horse's plasma, diluted six times in each case.

1.	Dilution with water	Coagulation occurred in		154	minutes
2.	Dilution with an extract of dried alcoholic precipitate of cat's muscle made with cold water	"	"	180	"
3.	Similarly diluted, but the extract was made with water at the temperature of 45° C. and the extract cooled before being used	"	"	150	"
4.	Dilution with an extract of dried alcoholic precipitate of rabbit's muscle made with cold water	"	"	150	"
5.	Similarly diluted, but the extract was made with water at the temperature of 45° C. and the extract cooled before being used	"	"	140	"

To a certain extent this experiment confirms Grubert's statement that the fibrin-ferment is present in muscle; that is, the muscle extract hastens the coagulation of blood-plasma slightly; thus showing that the fibrin-ferment if present at all is in very small quantities; but it is not conclusive, as it has already been shown that as great differences in the time of coagulation occur in specimens which apparently are under similar circumstances, i.e. diluted to the same extent and with the same liquid. It would therefore not be fair to draw conclusions from a single experiment. Other experiments showed however that there was always a slight hastening of the coagulation as in the experiment just recorded.

Fresh muscle-juice however has a very marked influence indeed upon the coagulation of blood-plasma. On adding a few pieces of fresh muscle (from which the blood has been removed), or the juice expressed from it, to specimens of diluted salted plasma the coagulation of the plasma occurs in a few minutes, whereas, if water was employed alone as a diluent, hours might elapse before coagulation set in.

This is illustrated by the following experiment.

Rabbit's sodium sulphate plasma. Each specimen was diluted to six times its volume, and all were allowed to remain at the atmospheric temperature.

1.	Diluted with water	Coagulation occurred in		95	minutes
2.	Diluted with water; a few pieces of cat's muscle free from blood added	"	"	45	"

When the juice expressed from the muscle was mixed with water, and this diluted muscle-juice added to the plasma, the hastening of coagulation was even more marked; as is seen in the following experiment.

Horse's sodium sulphate plasma. Diluted in each case with six times its volume of liquid.

1.	Diluted with water	Coagulation occurred in 96 minutes
2.	Diluted with a watery extract of fresh muscle	„ „ 18 „

This was always found to be the case, and the hastening of the coagulation is so pronounced, that it is remarkable if such hastening is due to fibrin-ferment, that it is not possible to obtain a very active fibrin-ferment from the alcoholic precipitate of muscle.

The action of fresh muscle upon blood-plasma must either be due to fibrin-ferment, or to something else. It seems improbable that it is due to fibrin-ferment because so little fibrin-ferment is obtainable from it. What then can be the other substance to which the action of muscle-juice is due? Grubert as we have already seen suggests that it is the problematical precursor of the ferment, which is converted into the ferment by the action of blood-plasma.

I sought to elucidate the question by seeing at what temperature the power of the muscle-juice to exert this power is destroyed.

A large quantity of diluted muscle-juice was obtained by pounding finely divided muscle (freed from blood in the usual way) with water. The water was found to contain all the proteids of the muscle-plasma in small but quite appreciable quantities: they could for instance be separated from one another by the process of fractional heat coagulation, the series of precipitates occurring at the successive temperatures 47°, 56°, 63°, and 73° C. The proteids of the globulin class contained in this series were enabled to enter into solution by the salts present in the muscle, which were dissolved at the same time. This aqueous muscle extract was heated to various temperatures; the precipitate which occurred was filtered off in some cases, not in others, and the time at which coagulation occurred in the specimens of salted blood-plasma to which it was added accurately noted.

The following illustrates the result obtained:—

Horse's plasma. Diluted to six times its volume in each case, and allowed to stand at the atmospheric temperature. In the cases in which the diluting fluid was heated, it was always cooled down to the temperature of the air before being added to the plasma.

		Coagulation occurred in
1.	Diluted with water	96 minutes.
2.	Diluted with fresh muscle extract	18 ,,
3.	,, Previously heated to 40° C.	23 ,,
4.	,, ,, ,, 50°	23 ,,
5.	,, ,, ,, 55°	23 ,,
6.	,, ,, ,, 55°	23 ,,
7.	,, ,, ,, 60°	108 ,,
8.	,, ,, ,, 65°	104 ,,
9.	,, ,, ,, 70°	96 ,,

From which it is seen that the activity of the substance in muscle which brings about the coagulation of blood-plasma is destroyed by a temperature between 55° and 60°. In all the foregoing specimens, if any proteid were precipitated by heating the muscle extract, the precipitate was removed and the filtrate used as the diluting fluid.

In two specimens the process of filtration was omitted, and the flocculi of suspended coagulated proteid were mixed in with the plasma. The particulars of these two cases are as follows:—

		Coagulation occurred in
10.	Diluted with fresh muscle extract previously heated to 60°	46 minutes.
11.	,, ,, ,, 80°	55 ,,

In which it is seen that the coagulation of the blood-plasma did not occur so quickly as when the muscle extract had not been heated, but still coagulation was more rapid than when water was used for the purpose of dilution.

The following experiment illustrates the same facts. It was performed in a similar way; the muscle extract was heated to various temperatures, and cooled before being added to dilute the horse's salted plasma employed in this as in the preceding experiment. With each however a control experiment was done in which water was used to dilute the plasma to an equal extent (six times the volume of the plasma employed), which had been heated to the same temperature and then cooled.

The following table gives the results.

A. Diluted with water	Coagulation occurred in	B. Diluted with muscle extract	Coagulation occurred in
1. At temperature of air 13·5° C.	56 minutes	1. At temperature of air 13·5° C.	13 minutes
2. Heated previously to 40° C.	46 „	2. Heated previously to 40° C.	14 „
3. „ „ 50°	47 „	3. „ „ 50°	14 „
4. „ „ 55°	46 „	4. „ „ 55°	13 „
5. „ „ 60°	49 „	5. „ „ 60°	53 „
6. „ „ 70°	54 „	6. „ „ 60°	48 „
7. „ „ 80°	49 „	7. „ „ 80°	55 „
		8. „ „ 60° but the precipitated proteid was not filtered off	24 „

This again shows, that the substance which is the active agent in producing coagulation can be destroyed by heating the muscle extract to 60° C.

At what temperature is fibrin-ferment destroyed? The text books state that it is destroyed by a temperature of 80° C.; and this I have confirmed in a few experiments. The following will serve as illustrations:—

Horse's plasma. Dilution in each case six times the volume of salted plasma used.

			Coagulation occurred in
1.	Diluted with solution of fibrin-ferment	Previously heated to 40° C.	81 minutes.
2.	„	„ „ 50°	92 „
3.	„	„ „ 60°	84 „
4.	„	„ „ 70°	91 „
5.	„	„ „ 75°	135 „
6.	„	„ „ 80°	143 „

It is between 75° and 80° then that the activity of the fibrin-ferment is destroyed. The plasma used in this experiment will be seen to be one in which coagulation occurred very slowly; three further experiments were performed with it which illustrate that a heightened temperature has no effect in altering the ability or rather inability of myosin-ferment to hasten the formation of fibrin.

