"Muscle Plasma and its Co-agulation"

Thesis presented in 1895 by Robert Main Horne M.B. & C.M., Edin., to the Medical Faculty of the University of Edinburgh for the Degree of M.D.

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I.

Introductory and Historical

This research was commenced in October 1893 and has occupied me during the last eighteen months. All the work has been done in the Physiological Laboratory of the Owens College, Manchester.

The system of references adopted is that now commonly employed - namely by the use of numbers. The numbers in the text refer to the works mentioned under the corresponding numbers in the Bibliography at the end of the Thesis.

Much work has been carried out within recent years which has for its object the elucidation of the nature of the substances concerned in, and the nature of the chemical changes going on during the process of the

coagulation of the blood. In consequence of these researches notably those of Hammarsten, Wooldridge, Halliburton and Lilienfeld on the one hand, and of Green, Ringer and Arthus + Pagés on the other, our knowledge of those substances and chemical changes has been much advanced. New facts have been established which have to some extent modified the older views on the subject.

It is not my intention to enter upon an exhaustive review of this work, but a short account of our present position on this question is necessary in order that we may compare with it the state of our knowledge on the subject of coagulation of muscle.

Schmidt's theory postulated that three substances were

necessary for blood coagulation, namely, Fibrinogen, Serum-globulin and Fibrin Ferment. Haammarsten[14] was the first to shew that Serum-globulin was unnecessary and that Fibrinogen alone, freed from Serum-globulin by preparation with the Sodium Chloride method, coagulated on the addition of Fibrin ferment. Haammarsten's[14] researches led Schmidt[36] to modify his theory. He admitted that coagulation could occur without the presence of Serum globulin but still held that this substance played an important part in the process. He found that more Fibrin was obtained from the coagulation of a given quantity of Fibrinogen when Serum-Globulin was also present than from an equal quantity of Fibrinogen coagulated alone.

Hammarsten's work and the more recent work of Frederikse,[5] however, shew that we cannot ascribe to Serum-Globulin even this rôle in coagulation and that indeed Serum-globulin plays purely a passive part in the process.

Much of the recent work has had for its object the determination of the nature of Fibrin Ferment. Schmidt and his pupils, on various grounds, believed that the Fibrin Ferment was derived from the breaking down of the white corpuscles of the blood. Schmidt was able to prepare it, by means of absolute alcohol, from blood serum. Gaugee obtained it from the "washed blood clot" of Buchanan. The point emphasised by Schmidt was that the ferment was derived from the formed elements.

of the blood. and only when these, be it white blood corpuscles or the more recently discovered blood platelets of Bizzozero, were breaking down. No Fibrin Ferment was obtained from blood which was allowed to pass directly from a blood-vessel into a large quantity of absolute alcohol.

Wooldridge[6] combated this view very strongly. He shewed that peptone plasma coagulated if Carbonic acid gass were passed through it, if it were diluted with water or neutralised with acetic acid. This he took to indicate that the ferment was already present in the plasma and could not have been obtained from the formed elements of the blood as these had been removed by Centrifugal machine.

If however the plasma were

~~previously~~ cooled, a precipitate was produced and the plasma, freed from this precipitate, no longer coagulated after having been treated with Carbonic acid gas, water or acetic acid. If the precipitated substance were added to the filtrate so treated, coagulation occurred. Cooling according to Wooldridge had removed a substance which was ~~the~~ determining factor in coagulation. This he called A Fibrinogen. He found further that Lecithin acted like A Fibrinogen and, as the latter contained some organic Phosphorus, he argued that its activity was due to admixture with Lecithin.

A Fibrinogen has no effect in inducing the coagulation of Hammarsten's Fibrinogen - ie Fibrinogen prepared by the Sodium Chloride method.

Wooldridge[6] was therefore led to assume the existence in the plasma of another Fibrinogen - B Fibrinogen - which he regarded as the mother substance of Hammarsten's Fibrinogen - the "C" Fibrinogen of Wooldridge. He believed that coagulation was due to an exchange of Lecithin from "A" through "B" Fibrinogen and regarded the Fibrin Ferment as a product, and not the determining factor, of coagulation.

Wooldridge[6] also obtained from various organs certain substances, called by him Tissue-Fibrinogens, which acted like "A" Fibrinogen. They produced intra-vascular coagulation when injected into the blood vascular system and did not coagulate Hammarsten's Fibrinogen. These substances

he therefore concluded, did not contain Fibrin Ferment and, inasmuch as they also contained Lecithin, their action was similar to that of A. Fibrinogen. These Tissue-Fibrinogens he also regarded as existing in the fluid and not in the formed elements of the tissues

Halliburton[32] has obtained from the lymphoid cells of the thymus gland three varieties of Globulin - one of which "C" Globulin he shewed to be identical with "A" Fibrinogen of Wooldridge. Subsequent work, however, enabled him to determine that this all-Globulin was in reality a Nucleo-albumen. When injected into the vascular system it produced intra-vascular coagulation like the Tissue-Fibrinogens of Wooldridge. As a rule it is mixed with

a greater or less amount of Lecithin but, after thorough purification from this substance by means of hot alcohol and ether, it is still capable of causing intravascular coagulation. It has no influence in the coagulation of Hammarsten's Fibrinogen.

Halliburton's researches corroborate and amplify to some extent those of Wooldridge but refute these to this extent, that they bring us back to the formed elements for the origin of the Fibrin Ferment. Halliburton[2] does not regard his Nucleo-albumen as identical with the Fibrin Ferment but believes that it has a close relation with it.

Pekelharing[12]. has shewn that after having been treated with Calcium Chloride nucleo-proteids – as he has designated them – can induce

or hasten extravascular coagulation. He therefore regards nucleoproteid as the Zymogen of Fibrin Ferment.

Lilienfeld's[4] researches on blood coagulation corroborate those of Halliburton but carry us much further. The Nucleo-albumen of Halliburton is called by Lilienfeld Nucleo-Histon. He has been able to separate this substance into its two constituents - Nuclein and Histon. The Nuclein part of Nucleo-Histon, called by Lilienfeld, Leuko-nuclein, is a compound of nucleic acid with a proteid substance. It produces intravascular coagulation when injected into the blood vascular system and also induces coagulation in Hammarsten's Fibrinogen.

The Histon, on the other

hand, delays or prevents coagulation of the blood and is equally effective when injected into the blood vascular system or when added to the blood as it is being shed. Further, it prevents coagulation when added to a coagulable mixture of Fibrinogen, Fibrin Ferment and Calcium Chloride. Lilienfeld believes that Histon is a proteid probably of the nature of an albumose.

This observer has also found that if acetic acid be added to a solution of pure Fibrinogen, - that is a Fibrinogen which does not coagulate of itself, a precipitate is produced. In the filtrate there is found only a small quantity of a proteid substance. The precipitate, dissolved in a small quantity of a dilute alkali, coagulates readily on the addition of a Calcium salt.

alone without the addition of any Fibrin Ferment.
Nuclein, nucleo-histon, and nucleic acid act on pure Fibrinogen in a manner similar to acetic acid.
The substance so precipitated is called "Thrombosin" by Lilienfeld[4].

Frederikse[5] has confirmed this observation of Lilienfeld and, further, suggests that the proteid left in the filtrate — the non coagulable part of the Fibrinogen — is identical with the Globulin known to be split off from the Fibrinogen in the ordinary process of coagulation with fibrin ferment.

Lilienfeld believes his Thrombosin to be the same substance as Wooldridge's A Fibrinogen

Schäfer[11] is inclined to doubt whether "Thrombosin" is

after all a different substance from Fibrinogen. He would explain the coagulation of it by supposing that it is Fibrinogen mixed with a small quantity of a Nucleo-albumin. Liebenfeld's own statement, that Fibrinogen yields an insoluble residue on gastric digestion, rather lends itself to this view. On the other hand Frederikse obtained no such residue with the Fibrinogen with which he made his corroborative experiments. Several other objections might be urged against this view of Schäfer but it is unnecessary for our purpose to discuss this matter further.

It is apparent that Liebenfeld regards nuclein as the active, coagulation-determining principle of Nucleo-Histon. He has found, however, that a solution of

Fibrin Ferment yields no insoluble residue on artificial gastric digestion. The activity of Fibrin Ferment is not due, therefore, to nuclein.

It is evident that, interesting and important as all these researches are, they have not clearly solved the problem of the nature of the Fibrin Ferment.

Reference has already been made to the importance of Calcium salts in coagulation of the blood. Virchow[16] first pointed out that Fibrin contained a quantity of Calcium Brücke[17] has also shewn that Fibrin is a Calcium compound.

Hammarsten[14] shewed that Calcium Chloride hastens the coagulation of a mixture of Fibrinogen and Fibrin Ferment.

Green[10] found that Calcium Sulphate hastened the coagulation

of salted blood plasma ~~and~~
but could obtain no such
effect with Calcium Chloride.
He concluded that Calcium
Sulphate was the salt of Calcium
most probably concerned in
coagulation of the blood.

Ringer and Sainsbury[7] have
shewn, however, that not only
Calcium Chloride but also
Strontium and Barium Chloride
are efficacious in hastening
coagulation although not so
effective as Calcium Sulphate.

The importance of ~~coagulation~~ Calcium
for coagulation has received
further confirmation by the
work of Arthus & Pagès[31] on
the effect of oxalates on shed
blood. These observers have
shewn that if oxalate of
Potassium be added to blood
while it is being shed coagulation
is delayed or absolutely prevented
according to the amount

of oxalate added. This result is brought about by the removal of the Calcium salts - these being precipitated as the insoluble Calcium Oxalate. Coagulation can be induced in this oxalated blood or in oxalated plasma on the addition of a proper quantity of a soluble Calcium salt. Fluorides and Soaps act in a similar manner to oxalates.

Halliburton and Brodie[2] have shewn that oxalates are also efficacious in preventing coagulation of the blood when injected into the blood vascular system.

Schäfer[11] finds that oxalates even in large amounts do not entirely prevent coagulation. In the plasma obtained from such blood there appears within a day or two a precipitation of some substance, believed by Schäfer to be Fibrin,

which must therefore have been able to obtain its Calcium from Calcium Oxalate.

To sum up, then, recent researches have shewn that three substances are required for & found are concerned in the coagulation of the blood. These are Fibrinogen, Calcium salt probably Calcium Sulphate, and Fibrin Ferment which bears some relation to, but is not identical with, Nucleo-albumen, or its synonyms Nucleo-proteid and Nucleo-Histons.

The resulting insoluble compound, Fibrin, is a chemical combination between Calcium and a part of the Fibrinogen, perhaps Thrombosin, the other part of the Fibrinogen remaining in solution as a Globulin.

Putting it as an equation it reads thus;,

Fibrinogen + Calcium Salt +

Fibrin Ferment = Fibrin + Globulin.

Our knowledge of the chemical changes, underlying the process of coagulation of muscle and even of milk, lag behind what has been made out in the case of blood. With regard to milk we know that in this, as in blood, three substances are necessary for coagulation - namely Caseinogen, Calcium Salt probably Calcium Phosphate and a Ferment, Rennin. The chemical nature of the Ferment has not been accurately determined.

The substance, produced as the result of coagulation of milk, is also a Calcium compound, Casein, which differs from Fibrin in being much more soluble in Saline solutions. Its solutions also possess the property of recoagulating on the addition of Ferment.

The condition of muscle in Rigor Mortis is now generally assumed to be the result of a process of coagulation of the muscle juice or muscle plasma. Kühne, by means of a muscle press kept at a low temperature, was able to obtain from the cooled muscles of frogs some of this muscle plasma still uncoagulated. It was a slightly opalescent, faintly alkaline fluid, which remained fluid so long as the temperature was kept sufficiently low. On the temperature being raised, coagulation occurred with great rapidity. The liquid plasma set into a solid jelly or clot which then contracted and squeezed out the muscle serum. This clotting was accompanied, as in the onset of Rigor Mortis, by an alteration of the reaction from alkaline

to acid

Halliburton, by a somewhat similar method, has been able to obtain muscle plasma from the muscles of warm blooded animals. The plasma remains fluid so long as the temperature is kept sufficiently low; clotting occurs when the temperature is raised, and is accompanied by a change in the reaction as in the case of frog's muscle plasma.

In speaking of these changes a similar terminology is employed as in the analogous processes of coagulation in blood and in milk. The coagulable proteid in living muscle is spoken of as myosinogen. The coagulated proteid in muscle which has passed into a state of Rigor mortis, or in clotted muscle plasma, is analogous to the Fibrin of

blood and the Casein of milk coagulation and is therefore known as myosin. But our knowledge of the differences between myosinogen and myosin is not so exact as in the case of Fibrinogen and Fibrin or of Caseinogen and Casein. The coagulated myosin is even more readily soluble in saline solutions than Casein and in such solutions presents all the characters of myosinogen even to the extent of being coagulable. The only difference seems to be that whereas, as Halliburton has shewn, cooled saline solutions of myosinogen from fresh muscle are feebly alkaline or neutral, similar solutions of myosin prepared from muscle in a state of Rigor mortis are faintly acid.

It is generally assumed, also, carrying out the analogy

with blood, that there is in muscle a ferment which plays, in the coagulation of muscles, a part su
to that played by Fibrin Ferment in the coagulation of blood or by Rennin in the coagulation of milk. There are some grounds for this assumption, among which may be cited the observations of Kühne[9] and Halliburton[1] on the influence of temperature on the coagulation of muscle plasma; it is retarded by prevented by a sufficiently low temperature & favoured by a temperature between 30° & 40° C. The effect of temperature on the activity of fibrin ferment is exactly similar — indeed all the body ferments have their activity similarly modified by temperature.

Direct observations on the existence of such a ferment have been made by Halliburton

Applying to muscle Schmidt's method for the preparation of fibrin ferment from blood, Halliburton obtained a solution of some substance which promoted the coagulation of saline solutions of myosinogen or of myosin. This he designated the muscle ferment.

We are still ignorant of the part played by Calcium in the coagulation of muscle. Experiments made on frogs and rabbits to determine the effect of oxalates on the rapidity of onset of Rigor mortis are contradictory. The delay and prevention of Rigor obtained by Cavazzani[28] have not been observed by Howell[30] and Locke.[29]

In this brief outline of some of the work which has been done on this subject I have necessarily omitted

any reference to many points regarding the onset and duration of Rigor Mortis which are of interest and importance. I have said enough however, to indicate that many gaps still exist in our knowledge of the process of coagulation of ~~blood~~ muscle as compared with our knowledge of the analogous process in blood. It was with the object of assisting to fill up some of these gaps that this research was undertaken.

II.

Is there in Muscle a Myo-albumose?

Halliburton[1] first stated that muscle contained an albumose allied to Deutero-albumose of Kühne. Fischel[18] and Muira[19] had previously described the presence of peptone in muscle and in a footnote to his paper Halliburton suggests that the peptone of Fischel and Muira is identical with his albumose.

The albumose according to Halliburton may be prepared in two ways.

I. Chopped muscle is placed in absolute alcohol for several months, the residue dried over sulphuric acid and pounded. A watery extract of the powder contains a substance which

promotes coagulation of a saline extract of muscle. This substance possesses the properties of an albumose.

II.

A saline extract of muscle is heated to 73°C. At this temperature all the proteids in muscle are coagulated except the albumose which remains in solution

The characters of this albumose given by Halliburton are as follows;

1. Nitric acid in the cold gives a slight precipitate soluble on heating and reappearing on cooling.
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 yields no precipitate unless the solution is first saturated with Sodium Chloride

4. Glacial Phosphoric acid acts in the same way as Glacial Acetic acid.
5. The albumose is completely precipitated by Ammonium Sulphate and by Sodio-Magnesium sulphate.
6. It is not precipitated by saturation with Sodium Chloride in a weak alkaline or acid solution — partially precipitated when a strong acid is added.
7. Precipitated by Mercuric Chloride in neutral as well as in acid solution.
8. It is not precipitated by Copper Sulphate.
9. When concentrated it gives the Biuret reaction faintly. The relative amount of albumose in unconcentrated extracts is never sufficient to give any colour with Caustic potash and Copper Sulphate.
10. It is not precipitated by dialysing the salt away from

its solutions.

These reactions, he concludes, show that is an albumose resembling especially the Deutero-albumose of Kühne and Chittenden.

Whitfield,[15] in his experiments, adopted a different method. He made an extract of chopped rabbit's muscle with as small a quantity of normal saline as possible, hoping thereby to obtain a concentrated solution of any albumose that might be present. He removed the proteids, other than albumoses or peptones, from this extract by means of a 10 per cent solution of Trichlor-acetic acid, and tested the filtrate for albumoses and peptones with the nitric acid and Biuret tests. His results were negative and were equally negative whether the precipitated proteids were

filtered off in the cold or, after warming, while still hot. The filtrate however always gave a faint Xanthoproteic reaction indicating that there was still some proteid substance present in it.

In my own experiments I have invariably used rabbits' muscles and generally those of the hind limbs but occasionally also those of the back. The animal was killed by a blow on the back of the head and its abdomen rapidly opened. The blood vessels of the hind limbs were then washed out with normal saline through the abdominal Aorta.

In my first series of experiments extracts were made with distilled water, normal saline and 10 per cent Sodium Chloride. From these extracts the proteids were

precipitated by several methods. and the filtrates tested for albumoses and peptones by the ordinary tests.
The filtrates were then concentrated by evaporation at a low temperature and again tested for these substances.

The methods used for precipitating the proteids other than albumoses and peptones. were. 1. Acidulation and boiling.
2. Salicyl-sulphonic acid
3. Trichloracetic acid.
The first method is now generally considered to be unsatisfactory. It undoubtedly fails to remove all the albumens and globulins from these muscle extracts.

Salicyl-Sulphonic acid in watery or alcoholic solutions has been highly recommended by McWilliam[38] as a delicate means of separating and

distinguishing the different proteids from each other.

Trichlor-acetic acid, first recommended by Raabe[26] was used by Fraenkel[23] to remove proteids from his liver extracts in his estimations of the amount of Glycogen in liver.

Its value has been contested by Weidenbaum[24] who states that it does not remove all the proteids from an extract of liver. From the controversy between these two observers on this point it would appear that the difference in their results is due to a difference in the quality of the acid used. Its efficacy is variable in different preparations — the absolutely pure being less efficacious than other preparations.

Frentzel[25] also discarded its use, because he found that the Glycogen, so obtained, was

mixed with some proteid substance.

Halliburton[2] believes it is the most delicate method we have for removing other varieties of proteids from ~~Albu~~ albumoses and peptones.

I ought to state in view of the recent appearance of Whitfield's paper that I had commenced my own investigations on the existence of a myo-albumose long before that paper was published.

The following are examples of some of the experiments made in this first series.

Experiment 1.

Extract with distilled water.

Muscles of hind limb of a rabbit, chopped finely and boiled for a few minutes in distilled water. The coagulated pieces of muscle then pounded with white sand, replaced in

the water and again boiled. The extract, allowed to cool, was then filtered. Filtrate neutral in reaction. Albumens and Globulins removed from three different portions of the filtrate by the following methods;

A. Acidulation and boiling. To this portion a little dilute acetic ~~acid~~ was added and the whole boiled and filtered. The filtrate reboiled and reacidified remained clear. Copper Sulphate and caustic soda gave with this clear filtrate a dirty reddish tint.

B. Salicylsulphonic acid. A solution of the reagent in alcohol was used. This portion of the extract was boiled after the addition of the reagent and filtered hot. More of the reagent added and the filtrate reboiled and

refiltered hot. The solution came through clear but became clouded on cooling. On standing a white deposit falls to the bottom of the test tube. This deposit was soluble in caustic soda and, on the addition of a small quantity of Copper sulphate to this solution, a faint reddish tint was developed.

