THE STROMATA OF THE RED CORPUSCLES.

By W. D. HALLIBURTON, M.D., B.Sc., *Assistant Professor of Physiology, University College, London,* AND WALTER M. FRIEND, M.D., (*Boston*).

(*From the Physiological Laboratory, University College, London.*)

THE constituents of the mammalian red blood corpuscles are haemoglobin, lecithin, cholesterin, a small amount of proteid material, and a small amount of inorganic salts of which the most important is potassium phosphate. The blood-pigment is by far the most abundant of these constituents; about 80—90 per cent. of the dried corpuscles being composed of it. It can be dissolved out from the corpuscles by numerous reagents, such as water, ether, dilute acids, etc.; and the colourless, less soluble portion of the corpuscle which is left is called the "stroma." The stromata retain approximately the shape of the original corpuscles, but they are swollen by the reagent used to dissolve out the haemoglobin, and in the case of some reagents are ultimately dissolved.

The stromata are composed of lecithin, cholesterin, proteid, and inorganic salts. They have been the subject of research by Schmidt, Kühne, Hoppe-Seyler and Wooldridge. The present investigation was undertaken for the purpose of determining the nature of the proteid contained in them, and the plan of the research has followed very much the lines of previous experiments by one of us[1], in an investigation of the proteid constituents of the white corpuscles.

The methods which have been adopted for the preparation of the stromata are the following:—

Hoppe-Seyler's method[2]. Defibrinated blood is mixed with ten times its volume of a solution of sodium chloride (made by mixing one volume of a saturated solution of sodium chloride with nine volumes of water) and set aside; the mixture is allowed to stand for a day; the

[1] Halliburton, *Brit. Assoc. Reports*, 1887, 1888. *Roy. Soc. Proc.* Vol. XLIV. p. 255. This *Journal*, Vol. IX. p. 229.

[2] *Hoppe-Seyler's Handbuch*, 5te Aufl. 1883, p. 429.

fluid is decanted from the subsided corpuscles, which are again mixed with the salt solution, and allowed to stand another day; the process may be then repeated. This separation can be effected in a much shorter time by the use of the centrifrugal machine. The mass of corpuscles ultimately obtained is treated with water to remove the haemoglobin, then shaken with water and ether, and lastly the stromata are collected on a filter.

Kühne's method[1]. The corpuscles are obtained free from serum as in the previous case; they are dissolved in a large excess of water, and the solution so obtained is treated with a stream of carbonic acid as long as white flakes continue to separate.

Wooldridge's method[2]. Freshly whipped blood is mixed with many times its volume of 2 per cent. sodium chloride solution, and the corpuscles separated from the salted serum by the use of the centrifugal machine; the mass of corpuscles is then mixed with more salt solution of the same strength, and the process repeated several times. The corpuscles which are thus obtained free from serum are then mixed with five or six times their volume of water, and a little ether added until the solution is perfectly transparent. The leucocytes which are unaltered by this treatment sink to the bottom of the vessel, and again the separation may be hastened by the use of the centrifuge. The supernatant fluid is then treated with a few drops of a one per cent. solution of acid sodium sulphate, until the fluid, at first clear becomes as thick as the original blood. The precipitate consists of the stromata which soon collect together to form coarse flocculi, and may be collected on a filter.

We first tried Hoppe-Seyler's method; we used sheep's blood, and the corpuscles subsided so slowly without the use of the centrifugal machine that even in the winter time putrefaction had invariably commenced before the process was completed. Even with the centrifugal machine we found that the corpuscles did not at all readily settle when we used the strength of saline solution recommended by Hoppe-Seyler. We found too, that the subsequent processes did not yield an abundant supply of stromata so readily as the method we ultimately adopted. We did not try Kühne's method, as it seemed to us adapted to obtaining the globulin-like substance of the red corpuscle, rather than the whole stroma.

[1] Kühne's *Lehrbuch*, p. 193.

[2] Wooldridge, *Zur Chemie der Blutkörperchen. Du Bois Reymond's Archiv*, p. 387, 1881.

The method we ultimately adopted was Wooldridge's with one slight modification. Instead of using a two per cent. solution of sodium chloride, we employed a solution of half that strength, as we found that in it the corpuscles settled more quickly. We employed the centrifugal machine throughout: but even then the processes are so prolonged, that it took us as a rule from seven to ten days to obtain a quantity of stromata sufficient to work with.