			Coagulation occurred in
7.	Diluted with solution of myosin-ferment	at temperature of air 13·5° C.	226 minutes.
8.	,,	previously heated to 80° C.	219 ,,
9.	Diluted with water		230 ,,

The fibrin-ferment in experiments 5 and 6 above was no doubt exposed to the temperatures of 75° and 80° C. for too short a time (2 or 3 minutes) to completely destroy its activity, as is seen by comparing them with experiment 9 in the above list.

A second experiment which illustrates the destruction of the activity of the fibrin-ferment by a temperature of 80° C. is the following :—

Horse's salted plasma. Diluted to six times its volume in each case, and each allowed to stand at the atmospheric temperature.

1.	Diluted with water	Coagulation occurred in	263	mins.
2.	Diluted with fibrin-ferment solution	,, ,,	19	,,
3.	Diluted with fibrin-ferment solution previously heated to 80° C.	,, ,,	100	,,
4.	ditto	,, ,,	167	,,

This is an experiment which illustrates the effect of temperature very forcibly, as the plasma employed was one on which the activity of fibrin-ferment could be very well demonstrated.

At first sight then it seems that the point is so far settled: the activity of the fibrin-ferment is destroyed at a temperature of 80° C., that of the substance in muscle which induces coagulation at a temperature between 55° and 60° C.; therefore the two cannot be identical. But the question cannot be answered in quite so rapid a fashion, for there is yet a third possibility. It may be that the precipitate which occurs at 56° C. carries down with it mechanically the ferment, and that this is removed when the coagulated proteid is filtered off; and thus it appears that whatever it is that induces coagulation has its activity destroyed at that temperature. At first sight this is a valid objection; for it is seen that when the coagulated proteid is not removed by filtration, there is still some acceleration of the process of coagulation of the blood-plasma. But on examination this objection will not be found to hold good; for the presence of particles of coagulated proteid suspended in the blood-plasma might also by mechanical means hasten the formation of fibrin, just as solid particles of carbon will. I am the more inclined to accept this explanation, because these solid particles of coagulated proteid are equally efficacious in producing a slight hastening of the formation of fibrin after they have been heated

to 80° C., i.e. above the temperature at which fibrin-ferment is destroyed. If fibrin-ferment was mechanically carried down by the proteid which is precipitated at 56° C., why should it not be equally mechanically carried down by the proteid which coagulates at 47° C.? But we have seen that after filtering off the 47° coagulum, the ability of muscle-juice to hasten the formation of fibrin remains as great as before.

Experimental proof is however better than theorising, and therefore I performed the following experiment of mixing muscle-juice with real fibrin-ferment, and then seeing whether the proteid which is precipitated in the former by the temperature of 56° C. carried down with it the ferment or not; and the result of the experiment showed that it did not. The details are as follows:—

Horse's sodium-sulphate plasma. Each specimen was diluted to six times its bulk. The specimens were all allowed to stand after dilution at the atmospheric temperature (14° C.).

1.	Dilution with fibrin-ferment solution	Coagulation in	18 mins.
2.	,, ,, ,, ,,	,,	19 ,,
3.	,, ,, ,, ,,	,,	15 ,,
4.	,, ,, aqueous extract of fresh muscle	,,	10 ,,
5.	,, ,, aqueous extract of fresh muscle previously heated to 60° and filtered	,,	40 ,,
6.	,, ,, equal parts of extract of fresh muscle and fibrin-ferment solution	,,	14 ,,
7.	,, ,, ditto	,,	14 ,,
8.	,, ,, equal parts of extract of fresh muscle and fibrin-ferment heated to 60° C. filtered and cooled	,,	14 ,,
9.	,, ,, ditto except that filtration was not performed	,,	12 ,,
10.	,, ,, equal parts of extract of fresh muscle and fibrin-ferment heated to 80° C. filtered and cooled	,,	38 ,,

We have now reached this point in the argument. An extract of fresh muscle has a very great power of hastening the formation of fibrin. This is either due to fibrin-ferment or to something else. It is probably not fibrin-ferment, first because little or no fibrin-ferment can be obtained from the muscle (what little there is could be quite well explained by

supposing its source to be some small amount of lymph left in the muscle after the blood has been washed out), and secondly, because its activity is destroyed at a much lower temperature than that at which the activity of fibrin-ferment is destroyed.

We also see that the substance in muscle which hastens fibrin formation is one that has its activity in this direction destroyed by the prolonged action of alcohol, and also by a certain high temperature. What substance is this more likely to be than a proteid which is coagulated by alcohol, and by heat? Now we have four of such proteids in muscle; which of them is it? Surely the one which coagulates between 55° and 60° C. the temperature at which the activity of the substance in question is destroyed. In other words, it is myosin, or rather that constituent of myosin, or myosinogen which coagulates at 56° C., with which we have to deal. So much might be concluded by reasoning alone; the next proceeding was to put this hypothesis to the test of experiment.

Myosin was prepared by taking a magnesium sulphate extract of muscle, and diluting it with a large amount of water, so causing precipitation of the myosin; this was washed by decantation with water, dissolved again in 5 per cent. magnesium sulphate solution, again precipitated and again redissolved; this process was then repeated for a third time; the final solution of myosin so obtained was used in the following experiment.

Pig's sodium sulphate plasma was diluted in each case to six times its volume.

1. Diluted with 5 per cent. magnesium sulphate solution.
2. Diluted with solution of myosin in 5 per cent. magnesium sulphate solution.
3. Similarly diluted, except that the diluting liquid was first heated to 47° C. and the heat coagulum which occurred at that temperature (and which was in this case a very small one) filtered off.

Coagulation not having occurred after 16 hours at the temperature of the air (14° C.) in either specimen, each was again diluted with an equal volume of water; the first specimen coagulated in four hours, the second, that to which the myosin was added, coagulated in 30 minutes, the third coagulated in 35 minutes.

Two other specimens of myosin were also prepared, and they gave corroborative results when tested on the blood plasma of the pig, horse, rabbit, and pigeon.

I will quote the experiment with horse's plasma.

Horse's sodium sulphate plasma. Diluted in each case to six times its volume; each specimen allowed to stand at the temperature of the air.

1. Diluted with water: coagulation occurred in 20 minutes.
2. Diluted with solution of myosin in 2 per cent. sodium chloride solution: here in spite of the salt present, coagulation occurred in 8 minutes.

This therefore confirms the hypothesis advanced that it is the proteid in muscle-juice coagulating at 56° C. which gives that juice its power of hastening the formation of fibrin, such power not being due to fibrin-ferment.