C. Strong Nitric acid

A few drops of strong nitric acid, having been added to this portion, the solution was boiled and filtered hot. It was reboiled with the addition of a little more nitric acid and refiltered hot. The solution came through clear and remained clear on cooling.

Concentrated Extract.

The remainder of the original filtrate was concentrated by evaporation and the concentrated solution filtered. The filtrate

was divided into four equal portions and treated in the following manner.

D. Salicyl-sulphonic acid.

This half was treated with salicylo-sulphonic acid in alcohol as before and filtered hot. The hot filtrate came through clear but became cloudy on cooling. This cloudiness disappeared on heating and reappeared on cooling

E. Trichlor. acetic acid.

This half was treated with 4.6 per cent solution of Tri-chlor. acetic acid until no further precipitation could be obtained. The filtrate was then tested for albumoses and peptone.

1. Biuret negative
2. Nitric acid negative
3. Acetic acid and Potassium Ferrocyanide - negative
4. Salicyl. sulphonic acid negative.

Experiment. 2.

Extract with .6 per cent Sodium Chloride

In this experiment a similar method was adopted to that of Fränkel in his glycogen experiments. The muscles of the hind limb of a rabbit were chopped and pounded with 150 c.c. of a 3 per cent solution of Trichloracetic acid in .6 per cent Sodium Chloride. The pieces of muscle rapidly became opague and hard. The muscle was extracted in this liquid for about ~~thirty~~ six hours. The mixture was then filtered until the filtrate was quite clear. The residue was then washed and the washings plus the filtrate were evaporated at a low temperature to a small bulk. A whitish flocculent precipitate, thrown down during concentration, was removed by filtration.

To the filtrate 2 per cent solution of Trichloracetic acid was added until no further precipitation occurred. The final filtrate was then tested for albumose and peptone.

1. Buiret - a dirty reddish tint
2. Nitric acid. perhaps a faint cloudiness disappearing on heating but not reappearing on cooling.
3. Acetic acid and Potassium Ferrocyanide. a whitish cloudiness, on boiling a faint coagulation. Filtered hot; filtrate remained clear on cooling.

Experiment 3.

Extract with 10 per cent Sodium Chloride.

Extracts were made of the muscle residues in Experiments 1 and 2. after extraction with distilled water and 6 per cent Sodium Chloride respectively.

The albumens and Globulins were removed from these extracts by means of Trichloracetic acid and the filtrates tested for albumoses and peptones by the Biuret, nitric acid and Acetic acid and Potassium ~~Ferro~~ cyanide tests, with negative results even after concentration

Experiment 4.

Extract with 10 per cent Sodium Chloride

An extract of fresh rabbits muscle from the hind limbs was made with a small quantity of 10 per cent Sodium Chloride. To the filtered extract was added some 10 per cent Trichlor-acetic acid to remove the albumens and Globulins. The final clear filtrate tested with the Biuret and Nitric acid tests yielded

negative results. This is a repetition of Whitfield's method. using however a 10 per cent solution of Sodium chloride for extracting instead of normal saline solution. The results are however the same.

The results of these experiments are unsatisfactory and, indeed, contradictory. In some cases positive and in others negative they still leave the question undecided. The results of the other experiments of this series conducted on similar lines are somewhat similar. The nature of these results is no doubt to be accounted for by two considerations. 1. The difficulty of removing all the proteid other than albumoses and peptone from the solutions by the methods employed. 2. If albumose or peptone is present at all it is present in such minute quantity as to be

unrecognisable with any degree of certainty by the ordinary tests.

The fact, that the filtrates in these experiments gave a faint but distinct Xanthoproteic reaction when other tests, employed for the detection of Albumoses and peptones, gave negative results, shews that they still contain some proteid.

A similar series of experiments were carried out on muscle which had passed the stage of Rigor mortis. The results obtained in these were no more definite than in those made on fresh muscle. In two cases putrefaction was allowed to set in before the experiments were made but these yielded results no more definite than in the previous cases.

The only conclusion then which can be drawn from

This series of experiments is that, if there be an albumose in muscle, it is not appreciably increased by the onset and disappearance of Rigor-mortis.

It is evident that in order to obtain a more definite answer to our question we must adopt more delicate tests than have hitherto been employed.

I have, therefore, made a few preliminary experiments with a solution of Hemialbumose prepared from Witte's peptone in order to determine the relative delicacy of various tests. The tests so compared have been chiefly the Biuret, Nitric acid, Ferrocyanide of Potassium and acetic acid, Potassio mercuric Iodide, Phosphotungstic acid and Tannic acid. The last three are well known to be extremely delicate, general tests for proteids

Hofmeister[20] places them first in order of delicacy as capable of recognising one part of proteid in 100,000, the Biuret test is capable, according to Hofmeister, of recognising one part in 2,000, Nitric acid one in 20,000 and Ferrocyanide + acetic acid one in 50,000.

I have been unable to see Hofmeister's original paper and Hoppeseyler[20], from whose book the above quotations have been made, does not state with what form of proteid Hofmeister made his experiments upon which these conclusions are based. I have, therefore, made ~~several~~ experiments with hemi-albumose to determine how far these conclusions apply to it. The following is an example of these experiments. It is to be remarked that all of the hemialbumose did not dissolve; consequently the results are not to be regarded as absolute

although in the Protocol of the experiment it is put as if all the hemialbumose had been dissolved this makes no difference in the results considered from the point of view of relative delicacy.

Experiment 5.

Delicacy of tests for albumose

Hemialbumose from Witte's peptone, ·5 gramme in 100 cc. of a 10 per cent solution of Sodium chloride and deluted as below. All the albumose did not dissolve

Strength per cent.	·5	·125	·0625	·015625	·00390625
1 Biuret	+	+	+	+(?)	—
2 Nitric acid	+	faint +	—		
3 Ferrocyanide	+	faint +	—		
4 Pot. Mercuric Iodide	+	+	+	+	+
5 Phospho-Tungstic	+	+	+	+	+
6 Tannic	+	+	+	+	+

This experiment is corroborated by others I have performed with other Hemialbumoses, prepared from both Witte and Grübler's,

peptone. It seems that the Biuret is a more delicate test for albumose than nitric acid or Ferrocyanide of Potassium and acetic acid. With about .015 gramme per cent there is still a feeble pink colour but so feeble that one could not have been certain, with this test alone, that albumoses were present, unless as in this case we knew that we were dealing with a solution containing albumose.

In using the nitric acid and Ferrocyanide of Potassium tests it was not considered sufficient to obtain a precipitate or cloudiness. The essence of these tests for albumose consists in this, that the precipitates must disappear on heating and reappear on cooling. It is the difficulty of determining this accurately which places these tests somewhat lower in the scale than the Biuret.

There can be no doubt that Potassio-mercuric Iodide, Phosphotungstic and Tannic acids surpass the others in delicacy as tests for albumoses. It only remains to be decided whether, by these tests, one can differentiate between albumoses and other forms of proteid.

Albumens and Globulins are coagulated by each of these reagents but the coagulum does not disappear on heating.

The precipitate of albumose produced by them is, in each case, readily redissolved on heating and reappears on cooling. A similar result is obtained with peptone.

The precipitate is further insoluble in cold water and can therefore be washed almost completely free from the reagent. It can then be dissolved in some caustic soda solution in which it is readily soluble.

we thereby obtain a comparatively strong solution of the albumose in a medium which allows one to perform the Biuret reaction. On the addition of a drop or two of weak Copper Sulphate solution the characteristic pink colour is developed. For this test the precipitates obtained with Potassio-mercuric Iodide and Phospho-tungstic acids are best suited for this ~~reagent~~ reason. — with Tannic acid it is more difficult to get rid of the reagent and on the addition of Caustic soda, not a clear colourless solution forms as in the other two but a brownish solution the colour of which obscures the delicate pink of the Biuret reaction.

In these tests, we have the means of detecting an extremely small quantity of albumoses & peptones in weak solutions.

In my second series of experiments

on the existence of a Myo-albumose I have used these tests.

Second Series of Experiments on Myo-albumose.

I have used in this as in the first series only rabbits muscle. The animals were killed and the blood vessels of the hind limbs were washed out as before. The chopped muscles were extracted with 10 per cent Sodium Chloride, 13 per cent Ammonium Chloride, 5 per cent Magnesium Sulphate, .6 per cent Sodium Chloride or with distilled water. The results obtained were similar, no matter which of these methods was used.

As a rule extraction was allowed to proceed for several hours, although here again one can obtain similar results even after an hour's extraction. Trichlor-acetic acid 10 per cent was generally used to remove

the albumens and globulins. This may be added to the filtered extract but is best added to the mixture before filtration. In this way, in consequence of the coagulation of the large bulk of the proteids, filtration is rendered more easy & more filtrate is also obtained. It is best also to filter first of all through muslin; this keeps back the coarser fragments of muscle which can then be thoroughly squeezed in the muslin to remove as much fluid as possible. To this filtrate I generally added more of the trichloracetic acid before filtering through filter paper. This filtrate was again tested with the reagents and if necessary again filtered.

The Biuret and nitric acid tests now yielded negative results.

On the addition of one or two drops of Potassio-mercuric

Iodide, even without the addition of Hydrochloric acid, a cloudiness is produced which is intensified when a drop or two of Hydrochloric acid is added. If the solution is allowed to stand for some hours the cloudiness resolves itself into a white flocculent precipitate which settles at the bottom of the vessel. The supernatant fluid can now be decanted off, leaving the precipitate in a small quantity of fluid. On heating this the precipitate disappears more or less completely - depending probably on the Trichloracetic acid used.

In some cases it disappeared entirely on heating forming a clear solution which became clouded on cooling. In those cases in which a faint coagulum appeared on heating it was removed by filtration while still hot. The hot filtrate, at first clear, became

clouded on cooling.

It is not necessary to allow ~~the reaction~~ the original cloudiness to resolve itself into a flocculent precipitate before observing the effect of heating and cold upon it. The same result, disappearing with heat and reappearing with cold, can be observed with the cloudiness itself.

If the whole extract from the hind limbs be treated in this manner one obtains a comparatively large quantity of the precipitated proteid. After washing this with water one can dissolve it in caustic soda. On adding a drop or two of dilute solution of Copper Sulphate to this, a fairly well marked pinkish colour is developed.

Finally the substance not precipitated with Trichlor-acetic acid does not dialyse. I have dialysed the filtrates for three

days in a Kühne dialyser with a running stream of water and, at the end of that time, found that it still gave the reaction with Potassio mercuric Iodide although no further precipitate could be obtained with Trichlor-acetic acid.

Tannic acid and Phospho-tungstic acid give results similar to those obtained with Potassiomercuric Iodide, ~~for the~~ before and after the filtrate has been dialysed. On dissolving the precipitate, produced by Phosphotungstic acid, in Caustic soda and adding some dilute copper Sulphate solution a pinkish colour is developed.

Neither Potassio mercuric Iodide, Tannic acid nor Phosphotungstic acid give a precipitate when added to 10 per cent solution of Trichloracetic acid alone.

The same result can be obtained in another way. To the filtered extract or to the mixture before filtration is added some Potassio-mercuric Iodide and Hydrochloric acid, or Phospho-tungstic acid. These precipitate all the proteids. The whole, having been boiled, is now filtered hot and kept hot during filtration by the hot-water funnel.

If sufficient reagent has been added the hot filtrate comes through clear but becomes clouded on cooling. The cloudiness disappears on heating and reappears on cooling.

Potassio-mercuric Iodide gives better results than Phosphotungstic acid by this method.

If to the dialysed filtrate obtained by the Trichloracetic acid we add some Sodium Carbonate so as to make the solution faintly alkaline a cloudiness is

produced. I thought at first that this might be some acid albumen, formed by the action of Trichloracetic acid on the albumens & Globulins, and that the results, obtained by Potassiomercuric Iodide, Tannic acid and Phosphotungstic acid, were due to the presence of this substance. But even after the precipitate obtained with Sodium Carbonate has been removed by filtration, these reagents still give the characteristic precipitates. Experiments also, which I have made on acid-albumen prepared from egg-albumen, shew that the action of these reagents on acid albumen is to cause a coagulation which does not disappear on heating.

We shall see reasons for believing that the precipitate produced by Sodium Carbonate is in reality

a precipitate of earthy phosphates thrown down by the alkalinity of the solution so brought about.

There can be little doubt that the substance precipitated by Potassio-mercuric Iodide, Tannic and Phosphotungstic acids from the filtrate prepared as described above is proteid in nature. Two facts indicate this. 1. It does not dialyse 2. Its precipitates with these reagents can be made to give a pinkish colour with Caustic soda and Copper Sulphate.

This reaction also together with the fact that the precipitates with these reagents disappear on heating and reappear on cooling indicates that the substance is either an albumose or a peptone. The reactions already described do not differentiate between these two.

To determine this point

I have saturated a portion of the dialysed filtrate with Ammonium Sulphate. A precipitate was produced. After complete saturation I tested the filtrate with Potassio-Mercuric Iodide and Hydrochloric acid with negative results. Phosphotungstic acid is not available for this experiment as it yields a precipitate with the Sulphate.

The precipitate, obtained with Ammonium Sulphate and dissolved in a little water, gave with Potassiomercuric Iodide a precipitate disappearing on heating and reappearing on cooling.

The substance is therefore an albumose.

The question now arises whether this albumose is originally present in the muscle or is the result of a certain amount of digestion

of the muscle produced by the pepsin known to be present in it. To determine this point I have made the following experiment.

Experiment 6

Rabbit's muscle chopped finely and added to 150 c.c. of a 3 per cent solution of Trichloracetic acid in water. This was at once boiled and then cooled rapidly by holding the flask under a tap of running water. This was done in order to avoid the conversion of some of the proteid into the primary albumoses by the action of heat alone.

After about two hours the extract was filtered. To the filtrate some 10 per cent Trichloracetic acid was added until no further precipitation was produced.

The final filtrate tested with Potassio mercuric Iodide gave a precipitate soluble on heating and reappearing on cooling. Tannic acid and Phosphotungstic acid gave similar results but not so well marked as Potassio-mercuric Iodide +

The remainder of the filtrate was dialysed in a Kühne dialyser for 3 4 days and at the end of that time still gave the same reactions. Biuret and tutric acid yielded negative results.

This experiment shows that the albumose in muscle is not the result of the digestive action of the pepsin present in muscle as this must have had its activity destroyed by the boiling, if not by the Trichloracetic acid, to which it was subjected.

The nature of myo-albumose.

I have endeavoured to obtain this substance in a more concentrated condition in order the more readily to determine its nature.

The filtrates, obtained by the Trichlor-acetic acid method, were first dialysed in a Kühne dialyser for several days and then evaporated over a water bath at a temperature below 35° C. to dryness. The drying was completed in some cases over sulphuric acid in a desiccator.

A solution of this residue was made in distilled water. All the dried substance did not dissolve in this medium even after several hours. The solution was therefore filtered and the filtrate tested for albumose.

The following are the results

obtained with two different samples of the dried product — both obtained from rabbits' muscle.

Experiment 7.

Tests for Myo-albumose.

1. The reaction of the solution is acid
2. Boiling — negative
3. Trichloracetic acid 10 per cent. — negative
4. Nitric acid — a faint cloudiness - doubtful if disappears on heating and reappears on cooling.
5. Biuret — a pinkish colour differing somewhat from the rosy-pink of ordinary albumoses.
6. Glacial acetic acid — negative
7. Glacial acetic acid and saturation with Sodium chloride — a well marked precipitate
8. Trichloracetic acid and saturation with Sodium chloride — a well marked precipitate.
9. The addition of an alkali to the precipitates obtained in 7 and 8, so as to make

the solution faintly alkaline causes almost complete disappearance of the precipitate. It is then not brought down on further addition of Sodium Chloride and only faintly on the addition of a strong acid.

10. Copper Sulphate — negative
11. Xanthoproteic — well marked

Experiment 8.

Tests for Myo-albumose

Extract in distilled water another sample of dried residue. Filtered and tested.

1. Reaction of solution — acid
2. Boiling — negative
3. Trichlor. acetic acid 10 per cent — negative
4. Xanthoproteic — well marked
5. Biuret — well marked pinkish colour — again different from the rosy pink of other albumoses.
6. Nitric acid — faint precipitate but better marked if the solution be neutralised & previously saturated with Sodium Chloride

7. Glacial acetic acid — negative
8. Glacial acetic acid and saturation with Sodium chloride — a well marked precipitate
9. Trichloracetic acid and saturation with Sodium chloride — a well marked precipitate
10. A portion made faintly alkaline and then saturated with Sodium Chloride gives only a faint precipitate
11. Copper Sulphate — negative
12. Potassio mercuric Iodide — a well marked precipitate disappearing completely on heating and reappearing on cooling
13. Tannic acid — similar result
14. Phosphotungstic acid — similar result
15. Neutralisation and saturation with Ammonium sulphate — a well marked precipitate
16. After complete saturation with Ammonium Sulphate in the ordinary manner the filtrate only gives a

faint precipitate with the Potassio-mercuric Iodide test. This is probably only some albumose not removed by the Ammonium Sulphate. It is known that saturation with Ammonium Sulphate in this manner is insufficient to remove all the albumose from its solution. Devoto's method of saturation has not been tried in consequence of the small quantity of fluid at available.

14. <u>Caustic Soda</u> — a precipitate insoluble in excess. This precipitate is flocculent and is probably phosphatic. It is evidently not due to acid-albumen because it is insoluble in excess.

That this precipitate produced by Caustic Soda is phosphatic is shewn also by the fact that Ammonio-nitro-molybdate gives, with the original solution, the

reaction for Phosphorous. On addition of the reagent a heavy precipitate is thrown down of the proteid. If this be filtered off and the filtrate be put into the water bath a faint canary yellow precipitate comes down. shewing the presence of phosphate.

This explains the precipitate obtained with Sodium Carbonate in the unconcentrated, dialysed extract previously referred to.

These experiments shew that the substance we have been dealing with is an albumose what has close affinities with deutero-albumose with this, however, it does not seem to be identical. The peculiar pinkish colour obtained by the Biuret reaction is not a typical rosy pink. With the exception of this difference, and also the slight precipitate obtained

by saturation with Sodium Chloride in a faintly alkaline medium, the reactions, obtained with the tests employed, are those given by deutero-albumose.

Conclusion

The answer to our first question is therefore an affirmative. There is in muscle a small quantity of an albumose — myo-albumose — unrecognisable by the ordinary tests in the solutions obtained by Whitfield, but recognisable in these solutions and uniformly recognisable by the more delicate tests I have employed. Further myo-albumose is closely related to Deutero-albumose.

This was the conclusion of Halliburton which, however, he has recently given up. (Halliburton and Brodie)[2]

III.

Is there in Muscle a Nucleo-albumen?

Whitfield[15] is the only one who has investigated this question. Adopting the two methods now employed for the preparation of Nucleo-albumens he states that the substance obtained from muscle by these methods is not a Nucleo-albumen but in reality Myosin.

I had already begun my investigations on this question before the publication of Whitfield's paper.

The rôle ascribed to Nucleo-albumens in the coagulation of the blood is a highly important one. Halliburton's work on Muscle plasma was carried out before their importance was recognised and he consequently made no observations on this point.

My own investigations lead me to believe that there is in muscle a nucleo-albumen.

Methods.

There are two methods for the preparation of nucleo-albumens. One of these is associated with the name of Woldridge[6], the other we owe to Halliburton[2]. The substance obtained by either of these methods is, according to Halliburton, identical when an organ, such as the Thymus, has been exhausted of its nucleo-albumen by one method, the other method applied to the residue fails to produce more of the Protein. It is to be remarked, however, as an exception to this that from some organs the yield of nucleo-albumen by one method is greater than that by the other.