On one point we feel inclined to differ from Wooldridge. He states that the mixture of ether and water does not dissolve the stromata, but renders them so swollen and transparent that they escape detection; the action of the acid sodium sulphate is to cause them to shrink together again.

We made several observations on this point. We took some of the watery ethereal liquid, and added it to various kinds of blood (human, sheep's, frog's) under the microscope. We found that the red corpuscles were quickly rendered colourless, and the stromata which were at first swollen, became smaller and smaller until they were invisible and apparently dissolved; the nuclei in the case of frog's blood were unaffected, and the white corpuscles in all three kinds of blood were, as Wooldridge states, apparently unaffected also.

In adding the acid sodium sulphate to precipitate the stromata we found it must be added with great care; as very slight excess renders the stromata very insoluble, and decomposes the haemoglobin. It changes the oxy-haemoglobin into methaemoglobin. By spectroscopic examination of the filtrate after separating the precipitated stromata, we usually discovered a trace of methaemoglobin; a faint band between C and D being present. This was not the band of acid-haematin. We therefore think we can safely say that the precipitate contained no globin, the proteid constituent of haemoglobin; otherwise we should have found spectroscopic evidence of haematin.

The question then arises is the whole of the stroma contained in the precipitate? Wooldridge considered that it was; as we have already seen he looked upon the action of acid sodium sulphate not as a precipitation, but as a shrinkage action upon the previously swollen stromata, and in this view of the case he is supported by his finding lecithin and cholesterin as well as proteid matter in the precipitate. If however we regard the action of the sulphate as a precipitation of something that was previously dissolved, we cannot be certain that the whole stroma is contained in the precipitate. Acid sodium sulphate is a very powerful precipitant of proteid material, as we determined in a few control experiments with diluted white of egg, and it is therefore possible that it may merely precipitate the proteid matter of the dissolved stromata in a similar way. If we adopt this view of the way in which it acts, we are no doubt confronted with the objection we just urged

against Kühne's method, namely, that ours is a method which gives us not all, but only a part of the stroma. Kühne's carbonic acid method is obviously one which would precipitate only globulin, and only part of that; Wooldridge's method is one which would at least precipitate all the proteids. We have devoted considerable attention to this objection and have arrived finally at the conclusion that, in whichever way we regard the action of acid sodium sulphate, we have in the precipitate ultimately obtained, virtually all the constituents of the stroma; even although the action of the sulphate is a mere precipitation of proteids, the precipitate carries down with it at least part of the lecithin and cholesterin; and as we were particularly concerned with the proteids, it really mattered but little to us if some of the lecithin and cholesterin remained in solution.

Having thus obtained a precipitate of the stromata upon the filter, it was quickly washed from adherent oxyhaemoglobin with distilled water containing a trace of acid sodium sulphate in solution. The operation of washing must however be rapidly performed, as prolonged exposure to water or still more to water containing acid sodium sulphate in solution, makes it very insoluble. When freshly prepared the stromata are readily soluble in 0·2 per cent. hydrochloric acid, but after a time, more and more insoluble residue remains behind after treatment with that reagent.

Our investigations of the proteids contained in the stromata consisted in making extracts of the precipitate with various saline media. In a previous research made in a similar way on lymph cells, one of us was able to distinguish in those cells the following proteids:—

1. A globulin present in small quantities only, which coagulates at the temperature of 48°—50° C. (*Cell-globulin a.*)

2. A globulin occurring in large quantities which coagulates in a 5—10 per cent. sodium chloride solution at 60°—65° C.; and in solutions containing a minimal quantity of sodium chloride, or 5 per cent. of magnesium sulphate at 75° C. This proteid (*Cell-globulin β*) is either identical with or closely associated with the fibrin-ferment.

3. An albumin which coagulates at 73° C. (Cell-albumin.)

4. A mucin-like proteid similar to that described by Miescher in pus, and called hyaline substance by Rovida. This swells up into a jelly-like substance with solutions of sodium chloride, or magnesium sulphate but not of sodium sulphate. It is insoluble in water, and is precipitated by salts like globulins. It is not mucin, as it yields no reducing sugar after treatment with dilute mineral acids. It is however rich in phosphorus, and yields nuclein on gastric digestion

in addition to albumoses and peptones. It thus belongs to the class of proteids called nucleo-albumins.

5. If the lymph cells are not examined perfectly fresh, they become acid from the development of sarcolactic acid, and the proteolytic action of pepsin, or a closely allied ferment found in the cell, comes into play with the formation of albumoses and peptone.