How myosin acts in this way, I do not pretend to be able to say. It may be, as Grubert suggests, that fibrin-ferment is formed from it by the action of blood-plasma. Myosin is not however the only proteid the presence of which favours the conversion of fibrinogen into fibrin; Hammarsten[1] has shown that serum globulin and casein act in a similar way.

Before concluding this section on muscle-ferments, it is necessary to allude to the existence of yet other ferments which have been shown to exist in muscle. Brücke has shown that muscle in common with most of the tissues of the body contains a small quantity of pepsin. This will be more fully entered into in a future paper. O. Nasse[2] showed that the muscle-juice also contains an amylolytic ferment which he supposes to act in the transformation of glycogen into sugar after death. I have made a few experiments on this subject, and can fully confirm Nasse's statement of the existence of this ferment; a watery extract of the dried alcoholic precipitate of muscle changes glycogen into a reducing sugar; it will also act upon starch in a similar way, and in both cases an intermediate product of the nature of dextrin is formed. The action on starch is however slow: at the temperature of 40° C. grape sugar is not discoverable by Fehling's test till after the ferment has acted upon it for five or six hours.

It now only remains to summarize the conclusions arrived at as the result of the observations recorded in the present section:—

1. By keeping muscle under alcohol for some months, most of the proteids are coagulated. Water will however extract from the alcoholic precipitate a proteid which has the characters of an albumose.

[1] "Ueber das Fibrinogen." *Pflüger's Archiv*, xix. 563.

[2] *Zur Anat. und Physiol. der quergestreiften Muskelsubstanz.* Leipzig, 1882.

2. This albumose has the properties of a ferment in causing the coagulation of muscle-plasma; or it may be that the ferment is in very close combination with the albumose.

3. This myosin-ferment as it may be termed does not hasten the coagulation of blood-plasma; nor does fibrin-ferment hasten the coagulation of muscle-plasma; the two are therefore not identical.

4. The juice expressed from muscle however hastens very markedly the coagulation of salted blood-plasma. This is not due to its containing fibrin-ferment, but it is due to the proteid substance myosinogen, which enters into the condition of a heat coagulum at 56° C. Fibrin-ferment is absent, or only present in exceedingly small quantities.

5. The activity of fibrin-ferment is destroyed at 75°—80° C.; the activity of myosin-ferment is not destroyed till the temperature of 100°C. is reached.

VII. The Proteids of Muscle-plasma and Muscle-serum.

Muscle-plasma differs from muscle-serum in the same way that blood-plasma differs from blood-serum; the serum in each case is the liquid residue after the removal of the clot, of myosin in the case of muscle, of fibrin in the case of blood.

Kühne stated that the muscle-serum contains three proteids, as follows:—

1. A proteid which coagulates at 45° C.

2. A proteid which coagulates at 70°—73°, and which has the properties of serum-albumin.

3. An alkaline albuminate, or as we may term it muscle-casein, which is precipitable by acids.

Muscle-plasma would then contain these same three proteids, with the precursor or precursors of myosin in addition. I am not aware of any previous research directed to ascertain the properties of such precursors of the muscle-clot. The name myosinogen, which I have used throughout this paper, was first suggested by Nasse.

This section will treat of my experiments directed to determine, by the processes of heat coagulation and saturation by certain neutral salts, more accurately the differences between the proteids of the muscle-plasma and those of the muscle-serum.

In the first place the salted muscle-plasma[1] was subjected to a process of fractional heat coagulation.

[1] Of rabbit, cat, dog and pigeon.

Precipitates occurred at the following temperatures: 47°, 56°, 63°, 73° C.; that is, four proteids, precipitable at the above temperatures, are present; after filtering these off, a proteid with the properties of an albumose is present; this is not coagulable by heat.

The next liquid investigated was the saline extract of muscle which had undergone rigor mortis. Heat coagula occurred in this at the same four points, and the albumose also was present, remaining unprecipitable by heat.

The third liquid investigated was the liquid which remained after the clot had been separated off by the process of dilution and filtration already described. This liquid, which may be called salted muscle-serum, gave heat coagula at 63° and 73° C., and contained the albumose. The two first (i.e. those coagulating at 47° and 56° C. respectively) had disappeared to form the clot.

The fourth liquid investigated was the myosin redissolved in a saline solution; this gave coagula at 47° and 56°. That is, the two proteïds present in the muscle-plasma, which had gone to form myosin, can be also recognised by heat coagulation when that myosin is redissolved by a 10 per cent. sodium chloride, or 5 per cent. magnesium sulphate solution. The facts ascertainable by heat coagulation can be represented as follows in a tabular form.

Proteids of the muscle-plasma	Proteid precipitated by heat at			47° C.	Proteids which go to form the muscle-clot.	
	,,		,,	56°		
	,,		,,	63°	Proteids of the muscle-serum.	
	,,		,,	73°		
	,,	not	,,	albumose		

No substance of the nature of an alkali-albumin or muscle-casein was discoverable. The reason why it has been stated to exist rests in the fact that one of the above proteids is readily precipitated by acids, and is slightly soluble in excess, as will be stated in its proper place. But after the separation of the heat coagula, there is no proteid present which is precipitated by acid in this way. Acid-albumin or syntonin in extracts of muscle which had undergone rigor mortis was also absent.

Next, the question was investigated as to whether peptone is present. W. Fischel[1] has stated that peptones are present in myomata of the uterus, though absent from the normal uterine walls. M. Muira[2] has found peptone in the heart, liver, and spleen in cases of puerperal

[1] *Zeit. physiol. Chem.* x. 14, 15.
[2] *Virchow's Archiv*, Bd. 101, 316.

fever and phosphorus poisoning. These are the only researches I can find designed to investigate the presence or absence of peptone in muscular tissue. These investigators however did not use the latest and best method of separating peptones from other proteids, viz. the method of saturation with ammonium sulphate; it is therefore possible that they may have been dealing with excess of the albumose normally present in muscle.

The method of separating peptones from other proteids by means of ammonium sulphate was first devised by J. Wenz[1]. He found that by saturating a solution of a mixture of proteids of all kinds with this salt, peptones alone remained unprecipitated, thus correcting the previous observation of Heynsius[2], that peptones are also precipitated by this method. The method has since been very largely employed by Kühne and Chittenden in their researches on the albumoses, globuloses, and other intermediate products of digestion. I have myself[3] previously described a salt which acts in the same way as ammonium sulphate, namely sodio-magnesium sulphate ($MgSO_4 . Na_2SO_4 . 6H_2O$). After many comparative experiments however with these two salts, I am convinced that ammonium sulphate is to be preferred for the purpose, since it effects the precipitation more rapidly; with the double sulphate of magnesium and sodium, the separation of the last portions of the precipitable proteids, especially of the albumoses, is very slow, involving the shaking of the solution with the powdered crystals of the salt for many hours. Ammonium sulphate has also the advantage of being cheaper, and of not undergoing decomposition in the air, as sodio-magnesium sulphate is apt to do.