Wooldridge's method.

This consists in extracting the organ with a large quantity of distilled water. The organ is first of all chopped as finely as possible and then put into a large quantity of distilled water. It is allowed to remain there for twenty-four hours. The supernatant fluid is then decanted off and filtered. The addition of a small quantity of dilute acetic acid to the filtrate causes a precipitation of the nucleo-albumen. This is the tissue-fibrinogens of Wooldridge.

Halliburton's method.

The organ is chopped finely and then pounded in a mortar with an equal bulk of Sodium chloride and a small quantity of distilled water, until the whole is of a syrupy consistence. It is then put into a large quantity of

distilled water. The mass sinks to the bottom of the vessel and from it there soon rises the nucleoalbumen in strings which may be seen to contract somewhat in rising to the surface of the liquid. Soon a layer of a slimy substance has formed on the surface. This is the nucleo-albumen.

Halliburton's method is quicker than Hofmeister's Woodridge's and by it one can obtain from such an organ as the Thymus a fairly large quantity of nucleo-albumen in a short time.

Characters of nucleo-albumens.

nucleo-albumens possess four main characteristics which serve to distinguish them from other forms of Proteid. These are;

1. They are readily soluble in a weak alkaline solution as

for instance, in a 1 per cent solution of Sodium Carbonate. Their solutions are opalescent and more or less viscid.
From these solutions in Sodium Carbonate they are readily precipitated on acidification with a dilute acid such as 2 per cent acetic acid.

2. After thorough purification from inorganic phosphates and from Lecithin they still contain a large proportion of Phosphorus. This resides in the Nuclein part of the Nucleo-albumens.

3. Digestion of Nucleo-albumens in an artificial gastric juice breaks it up ~~the Nucleo-albumen~~ into its two constituents Nuclein and Proteid. The latter in the course of digestion becomes converted into albumoses and peptone. The former remains as an insoluble residue. Salkowski[34]

prefers to call this residue paranuclein.

4. When a solution of Nucleo-albumen in Sodium Carbonate is injected into the blood vascular system of an animal death supervenes in a short time with symptoms of dyspnoea and stretchings. On Post mortem examination, made at once, extensive clotting of the blood is found to have occurred. The right side of the heart is engorged - the left side empty. Clots are found usually in the right ventricle extending into the pulmonary artery, in the right auricle and also in most of the large veins, more especially those of the portal system.

Method adopted.

The method which I have chiefly adopted in my experiments

on muscle is that of Wooldridge.

I have also used Halliburton's method in some of my experiments but to these I shall refer later.

In carrying out Wooldridge's method I have adopted the following procedure as a general rule.

Rabbits muscle only has been used.

The animal was killed by a blow on the back of the head, the abdomen immediately opened and the blood-vessels of the hind limbs washed out through the abdominal aorta with normal saline at the ordinary temperature. The washing was continued until the fluid coming from the Vena Cava was clear.

The muscles of the hind limbs were then removed and freed from fat, nerves, tendon & fibrous tissue as far as

possible. They were then chopped finely - and. sometimes, in addition, pounded with sand in a mortar. This mass was put into a large quantity of distilled water, as a rule ~~three~~ to four litres being used. This was stirred well and allowed to stand with occasional stirring from 24 to 48 hours. At the end of that time the supernatant liquid was syphoned off. and filtered

The filtrate is generally slightly opalescent. On the addition of dilute acetic acid the fluid first becomes distinctly clouded and after standing for several hours deposits a white flocculent precipitate. One has to be careful not to add an excess of acid as the cloudiness readily clears away with excess and no precipitate

is produced.
As a rule I have used a solution of acetic acid in distilled water containing 2 per cent of the glacial acid. About 2 to 4 cc. of this solution is sufficient to cause precipitation in a litre of the filtrate. When too much acid has been added precipitation can be brought about by reducing the acidity with dilute Sodium Carbonate.

There is no particular difficulty in obtaining this precipitate I have not failed to obtain it in any of my experiments. The best method to obtain this precipitate as free from the mother liquor as possible is by successively syphoning off the supernatant liquid, transferring the residual precipitate + fluid after each operation into a smaller

vessel until we have the precipitate finally settling in a fairly large sized test tube when the supernatant liquid can be pipetted off.

Nature of this substance.

Whitfield[15] makes the statement that the substance so precipitated is myosin.

1. When a small quantity of water, say a litre, is used to extract with, it is possible that a portion of the precipitate is myosin or perhaps more probably Paramyosinogen. The amount of precipitate, obtained by the use of a small quantity, is certainly greater than when a larger quantity of water is used. But it is difficult to understand how any Globulin - Myosin included - can have passed in this quantity into solution when 3 to 4 litres have been

used for extracting. The proportion of salines in such a solution must be infinitesimally small.

2. Solubility in Sodium Carbonate.

The precipitate – the supernatant fluid having been removed as far as possible in the manner described – is readily soluble in 1 per cent solution of Sodium Carbonate. ~~in~~ distilled water. Sometimes all of the precipitate does not so dissolve but the larger portion invariably does so and forms an opalescent solution. In its behaviour to Sodium Carbonate solutions of this strength it shews a marked difference to myosin or myosinogen.

If we endeavour to dissolve myosin or myosinogen – prepared by extraction with saline solutions and precipitated further by acid or by throwing into a large quantity of water – in ~~other~~

per cent Sodium Carbonate solution we find that the myosin or myosinogen remains for the most part undissolved. They retain their flocculent character and in a short time either fall to the bottom of the vessel or rise to the surface of the liquid leaving a clear non opalescent fluid. They are not dissolved even after standing for sixteen to thirty six hours. Further if the clear fluid be tested with dilute acetic acid only the faintest cloudiness is produced, showing that there is very little myosin - myosinogen or Paramyosinogen in solution.

With the opalescent solution in Sodium Carbonate if the deposit obtained by Wooldridge's method we get a well marked precipitation on acidifying with dilute acetic

or dilute Hydrochloric acids. This can be redissolved in Sodium Carbonate and reprecipitated on acidification
In this respect its behaviour is that of a nucleo-albumen and not of myosin.

3. Does it contain Phosphorus?

After being purified from inorganic Phosphates and from Lecithin it still gives the reaction for Phosphorus. As an illustration of this I shall describe in some detail one of my experiments.

Experiment 9.

Deposit obtained by Wooldridge's method from Rabbit's muscle. Washed in acidulated (with acetic acid), distilled water several times by decantation Dissolved in 2 per cent Sodium Carbonate. Reprecipitated by acetic acid.

Precipitate separated as far

as possible by pipetting off the supernatant liquid. absolute alcohol added and warmed. Allowed to remain under alcohol over night. Rewarmed and alcohol decanted. Fresh alcohol added, warmed, allowed to stand several hours and decanted.

In same way residue treated with two supplies of ether, warmed and decanted. - it was in contact with ether also for several hours. The alcohol from the second extraction & the ether yielded a faint brownish residue on evaporation.

After treatment with ether the residue was extracted with .06 per cent solution of Hydrochloric acid in distilled water, several times until the decanted fluid

yielded, after concentration, no evidence of Phosphorus with the Ammonium Molybdate test.

Finally the residue was washed several times with boiling absolute alcohol and several times with boiling ether and with a mixture of both.

Dried over Sulphuric acid and weighed. to give one an indication of the amount of the substance obtainable from muscle. The weight after drying was a little under .34 grammes.

This was pounded with one gramme of a mixture containing 4 parts of Potassium Nitrate to 1 part of Sodium Carbonate. and the mixture fused for some time in a platinum capsule.

The fused mixture was dissolved in 2.5 cc. of distilled water and to it was added

1 c.c. of pure strong nitric acid.

On the addition of ammonio nitro-Molybdate to this, a well marked, yellow, crystalline precipitate appeared at once in the cold.

Care was taken that the reagents used were free from Phosphorus and Arsenic. The nitric acid had been carefully distilled and pure Potassium ~~Nitrate~~ and Sodium Cartonate only used.

<u>A control experiment</u>

in which 1 gramme of the mixture of nitrate and cartonate was fused, dissolved in 2·5 c.c. of distilled water plus 1 c.c. of the nitric acid gave no reaction with Ammonio-nitro-Molybdate even after having been kept at a temperature between 30° & 40°C. for several hours.

This experiment, conformed

by others on similar lines, shews that organic Phosphorus is present in this substance.

4. Effect of digestion with Artificial Gastric juice.

This is shewn in the following example of my experiments.

Experiment 10.

Some the deposit prepared by Wooldridge's method put into .2 per cent Hydrochloric acid made with distilled water. To this was added some of Benger's Liquor Pepticus. Vessel put into a water bath at a temperature of 40° C. Digestion allowed to go on for three days. The supernatant fluid was then decanted and, tested with the Biuret reaction, gave a rosy pink colour. To the residue fresh .2 per cent Hydrochloric acid and fresh Liquor Pepticus and digestion continued at the same temperature

for two days longer. There is still a portion undigested This residue consists of nuclein.

Experiment 11.

This experiment was performed on the residue obtained in Experiment 10.

The fluids from the second digestion decanted and the residue washed first with .2 per cent hydrochloric acid and then with distilled water until the washings gave no reaction for Phosphorus, after being concentrated

The residue then washed for several days with absolute alcohol and with ether and finally with a mixture of both. It is insoluble in boiling absolute alcohol & in boiling ether.

The residue then put into a platinum capsule, the ether and alcohol burned off,

a and the final residue fused with a quantity of the same mixture of Potassium nitrate, and Sodium Carbonate as in the previous Experiment. 9. The fused mass dissolved in distilled water, neutralised and tested with Ammonio-nitro-molybdate, yielded a small amount of a yellow crystalline precipitate.

Control test performed as before gave negative results. These experiments are confirmed by several others which I have performed. They shew that this substance we are dealing with yields an insoluble residue on gastric digestion which contains organic Phosphorus, after being purified from inorganic Phosphates and from Lecithin, and is therefore nuclein - or according to Salkowski, Paranuclein.

5. Effect of injecting this substance into the blood-vascular system

I have made several experiments in order to determine whether the product, obtained from rabbits muscle by Wooldridge's method, caused intravascular coagulation when injected into the blood vascular system of rabbits.

I shall first detail the experiments I have made and then discuss the results.

Experiment 12.

Rabbit - killed & blood vessels of hind limbs washed out with normal saline.

muscles of hind limbs chopped & pounded in a mortar with white sand.

Extracted for about three days with from two to three litres of distilled water.

Fluid decanted off and filtered

Filtrate precipitated with 2 per cent acetic acid and precipitate

dissolved in 1 per cent Sodium Carbonate and filtered.
Filtrate is faintly opalescent.
50 c.c. of this were warmed to a temperature of 30° C. and injected into the left ~~External~~ jugular vein of a male rabbit. All the fluid had not passed into the circulation when symptoms of dyspnoea set in accompanied by stretchings and finally cessation of respiration.
Thorax immediately opened.
Heart still beating.
Right side of the heart engorged left side empty.
Clots present both in the right ventricle and right auricle and extending into the large veins.
The Inferior Vena Cava filled with one large, elongated clot.
Clotting also in the Hepatic and Portal veins.

Experiment 13.

The remainder of the solution used in experiment 12. together with a further quantity of the proteid, obtained by a second extraction of the same muscle with distilled water, in all 65 cc., were injected on the following day into the external Jugular vein of a male albino rabbit.

The whole of the fluid was injected.

The animal died but no intravascular coagulation observed post-mortem either in the heart or in the large veins.

Experiment 14.

Rabbit killed as before + blood vessels of hind limbs washed out.

Muscles of hind limbs chopped - not pounded with sand -

extracted with 1500 c.c. of distilled water.
Precipitated filtrate with 2 per cent acetic acid.
Precipitate dissolved in 1 per cent Sodium Carbonate and filtered.
90 c.c. of this solution injected into the left external jugular vein of a female albino rabbit
The animal died with dyspnoeac symptoms several minutes after the injection was finished.
Post-mortem. No intravascular coagulation in the heart or in the large veins. The right side of the heart distended with fluid blood. left side empty.

The blood, on being shed, was allowed to run into the pleural and abdominal cavities and ultimately got on to the table

Part of it coagulated readily enough but the larger portion was observed to retain its fluid character. This was transferred to a test tube, by means of a pipette, about 10 to 15 minutes after it had been shed. Time noted then 8.25 pm. At 8.40 pm it was still uncoagulated.

By 10 am. next morning it had coagulated into a firm jelly clot with a "buffy-coat" on the surface.

Experiment 15

Rabbit killed as before and Blood vessels of hind limbs washed out. Muscles chopped not pounded. Wooldridge's method.

The solution in Sodium Carbonate precipitated with acetic and redissolved in 1 per cent Sodium

Carbonate and filtered.

All of the fluid injected into the left external jugular vein of a Rabbit, non albino without effect.

There was then injected a solution of nucleo albumen prepared by a second extraction of the same muscles with distilled water.

Symptoms of dyspnoea but animal recovered.

Killed by asphyxiation

Post mortem. Blood fluid, no clots in heart or veins.

Experiment 16.

Nucleohistone prepared as before from Rabbit's muscle. Muscles chopped only.

Precipitate dissolved in 1 per cent Sodium Carbonate to form 100 cc. of solution.

The whole of this injected into the external jugular vein

of a large non-albino rabbit (male)

Slightly increased rapidity of respiration
Animal did not die.
Several minutes after completion of the injection animal killed by asphyxiation

Post mortem.

Blood fluid. No clotting in the right ventricle, right-auricle nor in the veins.
Some of the blood which had escaped into the pleural cavity, on opening the heart, was transferred to a test-tube at 11.30 am. Only a very partial clotting at 1.10 pm. becoming almost complete by 2.30 pm., but-even at-this time the clot was not quite firm.

Experiment 17.

Rabbit killed and blood vessels of hind limbs washed out.
Muscles of hind limbs chopped

and pounded with sand
Extracted twice with large quantities
of distilled water
Both precipitates dissolved in
1 per cent Sodium Carbonate
and filtered.
Filtrate injected into the left
External jugular vein of a
rabbit - non-albino
Animal did not die.
Several minutes after
completion of the injection the
animal was bled to death
from the Carotid artery, the
blood being collected in a
flask.

Post-mortem.

No evidence of clotting in heart
nor large veins.

The blood received from the
Carotid artery at 3.40 p.m. was
still fluid at 8 p.m. of the
same evening. Only a very thin
filmy clot on the surface
next morning at 9.35 a.m.

otherwise perfectly fluid.
At 4.5 p.m. blood thicker and lumpy on pouring into another flask.
At 7.15 p.m. little further change. Most of the blood still fluid.
Next morning coagulation practically complete.

Experiment 15.

Rabbit - killed + blood vessels washed out as before.
Muscles of hind limbs chopped, pounded with sand.
Extracted for 20 hours with distilled water.
Supernatant liquid filtered + filtrate allowed to remain until next morning. Then precipitated with acetic acid.
At 9.30 p.m. same evening precipitate dissolved in some caustic soda 1 per cent solution.
Further precipitates obtained from the same extract dissolved

in Caustic soda - reprecipitated with .8 per cent Hydrochloric acid. This precipitate added to the solution of the first precipitate in Caustic soda. and the whole made faintly alkaline.
3 cc. of a 10 per cent solution of Sodium chloride were added to 100 cc. of nucleo-albumen solution. This, together with some Sodium chloride formed in reducing the alkalinity with Hydrochloric acid, would give about .5 per cent Sodium Chloride.
The solution is therefore one in dilute Caustic soda w. .5 per cent Sodium chloride. It measured a little over 100 cc.
About 30 cc. of this injected into the left external jugular vein of a rabbit - non albino - caused dyspnoea and death
Post mortem.
Well marked coagulation

in the right ventricle and right auricle - and also in the Inferior Vena Cava.

Experiment 19.

Half of the remainder of the solution used in Experiment 18 was reprecipitated with .8 per cent Hydrochloric acid Precipitate was redissolved in 1 per cent Sodium Carbonate. Solution measured 55 cc. ~~to~~ This, injected (on the day following Experiment 18). into the jugular vein of a rabbit - non albino., produced no effect.

Some blood, drawn from the Carotid artery. after the injection was completed, coagulated immediately into a solid mass.

Experiment 20

This experiment was made on the same rabbit used in

Experiment 19. Immediately after the sample of blood had been drawn from the Carotid
There was then injected into the same rabbit 80 cc. of a .5 per cent solution of Caustic soda in normal saline.
This caused death of the animal
Post. mortem.

Right side of the heart filled with fluid blood. No blood clots in the right ventricle or right auricle - nor in any of the large veins.
The blood collected in a flask coagulated in a few minutes.

Experiment 21

Rabbit killed and blood vessels washed out as before.
Muscles of hind limbs chopped and pounded with sand.
Extracted with 3 - 4 litres of

distilled water. for eighteen hours.
Fluid decanted and precipitated with 2 per cent acetic acid.
Precipitate dissolved in some 2 per cent Sodium Carbonate at 9 pm of same evening.
and a further precipitate dissolved in the same solution at 9.40 pm.
This allowed to stand overnight.
And ~~heat~~ morning the opalescent fluid was decanted from the slight deposit which had settled at the bottom of the vessel.
In all there were 180 cc. of the solution.

50 cc. of this injected into the left external jugular vein of a rabbit - female non-albino - caused dyspnoea and death.

Post mortem.

Clot in right auricle - none in right ventricle. Clotting.

in the Inferior Vena Cava and in the Hepatic and Portal system. Slight clotting in the right external jugular. No clot in the Subclavians.

Part of the blood had remained fluid. This collected in a flask and observations made at intervals. *

First observation made at 11 am. - animal died and blood shed ~~about~~ 5-10 minutes previously.

No coagulation at 11 am, 11.30 11.30 - 11.40 - 12.45 pm 1.5 - 1.15 2 - 3.15 nor at 7.15 pm of the same day. It was still fluid on the following day at 4.15 pm. and also on the day succeeding this. Delayed thereafter certainly for 48 hours.

Experiment 22

The remainder of the solution used in experiment 21.

measured 130 cc.
This was precipitated with
some .8 per cent Hydrochloric
and
Precipitate dissolved in 1 per cent
~~Sodium~~ Carbonate.
Solutions measures 135 cc.
65 cc. of this injected into
a rabbit - male non-albino -
through the external jugular
vein.
Experiment made on same day
as Experiment 21.

The injection of 65 cc. caused
death with dyspnoea.

Post mortem

a clot in the right-ventricle
extending into the right-auricle
and thence into one of the
large veins.
Slight clotting in the Inferior
Vena Cava.
Well marked clot in the
left Subclavian and right
jugular veins. Well marked

clotting also in the Portal system.

The fluid part of the blood, collected at 12·40 a.m., was fairly large in amount. It was still perfectly fluid at 4·15 p.m. of the same day & was not completely coagulated until 4·15 p.m. of the following day.

Experiment 23.

The remaining 70 cc. of the solution used in Experiment 22. were again precipitated with ·8 per cent Hydrochloric acid The precipitate redissolved with 8 cc. of a ·5 per cent solution of Caustic soda. To the solution 1 cc. of a 10 per cent Sodium chloride added. The whole measured 25 cc. so that the solution contains ·15 per cent of Caustic soda and about ·5 per cent of Sodium Chloride.

It was allowed to stand for nearly 60 hours - then filtered and deluted with an equal volume of normal saline

This was injected into the External jugular vein of a rabbit.

Animal did not die.

Samples of blood removed from the Carotid artery 5 - 10 - and 15 minutes after completion of the injection clotted steadily.

Post mortem.

No clotting in any of the veins nor in the heart chambers.