We may take the foregoing as giving us a list of the proteids found in typical cells, in fact the proteids of protoplasm.

The proteids of liver cells were investigated by Plósz[1] in a very similar way, and with very similar results; there are present in liver cells:—

1. A proteid coagulating at 45° C.
2. A proteid of the nature of a globulin coagulating at 75° C.
3. A nucleo-albumin.

The proteids of muscular fibres, which are elongated and somewhat modified cells, show differences from the condition of original protoplasm: the chief modification from the chemical point of view being the appearance of myosinogen, a proteid convertible by ferment action into myosin.

The red corpuscles are cellular in origin also, and the question arises, how far do they resemble, and how far do they differ from unaltered protoplasm? The presence of haemoglobin is undoubtedly something entirely different from what we find in typical cells. We must therefore look for resemblances if any exist, not in the pigment, but in the uncoloured residue, the stroma.

In view of finding an answer to this question, we made extracts of the precipitated stromata with solutions of three salts:—

1. Half saturated solution of sodium sulphate.
2. Five per cent. solution of sodium chloride.
3. Five per cent. solution of magnesium sulphate.

The first named solution (sodium sulphate) we used at first, because one of us had found it the most convenient to use in connection with the white blood corpuscles. Sodium chloride and magnesium sulphate solutions swell up the white blood corpuscles into such a slimy mass, that subsequent manipulations are greatly hindered. Sodium sulphate has not this objection. We found subsequently that sodium chloride and magnesium sulphate solutions do not cause the stromata of the red corpuscles to swell in this way, and we consequently in our later experiments used them in preference to solutions of sodium sulphate, because

[1] *Pflüger's Archiv*, Vol. VII. p. 371.

they are much more powerful solvents. We found also that the more freshly the stromata were prepared, the more readily did they dissolve in these saline media; but there was in all cases a certain amount of insoluble residue. These solutions and this residue were then examined.

It will be most convenient to describe the examination of these materials, and the results obtained therefrom, by subdividing the general question, 'How far do the stromata of the red corpuscles resemble the white in regard to their proteid constituents?' into the following five subsidiary questions.

1. Do the stromata contain cell globulin *a*?
2. Do the stromata contain cell globulin *β*?
3. Do the stromata contain cell albumin?
4. Do the stromata contain nuclein or nucleo-albumin?
5. Do the stromata contain albumoses or peptone?

1. **Do the stromata contain cell-globulin** *a*? We examined the temperature of heat-coagulation of the proteids in a good many saline extracts of the stromata, but there was never any appearance of a cloudiness, much less precipitate, below the temperature of 60° C. We can therefore safely say, that the stromata do not contain cell-globulin *a*.

2. **Do the stromata contain cell-globulin** *β*? This proteid will in the subsequent portions of the paper be spoken of simply as cell-globulin.

Cell-globulin as obtained from the white corpuscles resembles paraglobulin or serum-globulin very closely. Its characteristics are as follows:—

a. In solutions containing a minimal amount of salt, or from 5 to 10 per cent. of magnesium sulphate, it is coagulated at a temperature of 75° C.

b. In solutions containing 5—10 per cent. of sodium chloride, it is coagulated at a much lower temperature, 60°—65° C.

c. It is precipitable from its solutions by carbonic acid, by dialysis, and by saturation with sodium chloride incompletely; by saturation with magnesium sulphate or ammonium sulphate completely.

d. It possesses fibrinoplastic activity; i.e. it has the power of hastening the formation of fibrin in dilute salted plasma, or in pure plasma (i.e. vein plasma, or pericardial and similar fluids). It is thus either identical with, or closely connected to the fibrin ferment.

Cell-globulin resembles pure serum-globulin in characteristics *a* and *c*; they differ from one another in characteristics *b* and *d*.

From our examination of saline extracts of the stromata of the red corpuscles, we find that they contain abundance of cell-globulin. The following are the facts upon which this conclusion rests.

i. *Heat coagulation.* On heating a faintly acidified extract of the stromata made with 5 per cent. magnesium sulphate solution, it becomes opalescent at 70° C. and a flocculent precipitate occurs at temperatures varying from 73° to 77°, but most frequently at 75° C. On filtering off this precipitate, there is practically no proteid found in the filtrate.

On heating a faintly acidified extract of the stromata made with 5 per cent. sodium chloride solution, it becomes opalescent at 60° and a flocculent precipitate occurs at 65°—66° C.