By saturating salted muscle-plasma, or saline extracts of rigid muscle, with either ammonium sulphate, or sodio-magnesium sulphate, an abundant precipitate was produced; but no proteid remained in solution. Peptone was therefore absent. The precipitate produced by saturation with these salts, which was a very fine one, was collected on a filter, washed with saturated solution of ammonium sulphate, and redissolved by the addition of water. Heat coagulation showed that by the fractional method, the same five proteids (four precipitable by heat, and the fifth an albumose) can be separated from one another.

The action of other neutral salts was then tried. Saturation with magnesium sulphate, or sodium chloride, produced an abundant precipi-

[1] *Zeit. Biol.* XXII. 1.

[2] *Pflüger's Archiv*, XXXIV.

[3] "Report on the Proteids of the Blood." *Brit. Med. Journal*, July 25, 1885.

tate; this precipitate was washed with saturated solution of magnesium sulphate or sodium chloride respectively, and then on applying the method of fractional heat coagulation to this solution, it was found to contain three proteids: viz. those which coagulate at 47°, 56°, and 63 respectively. In the filtrate after the separation of the precipitate, produced by saturation with either of these salts, two proteids were found, namely that which coagulates at 73° C. and the albumose.

If one saturates the salted muscle-serum with either magnesium sulphate or sodium chloride, a precipitate is produced which consists of the proteid which coagulates at 63° C., while that which coagulates at 73° C. and the albumose remain in solution.

It is one of the chief properties of the globulin class of proteids that they are precipitable either wholly or partially by saturation with magnesium sulphate or sodium chloride, while those of the albumin class are not. The three proteids precipitable by these salts are therefore members of the globulin class. This was subsequently confirmed by other tests. The following table represents the result of these experiments of saturating the salted muscle-plasma, or saline extracts of rigid muscle (for the results were the same in each case), with these salts, together with the names I would suggest for the individual proteids.

Proteid precipitated by heat at	Name	Saturation with sodium chloride or magnesium sulphate
47° C.	Paramyosinogen	causes precipitation
56°	Myosinogen	causes precipitation
63°	Myoglobulin	causes precipitation
73°	Albumin	does not cause precipitation
Proteid not precipitated by heat	Myoalbumose	does not cause precipitation

The following scheme represents the manner by which these proteids may be separated from the muscle-plasma, or from a saline extract of rigid muscle, or from a saline extract of muscle in which rigor has passed off, and from one another, by the combined methods of fractional heat coagulation and saturation with salts:—

MUSCLE-PLASMA. Saturate with ammonium sulphate. A precipitate is produced. Filter.

Precipitate: consists of all the proteids. Wash with saturated solution of ammonium sulphate; redissolve by adding water. Saturate this solution with either magnesium sulphate or sodium chloride. A precipitate is produced. Filter.

Filtrate: contains salts, extractives etc., no proteids.

Precipitate: consists of globulins. Wash with saturated solution of magnesium sulphate or sodium chloride, and redissolve by adding water. Heat this solution to 47° C., a precipitate is produced. Filter.

Filtrate: contains albumin and myoalbumose. Heat to 73° C., a precipitate is produced. Filter.

Precipitate: consists of albumin

Filtrate: contains myoalbumose

Precipitate: consists of paramyosinogen

Filtrate: contains myosinogen and myoglobulin. Heat to 56° C., a precipitate is produced. Filter.

Precipitate: consists of myosinogen B.

Filtrate: contains myoglobulin which is precipitated by heating to 63° C.

Or the following method may be adopted:

MUSCLE-PLASMA. Dilute to six times its volume, and expose this to a temperature of 35° C. for 1 or 2 hours. It separates with clot, and salted-muscle serum. Filter.

Clot: consists of myosin. Wash with water, redissolve in 5% magnesium sulphate solution. Heat to 47°, a precipitate is produced. Filter.

Salted Muscle-serum: contains myoglobulin, albumin, and myoalbumose. Saturate with magnesium sulphate or sodium chloride. A precipitate is produced. Filter.

Precipitate: consists of paramyosinogen

Filtrate: contains myosinogen which is precipitated at 56° C.

Precipitate: consists of myoglobulin

Filtrate: contains albumin and myoalbumose. Heat to 73°, a precipitate is produced. Filter.

Precipitate: consists of albumin

Filtrate: contains myoalbumose

We have now to take up these proteids one by one, and discuss the method of preparation, and reactions of each.

We shall also have to take into account two other substances allied to proteids in composition which form the pigments of the muscle-plasma, and which are haemoglobin and myohaematin. The remainder of this section will therefore be divided into seven paragraphs, the subjects of which will be

1. Paramyosinogen.
2. Myosinogen.
3. Myoglobulin.
4. Albumin.
5. Myoalbumose.
6. Haemoglobin.
7. Myohaematin.

1. *Paramyosinogen.* That a proteid substance which coagulates between 45° and 50° C. can be extracted from muscle was first shown by Kühne in his work on the muscle-plasma. This substance has been also worked at by B. Demant[1]. He found that by heating a watery extract of muscle to 40°—45° C. a cloudiness appears, which becomes a flocculent precipitate at 47° C. and this only when the reaction of the liquid is neutral or acid. He states that it is partially precipitable by saturation with magnesium sulphate. The amount in which this proteid was present was estimated by collecting and weighing the precipitate which occurred in extracts of the muscle. The muscle was repeatedly extracted with water, until by heating the extract no precipitate was found to occur at 47° C. In many cases the merest traces, too small for weighing, were present. The average amount found in the muscles, voluntary and involuntary, of the dog, rabbit, and pigeon, was a half per cent. In a later paper Demant[2] speaks of this proteid as an albumin, and it has been very generally spoken of as a constituent of the muscle-serum; whereas it is in reality a globulin, and a constituent of the muscle-clot.

Although it can be extracted from muscle by means of water, this is simply due to the fact that the soluble salts of muscle enter into solution at the same time; in fact, as has been already shown in this paper, all the proteids in muscle will similarly enter into solution. It is

[1] *Zeit. physiol. Chem.* III. 241.
[2] *Zeit. physiol. Chem.* IV. p. 386.

moreover completely removed from a saline extract of muscle by saturating that extract with either magnesium sulphate or sodium chloride. It is also precipitated by dilution, or after the salts have been removed by dialysis, from a salted muscle-plasma, or saline muscle extract. These two facts clearly show that it is a member of the globulin class of proteids.

This proteid has also been counted as one of the constituents of the muscle-serum, since an extract of rigid muscle is found to contain it; but as we have already seen this is no proof of its being in the serum, since the liquid employed to extract the muscle is able to dissolve it out of the muscle-clot. This is most conclusively proved by the fact, that if one takes the muscle-clot, and redissolves that, it is possible to obtain from it its two constituents—paramyosinogen and myosinogen. It is however found that after redissolving and reprecipitating myosin several times, the amount of paramyosinogen which ultimately goes into solution is very small; it seems to be more and more held closely in combination with myosinogen. This is perhaps the change which simultaneously results in the formation of sarkolactic acid.