Discussion of the results.

The first point that strikes one about these experiments is the want of uniformity of results. Experiments 13 - 14 - 15 - 16 - ~~17~~ and 23. yielded negative results so far as intra vascular coagulation is concerned. Experiments 12 - 18 - 21

and 22. yielded positive results.
In Experiment 19. death was not
produced by the injection of the
solution but the sample of blood
taken from the carotid clotted with
great rapidity.
Experiment 20. was made in order
to determine whether the injection
of Caustic Soda alone was capable
of producing intra-vascular
coagulation. The result was negative
and consequently the positive result
obtained in Experiment 18. was
not due to the Caustic Soda
in which the protein was dissolved.

The proportion of failures
to successes at first sight seems
large. It has been shewn, however,
by Halliburton and Brodie[2] that
nucleo-albumens do not always
produce intra-vascular coagulation
Working with nucleo-albumens
prepared from various organs, they
found that in a certain proportion
of cases extra-vascular coagulation

was not induced.

They have endeavoured to determine the causes of these failures and have classified them in four groups.

1. Idiosyncrasy of the animals
2. Changes of the proteid due to its having been kept some time in solution
3. Too rapid preparation of the proteid
4. Purification of the proteid.

It is evident that, where such possibilities of failure exist, one positive result is of more value than several negative results.

Under "Idiosyncrasy of the animals" they find that "albino" rabbits are not susceptible to the action of Nucleo-albumens. While they have had occasional negative results with coloured rabbits they have never been able to produce intravascular coagulation in an "albino"

how in experiments 13 and 14. "Albino" rabbits were used. — unintentionally because the fact of their ~~an~~ insusceptibility had not, by that time, become fully impressed upon me. The negative results obtained in these experiments is therefore at once explained,

There remain now the negative results obtained in experiments 15, 16, 17 and 23. These, I think, are to be explained either by changes of the proteid from too long standing or by purification of the proteid As Wooldridge's method was adopted there can be no question of too rapid preparation.

Halliburton and Brodie have found that a solution of nucleo-albumen in Sodium Carbonate, which, when freshly prepared causes intravascular coagulation, loses this power gradually in the course of a

few days and, in some cases, even in a few hours. Similarly too long contact with dilute acid in Wooldridge's method causes a similar injurious effect on the proteid

Further they have found if the proteid be purified too much by frequent precipitation and resolution its activity is destroyed.

Experiment 23 was made specially to determine these points. The solution injected in this experiment was made from a precipitate known to be active as shewn by Experiments 21 and 22. But it was reprecipitated and redissolved and further allowed to stand for about 60 hours. The results of the experiment were negative

Experiment 19. illustrates the same point. This solution was originally active in Experiment

18. but, by reprecipitation and standing, its activity was lost to a certain extent. No intravascular coagulation was produced but the blood clotted very rapidly on being shed.

The same explanation accounts for the failures in Experiments 15, 16 and 17. No special haste was used in preparing the substances employed in these experiments. Wooldridge's method is slow at all times, but, in the case of muscle, many hours are required before the precipitate obtained by acetic acid has settled sufficiently to allow the supernatant liquid to be syphoned off. In these three experiments the injection was not made until the 4th day after the acetic acid had been added. The substance used in Experiment 15. had also been

reprecipitated and redissolved before injection.

On the other hand, where special haste was used, as for instance, more especially in preparing the substance used in Experiments 21 and 22, in which the injections were made within twenty-six hours after the acetic acid had been added to the filtrate, the results were positive, and a positive result was obtained in experiment 22 in spite of the fact that the substance had been reprecipitated and redissolved before injection.

There was also less delay in Experiments 12 and 18 than in the case of the others with negative results.

There is another difference noticeable in these experiments which may have had some influence on the results. In Experiments 13, 14, 15 and 16 the

substances injected was prepared from muscles which had been chopped only. The substance used in the other experiments, was prepared from muscles which had not only been chopped but also pounded with sand in a mortar. If this had anything to do with the better results obtained in these experiments it would seem to indicate that the active substance was derived, not from the fibrous tissues, but from the sarcous substance of the muscle itself - the pounding with sand allowing more of the protein to pass into solution by breaking up the muscle more finely than can be accomplished by mere chopping.

On all of these grounds one must conclude that the positive results outweigh the negative results obtained in these experiments. The decision

arrived at is, therefore, that the substance obtained by Wooldridge's method from rabbits muscle does produce intravascular coagulation.

Reviewing the whole question of the nature of this substance it has been found to possess all the properties characteristic of nucleo-albumens. 1. It is soluble in Sodium Carbonate solutions containing 1 per cent of the salt.
2. It contains organic Phosphorus after being freed from Lecithin
3. It yields a residue of Nuclein or paranuclein on digestion with artificial gastric juice
4. When injected into the blood vascular ~~system~~ of rabbits it produces intravascular Coagulation.

Conclusion

This substance is a nucleo-albumen.

Wooldridge's "Negative Phase"

There is one other point brought out by the experiments on intravascular coagulation to which I have not yet referred - namely the delayed coagulation observed in some of these experiments.

No observations on this point were made in experiments 12, 13, 15, and 18; in experiments 12 and 18 because in these intravascular coagulation had occurred, in 13 because I was not then aware that such delay could be induced in rabbits by this ~~substance~~. I was inclined to ascribe the delay, observed accidentally in Experiment 14., to the fact that the rabbit was an "albino" and consequently no observation was made in Experiment 15 immediately following it as the rabbit used was not an albino. In all the other experiments

observations were made on this point and delay in coagulation of all or of part of the blood, was made out in all of them except in Experiments 19 and 23.

The length of time by which coagulation was delayed varied from 4 to 48 hours. Coagulation was observed ultimately to occur in all of the experiments except in the fluid portion of the blood obtained from the rabbit used in Experiment 21.

Some of those experiments, then, which yielded negative results so far as intravascular coagulation is concerned, gave a positive result of quite the opposite nature. This was best marked in Experiment 17. In this experiment no intravascular coagulation occurred and the blood, drawn in this case from the carotid, remained fluid

for at least twenty four hours after being shed.

In experiments 14 and 16 the blood coagulated within a few hours after being shed. In these experiments also no intravascular coagulation had occurred.

In experiments 21 and 22 there is a mixed state of affairs - part of the blood coagulated intravascularly - and part remained fluid for many hours after being shed.

Explanation of these results.

It was first observed by Wooldridge[6] that, in dogs, when the tissue fibrinogens failed to produce intravascular coagulation the blood on being shed coagulated with extreme slowness. This he designated the "negative Phase of coagulation".

Groth[21] had previously

observed the same thing in his experiments on the injection of cells into the blood system.

Wright[27] has confirmed the observation of Wooldridge, and suggested that it may be due to the splitting off of a peptone-like substance from the nucleo albumen which, therefore, acts like other albumooses, towards coagulation.

A peptone like substance has been found in the urine in animals which show this negative phase and Pekelharing[12] by Devoto's method has demonstrated the existence of peptone in the blood.

Lilienfeld's[4] work on nucleo-histon indicates that this negative phase may be due to the Histon - which he regards as allied to peptone.

Halliburton and Brodie[2] do not agree with this

explanation. They point out Lilienfeld's Histon is coagulated by heat and cannot therefore be peptone. after the injection of Nucleo-albumen active and inactive they have never found protease or peptone in the blood or urine.

They have never obtained this negative phase in their experiments in rabbits (apparently they never obtained even a mixed result). although they remark that this is not to be wondered at as rabbits are peculiarly insusceptible to the action of commercial peptone.

One experiment they made on a dog did not show the negative phase.

Martin[13] has repeated the experiments on dogs and has obtained the negative phase but was not able

to detect protease or peptone in the blood or urine.

The question arises whether this delay in coagulation observed in my experiments on rabbits is the same or, rather, is due to the same cause as Wooldridge's negative phase observed in dogs. I have not been able to make any experiments to determine this point.

If it is agreed that the substance obtained from rabbit's muscle is, as I believe it to be, a nucleo albumen the conclusion naturally follows that the phenomenon now under consideration is the "negative phase of Wooldridge" observed in rabbits.

Opposed to this, however, is the statement of Halliburton and Brodie[2] that they have never obtained this negative phase in their experiments

on rabbits, carried out with several varieties of ~~Nucleo~~ albumens and, from the protocols of this experiments, they do not appear to have obtained a mixed result similar to that observed in Experiments 21 and 22.

On the other hand Lilienfeld[4] describes such a mixed result in one of his experiments on rabbits with Nucleo-Histon. Further he has isolated from this Nucleo-Histon (which is identical with Halliburton's nucleo-albumen) a substance "Histon" which absolutely prevents coagulation when injected into the blood-vascular system of rabbits and also when added to shed blood.

It is really a matter of indifference whether Histon is an albumose or peptone, as Lilienfeld is inclined to regard

it, or a Globulin, as Halliburton asserts — the important fact from my point of view is that it is a substance which delays or prevents coagulation when injected into the vascular system of rabbits as well as of dogs.

There would appear to be no reason therefore why, ~~ought not~~ under the appropriate conditions, the injection of a nucleo-albumen ought not to show a complete negative phase in rabbits as well as in dogs.

I am inclined to regard the results in Experiments 14, 16, and 17, as illustrations of a complete negative phase of coagulation in rabbits produced by the injection of Muscle-Nucleo-albumen.

If this view be correct it furnishes strong confirmatory evidence that the substance

obtained from muscle by Wooldridge's method is a nucleo-albumen.

Halliburton's Method.

I have made several attempts to obtain nucleo-albumen from muscle by this method of which the following are examples.

Experiment 24.

Rabbit killed by blow on head - blood vessels of hind limbs washed out with normal saline. Muscles of hind limbs freed from fat, nerve etc and chopped finely and then pounded with an approximately equal quantity of Sodium Chloride, with a little distilled water.

The mass then put into a large quantity of water. A precipitate rose to the

surface of the liquid and was somewhat shiny in character.

This was removed and attempted to be dissolved in Sodium Carbonate. - about an equal quantity of 2 per cent Sodium Carbonate being added so that the mixture would contain about 1 per cent. This was shaken up and allowed to remain for several hours. At the end of that time the mixture had separated into two parts - an upper consisting of the bulk of the deposit still undissolved and a lower of clear, transparent solution. A little of this clear solution, tested with acetic acid gave only a faint cloudiness.

It is evident that the bulk of the precipitate in this

experiment is not nucleo-albumen.

Experiment 25.

Rabbit killed + washed out as before. Muscles of hind limbs chopped + pounded well with about an equal bulk of salt. Allowed to remain thus for fourteen hours and then put into a large quantity of water. A deposit rose to the surface. This removed and some 2 per cent Sodium Carbonate solution added. The larger part remained undissolved even after two or three hours. Filtered through fine muslin. Filtrate only slightly turbid. Acetic acid added gives a fairly well marked precipitate which rises to the surface. This separated

and dissolved in some 2 per cent Sodium Carbonate. This was used in the next Experiment.

Experiment 26

The Solution prepared as described above measured 240 c.c. (Experiment 25).

Rabbit - large - male - non albino.

Sample of blood drawn from the Carotid before injection coagulated in about two minutes

170 c.c. of solution were then injected into the external jugular vein -- the injection taking about 10 minutes. The animal was then evidently dying with symptoms of dyspnoea.

A little blood drawn from Carotid coagulated in a minute or two, but not immediately.

Post mortem.
No clots in the right-side of the heart nor in the veins
-The blood, which first escaped on opening the right ventricle, did not begin to coagulate until about fifteen minutes afterwards. But coagulation was complete within half an hour from the time of its being shed.
A second sample, taken somewhat later, as it escaped from the right ventricle, coagulated in a few minutes
Another sample from the Inferior Vena Cava also coagulated in a few minutes.

The result of this experiment is negative ~~as~~ regards intravascular coagulation and practically also as regards delay in coagulation.
The slight delay observed in

the first sample of blood from the right ventricle is not present in the samples obtained somewhat later nor in the samples from the Carotid artery and from the Inferior Vena Cava, and is probably due to a greater dilution or admixture with newly injected ~~substance~~.

There seems little doubt that the substance obtained from the muscles of rabbits by Halliburton's method is largely composed of myosinogen or Paramyosinogen. There may be some nucleo-albumen mixed with it but apparently that is not taken up in sufficient quantity by the Sodium Carbonate to cause intravascular Coagulation.

Note. In all the intravascular coagulation experiments the rabbits were anaesthetized with Chloroform

Amount of Phosphorus in muscle Nucleo-albumen.

I have made some ~~estimations~~ of the amount ~~of~~ Phosphorus present in the nucleo-albumen of muscle ~~prepared~~ prepared by Wooldridge's method, of what the following is an example.

Experiment 27.

·1252 grammes of nucleo-albumen prepared and purified from inorganic Phosphates and Lecithin - weighed after drying over Sulphuric acid,
Fused in a test tube, made of Combustion glass tubing and provided with a stopper to prevent spirting, with 1 gramme of the mixture of Potassium Nitrate 4 parts and Sodium Carbonate 1 part.
Fused mass dissolved in distilled water, - neutralised

with nitric acid, and some Ammonio-nitro. Molybdate added. Tube put into the water bath and left there for several hours - until precipitation complete. Filtered through a pure filter paper.

Tube and precipitate washed with a 10 per cent solution of Ammonium Nitrate in distilled water.

Tube then washed with a 2 per cent solution of Ammonia also in distilled water. to dissolve any of the precipitate still adhering to ~~the~~ it. Precipitate on the filter paper also dissolved out with the same solution.

To this ammoniacal filtrate Ammonio-magnesia mixture added and allowed to stand for several hours until precipitation complete.

Precipitate collected on an ash-free filter paper and washed with weak Ammonia solution in distilled water. Finally heated ~~and~~ dried over sulphuric acid and weighed It gave .0060 grammes of Magnesium Pyrophosphate which has the formula $2MgO, P_2O_5$ and contains therefore .31 grammes of Phosphorous in 111 grammes of the salt.

A simple calculation therefore shews that the .1252 grammes of Nucleo albumen contain .00167 grammes of Phosphorous. or 1.33 per cent.

The other estimations gave slightly different results - the average coming to nearly 1.7 per cent.

In consequence of the small quantity of the substance available for these estimations

this result can only be regarded as approximate.

Conclusion.

There is in muscle a nucleo-albumen so that the answer to our second question is also an affirmative. This is best prepared by Wooldridge's method. The large bulk of the substance obtained by Halliburton's method is not nucleo-albumen. Muscle nucleo-albumen possesses all the characters of other nucleo-albumens. It contains approximately 1.7 per cent of Phosphorus.

IV

The Conditions of Coagulation of Salted muscle Plasma

I have already stated that Kühne[9] was the first to prepare muscle Plasma. This he did by squeezing cooled frog's muscle in a specially designed muscle press also cooled. In this way a clear fluid is obtained which remains fluid so long as the temperature is kept low, but sets into a solid jelly when this becomes elevated. The ~~plactiop~~ previously alkaline becomes acid on coagulation.

Halliburton[1] has applied the method to the muscles of warm blooded animals and has succeeded in obtaining an alkaline plasma which coagulates when the temperature is not kept low enough — the reaction then becoming acid.

I have prepared the plasma in this way both from frog's muscle and from rabbit's muscle.

The method of obtaining plasma is not practicable for a continued series of experiments in consequence of the small quantities obtainable

Halliburton[1], in his work on muscle plasma, adopted a method of preparation which, in principle, is identical with the preparation of salted blood plasma

He found that for the preparation of salted muscle plasma a 5 per cent solution of Magnesium Sulphate is most suitable

The animal is killed, the blood vessels washed out with normal saline at a low temperature, then

quickly removed to a freezing mixture and frozen; then sliced finely and extracted at a low temperature with the saline.

A faintly opalescent solution is thereby obtained which remains uncoagulated when elevated to the ordinary temperature.

This is the salted-muscle plasma of Halliburton.

It is faintly alkaline in reaction.

When diluted with four to five times its volume of water, a coagulation of the myosinogen takes place, slowly at the ordinary temperature, more quickly at a higher temperature.

The liquid first becomes opalescent, then a jellying occurs throughout and finally contracts to form a

clot which floats in the clear fluid, the muscle serum.

This process is accompanied by a change in the reaction of the fluid from alkaline to acid.

One point in favour of this being a coagulation or clotting is the influence of temperature upon it. At the ordinary temperature coagulation occurs very slowly or not all, It is coagulated more quickly at a temperature of 30° - 40° C.

Method adopted.

I have used to prepare muscle plasma both a 5 per cent solution of Magnesium Sulphate and also a 1/3 and ¼ per cent solutions of ammonium chloride.

The latter salt was chiefly used because it was intended to test the effect

of various substances upon coagulation which ~~might~~ might produce a chemical change in the Magnesium Sulphate e.g. alkalies ~~etc~~.

In my earlier experiments I prepared the salted muscle plasma according to the method described by Halliburton, the essence of which is to carry on all the processes at a low temperature.

I gave this up however as being tedious and unnecessary for my purpose, and I have prepared the plasma from perfectly fresh, living muscle with Salines at the ordinary temperature.

Rigor mortis does not set in until an hour or two after death and by that time the saline will have

penetrated the muscle sufficiently to have prevented any extensive conversion of myosinogen into myosin. Such an extract, at any rate, when freshly made has a neutral or faintly alkaline reaction.

An extract of dead muscle is acid in reaction but otherwise, so far as coagulation is concerned, it possesses all the properties of salted muscle plasma. It coagulates on dilution & more especially when the temperature is suitable. The clot, which forms, is apparently identical with that produced in the coagulation of salted muscle plasma.

An extract of living muscle made, in the manner I have generally made it, presents all the characters of Halliburton's muscle plasma. Its reaction

differentiates it from an extract of dead muscle. This is the plasma to which I have applied the term "salted muscle plasma". There seems to be but little difference between it and Halliburton's plasma

In some of my experiments the myosinogen has been precipitated by throwing the plasma into water - the myosinogen washed and redissolved in the same strength of saline as before

Myosinogen is the important constituent of muscle plasma - it is the coagulable proteid in muscle plasma and saline solutions of myosinogen act like salted muscle plasma itself.

To avoid confusion therefore both of these saline solutions, used in my experiments, will be spoken of as plasma or salted muscle plasma

although in each case the exact character of the solution being experimented upon will be given.

The stages in coagulation of muscle plasma, prepared in the manner I have described, with ammonium chloride are similar to those observed in the coagulation of Halliburton's plasma.

I had some difficulty in obtaining a well marked jelly stage. This requires for its proper development a plasma which is rich in myosinogen. If the plasma be dilute there no jelly stage is observed - there is simply an increase of opalescence followed by a separation of flocculi which may ultimately cohere more or less completely to form a clot like mass. Even when a jelly stage is obtained one usually observes

these preliminary flocculi especially if the temperature be somewhat low -- say about 26° or 27° C.

There can be little doubt that the flocculi of dilute solutions and the jelly clot obtained in the coagulation of stronger solutions are but different expressions of the same process, - namely a process by which a more soluble proteid is converted into one less soluble.

Quite an analogous condition is observed in milk coagulation If the milk be diluted sufficiently the effect of rennet is to cause a flocculent precipitate. The flocculi may subsequently run together to form curdy masses but no jelly-up occurs. The flocculent precipitate consists of casein and in that respect the process is as much one of coagulation as if a true jelly clot had been formed.

with blood plasma it is different. No matter how much the plasma be diluted coagulation, if it occurs at all, never assumes the flocculent form. There is always an attempt at a jelly stage.

This difference must be ascribed to the structure of the clot in each case.

The organic structure of blood clot is well known. Its network of fibrin filaments is characteristic.