On saturating a saline extract of the stromata with magnesium sulphate, a precipitate is produced. If this precipitate be washed with saturated solution of magnesium sulphate, it can be redissolved on the addition of water, the adherent salt rendering it soluble. The solution so formed is faintly opalescent; or making this solution slightly acid with 2 per cent. acetic acid, and heating it gradually, it becomes more opalescent at 70°, and a flocculent precipitate occurs at 75° C.

If the precipitate produced by saturation with magnesium sulphate be dissolved in 5 per cent. sodium chloride solution, rendered faintly acid as before and heated, opalescence occurs at 60°, and a flocculent precipitate at 66° C.

ii. *Precipitation by salts, etc.* As just stated, a precipitate is produced by saturating a saline extract of the stromata with magnesium sulphate; saturation with ammonium sulphate causes an equally dense precipitate; saturation with sodium chloride causes a considerably less dense precipitate, showing that this salt does not cause such complete precipitation of the proteid as the other two just mentioned.

A stream of carbonic acid also causes a fine precipitate.

Dialysis of a saline extract of the stromata in a stream of running water, causes the contents of the dialyser to become turbid from the partial precipitation of the proteid originally in solution. In this as in all experiments in which dialysis was employed, putrefaction was prevented by the addition of crystals of thymol.

iii. *Fibrinoplastic powers.* The globulin which is thus proved to be present in the stromata of the red corpuscles possesses marked fibrinoplastic activity. The details concerning this power may be conveniently

stated in the form of a number of propositions, after the statement of each of which, we will quote illustrative experiments.

a. A saline extract of the stromata when added to dilute salted plasma hastens its coagulation.

EXPERIMENTS. Sheep's sodium sulphate plasma was diluted in each case to six times its volume, and allowed to stand in some cases at the atmospheric temperature of about 13° C. in other cases in a water bath at the temperature of 40° C.

One specimen diluted with water; coagulation occurred

at 13° C.	at 40° C.
in 50 minutes	in 40 minutes.

Another specimen diluted with a sodium sulphate extract of stromata, which had been freed from excess of salt by a few days' dialysis; coagulation occurred

at 13° C.	at 40° C.
in 10 minutes	in 5 minutes.

Other experiments were performed with sheep's magnesium sulphate plasma, which when diluted clots more slowly than does sodium sulphate plasma.

One specimen was diluted to six times its volume with water: coagulation had not occurred 48 hours afterwards.

Another specimen diluted to the same extent with the sodium sulphate extract of stromata mentioned above, clotted in a few hours both in the water bath at 40° and in the air at 13° C.

b. The globulin precipitated from saline extracts of the stromata, by saturation with magnesium sulphate, has when redissolved, marked fibrinoplastic properties.

EXPERIMENTS. Sheep's sodium sulphate plasma was used as before, and diluted in each case to six times its volume.

One specimen diluted with water coagulated

at 13° C.	at 40° C.
in 50 minutes	in 40 minutes.

Another specimen diluted with a solution of the globulin obtained from the stromata by precipitation with magnesium sulphate, and freed from excess of salt by a few days' dialysis, coagulated

at 13° C.	at 40° C.
in 10—12 minutes	in 6—8 minutes.

c. This same globulin causes the formation of fibrin in fluids like hydrocele and pericardial fluids.

EXPERIMENTS. A quantity of ascitic fluid was divided into two parts, A and B; A underwent no further treatment and had not coagulated even after 48 hours: to B a few drops of solution of the globulin were added, and in a few hours there was a marked formation of fibrin.

An exactly similar result was obtained with hydrocele fluid, using another preparation of the stroma-globulin; while in the control specimen there was no formation of fibrin, there was after about half-an-hour (at 40° C.) a firm clot in the specimen to which a few drops of the globulin solution had been added.

d. The fibrinoplastic power of this globulin is destroyed at the same temperature as that at which the globulin itself enters into a condition of heat-coagulation. (75°—80° C.)

EXPERIMENT. A solution of the globulin obtained from the stromata by precipitation with solid magnesium sulphate was rendered faintly acid and divided into three parts A, B and C.

A was not further treated.

B was kept at a temperature of 70° C. for 15 minutes, and subsequently cooled.

C was kept at a temperature of 77°—80° C. for 15 minutes, and subsequently cooled.

Sodium sulphate plasma (sheep) was diluted to six times its volume.