How can one separate paramyosinogen from the other proteids of muscle-juice? The simplest method is by heat coagulation. It coagulates at 47° C. The coagulum which forms is a finely flocculent one, quite unlike that of myosinogen which is sticky; this can be collected on a filter, and it is then found to have the usual characters of coagulated proteid. Although this is a good plan of getting myosinogen free from its admixture with paramyosinogen, it does not give us the latter in such a form as enables us to investigate its properties. The method of fractional precipitation by means of salts was therefore adopted.

Magnesium sulphate was the first salt investigated with this end in view. 100 parts of crystalline magnesium sulphate dissolve in 79 parts[1], or, in round numbers, in 80 of water. An extract of muscle in 5% magnesium sulphate was taken, and divided into portions of 80 c.c. each. To each portion was added a weighed quantity of magnesium sulphate, as follows:—

To (1) 21 grammes of sulphate were added; the 80 c.c. would therefore contain 25 grammes of the salt; i.e. the fluid was a quarter saturated. This produced no precipitate.

To (2) 26 grammes of the salt were added; this produced a very slight precipitate indeed.

[1] Watts' *Dictionary of Chemistry.*

To (3) 40 grammes of the salt were added. This produced a fairly abundant precipitate; it was collected, washed with water containing 44 grammes of magnesium sulphate to every 80 c.c. of water, and redissolved by adding water. This solution coagulated at 47° C. The filtrate, after separating the precipitate produced by this amount of salt, coagulated at 56°, 63° and 73°.

To (4) 46 grammes of the salt were added, i.e. the fluid was thus half-saturated: the precipitate consisted of proteids coagulating at 47° and 56°; the filtrate gave heat coagula at 56°, 63° and 73°. That is, the proteid coagulating at 56° was only partially precipitated by half-saturation; that at 47° wholly as in (3).

It will be seen from the foregoing that the addition to a 5% magnesium sulphate extract of muscle of 50 grammes of magnesium sulphate for every 100 c.c. of liquid (a proportion rather under half saturation) will effect the separation of paramyosinogen from myosinogen. This precipitation begins when the proportion of magnesium sulphate present is about 37 grammes to the 100 c.c. of extract.

The precipitate produced by magnesium sulphate occurs in white curdy flakes.

A similar separation can be brought about by sodium chloride, and the precipitated proteid has a similar curdy appearance. The precipitation of the proteid is accomplished when from 15 to 26 grammes of sodium chloride are present in 100 c.c. of solution. When more salt than this is added myosinogen is also precipitated. When less than 26 grammes to the 100 c.c. of solution are added, the precipitation of paramyosinogen is incomplete.

By either of the two foregoing methods then a solution of paramyosinogen not mixed with any other proteid can be obtained. By this method also the same fact is shown to which Demant drew attention, that the quantity of this proteid is exceedingly variable. In some cases, there is not more than a slight cloudiness produced either by heating the muscle extract to 47° or by adding the necessary proportion of salt[1].

The proteid obtained in this way free from other proteids gave the usual proteid reactions. It is completely precipitated from its solutions by saturating with either sodium chloride or magnesium sulphate; satura-

[1] In the foregoing experiments, the reaction of the fluid to which the salts were added was neutral or faintly acid; the temperature of the air at which they were performed varied from 13° to 15° C.

tion is not however necessary, as is seen by referring to the methods just described as used in its preparation. It is not precipitated from its saline solution by acetic acid. It is precipitated by dialysing out the salts from its solutions. It does not form a coagulum when its solution is diluted either with water, or with solution of the myosin-ferment. The precipitate of this proteid obtained by dialysis is not soluble in 10% sodium chloride solution, but it is soluble in weak acids and in alkalies. The precipitate produced by saturation with sodium chloride or magnesium sulphate is also rendered insoluble by prolonged washing with saturated solutions of these salts.

2. *Myosinogen.* This is the proteid in muscle-plasma which coagulates at 56° C. (the precipitate being a sticky one) and which is apparently the same as that which coagulates at the same temperature when myosin is redissolved. 56° C. is the usual temperature of coagulation given for redissolved myosin.

It can be prepared in several ways: (1) myosin is redissolved; the 47° C. heat coagulum of paramyosinogen is filtered off, myosinogen remains in solution. (2) By fractional precipitations with magnesium sulphate. Take muscle-plasma, and add sufficient salt to precipitate paramyosinogen, i.e. 50 grammes of magnesium sulphate to every 100 c.c. of muscle-plasma; filter off the curdy precipitate of paramyosinogen. Myosinogen remains in solution, and may be precipitated by adding magnesium sulphate in such a proportion that each 100 c.c. of liquid contains 94 grammes of the salt, that is the liquid is three-quarters saturated; this separates the myosinogen from myoglobulin which is not precipitated until the liquid is fully saturated with the salt. Sodium chloride cannot be used to separate these two proteids, as complete saturation with that salt is required to cause complete precipitation of both proteids (i.e. 36 grammes of sodium chloride present in each 100 c.c. of liquid).

The following table states succinctly these facts concerning the precipitation of these three globulins of muscle-plasma by the method of fractional saturation, the solution being neutral or faintly acid.

	Magnesium sulphate precipitates it when present in the following proportion	Sodium chloride precipitates it when present in the following proportion
Paramyosinogen	37 grammes of salt to the 100 c.c. of solution, precipitation begins	15 grammes of salt to the 100 c.c. of solution, precipitation begins
	50 grammes of salt to the 100 c.c. of solution, precipitation complete	26 grammes of salt to the 100 c.c. of solution, precipitation complete
Myosinogen	60 grammes of salt to the 100 c.c. of solution, precipitation begins	30 grammes of salt to the 100 c.c. of solution, precipitation begins
	94 grammes of salt to the 100 c.c. of solution, precipitation complete	complete saturation is necessary for the complete precipitation of this proteid, i.e. 36 grammes of salt to the 100 c.c. of solution
Myoglobulin	precipitation only occurs when the saturation is complete, i.e. 125 grams of salt to the 100 c.c. of solution	precipitation only occurs when the saturation is complete

It is found that, after filtering off the heat coagulum produced in muscle-plasma or saline extracts of muscle by the temperature of 47° C., dilution of the filtrate still produces the formation of a clot of myosin. The clot is formed under the same conditions as those in which both forms of myosinogen are present, i.e. most rapidly at a temperature of 35°—40° C. and especially when solution of myosin-ferment is used as the diluting liquid. The clot which is formed differs in amount from that formed when both varieties of myosinogen are present. This is illustrated by the following experiment:—

The weight of the clot (washed with distilled water and subsequently dried to constant weight at 115° C.) obtained from 100 c.c. of a 5 per cent. magnesium sulphate extract of muscle was 1·82 grains. The weight of clot obtained from 400 c.c. of the same extract from which the paramyosinogen had been just removed by heating to 47° C. and filtering was 1·58 grains.