No such organic structure is to be made out either in milk clot or in muscle clot.

I have tried to prepare cover-glass preparations of muscle clot in the manner used for blood and have also examined the clots themselves, stained and unstained, but have failed to observe any organic structure at all.

The same has been made out

by Fraser Harris for milk clot.
Muscle clot therefore resembles milk clot more closely than blood clot. I believe that both are due simply to an aggregation of closely cohering flocculi. The flocculi have less tendency to hold together in the case of muscle clotting than in milk clotting.

The essential feature of coagulation is the conversion of a more soluble protein into one less soluble.

Conditions

In carrying out experiments such as these one must avoid three sources of error.

1. over dilution of the plasma
2. Too high a temperature
3. Over acidity of the plasma.

1. Over dilution is to be avoided because precipitation of the myosinogen is thereby produced.

In my experiments with Magnesium Sulphate plasma I have diluted with three to four volumes of water. With Ammonium chloride plasma I have diluted so as to have in the dilute solution chloride to the extent of 2.5 to 3.5 per cent.

2. Too High a temperature is to be avoided because it may produce heat-coagulation. Halliburton gives 46°C. as the coagulating point of Paramyosinogen - the most easily coagulated of the muscle proteids.

It must be remembered however that salines have the power of reducing the temperature of heat coagulation of Proteids. I have found, in experiments with 13 per cent. Ammonium Chloride plasma, that the first coagulum is obtained about 35°C.

Of course after the plasma has been diluted there will be less saline present but it would

seem advisable to conduct coagulation experiments at a temperature not exceeding 35° C. As a rule my experiments have been made at a temperature about 30° C.

Coagulation of course occurs more slowly at this temperature than at 38°- or 40° C but this is rather an advantage because one can the better decide what influence is exerted by a given substance upon the rapidity of coagulation.

At a temperature of 30°- C. all possibility of a heat coagulation is avoided.

3. Over acidity is to be avoided because myosinogen and Paramyosinogen are precipitated by acids when added in the requisite quantity. Acetic- and Lactic acids act in this way. In very minute quantities these acids do not cause a precipitation of myosinogen or paramyosinogen Rigor mortis was at one time thought to be due

to the precipitation of myosinogen by the Sarcolactic acid

Influence of Variations in Reaction.

A degree of acidity which is insufficient to cause a precipitation of myosinogen, may exercise an important influence on coagulation. Slight variations in reaction towards acid hasten coagulation to an appreciable degree. A slight increase of alkalinity acts in quite the opposite direction, and when the reaction becomes fairly alkaline, coagulation may be absolutely prevented. The following Experiments among others bring out these points.

1 The Effect of an acid reaction

Experiment 28

Muscle plasma made with 5 per cent Magnesium Sulphate. Diluted with four times its volume of water.

8 cc of this diluted plasma into

each of thirteen test tubes.
and to each of these acetic
acid 2 per cent was added
in the quantities represented in
the table.

Tubes.	1	2	3	4	5	6	7	8	9	10	11	12	13
Acetic acid in drops.	0	1	2	3	4	5	6	7	8	9	10	11	12

All the tubes were kept
at the ordinary temperature
of the room.

Tubes 2 - 3 - 4 - 5 and 6 became
milky in a very short time,
and shortly afterwards flocculi
began to separate out in
tubes. 3 - 4 - 5 and 6.
Flocculi did not appear
in tube 2 until some minutes
afterwards and in tube 1. not
until several hours after.
The flocculent stage was
more slowly reached in tubes
7 - 8 - 9 - 10 - 11 - 12 and 13 than
in 3 - 4 - 5 and 6 but more quickly
than in tube 1.

One drop of a 2 per cent solution of acetic acid is capable of causing a precipitate in 8 cc. of diluted plasma in a very short time at the ordinary temperature. Counting twenty drops to the cubic centimeter. this would indicate .01 per cent of acetic acid or 1 in 10,000.

Experiment 29

Muscle plasma made with 3 per cent solution of Ammonium Chloride.

.1 per cent solution of acetic acid used to acidify

Tube 1. contains 2 cc plasma

" 2 " 2 cc. " + 3 drops acid

" 3 " 2 cc " + 5 " "

All put into the water bath at a temperature of 27° C. at 11.5 a.m. By 7.30 p.m. tubes 2 and 3 were coagulated. Tube 1 was still clear and uncoagulated but was coagulated by next morning.

The acid was present in tubes 2 and 3. to the extent of. about 1 in 14 000 and 1 in 10000 respectively.

Experiment 30.

Muscle plasma made with 4 per cent Ammonium Chloride Acetic acid .1 per cent.

Tube 1. 2 c.c. plasma

" 2. 2 c.c. " + 1 drop Acetic acid

" 3. 2 c.c. " + 3 drops " "

all put into the water bath at a temperature of 27° C. at 12.22 p.m.

They were still uncoagulated next morning at 9.30 a.m. Flocculi began to shew themselves in tube 3 about 5 p.m. and in tube 2. about 7.30 p.m. At this time Tube 1. still clear. It remained so until 8 p.m. It was coagulated next morning at 10.30 a.m.

The long delay in coagulation is due to the strong saline, in which the plasma is dissolved.

The acid was present in tube 2 to the

extent of 1 in 2,000, and in tube 3. 1 in 14,000.

The effect of acids in these experiments has been either to precipitate the myosinogen at once from its solutions or to hasten coagulation.

The same point is observed in the coagulation of solutions of myosin - or muscle plasma made from muscles in a state of Rigor mortis. This is, of itself, faintly acid in reaction and coagulates more quickly than muscle plasma made with the same saline from fresh muscle, which is of course neutral or faintly alkaline.

Effect of alkalies

The effect of alkalies is to retard or prevent coagulation. This is brought out in the following experiments.

Experiment 31.

Muscle plasma in 3 per cent Ammonium Chloride

Caustic Soda 1 per cent.

Tube 1 contains 2 c.c. plasma.
" 2 " 2 c.c. " + 1 drop NaOH.
" 3 " 2 c.c. " + 2 " "

Test tube 1. is neutral or faintly acid in reaction.
Tube 2 is faintly alkaline 1 in 4000.
Tube 3 is alkaline 1 in 2000.

All three put into water bath at a temperature of 27°C. at 12·7 pm. Tube 1. had begun to coagulate at 9·30 am. next morning. Tubes 2 & 3 were still clear at 10·30 am. ~~on the~~ following morning.

A similar result was obtained in experiments with muscle plasma, prepared with Magnesium Sulphate, to which Caustic Soda had been added.

Experiment 32.

Muscle plasma in 4 per cent Ammonium Chloride
Sodium Carbonate 1 per cent.

Tube 1 4 cc. plasma

" 2 4 cc " + 1 c.c. Carbonate

" 3 4 cc " + 1 cc "

All put into the water bath at a temperature of 27°C. Tube 1 was coagulated within 48 hours. Tubes 2 and 3 were uncoagulated at the end of four days.

These experiments are sufficient to show that alkalies even in small quantities have a strong hindering action on coagulation. Other examples on the same point will be found in the course of this work. Indeed, this variation in the rapidity of coagulation produced by slight differences in the reaction proves a great stumbling block in experiments, made to test the action of certain substances on coagulation of salted muscle plasma. It is extremely difficult to determine, by means of Litmus paper, that

the reactions of any two solutions are exactly alike and it is evident that unless the reactions are identical any conclusions, which may be drawn, from the rapidity of coagulation, regarding the action of any substance on salted muscle plasma, will be apt to lead one very much astray.

Conclusions

Among the conditions which influence the rapidity of coagulation are. 1. Dilution. Within limits. the greater the dilution the more rapid the coagulation of the salted muscle plasma. Over dilution immediately causes a precipitation of the myosinogen.

2. Temperature - Within limits the higher the temperature the more rapid the coagulation. Too high a temperature causes

heat-coagulation of the Paramyosinogen and then of the Myosinogen.
3. Reaction. This is one of the most important conditions. The two previous can be readily avoided. It is more difficult to avoid this one.
A slight increase in the acidity favours - a slight increase in the alkalinity of the plasma delays or prevents coagulation.

The <u>phenomena</u> observed in coagulation of salted muscle plasma are. 1. Increase in opalescence 2. Flocculiform. 3. Jelly stage of the diluted plasma is rich in Myosinogen 4. Contraction of the clot.

The <u>Myosin or muscle clot</u> has no organic structure — in this respect resembling milk clot. It is probably the result of cohesion of flocculi.

V

Relative solubility of myosinogen and myosin in saline solutions.

The essence of coagulation, whether of blood, of milk or of muscle, is the conversion of a more soluble proteid into one less soluble.

In experiments on diluted salted muscle plasma the substance which separates out in the process of coagulation is myosin, and is evidently less soluble than the myosinogen from which it has been derived.

Myosin ought therefore to be insoluble in dilute saline solutions which still contain sufficient saline to dissolve myosinogen.

I have made several experiments on this point of which the following may serve as illustrations of the method and of the results obtained.

Experiment 33.

Rabbit killed by blow on back of head
Blood vessels of hind limbs washed out with normal saline.
The muscles of one limb at once removed and chopped finely.
This divided into seven approximately equal portions. Each of which was added to a flask containing 80 c.c. of solutions of Ammonium Chloride of various strengths; varying from .5 to 4 per cent. Each was stirred up and tested at intervals for Paramyosinogen and Myosinogen with 2 per cent acetic acid.

Time	10.55 am.	11.10	11.25	12.40	1	10 am.
.5 per cent	—	—		√?	√faint (?)	Little
1 " "	—	—	—	√very faint	√still feeble	difference
2 " "	—	√faint	√faint	√faint	√better	from the
2.5 "	—	√better		√fairly marked		results at
3 " "	—	√well marked		√jelly	—	1 p.m. of
4 " "	—	√well marked		√jelly		previous day.

The precipitate, obtained by acetic acid, may however only consist of Paramyosinogen. To determine whether myosinogen also is present it is necessary to observe whether the fluid coagulates.

At 4 p.m. and at 5 p.m. therefore, samples were taken from each flask and kept at a temperature of about 35°C.

By 10 a.m. next morning well marked coagulation changes had occurred in the 2 per cent 2·5 and 3 per cent samples. And by 3·30 p.m. of that day a clot had formed in the 4 per cent sample.

Faint flocculi were present in the other tubes

It would appear from this experiment that myosinogen is soluble in 2 - 2·5 - 3 and 4 per cent solutions of Ammonium Chloride. The results

obtained in the other tubes by acetic acid ~~or~~ coagulation are so slight that for purposes of comparison with myosin they may be ignored.

Experiment 34.

The other hind limb of same rabbit used in Experiment 33 allowed to pass into a state of Rigor mortis next morning, while rigor was still present, the muscles were removed, chopped, and treated in exactly the same way as in Experiment 33. and tested with acetic acid.

Time.	12·40	12·55	1·10	2·55	4·30.
·5 per cent.	–	–	–	–	√ faint
1 " "	–	–	–	√ very faint	√ faint
2 " "	–	–	√ faint	√ faint	√ marked
2·5 " "	–	–	√ faint	√ fairly marked	√ very marked
3 " "	–	√ faint	√ marked	√ very marked	√ jelly
4 " "	–	√ faint	√ marked	√ very marked	√ jelly

These results do not indicate any striking difference between fresh and Rigor muscle.

There is little if any difference in the quantity of substance, precipitable by Acetic acid, which is obtained from fresh muscle and from Rigor mortis muscle.

Coagulation experiments were also made with samples of the fluid from each flask. It is only necessary to refer to the results with the four stronger solutions.

All put into the water bath at 11 a.m. By 11.20 a.m. flocculi had begun to appear in the 2. and 2.5 per cent- tubes. At 11.25 flocculi also in the 3 per cent. The 4 per cent only opalescent at 1 p.m. By 4 p.m. a firm clot in the 4 per cent - marked flocculi in the 3 per cent + also in the 2.5- and 2 per cent- samples.

The coagulation may have been due to the acid reaction of these fluids. Another experiment was therefore made in which

the fluids were neutralised with Sodium Carbonate solution. Coagulation also occurred in these tubes in this experiment.

Experiment 35

Rabbit killed and blood vessels washed out as before.

Muscles of one hind limb chopped fresh & portions extracted with 2·5 - 3· and 4 per cent solutions of Ammonium Chloride.

Muscles of the other hind limb, after Rigor had set in, chopped and portions extracted in the same way as the fresh muscle

Equal volumes of solution used for extraction in each case.

10 c.c. from each flask into a test tube.

To each of the Myosin tubes were added 4 drops of a ·1 per cent solution of Sodium Carbonate.

Reaction of all the tubes made

as nearly neutral as possible
All put into water bath at
a temperature of 27°C.

Time.	Myosinogen.			Myosin.		
	2·5	3	4	2·5	3.	4.
11·30 am	—	—	—	—	—	—
2·55 pm	—	—	—	—	—	—
3·55.	—	—	—	Flocculi	opalesc.	clear.
4·25	opalesc	opalesc.	clear	Coag.	"	opalesc.
5	"	"	"	"	"	"
7·15	"	"	"	"	"	"
9·25 am.	Coag.	Coag.	opalesc.	Coag	Coag.	"
12·15 pm.			"			"
7·30 pm.			"			Coag.
10·30 am			Coag.			

Coagulation occurred therefore somewhat earlier in the myosin tubes. Practically no difference was observable in the quantity of clot in the corresponding tubes of the two series.

An experiment on the same lines was made with ·8, 1 and 1·5 per cent

solutions of Magnesium Sulphate. Similar results were obtained. But neither in the myosinogen nor in the Myosin tubes was the clot so marked as in the 2·5 - 3 + 4 per cent Ammonium chloride plasmas.

If a muscle plasma, prepared with ,5 per cent Magnesium Sulphate, be diluted with four times its volume of water the diluted plasma contains 1. per cent of the Sulphate.

Conclusion from these Experiments

The conclusion I would draw from these experiments is that there is little appreciable difference between the solubility of myosinogen and of myosin, as it exists in muscle during Rigor mortis, in weak saline solutions.

On the other hand the myosin, produced during coagulation of these plasmas, is insoluble in

the same saline solutions.
The myosin, separated out in coagulation of salted muscle plasma, is therefore less soluble than myosinogen and less soluble also than myosin existing in muscle in a state of Rigor.

This may be accounted for in two ways.

1. Myosin obtained in the coagulation of salted muscle plasma may be slightly different from the myosin in Rigor muscle.
2. The relative insolubility of salted plasma myosin may be due to the difference in temperature. The solution of myosin from muscle in a state of Rigor mortis is made at the ordinary temperature 13°-16°C the coagulation of salted muscle plasma in the above experiments at a temperature between 27°-30°C.

That myosin may be soluble at one temperature and insoluble at another, slightly more elevated but not elevated sufficiently to cause heat coagulation, is not impossible.

Ringer & Lea has obtained a Caseate of Lime which is soluble at a low temperature and separates out in a curdy form when the temperature is elevated. On cooling this again, the curd redissolves. and so in this way one can precipitate and redissolve the substance several times.

I have put this question with regard to Myosin to the test of Experiment.

Experiment 36.

Muscle plasma made from fresh muscle with 3·5 per cent Ammonium Chloride. Put into water bath at a temperature of 30°C. in the afternoon. Was coagulated into

a jelly next morning.
Flask removed from the water bath to the ordinary temperature of the room. and shaken slightly. Two hours afterwards clot still undissolved.
50 c.c. of 3·5 per cent solution of Ammonium Chloride added to it and allowed to remain for 24 hours. The larger bulk of the clot had not dissolved
Then added an equal volume of ¼ per cent ammonium Chloride. This dissolved the larger part of the clot forming an opalescent, somewhat muddy, solution.

Other experiments corroborate this one. so that the relative insolubility is not, ~~absolutely~~ at any rate, the result of the difference in temperature.

It would seem to be indicated

that the myosin of coagulated salted muscle plasma is not quite identical with the myosin of Rigor mortis muscle.
A certain amount of caution is necessary in applying results, obtained in the coagulation of salted muscle ~~plasma~~, to Rigor mortis.

Halliburton[1] considers that the two myosins are identical but the evidence brought forward in this section regarding the slight difference in their solubilities can, I think, only be explained by supposing that they are not quite identical.

VI.

Is Myosin a Calcium compound?

Fibrin and Casein are, both, generally believed, on good grounds, to be chemical compounds of Calcium with a proteid.

So far as I am aware no such grounds exist for regarding Myosin as similarly a Calcium compound.

Halliburton[3] gives a formula for Myosin which makes no mention of its containing Calcium.

I have made some experiments, with the object of determining, by comparative volumetric estimations, whether there was any difference in the amount of Calcium obtainable from Myosinogen and Myosin.

I prepared three series of samples of these substances

from Rabbits muscle - the myosinogen from fresh muscle and the myosin from muscle in Rigor. In one sample the myosin was prepared from Coagulation of salted muscle plasma.

The samples so obtained were well washed both with distilled water containing a little acetic acid & with ~~distilled water~~ alone, then dried over sulphuric acid & weighed. They were afterwards incinerated in a porcelain or platinum capsule. The ash dissolved ~~with~~ with strong Hydrochloric acid, ~~diluted~~ with distilled water and filtered through a pure filter paper, After neutralising with Ammonia some Ammonium oxalate added and the solution either warmed or left to stand for many hours until precipitation was completed.

Filtered - precipitate washed and finally washed through a hole made in the bottom of the filter with hot dilute Hydrochloric acid.

A decinormal solution of permanganate was used to estimate the Calcium.

An example will best show the kind of result obtained.

Experiment 37.

2·2029 grammes of Myosinogen incinerated and the Calcium oxalate obtained - only a faint precipitate - dissolved with warm dilute Hydrochloric acid Solution measured 150 c.c.

First estimation made with 50 cc.

Second estimation made with 100 cc.

To the 100 cc. some strong Sulphuric acid added - the whole then heated and while hot the permanganate dropped in.

Four drops were sufficient

This is equal to ·2cc

The 150 cc. required therefore ·3cc

of the decinormal permanganate to oxidise the oxalic acid contained in it.

The corresponding myosin Sample - 1·2006 grammes incinerated and treated in the same way.

Final solution measured 150 cc.

First estimation made with 50 cc.

Second " " " 100 cc.

3 drops were sufficient to oxidise the oxalic acid in 100 cc.

This is equal to ·15 cc.

150 cc. therefore require ·225 cc.

2·2019 grammes myosinogen - ·3 cc. permanganate

1·2006 " myosin ·225 cc. "

2·2019 grammes of the myosin - ·4 cc. permanganate.

It is unnecessary to calculate the result in terms of Calcium Oxalate, or Carbonate or oxide. It was only intended to compare the amounts in different samples of the two proteids prepared under

as nearly similar conditions as possible:
The difference is not a great one and might easily be ~~explained by sources of~~ error in the method.

With these quantities of the substances, as shewn also in the other experiments - only a slight difference can be detected.

To obtain a larger quantity of the substance I made the following experiment.

Experiment 38.

Muscles of both legs chopped together at once.
One half put immediately into 350 c.c. of a .5 per cent solution of acetic acid in distilled water. The flask and water were previously weighed together and by weighing the whole it was found that the amount of muscle added

was about 5·4 grammes.

The other half of the chopped muscle allowed to stand for several hours, until rigor had become well marked in the rabbit, and 5·4 grammes then added to 350 cc. of same solution of acetic acid.
Extraction in each case was allowed to go on for 15 hours 35 minutes.
Filtered through a porous glass plate and the residue in each case washed with 150 cc. of the same solution of acetic acid - i.e. 5 per cent.

The residues dried over a water bath, at the same temperature, and finally over sulphuric acid. Mass weighed, incinerated and treated as in the previous experiment. A more dilute solution of permanganate used in

making these estimations
namely 5 c.c. of the decinormal
solution in ~~100~~ c.c. of distilled
water.