But not only is the clot formed under these circumstances less in amount than normal, but its solution in weak magnesium sulphate

solution coagulates at 56° C. without showing any trace of a 47° precipitate; otherwise its properties are those of normal myosin. It would thus appear that myosinogen is essential to the formation of myosin, whereas paramyosinogen is not so essential, but, when present, is carried down by the change of myosinogen into myosin, and thus constitutes part of the muscle-clot.

It has been previously shown that attempts to obtain myosin free from ferment by washing the clot with distilled water failed because prolonged washing with water renders the myosin ultimately insoluble in saline liquids. Hence it was always found that solutions of redissolved myosin coagulated after dilution when exposed to the temperature of 40° C. even though no ferment was added; the addition of ferment however hastened the coagulation.

The method of saturation of muscle-plasma with magnesium sulphate or sodium chloride was therefore used to see whether by this means myosinogen might not be obtained free from ferment; and the result showed that it could. The details of one experiment are as follows. Two others were performed with confirmatory results:—

An extract of rabbit's muscle (freed from blood in the usual way) was made with 5 per cent. magnesium sulphate solution, and also with 10 per cent. sodium chloride solution.

a. The magnesium sulphate extract. This was saturated with magnesium sulphate, and the precipitate so produced washed by decantation with a saturated solution of magnesium sulphate three or four times.

The precipitate suspended in a small quantity of saturated solution of this salt was then dissolved by diluting the saturated solution with distilled water, until the strength of the magnesium sulphate solution was 5 per cent. The precipitate dissolved completely. It was then further diluted to four times its bulk, in one specimen with water, in another with solution of myosin-ferment. No coagulation occurred in the first, all the ferment having been removed from the myosinogen by washing it with the saturated solution of magnesium sulphate. This specimen was watched for 24 hours, half of it being kept at the atmospheric temperature, the other half at the temperature of 35° C.; but after the lapse of this time no coagulation had occurred in either specimen. The specimen on the other hand to which the myosin-ferment solution had been added gave in 3 to 4 hours at the temperature of 35° C. a very distinct clot of myosin.

b. The sodium chloride extract. This was treated in the same way as the magnesium sulphate extract; sodium chloride was however used to saturate the fluid instead of magnesium sulphate. Here however the ferment

was not all washed away from the myosinogen so successfully, as is seen from the result of diluting the 10 % sodium chloride solution of myosin ultimately obtained :—

1. Diluted to four times its volume with water. Kept at the temperature of the air for 24 hours. No clot formed.

2. Diluted similarly, but kept at the temperature of 35° C. after 12 hours. A very small clot had formed.

3. Diluted to a similar extent with solution of myosin-ferment. At the temperature of the air a distinct clot formed in about 15 hours.

4. Diluted similarly, but kept at the temperature of 35° C. A distinct clot formed in from 3 to 4 hours.

In this way then, it is possible to obtain a pure solution of myosinogen, which will not clot and form myosin unless the ferment be added to it.

But as will be seen both paramyosinogen and myosinogen are present in such solutions obtained by the method just described. The question which arises here is, Does the ferment act on both, or only one of these two proteids? This was put to the test of experiment.

A solution of paramyosinogen in 5 % magnesium sulphate solution was prepared as described a few pages back. This was diluted with water, and also with solution of myosin-ferment, but in neither did coagulation occur.

A solution of myosinogen was then prepared by heating a mixture of the two proteids in 5 % magnesium sulphate solution to 47° C. and filtering off the precipitated paramyosinogen. To this solution of myosinogen, water was added to dilute it; the merest trace of clot formed after 24 hours' exposure to the temperature of 35° C. Another specimen of the same solution was diluted with solution of myosin-ferment to the same extent; in this, coagulation occurred after about 2 hours' exposure to 35° C. These experiments furnish an additional proof of the statement previously made, that myosinogen is the essential precursor of myosin, paramyosinogen being a constituent of the muscle-clot, but only accidentally so, as it were. That this is the case is also supported by the fact that paramyosinogen is sometimes absent or nearly so from salted muscle-plasma; heating to 47° C. producing sometimes the merest cloud.

Another difference was brought out between the two forms of myosinogen by treatment with magnesium sulphate. It was found that mere saturation with this salt produced when added to a muscle extract in all cases an abundant precipitate; but this precipitate varied in appearance, being in some cases a fine precipitate which collected on

the filtering paper as a slimy almost gelatinous scum, while at other times this was mixed to a varying extent with a curdy precipitate which settled in large flocculi. The explanation of this was made clear by the fractional saturation experiments. The curdy precipitate consists of paramyosinogen; the slimy gelatinous precipitate of myosinogen. By means of sodium chloride a similar difference is seen, but it is not nearly so marked.

Both forms of myosinogen however resemble each other in this:— that after prolonged washing with saturated solutions of salt they became insoluble in dilute solutions of salt. This reminds one forcibly of the way in which the muscle-clot becomes insoluble after prolonged washing with water. It is not the mere exposure to a saturated saline solution, but the washing with that solution that makes the myosinogens insoluble. This is illustrated by the following experiment.

Rabbit. A 10 per cent. sodium chloride extract of muscle prepared and saturated with sodium chloride. The liquid with the precipitate so produced suspended in it was divided into two parts *a* and *b*.

a. In this the precipitate was allowed to settle. After ten days it was found to be easily soluble by diluting the concentrated solution of sodium chloride till its strength was 10 per cent.

b. In this the precipitate was allowed to settle; and then it was washed by decantation with saturated sodium chloride solution, ten times. After ten days, the concentrated saline solution was diluted till its strength was 10 per cent. The greater part of the precipitate was insoluble: that which did enter into solution was myoglobulin as shown by the fact that it coagulated (after dialysing away the greater part of the sodium chloride) at 63° C.

A similar experiment performed with a magnesium sulphate extract of pigeon's muscle showed that washing the precipitate of paramyosinogen and myosinogen with a saturated solution of magnesium sulphate also rendered it insoluble. It need hardly be said that in previous experiments, which described the re-solution of precipitated myosinogen, the process of washing with a saturated saline solution was not carried so far as to render that precipitate insoluble.

It is noteworthy in this connection to find that the vegetable myosins described by Dr Sidney Martin[1] become insoluble in saline solutions after prolonged dialysis, in this way resembling the myosin of muscle.

We now come to the way in which myosinogen behaves to acids.

It is precipitable like other proteids by the strong mineral acids.

[1] *Proc. Physiological Society*, 1886, p. viii.

It is also precipitable from its saline solutions by very weak acetic acid. A salted muscle-plasma or a saline extract of muscle gives a very copious precipitate on the addition of a few drops of 2 % acetic acid; so does a solution formed by redissolving the muscle-clot. This precipitate has in all cases a stringy character, very like the precipitate formed by adding acetic acid to saliva containing mucin, and it is but little soluble in moderate excess; in excess of stronger acetic acid, it readily dissolves. This precipitate is not given by a specimen of salted muscle-plasma after it has been heated to 56° and the precipitated proteid filtered off. This fact, together with the fact that it is produced in a solution of the muscle-clot, shows that it must be due to either paramyosinogen or myosinogen. As has been already stated, it is not given by a solution of paramyosinogen; it must therefore by the process of exclusion be due to myosinogen. This is confirmed by the fact that a solution of myosinogen does give this characteristic stringy precipitate with acetic acid.

It is probably this precipitation by weak acid that led previous observers to the conclusion that an alkali-albumin or casein is present in muscle. But just as the so-called 'serum-casein' was shown to be serum-globulin partially precipitated by the acid, so can the 'muscle-casein' be shown to be similarly part of the proteid precursor of myosin.

I have made experiments with other weak acids; and the following is the result:—

2 % phosphoric acid gives a precipitate which is not stringy and readily dissolves in excess.

1 % sulphuric acid gives a somewhat stringy precipitate, not soluble in excess.

1 % oxalic acid gives an abundant precipitate not so stringy as with weak acetic acid, not soluble in excess.

1 % nitric acid gives a precipitate, stringy only to a slight extent and slightly soluble in excess.

The fact that weak acids do give such a precipitate renders it impossible to add more than the faintest trace of acid to a liquid containing myosinogen before one determines the heat coagulation temperatures in that liquid.

In the case of saline extract of rigid muscle, such addition of acid is not necessary, as the lactic acid present confers sufficient acidity on the liquid for the purpose.

3. *Myoglobulin.* This can be best prepared from salted muscle-serum by saturation with either magnesium sulphate or sodium chloride. Precipitation does not occur until saturation is complete. This fact has been already dwelt upon in connection with the separation of this substance from the other globulins of muscle-plasma by the method of fractional saturation with salts. The precipitate as produced is then washed with saturated solution of the salt, and redissolved by the addition of water, the salt adherent to the precipitate enabling it to dissolve. By dialysing away the salt from a solution of this proteid, the latter is precipitated, so showing it to be a true globulin. In its reactions it thus resembles paraglobulin or serum-globulin very closely; it differs from serum-globulin in its coagulation temperature which is 63° C. while that of serum-globulin is 75° C. Myoglobulin differs from myosinogen, in not being rendered insoluble in saline solution either by prolonged dialysis or by washing with saturated solution of salt.

It takes no part in the formation of the muscle-clot; and as has been before stated (p. 149) it is not a result of the formation of myosin, as serum-globulin in part is stated to be a result of the formation of fibrin.

4. *Albumin.* This is apparently identical with serum-albumin as stated by Kühne, and more recently by Demant[1]. It coagulates at the same temperature 73° C., and is the only proteid of muscle-plasma coagulable by heat which is not precipitable by saturation with sodium chloride or magnesium sulphate. It is not precipitable by ether. It is often present in exceedingly small quantities.

I have however elsewhere[2] shown that serum-albumin is really a mixture of three different proteids which I have named α, β, and γ, coagulating at 73°, 77° and 84° C. respectively. The muscle-albumin is identical with serum-albumin α.

5. *Myoalbumose.* The properties of this substance have already been partially described in dealing with the myosin-ferment. It may be prepared by heating a specimen of salted muscle-plasma, or a saline extract of dead muscle, to 73° C. The proteids coagulable by heat are then filtered off, and muscle-albumose remains in solution; or it may be obtained by extracting the dried alcoholic precipitate of muscle with water. Its properties are as follows:—

[1] *Zeit. physiol. Chem.* IV. 384.

[2] This *Journal*, Vol. V. p. 157.

(1) Nitric acid in the cold gives a very slight precipitate; this disappears on boiling and reappears on cooling the solution. If a solution be first boiled, and nitric acid added to the solution while it is still hot, no precipitate at all is produced, but on cooling, the precipitate forms, which on boiling again completely disappears. The precipitate produced by cooling the albumose solution to which nitric acid has been added when the solution was hot, is more abundant than the precipitate produced by simply adding nitric acid in the cold.

(2) If the albumose solution is first saturated with sodium chloride, nitric acid produces a more abundant precipitate than if no salt be added.

(3) Glacial acetic acid causes no precipitate unless the solution be first saturated with sodium chloride. Glacial phosphoric acid acts in the same way. The precipitation is in either case an incomplete one.

(4) It is completely precipitable by saturation with ammonium sulphate, and also by sodio-magnesium sulphate.

(5) It is not precipitated by saturation with sodium chloride or magnesium sulphate in a neutral alkaline or slightly acid solution. When the solution is made strongly acid with glacial acetic acid, there is partial precipitation. The fact that the albumose is not precipitated by these salts in a neutral or slightly acid solution enables one to separate myosinogen from the myosin-ferment in the manner already described (pp. 193, 194).

(6) It is precipitable by mercuric chloride in a neutral as well as in an acid solution.

(7) It is not precipitable by copper sulphate.

(8) When concentrated, it gives the biuret reaction faintly. The relative amount of albumose in unconcentrated extracts is never sufficient to give any colour with copper sulphate and caustic potash.

(9) It is not precipitable by dialysing the salt away from its solutions.

(10) It is precipitable by alcohol, but not converted into coagulated proteid by it. These reactions clearly show that the substance is an albumose, and that it resembles especially the deutero-albumose of Kühne and Chittenden[1]. I have used the word albumose in a generic sense, meaning by it proteids which have properties resembling those of the intermediate products of the conversion of proteids into peptones. But as the word albumose is now restricted to the intermediate products

[1] The most recent paper which gives the properties of deutero-albumose is by R. Neumeister. *Zeit. Biol.* XXIII. p. 381.

formed in the digestion of albumin, while the names globulose, vitellose, caseose, etc. have been given to similar products of the digestion of globulin, vitellin, casein, etc. respectively, the word proteose would perhaps be more correct as implying no theory as to which form of proteid this substance is most clearly allied. Albumoses, globuloses, etc. however resemble each other in their reactions, and differ but little in elementary composition.

6. *Haemoglobin.* The red muscles of animals as is well known contain a quantity of haemoglobin in the muscle-plasma. A few experiments were performed in order to determine whether the presence of this proteid alters in any way the behaviour of the plasma, or saline extracts, obtained from such muscles. It was found that there was no difference in their general properties. Extracts of the red muscles of rabbits, and of the breast muscles of pigeons freed from blood in the usual way, were prepared, and differed only from the extracts of the pale muscles, which were exclusively used in all experiments related up to this point, in giving the haemoglobin spectrum. The haemoglobin takes no part in the formation of the muscle-clot, and can be recognised by the spectroscope, in the salted muscle-serum, as one of its constituents. There is also no difference in the heat coagulation of preparations from red muscle: precipitates form at the same temperatures, as in those from the pale muscle. The liquid however becomes brownish about 70° C., and as haemoglobin is decomposed and precipitated by heat between 70° and 80° C. it is no doubt carried down with the serum-albumin which is precipitated at 73° C.