Myosinogen (muscle) 0.3225 grammes
Final solution measured 60 c.c.
First estimation made with 30 c.c.
required 1.1 c.c. of permanganate
Second estimation made with 30 c.c.
required 1.2 c.c. permanganate.
The 60 c.c. therefore required 2.4 c.c.

Myosin. Muscle. 6.6924 grammes
Final solution measured 60 c.c.
First estimation made with 30 c.c.
required 2.2 c.c. permanganate.
Second estimation made with 30 c.c.
required 1.7 c.c. permanganate.
The 60 c.c. therefor required 3.4 c.c.

The estimations were made at
the same time and the indicator
tint made of same depth as far
as possible in each.

4·3228 grammes Myosinogen muscle. 2·4 c.c. Permang.
6·6924 " Myosin " 3·4 c.c. ".

The difference is here also not great. Still it is, like the other experiment described, in favour of there being more Calcium in Myosin than in Myosinogen.

I hesitate to draw any conclusion from these experiments alone. The differences brought out are too slight to admit of this. But considered in conjunction with other results obtained by other methods they may be regarded as furnishing some evidence as an allied question - namely - whether Calcium plays any part in the coagulation of muscle plasma or of muscle.

The conclusion cannot be drawn from them that Myosin is a Calcium Compound.

VII

Effect of Gastric Digestion on Myosinogen and Myosin.

I have made several experiments on this point. of which the following are described in greater or less detail

Myosinogen

Experiment 39.

Myosinogen - prepared by extracting rabbit's muscle with 10 per cent. Sodium chloride filtered - Myosinogen precipitated from filtrate by acetic acid, washed with large quantities of water by decantation. Digested in a large quantity of .2 per cent Hydrochloric acid + some of Benger's Liquor Pepticus in water bath for several days. An insoluble residue still remained at the end of that time.

Experiment 40

Myosinogen - prepared from Rabbits muscle. with 5 per cent. Magnesium Sulphate - Blood vessels of rabbit previously washed out.
Myosinogen precipitated with Acetic acid and precipitate washed in a large quantity of distilled water.
Digested in .2 per cent Hydrochloric acid with Benger's liq. Pepticus.
Digestion fluid changed twice. after a week's digestion an insoluble residue.

Experiment 41.

Myosinogen - prepared with 7 per cent Ammonium Chloride precipitated by throwing into distilled water - washed with a large quantity of distilled water by decantation.
Digested in a large quantity of the ~~fluid~~ of artificial gastric

juice, prepared as before,
Digestion continued for several
days at a temperature of 30°C.
At the end of that time a
slight insoluble residue.

Experiment 42.

Myosinogen - prepared with 5
per cent Magnesium Sulphate
precipitated by throwing it
into a large quantity of distilled water.
Precipitate washed in large
quantities of distilled water.
Digested with the same artificial
gastric juice as before for
several days at a temperature
of 30° C.
A slight residue.

Experiment 43.

Rabbit killed - blood vessels of
hind limbs washed out.
Muscles chopped and extracted
with 5 per cent Magnesium
Sulphate.

filtered and precipitated by throwing into waters. Washed with water by decantation. Redissolved in Magnesium sulphate - reprecipitated and grew washed.

. Digested in three changes of digestive mixture for over a week at a temperature of about 30° C.

A well marked residue

Myosin.

Experiment 44.

Rabbits muscle in Rigor Mortis chopped and extracted with 13 per cent Ammonium Chloride. Filtered and myosin precipitated by throwing filtrate into a large quantity of water. Precipitate collected and washed twice by decantation with a large quantity of waters. Redissolved precipitate in

Ammonium Chloride. It dissolved completely. Filtered Reprecipitated by throwing into water and washed twice by decantation.

Precipitate put into a mixture of .4 per cent Hydrochloric acid and Liquor Pepsinus Digested for two days at a temperature of 30° C.
Fresh acid and liq. Pepsinus added to residue and digested at same temperature for two days longer.
Still a residue.

Experiment 45.

Myosin.

Muscles of hind limbs of a rabbit extracted first with a large quantity of distilled water as in the preparation of Nucleo-albumen then with 13 per cent Ammonium Chloride. Filtered, and Myosin precipitated by throwing the

filtrate into a large quantity of water.
Precipitate washed twice with a quantity of distilled water.
Digested with the mixture of Hydrochloric and and Liquor Pepticus for several days. The slight residue

Experiment 46

Myosin.
Muscles of back of rabbit
Extracted first for five days with large quantities of distilled water to remove as much Nucleo-albumen as possible.
Muscle then extracted with 13 per cent Ammonium Chloride. Extract filtered and thrown into a large quantity of water.
Myosin collected, washed twice with distilled water.
Put into mixture of Hydrochloric

acid and liquor Pepticus and digested for four days at a temperature of 40° C. Fresh acid and pepsin added to residue and digestion continued for four days longer.
Fresh acid and Pepsin again added to residue and digestion continued for 24 hours.
There is still a residue

Experiment 47

Myosin.

Muscles of rabbit extracted with large quantities of distilled water for seven days. Extracted then with 13 per cent Ammonium Chloride. Filtered and filtrate thrown into a large quantity of water. Precipitate collected and washed first with distilled water, then with .5 per cent solution of Sodium Carbonate

with which it was allowed to remain in contact ~~for~~ several hours.

Finally it was again washed with distilled water.

Digested for twenty four hours in mixture of Hydrochloric and and liquor Pepticus. in water bath

Fresh acid and liquor Pepticus added to the residue and dijestion continued for other twenty-four hours.

Again fresh acid and Pepticus addedd to residue and portion digested.

A residue still remained.

Experiment- 48.

Myosin.

Muscles of Hind limbs of rabbit chopped and poundedwith sand. Extracted twice with large quantities of distilled water. in the preparation of nucleo-albumen. Then extracted with 10 per cent

solution of Magnesium Sulphate.
Extract filtered and myosin
precipitated by throwing filtrate
into a large quantity of water.
Did not separate well and a
little acetic acid added.
Precipitate washed twice by
decantation with large
quantities of water
Redissolved in Magnesium
Sulphate with the aid of a
little alkali. Filtered and
reprecipitated by throwing into
water.
Washed twice by decantation.
Digested with .4 per cent
Hydro-chloric acid and Liquor
Pepticus for two days at a
temperature of 28°C.
Fresh acid and liquor Pepticus
added and digestion continued
for several days longer.
Again fresh acid and liquor
Pepticus added to residue
and digestion continued for

twenty four hours.
Still a residue.

Experiment 49.

Same myosin as that used in the previous experiment. Washed further with distilled water. It would not redissolve in 5 per cent Magnesium Sulphate. Digested in 4 per cent Hydrochloric acid with some liquor Pepticus for about a week at 30° C.
A slight residue.

Experiment 50.

Control.

A mixture of Hydrochloric acid and the liquor Pepticus used in the above experiments digested for 48 hours at a temperature of 30° C.
No residue.

The conclusion arrived at from these experiments is that both myosinogen and myosin yield a residue to gastric digestion.
In the foregoing experiments attempts of various kinds have been made to purify the myosinogen and the myosin as far as possible from nucleo-albumen but I have never failed to obtain a residue. This residue is not large in amount but is, nevertheless, well marked.

Nature of this Residue

This residue is insoluble in .4 per cent Hydrochloric acid. It is also insoluble in boiling alcohol and in boiling ether. It is readily soluble in .5 per cent Caustic soda and in 1 per cent Sodium Carbonate.
I have in several instances tested this residue for Phosphorus

For this purpose it was first washed with dilute Hydrochloric acid then with distilled water. and finally washed well with hot alcohol and hot ether.
It was then fused in a platinum capsule with the same mixture of Potassium Nitrate and Sodium Carbonate used in the previous experiments of the same kind.
In the three specimens examined in this way I obtained a slight but distinct precipitate with Ammonio nitro-molybdate.

This residue presents all the characters therefore of a Nuclein.

Is there Phosphorus in Myosinogen or Myosin?

I have examined several specimens of Myosinogen and Myosin prepared as in the foregoing experiments on the effect

of gastric digestion upon these substances.

The substances were purified first from inorganic phosphates and ~~then~~ from Lecithin by means of hot absolute alcohol and ether.

In one sample the substance was extracted with cold absolute alcohol for several weeks and with hot alcohol for several days and finally for several days with hot ether.

All these were fused with Potassium Nitrate and Sodium Carbonate.

On testing with Ammonio-Nitro Molybdate the characteristic canary yellow crystalline precipitate appeared in the cold.

I have not been able, therefore, to prepare Myosinogen or Myosin entirely free from Phosphorus nor from Nuclein.

These results are at variance with those obtained

by Whitfield[15]. He obtained an insignificant deposit on digestion of myosin ~~prepared~~ from Dog's muscle. This deposit did not contain Phosphorus.

A sample of myosin prepared from the muscles of the hind limbs and back of a rabbit did not contain Phosphorus.

Digestion of the muscles of the back and hind limbs of a rabbit for several days left a residue which did not contain Phosphorus.

Kühne and Chittenden,[35] on the other hand, state that myosin yields a considerable residue to gastric digestion. They did not investigate the question further.

My own experiments leave little room for doubt that both myosinogen and myosin yield a residue to gastric digestion, and that this residue possesses the

properties of a nucleïn. My experiments on the presence of Phosphorus in Myosinogen and Myosin are in harmony with these results.

I would not be understood to suggest that Myosinogen or Myosin are Nucleo-albumens. Certain reasons have already been adduced - such for instance as the behaviour of these substances to weak solutions of Sodium Carbonate - which indicate that they are not Nucleo-albumens. Further Whitfield finds that Myosin does not cause intravascular coagulation.

I think rather that the results obtained are due to an admixture with Nucleo-albumen.

Lilienfeld[4] has found that purified Fibrinogen yields a similar residue to gastric digestion.

Frederikse[5], however, has not

been able to confirm this observation and may probably, therefore, have worked with a purer substance than Lilienfeld.

Lilienfeld[4] has found also that fibrinogen contains Phosphorus and he regards it as a nucleo-albumen

Schäfer[11] thinks that the residue obtained by gastric digestion is so small as to render it probable that it is due to admixture with the small quantity of a nucleo-albumen. The results of Fredericse[5] favour this view.

It is much more difficult to purify myosinogen than fibrinogen in consequence of the fact that the former becomes insoluble with repeated washings. When such results therefore are obtained with a purified fibrinogen it is not to be wondered at that similar results are obtained.

with a purified myosinogen or with a purified myosin.

There seemed very little difference in the relative amounts of the residue obtained from myosinogen and myosin but I have made no accurate observations on this point.

Conclusion

Myosinogen and myosin yield a residue of a nuclein to gastric digestion but this is probably due to admixture with a nucleo-albumen

VIII

Action of nucleo-albumen on coagulation of salted muscle-plasma.

I have made several experiments on this question both with muscle and with thymus nucleo-albumen. I give examples of the experiments made with muscle nucleo-albumen.

Experiment 51.

Muscle plasma made with 7 per cent Ammonium Chloride nucleo-albumen of muscle dissolved in dilute Sodium carbonate solution.

Tube 1. 2 c.c. plasma + 2·5 c.c. Aq. dest.

Tube 2. 2 c.c. " + 2·5 c.c. nucleo-alb.

Both tubes put into water bath at temperature of 28°C. at 3·20 p.m. Both uncoagulated at 8·40 p.m. Tube 1 was coagulated at 9.25 a.m. of the next day. Tube 2 was still uncoagulated 48 hours after

the commencement of the Experiment.

This delay in coagulation may not be due to the presence of nucleo-albumen in tube 2. but may in reality be due to the addition of the alkali in which this proteid is dissolved. It has already been pointed out that an increase of alkalinity exercises a retarding influence on coagulation.

Experiment 5-2.

Same plasma as in the previous experiment and same nucleo-albumen solution.

Tube 1. 2 c.c. plasma + 2 c.c. Aq. dest.

Tube 2 2 c.c. " + 2 c.c. nucleo-alb.

To tube 1 was added some Sodium Carbonate so as to make the reaction of the two solutions as nearly as possible alike, as tested with litmus-paper.

Both tubes put into water bath, the temperature of which varied between 29° and 32° C. during the experiment.

Both tubes were still uncoagulated 48 hours after commencing the experiment.

This experiment shews that the delay obtained in the tube 2 of the previous experiment was due not to the Nucleo-albumen but to the alkali which was added.

Similar results were obtained in the experiments with nucleo-albumen prepared from Thymus Gland.

One cannot deduce any conclusion from these experiments as to the action of Nucleo-albumen on the coagulation of salted muscle plasma.

It is difficult to see how the alkalinity of the Nucleo-albumen solution is to be avoided.

Even with a solution of a nucleo-albumen in 5 per cent Sodium Carbonate similar results were obtained.

These experiments indicate however that if nucleo-albumens have any influence in ~~hastening~~ the coagulation of salted muscle plasma this is readily overcome by the presence of a small quantity of a weak alkali

IX.

Action of Leuko-nuclein on coagulation of salted muscle plasma.

The Leuko-nuclein ~~obtd~~ in these experiments was obtained from nucleo-albumen prepared by Halliburton's method from the Thymus gland of an ox. This nucleo-albumen was dried over sulphuric acid, pounded with .8 per cent Hydrochloric acid and allowed to extract for several hours.

The undissolved residue was dissolved in a small quantity of weak caustic soda, filtered and reprecipitated with .8 per cent Hydrochloric acid

It was redissolved and reprecipitated This precipitate was washed with distilled water and dried over Sulphuric acid.

The action of .8 per cent Hydrochloric acid on nucleo-

-Histon (nucleo-albumen). is, according to Lilienfeld[4], to break it up into its two constituents, Leuko-nuclein and Histon. The former is insoluble in this strength of Hydrochloric acid; the latter is soluble in it.

Leuko-nuclein invariably produces intravascular coagulation and also causes coagulation in a non coagulable mixture of fibrinogen and a Calcium salt.

Histon, on the other hand, is a powerful preventive of coagulation of the blood both intra- and extra-vascularly.

In experimenting with Leuko-nuclein on Muscle plasma the same difficulty meets us as in the case of Nucleo-albumen.

I have not been able to dissolve this Leuko-nuclein in a strictly ~~alkal~~ neutral medium. The solution was required to be distinctly alkaline before the

Leukonuclein dissolved to any extent.

The following are some of the experiments I have made with this Leuko-nuclein.

Experiment. 53.

Muscle Plasma made with ¼ per cent Ammonium chloride.
Leukonuclein .166 grammes in 4.3 cc. Aq. dest. + 4 cc. of a 1 per cent solution of Sodium Carbonate.
All the nuclein did not dissolve after several hours. 2 c.c. of the supernatant solution diluted with 8 cc. of distilled water.-
Tube 1. 2 cc. plasma + 2 cc. Aq. dest.
Tube 2. 2 cc " + 2 cc. diluted Leukonuclein

Both tubes put into water bath - temperature 28°C.
Tube 1. coagulated within 18 hours.
Tube 2. was still uncoagulated after 48 hours.

Experiment 54.

Muscle Plasma made with Ammonium Chloride ¼ per cent
A stronger solution of Leukonuclein used than in previous Experiment
Exact strength not determined.

Tube 1. 2 cc. plasma + 3 cc. Aq. dest.
Tube 2. 2 cc. " + 1·4 cc. Nuclein + 2·6 cc. Aq. dest.

Leuko-nuclein solution was made with .5 per cent Sodium Carbonate and was fairly alkaline.

Both tubes put into water bath - temperature varied between 28° and 32°C.
Flocculi were well marked in Tube 1. in about 90 minutes
Tube 2. remained unchanged for about twelve hours when flocculi began to appear.

Experiment 55.

Same plasma and same Leuko nuclein solution as in previous experiment.

Tube 1. 2 cc. plasma + 2 cc. Aq. dest.
Tube 2. 2 cc. " + 1 c.c. leukonuclein + 1 cc. Aq. dest.

Some Sodium carbonate added to tube 1. to make reaction of both as nearly as possible alike.

Both put into water bath - temperature 30°C. - at 10.30 a.m. - were uncoagulated at 6.55 p.m. when they were removed from bath and kept at the ordinary temperature overnight. at 9.40 a.m. returned to bath. temperature 28°C. They were uncoagulated at 6 p.m. and also at 9 a.m. on the following morning. Tube 1. had begun to coagulate at 9.50 a.m. but tube 2. remained fluid for many hours subsequently. It ultimately also coagulated.

The results of these experiments are exactly similar to those in the experiments with

Nucleo-albumen.

The results are here also to be ascribed not to the Leuko-nuclein but to the alkali in which this is dissolved.

If Leuko-nuclein exerts a hastening influence on the coagulation of salted muscle plasma it is obscured by the presence of a small quantity of a weak alkali.

X.

Action of Histon on coagulation of salted muscle plasma.

The Histon employed in some of these experiments was prepared from the same nucleo albumen from which the Leuko-nuclein of the last section was obtained.

The extract in .8 per cent Hydrochloric acid was treated with several times its volume of a mixture of alcohol and ether and well shaken and the Histon allowed to precipitate.

The supernatant fluid was then removed and the precipitate washed with fresh alcohol and ether and dried over Sulphuric acid.

This Histon ~~not~~, as Schenfeld calls it, Hydrochlorate of Histon, dissolved to some extent in distilled water, forming a solution with an acid reaction.

As all the dried substance did not dissolve the exact strength of the solution cannot be given.

The results obtained in experiments upon the action of Histon on coagulation of salted plasma are explainable by the variation in reaction.

When Histon is added, as it is prepared it acts in reaction, it hastens coagulation. When neutralised or made faintly alkaline it delays coagulation as shewn in the following Experiment.

Experiment 56

Muscle plasma prepared with ¼ per cent Ammonium Chloride.

Histon solution neutralised with 1 per cent Sodium Carbonate.

Tube 1. 2 cc. plasma + 2 cc. Aq. dest.

Tube 2. 2 cc " + 2 cc. Histon Sol.

Both tubes put into water-bath - temperature 28°-30° C at 3.10 p.m. and were still

uncoagulated at 8.40 p.m.
Tube 1. was coagulated at 9.25 a.m. next morning. Tube 2 was still uncoagulated at 4.40 p.m. on the day following. Here there is a distinct retardation.

Experiment 5th

Same plasma and same Histon solutions as in Experiment 5b.

Tube 1. 2cc plasma + 2cc. Aq. dest
Tube 2. 2c.c. plasma + 2c.c. Histon. solution

To both tubes a little sodium Carbonate added - Reaction of both faintly alkaline.

Both tubes put into water bath - temperature 29°C. - at 3.3 p.m.
Both still uncoagulated at 10 p.m. of the next day.
Tube 2. was coagulated at 9. a.m. of the following day - temperature now 36°C.
Tube 1 still uncoagulated at 5 p.m. of that day.

Another experiment, made with a second Histon prepared also from Thymus nucleoalbumen, in which at was attempted to make the control and Histon tubes of the same reaction yielded a similar result to experiment 56.

It is seen then that a solution of Histon, apparently reacting neutral to litmus, has retarded coagulation as compared with a control to which no alkali has been added. In an attempt to make the reactions of control and test solutions exactly alike by the addition of Sodium Carbonate to both no delay in coagulation is observed in the latter.

The variation in the results are due, in all probability, to the variations in the reaction

XI

Action of myo-albumose on coagulation of salted muscle plasma.

To determine this point I have made several experiments with this substance prepared in the following manner. Extraction was made with normal saline and the other forms of proteid got rid of by Trichlor-acetic acid. The filtrate having been dialysed for 3-4 days was evaporated at a temperature never exceeding 35°C. and dried over sulphuric acid.

The results will be best indicated by examples of the experiments.

Experiment 5-5.

Muscle plasma prepared with ¼ per cent Ammonium chloride.

Myo-albumose-residue prepared as above described .085 grammes.

in 10 cc. of distilled water.
It nearly all dissolved.
Reaction very faintly acid.
Tube 1. 2 cc. plasma + 2 cc. Aq. dest.
" 2. 2 cc. " + 2 cc. Nyo-albumose
Both put into water bath.
temperature 28°C — at 2.55 p.m.
Were uncoagulated at 8.40 p.m.
Both coagulated at 9.25 a.m.
next morning.
Coagulum in tube 2 more
marked & more complete than
in tube 1.

Experiment 59.

Same plasma and same
albumose solution as in
Experiment 58
Tube 1. 2 cc. plasma + 2 cc. Aq. dest.
Tube 2. 2 cc " + 2 cc. Albumose
Reaction of both made as nearly
alike as possible.
Both put into water bath,
temperature 29°C, at 10.25 a.m.
Tube 2 shewed well marked

coagulation at 10 p.m. of the same evening.
Tube 1. still clear at that time & was still uncoagulated 24 hours later.

Experiment 60.

Muscle plasma prepared with ¼ per cent Ammonium Chloride.
Myo albumose – of residue .082 grammes in 10 cc of distilled water and made faintly alkaline with some 1 per cent Sodium Carbonate.
Tube 1. 2 cc. plasma + 2 cc Aq. dest.
Tube 2. 2 cc. " + 2 cc. Myo-albumose.
No alkali added to tube 1.
Both put into water bath, temperature 28°C.; at 10.40 am. well marked flocculi in Tube 2. at 3.50 p.m.
Flocculi first appeared in tube 1. at 6.45 p.m.
Experiment repeated.

yielded a similar result.

Experiment 61.

Same muscle plasma - Same myo-albumose as in Experiment 60. only myo-albumose made more distinctly alkaline with Sodium Carbonate.

Tube 1. 2 cc. plasma + 2 cc. Aq. dest.
Tube 2. 2 cc. " + 2 cc. myo-albumose.

Both put into water bath, temperature 30° C., - at 11.40 a.m. No coagulation in either at 11.20 p.m. Removed from bath to ordinary temperature and returned to bath at 10.40 a.m. next day.

Tube 1. coagulated by 6.35 p.m.
Tube 2. only more opalescent with doubtful flocculi and had undergone no further change by 7.20 p.m.

Experiment 62.

Muscle plasma prepared with ¼ per cent Ammonium Chloride Myo-albumose - of residue .108 gramme in 15 c.c. of distilled water. Solution therefore filtered and filtrate made faintly alkaline with 20 drops of 1 per cent solution of Sodium Carbonate. These are equal to 1 c.c.

Part of this neutralised solution was boiled - a flocculent precipitate formed which was removed by filtration. Reaction of this solution still faintly alkaline. Precipitate probably phosphatic and is a further indication that the reaction is neutral or faintly alkaline

Tube 1. 1 c.c. plasma + 1 c.c. Aq. dest.
Tube 2. 1 c.c. " + 1 c.c. Myo-albumose
Tube 3 1 c.c " + 1 c.c do. boiled

All into the water bath at 1½ 10 p.m. temperature 31°C.

By 9.40 p.m. of same evening.

Flocculi had begun to form in tubes 2 + 3. Tube 1 still clear.
At 9.45 a.m. next morning coagulation was complete in tubes 2 and 3. Tube 1. only more opalescent.
Flocculi showed themselves first in tube 1 about 11.25 a.m.

Experiment 63.

Same plasma and same myo-albumose solution as in Experiment 62. Experiment began on following day.
The boiled myo-albumose was boiled a second time for several minutes.

Tube 1. 1 c.c. plasma + 1 c.c. Aq. dest.
Tube 2. 1 c.c. " + 1 c.c. myo-albumose
Tube 3 1 c.c. " + 1 c.c. di boiled.

All put into water bath - temperature 31°C - at 10.30 a.m.
At 5 p.m. no coagulation in any of the tubes.
At 11.30 p.m. faint flocculi in

tube 1. Very much better marked flocculi in tube 2. None in tube 3.

Still no flocculi in tube 3. at 8.25 p.m. and only faint flocculi at 9.30 a.m. next morning.

There is in this experiment a hastening in tube 2., as compared with tube 1., in spite of the fact that a faintly alkaline solution has been added to it and in spite also of the further fact that tube 1 has had its alkalinity reduced by standing.

Boiling has destroyed the activity in tube 3. and allowed the alkali to exercise an unrestricted action.

Experiment-64. Control.

This was made to determine whether trichloracetic acid after neutralisation with

Sodium carbonate exercised any influence on coagulation. The reaction of the albumose solution is acid probably due to some trichloracetic acid remaining after dialysis.

Muscle plasma prepared with ¼ per cent Ammonium Chloride.

Solution prepared as follows.

Trichloracetic acid 10 per cent .3 c.c.
Aq. destillata 4.7 c.c.
Sodium Carbonate 1 per cent. 1.7 c.c.
Reaction very faintly alkaline

Tube 1. 1 c.c. plasma + 1 c.c. Aq. dest.
Tube 2. 1 c.c. " + 1 c.c. above Solution.

Both put into water bath temperature 31°C. at 10.35 a.m.
Flocculi in tube 1. at 7.20 p.m. that evening. None in tube 2 at this time nor at 8.25 p.m. of same evening.
Faint flocculi next morning at 9.30 a.m.

Conclusion

These experiments show that, independent of variation in the reaction, within certain limits, myo-albumose exercises a hastening influence on the coagulation of salted muscle plasma. It can exercise this influence even when it is made faintly alkaline, This influence is not evident when its solutions are made more strongly alkaline

Boiling slightly prolonged destroyed its activity - less slightly prolonged appeared to have little effect.

XII

Action of Myo-albumose on the coagulation of blood – extra- and intra-vascularly.

The experiments, made on this point, were carried out with Myo-albumose prepared in the same manner as that used in the previous experiments on muscle-plasma.

It was unable to make many experiments because of the difficulty in obtaining large quantities of the material.

Extravascular blood

Experiment 65.

·272 gramme of dried residue dissolved as far as possible in 5 c.c. of a ·6 per cent. solution of Sodium Chloride + 5 c.c. of 1 per cent. solution of Sodium Carbonate.

Solution is alkaline in reaction.

Rabbit anaesthetised with Chloroform

10 cc. of blood drawn from the carotid artery clotted in a minute or two.

10 cc. blood allowed to flow from carotid into a test tube containing 5 cc. of myo-albumose solution. This also clotted very quickly.

To the remainder of the albumose solution (about 4 cc). in a test tube an approximately equal quantity of blood was allowed to flow. Shaken. Clotting occurred very quickly.

The myo-albumose solution tested with Nitric and gave a faint precipitate disappearing on heating and reappearing on cooling.

Experiment 66.

Unconcentrated myo-albumose prepared by normal saline and trichlor-acetic acid method. Dialysed and neutralised

To 50 cc. of this were added 150 cc. of blood of an ox as it was being shed. Shaken. Coagulation had occurred before the blood reached the laboratory. Fifteen minutes after being shed.

Experiment 67.

8 cc. of a solution of Myo albumose made from the dried residue Strength not determined but known to be fairly strong. It was made alkaline with Sodium Carbonate.

To this were added a little over 10 cc. of blood from a sheep and well shaken. Coagulation had occurred and a complete clot formed before the blood reached the laboratory a few minutes after being shed.

Intravascular.

Experiment 68.

Myo-albumose - .58 gramme of residue dissolved as far as possible in 60 cc. of 1 per cent. Sodium Carbonate. Filtered. Filtrate tested with nitric and gave a faint precipitate disappearing on heating and reappearing on cooling.

Young rabbit. - weight 850 grammes.

Anaesthetised - Carotid artery exposed. and cannula inserted in the external jugular vein.

A sample of blood taken from the carotid before the injection coagulated in about two minutes.

60 cc. of solution then injected Injection was finished in three minutes.

Some blood, drawn from the Carotid immediately the injection was finished, coagulated in about one minute
Blood, drawn 5 minutes after, clotted in one minute
Blood, drawn 10 minutes after, clotted in 4–5 minutes
Blood, drawn 15 minutes after, clotted in 1 minute
Animal bled to death 20 minutes after completion of the injection - the blood clotted readily.
Except then for a very slight delay in the blood, drawn 10 minutes after, the injection. has had no effect on coagulation.
This is the only experiment on intravascular ∧ Coagulation.

Conclusions.

Thyo-albumose exercises no influence ~~on~~ with coagulation of rabbits, or or sheep's blood to which it has been added while being shed.
It exercises no influence on the

coagulation of blood when injected into the blood vascular system of rabbits.

These would seem to indicate that it is, at any rate, not identical with Lehmfeldt's Histon which prevents coagulation when added to rabbit's blood either extra- or intra-vascularly.

XIII

Action of Calcium salts on coagulation of salted muscle plasma.

The importance of Calcium in the coagulation of blood and of milk is now firmly established No work so far as I am aware has been carried out to determine what part it plays in the coagulation of muscle plasma.

My own experiments have been made with Calcium Chloride and Calcium Sulphate.

Both of these salts are now known to be effective in hastening the coagulation of salted blood plasma.

Green[10] observed no hastening of coagulation of salted blood plasma with Calcium Chloride

Ringer and Saintsbury[11] have observed that, not only Calcium Chloride, but also the chlorides

of the allied metals Strontium and Barium hasten coagulation. The experiments of Hammarsten shew that Calcium Chloride materially hastens the coagulation of Fibrinogen ~~to~~ whilst Fibrin ferment has been added.

The following are the results obtained in some of my experiments.

Calcium Chloride

Experiment 69.

Muscle plasma prepared with 10 per cent Sodium Chloride 2 c.c. of this plasma added to 8 c.c. of Calcium Chloride containing respectively 0·8, 0·4, 0·1, 0·05, 0·025 per cent. Control tube to which no Calcium chloride was added contained 2 cc plasma diluted with 8 cc. of distilled water.

All tubes put in a water bath, temperature, 28°C. Flocculi appeared in about-

twelve minutes in all the tubes except those containing 0·8 and 0·4 per cent Calcium Chloride. Slight flocculi were present in the latter about six minutes later and in the former not until about half an hour later.

Experiment 70.

Same plasma as in Experiment 69.

Calcium chloride ·8 per cent

Tube 1. 4 cc. plasma + 8 cc. water.

Tube 2 4 c " + 8 cc. $CaCl_2$.

Tube 3. 2 cc. " + 6 cc. water.

Tube 4. 2 cc " + 6 c. $CaCl_2$

Tube 5 2 cc. " + 8 cc. water

Tube 6. 2 cc + 8 cc. $CaCl_2$

All tubes put into water bath. at 12. 10 am. Temperature varied during the experiment between 28° and 33°C. Coagulation had not commenced in tubes 1, 2. 3 + 4.

by 3.50 p.m. of same day. Coagulation was present in tubes 1 and 3. at 10 a.m. next morning. but absent in tubes. 2 and 4. These tubes were still uncoagulated many hours afterwards. Coagulation was complete in tube 5 within fifteen minutes but did not appear in the corresponding Calcium tube until 2.35 p.m. that is two hours afterwards.

Experiment 41.

Muscle plasma prepared with 7 per cent Ammonium Chloride. Precipitated, washed with water by decantation Dissolved with more Ammonium Chloride Calcium Chloride .2 per cent in distilled water.

Tube 1. 2 cc. plasma + 2 cc. Aq. dest

Tube 2. 2 cc " + .1 cc. $CaCl_2$ + 1.9 cc. " "

Tube. 3. 2 cc. " + .2 cc " + 1.8 cc. " "

Tube 4. 2 cc " + .3 cc " + 1.7 cc. " "

Tube 5. 2 cc " + .4 cc + 1.6 cc " "

All put into water bath, temperature 28°C, at 11.5-9 a.m. Flocculi began to appear in tube 4 at 2.25 p.m. No change in others at 7.50 p.m. same evening. + were then put at ordinary temperature of room. Returned to bath at 10.20 a.m. No coagulation at 2 p.m. all had coagulated by 6 p.m.

Experiment "y" 2.

Muscle plasma prepared with "y" per cent Ammonium Chloride. Precipitated by throwing into distilled water, washed with distilled water, and redissolved in "y" per cent Ammonium Chloride in distilled water.

Calcium Chloride · 2 per cent.

Tube 1. 2 cc. plasma + 2 cc. Aq. dest

Tube 2. 2 cc " + ·1 cc. $CaCl_2$ + 1·9 cc. " "

Tube 3. 2 cc " + ·2 cc. $CaCl_2$ + 1·8 cc " "

Tube 4 2 cc " + ·3 cc " + 1·7 cc. " "

All put into water bath temperature 29°C. at 2 p.m. At 11·30 p.m. no change. Tube 4 somewhat more opalescent than others.

At 6·35 p.m. next day, temperature meanwhile having been raised to 36°C., flocculi were well marked in tubes 2. 3 & 4. Absent in tube 1. and still absent the following morning.

Calcium Sulphate.

Experiment #3.

Muscle plasma prepared with Magnesium Sulphate 5 per cent.

Calcium Sulphate saturated solution in distilled water.

Tube 1. 1 cc. plasma +4 cc. Aq. dest.
Tube 2. 1 cc. " + 0.2 cc. $CaSO_4$ +3.8 cc. "
Tube 3. 1 cc " + .5 cc. $CaSO_4$ + 3.5 cc. "
Tube 4 1 cc " + 1 cc. " + 3 cc. "
Flocculi appeared in the first three within fifteen minutes after being put into water bath - temperature 26°C
No flocculi in 4 until 15 minutes afterwards.

Experiment #4.

Muscle plasma in 5 per cent Magnesium sulphate.
Reprecipitated twice, washed and dissolved in 5 per cent Magnesium sulphate
Tube 1. 2 cc plasma + 6 cc. water.
Tube 2. 2 cc " + 6 cc " + 2 drops $CaSO_4$.
Put into water bath at a temperature of 27°C.
Flocculi appeared in Tube 1. within an hour, were still absent in tube 2 twenty-five hours after, were present

within twenty-eight hours after the commencement of the experiment.

Experiment No 5.

Muscle plasma prepared with 5 per cent Magnesium Sulphate Precipitated, washed, and redissolved.

1/4 saturated Solution of Calcium Sulphate.

Tube 1, 2 cc Plasma + 3 cc. Aq. dest.

Tube 2. 2 cc " + 2.9 cc ... + .1 cc $CaSO_4$.

Tube 3. 2 cc " + 2.8 cc .. + .2 cc "

Tube 4. 2 cc. " + 2.7 cc " + .3 cc --

Tube 5 2 cc " + 2.6 cc " + .4 cc "

Tube 6 2 cc " + 2.5 cc " + .5 cc "

All put into water bath at 12.25 p.m. — temperature 28°C.

At 1.30 p.m. flocculi had appeared in tube 1.

Flocculi in tubes 2 + 3 at 2.10 p.m.

Flocculi in tube 4 at 2.20 p.m.

+ in tubes 5 and 6 at 3.15 p.m.

Experiment 76

Same plasma as in Experiment 75.

1/8 saturated Solution of Calcium Sulphate.

Tube 1. 2 cc. plasma + 3 cc. Aq. dest.

Tube 2. 2 cc " + 2.9 cc. " " + .1 cc. Ca SO_4

Tube 3. 2 cc " + 2.8 cc " " + .2 cc. "

Tube 4. 2 cc " + 2.7 cc " + .3 cc "

Tube 5. 2 cc " + 2.6 cc " + .4 cc "

Tube 6. 2 cc. " + 2.5 cc " + .5 cc "

All put into water bath temperature 27.° C. at 4.35 p.m.

At 6.25 p.m. fairly well marked flocculi in tubes 2 & 3. and absent in others

At 6.40 p.m. similar flocculi in tube 1. and fainter flocculi in tubes 4, 5 and 6

At 6.45 p.m. fairly marked in tube 4. still faint in 5 and 6

By 7.30 p.m. they were fairly well marked also in tubes 5 and 6.

Conclusions

Some of these experiments furnish evidence, by no means definite, however, that both Calcium Chloride and Calcium Sulphate in minute quantities exercise a hastening influence on the coagulation. The difficulty in obtaining a pure myosinogen owing to the change which this undergoes in the process of purification is a great drawback for experiments of this kind. With a fairly strong solution of a myosinogen free or practically free from Calcium more trustworthy results would be obtained.

These experiments however bring out another point very definitely - namely that slightly larger quantities of both Calcium Chloride and Calcium sulphate exercise a delaying influence on the coagulation

of salted muscle plasma. This is well illustrated in all of the experiments given and is obtained with apparently very small quantities of the salts.

It has been stated that, when myosino-gen has been rendered insoluble by repeated washing, it may be got to redissolve to some extent by adding a little calcium chloride to the saline used as solvent.

These two facts may be the results of the same cause but what this may be is not known.

XIV

Action of Calcium, Strontium and Barium salts in excess on coagulation of blood plasma and of blood.

The retarding effect, produced on the coagulation of salted muscle plasma by the addition of comparatively small quantities of calcium salts, led me to investigate the question whether a similar effect was produced on blood.

My first experiments were made on oxalated blood plasma. Calcium Chloride only was used in these experiments.

Blood plasma and Calcium Chloride

The plasma was prepared by centrifugalising blood to which Potassium oxalate had been added to the extent of 2 grammes to the litre.

It was clear, straw coloured.

Part of this plasma was used

undiluted, the other part was diluted first with an equal volume of .2 per cent Potassium oxalate and then with some distilled water.
These will be spoken of as "Plasma" and "diluted" Plasma", in the following experiments.
Calcium Chloride in 5 per cent solution used.

Experiment 77.

Tube 1. 10 cc. plasma + .4 cc. $CaCl_2$.
Tube 2. 10 cc. " + .3 cc. "

Half of each tube into water-bath., temperature 36°-37°C.
The other half of each tube kept at the ordinary temperature in this case 13°C.
A firm clot had formed within an hour in the two tubes in the water bath.
Two hours afterwards a firm clot had formed in tube 1

kept at the ordinary temperature. There were then in tube 2. only a few filaments of fibrin but in this also a firm clot next morning.

The plasma therefore coagulates on the addition of calcium chloride in this amount both at the ordinary temperature and at 36°-37°C.

Experiment 78.

Tube 1. 10 cc. plasma + 1 cc. $CaCl_2$.
Tube 2. 10 cc. " + 2 cc. "
Tube 3. 10 cc. " + 3 cc. "

Half of each at the ordinary temperature designated as tubes 1, 2. and 3.
The other half of each put into water bath temperature 38°-40°C. and designated as 1^a, 2^a, 3^a.

Within three hours a well marked clotting in

Tube 1. (ordinary temperature 13°C).
No coagulation in any of the other tubes even after twenty-nine hours.
Plasma therefore coagulated at 13° C. which would not coagulate at 38°-40° C.

Experiment 79

Tube 1. 10 c.c. diluted plasma + 1 drop $CaCl_2$.
" 2. 10 cc. " " + 2 drops "
" 3. 10 cc. " " + 3 " "
" 4. 10 cc. " " + 4 " "
" 5. 10 cc. " " + 5 " "
" 6. 10 cc " " + 6 " "
" 7. 10 cc " " + 7 " "
" 8 10 c.c. " " + 8 " "
" 9 10 cc " " + 9 " —
" 10 10 cc. " + 3 cc. $CaCl_2$.

All the tubes put into the water bath at a temperature of 38° C.