7. *Myohaematin.* This colouring matter has been recently described by MacMunn[1] as a constituent of muscle-plasma. It is considered by him to be a substance allied to a proteid, and it is therefore important to investigate its properties in connection with the subject of this paper. The investigation of this substance however opens up a wide field, which it would be rather beside the purpose of this paper to enter into. I intend at a future time to investigate myohaematin with a view to obtaining it in a pure state, for the purpose of elementary analysis. I will here only mention a few preliminary observations that I have made on the subject.

I was never able to prove the presence of this pigment in any of the saline extracts of muscle that I prepared even though I used the breast muscles of the pigeon which contain it in abundance. No doubt it was obscured by the bands of oxyhaemoglobin with which it was mixed. On

[1] *Phil. Trans.* Part 1, 1886, p. 267.

adding ammonium sulphide, the spectrum of reduced haemoglobin was seen, but no bands due to myohaematin. MacMunn found similar difficulties in getting the myohaematin into solution, and even in the expressed muscle-juice, the bands of it were so faint that the band at D was not visible, and the others were only rendered apparent by adding ammonium sulphide. No doubt myohaematin if dissolved in any saline solutions was present in too small a quantity to show any spectrum in the presence of oxyhaemoglobin.

In my attempts to get myohaematin pure, I have employed a method which was shown by MacMunn to the Physiological Society (1886, p. 1), but which so far as I am aware has not been published[1]. The breast muscles of the pigeon are chopped up finely and allowed to stand under ether for twenty-four hours; a yellow lipochrome (the nature and source of which I have elsewhere described[2]) is dissolved by the ether; a yellowish-red watery fluid floats below this. This watery fluid is withdrawn by a siphon or pipette, and filtered. It is acid in reaction. It contains a small amount of all the proteids of muscle-plasma (as tested by heat coagulation after neutralisation). It smells slightly of ether; it is deep yellowish-red in colour, and it shows with the spectroscope the bands of what MacMunn calls modified myohaematin. This gives a band half way between the D and E lines, and a fainter one on the red side of the B line[3]. There is no trace of haemoglobin bands visible. A question which will arise here is, What has become of the haemoglobin, which as has already been mentioned exists in these muscles? In order to determine this, I subjected several specimens of blood-clot to the same treatment as the pigeon's muscle; that is they were chopped into small pieces, and placed under ether. But no yellowish-red or any other coloured watery fluid floated under the ether: the pieces of clot set into hard solid lumps, the proteids and the haemoglobin being very firmly coagulated by the ether. And this no doubt is what happens to the haemoglobin in the muscle; it is coagulated *in situ*, leaving the myohaematin in what MacMunn calls its modified condition in solution.

By boiling the yellowish-red fluid so obtained, the coagulable proteids are precipitated and can be filtered off. Albumose remains in solution and so does the myohaematin. On boiling this solution, its colour becomes more yellowish, and its bands completely disappear. They reappear again however when the liquid cools.

[1] It has been published since the above was written. This *Journal*, Vol. VIII. p. 54.

[2] This *Journal*, VII. p. 325.

[3] *Phil. Trans.* Part 1, 1886, Plate 12. Chart IV. spectrum 18.

The separation of the myohaematin from the albumose I have not yet accomplished. Both are precipitated by alcohol; the precipitate so formed is white, and myohaematin cannot be removed from it. Both are precipitated by saturation with ammonium sulphate; the precipitate so obtained is soluble on adding water, and the solution shows especially after adding a drop of ammonium sulphide the modified myohaematin spectrum. Neither is precipitable by saturation with magnesium sulphate or sodium chloride. After saturation with sodium chloride, the fluid becomes slightly greenish in tinge; this is perhaps due to the formation of free hydrochloric acid from sodium chloride and lactic acid[1]; the liquid however still gives the same spectrum on adding a drop of ammonium sulphide. So far then attempts to separate the albumose and the myohaematin have failed. It is of course quite possible that myohaematin is a coloured albumose.

I shall conclude this section with a summary of the properties of the proteids of muscle-plasma.

Paramyosinogen.	Myosinogen.
1. It coagulates at the temperature of 47° C. The precipitate is flocculent.	1. It coagulates at the temperature of 56° C. The precipitate is sticky.
2. It is partially precipitated in a magnesium sulphate solution of the strength of 37 per cent.	2. It is partially precipitated in a magnesium sulphate solution of the strength of 60 per cent.
3. It is precipitated completely when the strength of the magnesium sulphate solution is 50 per cent.	3. It is precipitated completely when the strength of the magnesium sulphate solution is 94 per cent.
4. It is partially precipitated in a sodium chloride solution of the strength 15 per cent.	4. It is partially precipitated in a sodium chloride solution of the strength 30 per cent.
5. It is precipitated completely when the strength of the sodium chloride solution is 26 per cent.	5. It is completely precipitated when the strength of the sodium chloride solution is 36 per cent. (i.e. saturated).
6. The precipitate produced by these salts is curdy, and settles in coarse flocculi.	6. The precipitate produced by these salts is a fine precipitate which settles into a semi-gelatinous deposit.

[1] Landwehr. *Chem. Centralblatt.* 1886, p. 484.

7. It is rendered insoluble in dilute saline solutions, by prolonged washing with saturated saline solutions.	7. It is rendered insoluble in dilute saline solutions by prolonged washing with saturated saline solutions.
8. It takes part in the formation of the muscle-clot, but does not, by itself, coagulate under the influence of myosin-ferment.	8. It takes an essential part in the formation of the muscle-clot, and coagulates under the influence of the myosin-ferment.
9. It is not precipitated from its saline solutions by acetic acid.	9. Acetic acid gives when added to a solution of this proteid a characteristic stringy precipitate.
10. It has no power in hastening the formation of fibrin in blood-plasma.	10. It has a very marked power of hastening the formation of fibrin in blood-plasma.

Myoglobulin. This resembles serum-globulin very closely in its properties, the most marked difference being in its coagulation temperature which is 63°, while that of serum-globulin is 75° C.

Muscle-albumin is apparently identical with serum-albumin *a*.

Myoalbumose or *Proteose* gives the reactions of the deutero-albumose of Kühne and Chittenden, and to it is closely connected the myosin-ferment, with which it is probably identical.

These three bodies (together with haemoglobin in the case of the red muscles, which alone yield any appreciable quantity of that substance) constitute the proteids of the muscle-serum.