Tubes 3 & 4 coagulated in 7 minutes
" 2, 5, 6. 7. " "10 "
" 8 " 13 "
9 " 23 "

Tubes 1 and 10 had not coagulated and were still perfectly fluid three hours after the commencement of the experiment. They were then removed from the water bath to the ordinary temperature of the room. Tube 1 remained uncoagulated. Tube 10 had set into a solid jelly within half an hour.

Conclusion

There is a certain proportion of calcium most favourable to coagulation of this oxalated plasma. This proportion in the experiment I quoted, was 3 to 4 drops of a 5 per cent solution to 10 cc. of plasma. Beyond that amount there was delay in coagulation, the delay

increasing with increasing quantities of Calcium chloride until it may be made to extend over many hours.

Heat seems to increase this delaying action, at any rate, when the Calcium chloride is present in certain quantities This is shewn in Experiment 78 tubes 1. and 1a.

It is also illustrated in tube 10 of Experiment 79 in the coagulation which occurred so rapidly when it was removed from the water bath.

I have observed this effect of heat frequently in the course of these experiments on oxalated plasma.

Effect on blood.

I have made experiments to determine Calcium, ~~Strontium~~ and ~~Barium~~ salts in excess retard coagulation of blood.

Ringer and Saintbury ^ shewed that they are all capable of hastening the coagulation of salted blood plasma.

Experiment 80.

Calcium chloride

7/5 c.c. of an 8 per cent solution of Calcium Chloride (non anhydrous). + 1.75 cc. of blood received straight from the ox and mixed thoroughly Received at the laboratory at 4.35 p.m. It was then perfectly fluid. Still fluid at 7.35 p.m.

at 9.30 a.m. next morning all of it fluid except a small portion of clot adhering to the bottom of the flask. The corpuscles had subsided to some extent and there was a layer of clear plasma on the surface Coagulation became

only slightly more marked during the next four days and the layer of plasma increased in thickness. It was only two days subsequently, ie about a week after the blood was shed, that coagulation became complete.

Experiment 81.

This experiment was made with the blood obtained in experiment 80. on the day following that on which the blood was shed.

Tube 1. 2 cc. blood + 6 cc. water
" 2. 2 cc " + 4 cc "
" 3 2 cc " + 2 cc "
" 4 2 cc " + 1 cc "
" 5. 2 cc " + .5 cc "

all were kept at the ordinary temperature of the room. A firm clot had formed in 1, 2 and 3. in 1 about 43.

minutes,

Coagulation in tube 4 about 17 minutes afterwards. and in tube 5 about 20 minutes after tube 4.

Experiment 82.

Strontium chloride

A solution of 6 grammes of Strontium chloride was made in 10 c.c. of water. Blood was run in straight from a sheep to bring up solution to 200 cc.

In the mixture there is 3 per cent of Strontium Chloride (Non anhydrous).

There was absolutely no coagulation when putrefaction set in five days afterwards.

Experiment 83.

Barium Chloride

A solution of 5 grammes of Barium Chloride made.

in 10 cc. of water. It did not quite entirely dissolve. Made up to nearly 200 cc. with blood from a sheep. Received at the laboratory at 5.15 p.m. perfectly fluid. It was still fluid at 8.30 p.m. Remained fluid all next day. Was somewhat lumpy on pouring into another flask at 9.35 a.m. of following day. The greater portion was still fluid five days after the blood had been shed, when putrefaction began to set in.

Experiment 84.

Calcium Sulphate.

Saturated solution of Calcium sulphate in water. To 50 cc. together with some of the undissolved salt were added 150 cc. of blood received straight from a sheep.

It was still fluid when putrefaction had begun to set in five days later.

Conclusion

These experiments make it evident that not only Calcium, Strontium, and Barium chloride but also Calcium Sulphate retard or prevent the coagulation of the blood.

In looking up the literature subsequently I have only been able to find one reference to this question.

The influence of Barium chloride in retarding coagulation has been observed by Green[10] who believed that it was due to the precipitation of the Calcium Sulphate, considered by him to be necessary for blood coagulation.

In the light of further researches on the influence

of Calcium chloride on Coagulation this theory is no longer tenable.

It is outside the limits of this paper and would carry us too far to enter upon a discussion of this question now.

These experiments have only been introduced as corollary to those made on muscle plasma with Calcium chloride and Calcium Sulphate in which a similar retarding of coagulation was observed.

It is interesting to find that an observation first made on salted muscle plasma also applies to blood plasma and to blood

XV.

Action of oxalates on coagulation of salted muscle plasma.

I have used in my experiments upon this subject both Potassium, and Ammonium oxalates. In most of the experiments plasma prepared with Ammonium chloride was used.

The following are examples of some of the experiments.

Experiment 85.

Muscle plasma prepared with 13 per cent Ammonium Chloride.

Ammonium Oxalate of various strengths

Tube 1. 2 c.c. plasma + 8 c.c. water.

" 2. 2 cc " + 8 cc. oxalate .025 per cent

" 3 2 cc " + 8 cc " .05 " "

" 4. 2 cc " + 8 cc " .1 " "

" 5. 2 cc " + 8 cc " .2 " "

" 6. 2 cc " + 8 cc. " .4 " "

" 7. 2 cc " + 8 cc " .8 " "

All the tubes put into the water bath temperature 28°–32°C
Tubes 1, 2, and 3. coagulated in about 16 minutes
Tube 4. coagulated about 15 minutes later.
Tube 5 coagulated within 46 minutes after tube 4.
Tubes 6 and 7 were still uncoagulated three hours after tube 5 had coagulated. By 10 a.m. next morning tube 6 had also coagulated.
Tube 7 was somewhat milky but remained uncoagulated during this and following day.
The solution in tube 7 contains .64 per cent of ammonium oxalate.

Experiment 86.

Experiment similar to the previous one

Same number of tubes used & the same strength of Ammonium oxalate in each.
Only in each tube there were 2.5 c.c. plasma and 7.5 c.c. oxalate solution.
Tubes numbered corresponding to those in Experiment - 85
Put into water bath, temperature varied 30°-34°C.
Coagulation had not occurred in any of the tubes five hours afterwards.
Tubes 1, 2, 3, 4 and 5 more opalescent, 6 & 7 practically unchanged.
Tubes transferred to ordinary temperature of room for several hours. At the end of that time slight precipitation in tubes 1 and 2. and these coagulated within three hours after being returned to the water bath.

Tube 6 co-agulated in about three hours after tubes 1 + 2.
all the other tubes remained uncoagulated.
Why coagulation should have occurred in tube 6 it is difficult to say. Occasionally contradictory results are obtained in these experiments

Experiment 8/4

Muscle plasma prepared with 10 per cent- Sodium Chloride.
Potassium oxalate of various strengths.

Tube 1: 2cc plasma + 8 cc. water.
" 2 2cc " + 8 cc Oxalate .025 per cent-
" 3 2cc " + 8 cc " .05 "
" 4 2cc " + 8 cc " .1 "
" 5 2cc " + 8 cc " .2 "
" 6. 2cc " + 8 cc " .4 "
" 7. 2cc " + 8 cc " .8 "

All put into water bath temperature varying 28°-32°C. Flocculent-coagulation in tubes 1, 2, 3, 4, 5- & 6. within 15 minutes
no Flocculation in tube 7. six hours after the commencement of the experiment. Very faint flocculi next morning and a few hours after well marked.

Experiment 88.

Plasma made with 4 per cent. Ammonium Chloride in distilled water.
Ammonium Oxalate 1.6 per cent.

Tube 1. 2 cc plasma + 2.5 cc aq. dest.
" 2. 2 cc " + 2.3 cc " + .2 cc Oxalate
" 3. 2 cc " + 2.2 cc " + .3 cc "
" 4. 2 cc " + 2.1 cc " + .4 cc "
" 5- 2 cc " + 2 cc " + .5 cc "

Solutions measured from burettes.

All tubes put into water bath temperature 28°C.
Coagulation occurred in tubes 1 and 3. about the same time, within nine and a half hours after tubes put into bath.
Coagulation in tube 2 about 25 minutes later.
No coagulation in tubes 4 and 5 at 9 p.m. or 15 minutes after tube 2 had coagulated.
Incomplete coagulation in tube 4 at 9.45 a.m. next morning.
Tube 5 was still only opalescent.
Amount of oxalate in this tube is .17 per cent.

Experiment 89

Same plasma as in experiment 88
Ammonium oxalate 1.6 per cent.

Tube 1, 1 cc plasma + 1.5 cc. Aq. dest.
" 2 1 cc " + 1 cc " " + .5 cc. oxalate
" 3 1 cc " + .5 cc " " + 1 cc "

All put into water bath at a temperature of 30° C. at 5.19 p.m.

By 10 a.m. next morning there were faint flocculi in tube 1

Tubes 2 + 3 still clear

Coagulation complete in tube 1 at 11.40 a.m.

Tubes 2 + 3 were still unaltered, except for a slight increase of opalescence three days after the beginning of the experiment.

Amount of oxalate in tube 2 = .3 per cent.

Experiment 90.

Muscle plasma prepared with ¼ per cent. Ammonium Chloride in distilled water.

Potassium oxalate 2 per cent.

Tube 1. 1 cc plasma + 1.5 cc. Aq. dest.
" 2 1 cc " + 1 cc " + .5 cc oxalate
" 3 1 cc " + .5 cc " + 1 cc "

Put into water bath at 8.21 pm.
Temperature 30° C
By 10 a.m. next morning
coagulation in tube 1.
At 11.40 a.m. tube 2 was
more opalescent
neither tube 2 nor tube 3.
underwent any further
change within the next
twenty four hours.
Amount of oxalate in tube 2 is .66 per cent.

These experiments, along with others I have performed, show that both Ammonium and Potassium oxalates retard coagulation. It is difficult to determine the percentage of salt required as this varies with the different plasmas.

It is possible that the oxalates may simply act as salts per se and thereby retain the protein in solution.

That this is not the case is

evident from a consideration of some of the above experiments.

If we compare for instance Tube 1 of Experiment 88 with tube 2 of Experiment 89, as regards the amount of salts contained in them we get this result.

Tube 1. of Exp. 88. is made up thus.

2 cc. plasma + 2.5 cc. aq. dest.

The mixture therefore measures 4.5 cc. and contains 0.14 gramme of Ammonium Chloride = 3.1 per cent.

Tube 2 Exp. 89. is made with the same plasma thus:-

1 cc. plasma + .5 cc oxalate + 1 cc. aq. dest.

The mixture therefor measures 2.5 cc. and contains .07 gramme of Chloride and .008 gramme of oxalate or altogether .078 gramme of salts = about 3.1 per cent.

The difference in the proportion of salts in these tubes is exceedingly slight. Tube 1. Exp. 88 coagulated within three and a half hours

whereas Tube 2 Exp. 8.9 had only become slightly more opalescent in three days.

Both were subject to the same surroundings as regards temperature.

The question was made the subject of direct experiment and in the following manners.

Experiment 91.

Plasma prepared with 1.3 per cent Ammonium Chloride.

Potassium oxalate 1.6 per cent.

Tube 1. 10 cc. plasma + 30 cc. Aq. dest.

" 2 10 cc " + 22.5 cc " " + 12.5 cc. oxalate

" 3 10 cc " + 32.5 cc. " " + 12.5 cc " "

All three put into water bath, temperature 28°C.

Tube 1. coagulated within two hours.

Tubes 2 + 3 had not coagulated at the end of twenty four hours.

Tube 1 contained 3.25 per cent of salt
" 2 " 3.3 " " —
" 3 " 2.7 " " " "

Tube 3 therefore contained about .5 per cent less of salt than tube 1. yet this coagulated very much earlier.

Experiment 92.

Muscle Plasma in 13 per cent Ammonium Chloride (different plasma from that used in Experiment 91)
Ammonium Oxalate 1.6 per cent.
Solution of Ammonium Chloride 1.6 per cent in distilled water.

Tube 1. 10 cc. plasma + 30 cc. 1.6 per cent NH_4Cl
" 2. 10 cc " + 27.5 cc NH_4Cl + 2.5 cc oxalate
" 3. 10 cc " + 25 cc " + 5 cc "
" 4 10 cc " + 20 cc " + 10 cc "
These tubes all contain the same per centage of salts.

All the tubes put into the water bath temperature 36°C. at 10.40 a.m.
No coagulation in any tub 5 p.m.
Tube 1 more opalescent
At 6.55 p.m. all the tubes removed from water bath and kept at the ordinary temperature until 9.35 a.m. next morning.
Restored to water bath. 36°C.
Flocculi in tube 1 at 11.35 a.m.
No change in tubes 2.3 + 4.
at 5 p.m. of the following day.

Experiment 93.

Plasma prepared with ¼ per cent Ammonium Chloride in distilled water
Sodium chloride 1.5 per cent in Aq. dest.
Potassium oxalate 1.0 " " " "

Tube 1. 2 c.c. plasma + .5 c.c. NaCl + 2 c.c. Aq. dest.
2. 2 c.c. " + .5 c.c. $K_2C_2O_4$ + 2 c.c. " "
3. 2 c.c. + .75 c.c. " + .75 c.c. " ".

Tubes 1 and 3 contain the same proportion of salts - tube 2 contains less.

all put into the water bath
temperature 26°C. at
6.40 p.m.
No coagulation in any of them
at 10 p.m.
At 9.40 a.m. next morning
were present in tube 1 and
became well marked by 10.20 a.m.
Temperature now raised
to 30°C. at 11.10 a.m. At this
time beginning flocculi in
tube 2.
No change or only slight increase
in opalescence in tube 3.
Flocculi did not become
well marked in ~~tube~~ 2 until
about 12.30.
Flocculi did not appear
in 3 until about 1.40 p.m.
becoming well marked
about 2.20 p.m.

Conclusion

From the results of these
experiments it may be concluded

that Ammonium and Potassium oxalates do not owe their activity in preventing the coagulation of salted muscle plasma to any property they possess as salts per se.

Judging from analogy it seems probable that they act in virtue of their ability to precipitate Calcium.

I am not aware that any previous work has been done on the action of oxalates on the coagulation of salted muscle plasma. Several experiments have been made on frogs and on rabbits to determine what influence they exert on the onset and duration of Rigor mortis. Such experiments have been made by Cavazzani[28] Howell[30] and Locke[29].

I have made some experiments on pithed frogs and one on a

rabbit on the effect of injection of oxalates. My ~~results~~ in all the cases were practically negative.

A further discussion of this question is not properly included under the title of this Thesis. and it will suffice therefore merely to state that my results so far agree with those of Howell and Locke.

XVI

Summary of Results

Although the conclusions have already been summarised at the end of each section, a brief general survey of the whole results arrived at may be of advantage.

I There is in muscle an albumose which is unrecognisable by the Buret and Nitric acid reactions in ordinary extracts, but recognisable in these by the Potasso-mercuric Iodide Tannic acid and Phosphotungstic acid tests.

It is not due to digestion of the muscle proteids ~~by~~ & the Pepsin known to be present in muscle.

It is therefore a true myo albumose.

It presents close affinities with deutero-albumose. but is probably not identical with it

It hastens the coagulation of salted muscle plasma even when made faintly alkaline

It does not retard coagulation when added to rabbits blood as it is being shed.

Similarly it does not retard the coagulation of ox or of sheep's blood.

It does not retard the coagulation of the blood in rabbits when injected into the blood-vascular system

It is therefore not identical with Lilienfeld's Histon.

II. There is in muscle a nucleo-albumen.

This is best prepared by Wooldridge's method.

By Halliburton's method a large admixture with myosinogen or paramyosinogen occurs.

It is soluble in weak Sodium Carbonate, contains Phosphorus, yields a residue to gastric digestion and produces intravascular coagulation in rabbits.

When injected into the vascular system of rabbits it sometimes fails to produce intravascular coagulation. It may then produce Wooldridge's negative phase of coagulation.

It sometimes produces partly intravascular coagulation and partly the "negative Phase".

It is derived in all probability from the substance of the muscle itself. This is indicated by the fact that better experimental results are obtained with the precipitate from extract of muscle which has been previously pounded with sand. Again myosinogen and myosin are difficult to obtain free from Phosphorus, and even after being well purified they yield a residue to gastric digestion. This does not necessarily indicate that these proteids are themselves Nucleo-albumen. On various grounds, it seems more probable that the results obtained are due to an admixture with a nucleo-albumen. If this be*

so then the close association between the myosinogen or myosin and the nucleo-albumen is strong evidence that the latter is derived from the sarcous substance of muscle.

Muscle nucleo-albumen contains approximately 1.7 per cent of Phosphorus.

There is is no evidence that muscle-nucleo-albumen or Thymus nucleo-albumen exercises a hastening influence on coagulation of salted muscle plasma. The delay observed is due to the alkali in which the nucleo-albumens are dissolved

III. Conditions affecting coagulation.

Dilution. Within limits the

greater the dilution the more rapid is the coagulation. Over dilution causes at once a precipitation of the myosinogen.

Temperature. Within limits the higher the temperature the more rapid the coagulation. Too high a temperature causes heat coagulation. The temperature ought not to exceed 35° C.

Reaction of the Solution.

Slight variations in the reaction, not discernible with any accuracy by litmus paper, influence the rapidity of coagulation.

A slight increase in the acid direction hastens coagulation. A greater increase at once precipitates the myosinogen.

A slight increase in the alkalinity, on the other hand, retards, a greater increase prevents coagulation.

IV. Phenomena of Coagulation.

There is first an increase in the opalescence of the solution which is followed by a flocculent stage. The flocculi run together to form a more or less complete jelly stage and finally there is the stage of contraction.

The jelly stage is not always evident and is dependent on the amount of myosinogen in the plasma.

The muscle clot is unorganised and resembles milk clot rather than blood clot.

V Solubility of Myosinogen and Myosin.

There is little appreciable difference between the solubilities in saline solutions of myosinogen and myosin as it occurs in muscle in a state of Rigor.

The Myosin, obtained in coagulation of salted muscle plasma, is less soluble in saline solutions than the myosinogen from which it has been derived.

Myosin obtained from salted muscle plasma is also less soluble than Myosin in Rigor Mortis muscle.

This difference is not entirely due to the difference in temperature at which the solubilities of plasma Myosin

and Rigor muscle Myosin are compared.

Plasma Myosin does not redissolve when the temperature is reduced to the ordinary temperature of the room.

There is probably therefore some difference between Plasma Myosin and Rigor muscle Myosin. The two are apparently not quite identical

VI Myosinogen and Myosin both yield a residue of Nuclein to gastric digestion and contain organic Phosphorus.

They are probably, however, not themselves Nucleo-albumens but are mixed with a Nucleo-albumen

VII. Leuko-nuclein

There is no evidence that the Leuko-nuclein of Lilienfeld exercises a hastening influence on the coagulation of salted muscle plasma.

VIII. Histon

There is no evidence that the Histon of Lilienfeld delays the coagulation of salted muscle plasma.

IX. Calcium

The trend of evidence is in favour of the view that Calcium salts play a part in coagulation of salted muscle plasma.
In small amounts they have been observed to hasten coagulation.
Oxalates or the other hand retard coagulation.
Estimations of the relative

amounts of Calcium in Myosinogen and Myosin are in favour of this view.

There is no direct evidence, however, that Myosin is a Calcium Compound. in the sense that Fibrin and Casein are generally regarded to be Calcium compounds.

On the other hand Calcium salts in excess delay or prevent the coagulation of salted muscle plasma. The excess need only be slight to produce this result.

X Blood.

Calcium Chloride and Calcium sulphate retard or prevent the co-agulation of blood, if added in excess.

Strontium and Barium

Chlorides act in a similar manner.

XVII

Biblio-graphy.

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