	Coagulation occurred	
	at temperature of room, 14°—15° C.	at temperature of water bath, 40° C.
with water	96 minutes	53 minutes
with solution A	12 ,,	3 ,,
with solution B	12 ,,	3—4 ,,
with solution C		75 ,,

e. The globulin obtained from the stromata is precipitable by dialysis; on the removal of this precipitate by filtration, a good deal of globulin still remains in solution. The filtrate therefore still retains considerable but slightly diminished fibrinoplastic properties.

EXPERIMENT. A solution of the globulin was dialysed for 14 days. At the end of this time there was an abundant precipitation. The fluid with the precipitate suspended in it was divided into two parts, A and B; A was

filtered, and the filtrate used in the subsequent part of the experiment, B underwent no treatment.

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

	Coagulation occurred	
	at 13° C.	at 40° C.
Diluted with water	50 minutes	40 minutes
Diluted with filtrate from A	12 „	6—7 „
Diluted with B	10 „	4—5 „

f. When means are taken to completely separate the globulin from a saline extract of the stromata by thorough saturation with magnesium sulphate[1], all fibrinoplastic power is also removed from the solution.

EXPERIMENT. The globulin was precipitated from an extract of stromata by means of repeated saturation with magnesium sulphate, and removed by filtration. The precipitate was washed and then redissolved by the addition of water, and the solution so obtained was freed from excess of salt by dialysis. The filtrate from which the precipitate produced by magnesium sulphate had been removed was also freed from excess of salt by means of dialysis.

Sheep's sodium sulphate plasma was diluted to six times its volume with the solution of the globulin and coagulated in from 5—10 minutes. Another specimen of the same salted plasma was diluted to the same extent with the globulin-free filtrate, and had not coagulated even after 48 hours.

From all these experiments we can conclude:—

That the stromata of the red corpuscles contain a globulin that in heat-coagulation temperature, precipitability by salts and other reagents, and in ferment activity resembles the proteid called cell-globulin derived from lymph-cells or white blood corpuscles.

That therefore stroma-globulin and cell-globulin are probably identical.

The question whether the globulin and fibrin-ferment

[1] We found considerable difficulty in removing the last traces of globulin by this method; the precipitate is so fine that small quantities pass through the filter paper. However by repeated shakings with excess of the salt, and filtration many times through the same filter paper, we obtained in two specimens a final filtrate that contained no globulin and possessed no ferment activity. A similar difficulty was found by one of us in separating the last traces of globulin from the serum of horses' blood. (This *Journal*, Vol. IX. pp. 261, 262.)

are identical, or merely in close relationship with one another must still be left to a certain extent open; the balance of evidence appears to us however to be, that the fibrin-ferment is identical with cell-globulin.

Two possible objections might be urged against the last of these conclusions, namely, first that the ferment action is due to contamination with serum, and secondly that it is due to contamination with haemoglobin. In order to see whether it was possible that any constituents of serum might be present, we took a quantity of serum and treated it in exactly the same way as we had treated defibrinated blood in order to prepare the stromata, and the resulting solution gave no proteid reactions and possessed no ferment activity as tested with dilute salted plasma. This disposes of the first objection. With regard to the second, the solutions of stromata that we employed were always perfectly colourless; still in order to be quite certain, we made a solution of pure oxyhaemoglobin crystals from rat's blood, and on testing this with dilute salted plasma found that it had no power to hasten coagulation[1].

3. **Do the stromata contain cell-albumin?** We tested for the presence of cell-albumin in four different specimens of stromata. This we did by saturating saline extracts of the stromata with magnesium sulphate, filtering off the resulting precipitate and then examining the filtrate for albumin. The results obtained were as follows:—

Preparation 1. The filtrate obtained as just described contained no proteid.

Preparation 2. The filtrate gave a faint xanthoproteic reaction, but gave no precipitate on acidulating and boiling, or any other evidence of the presence of albumin.

Preparation 3. The filtrate gave a fairly well-marked xanthoproteic reaction, but no precipitate on acidulating and boiling.

Preparation 4. The filtrate gave not only a well-marked xantho-proteic reaction, but on acidulation and heating a precipitate occurred at about 70° C. This preparation however contained a small quantity of impurity in the shape of haemoglobin.

From the examination of the first three preparations which are the most trustworthy, we conclude that cell-albumin is either absent

[1] A. Schmidt originally supposed that haemoglobin was fibrinoplastic, but further researches by his pupils Rauschenbach (*Inaug. Diss.*, Dorpat, 1882) and Nauck (*Inaug. Diss.*, Dorpat, 1886), and by Wooldridge (*Practitioner*, p. 187, 1886) have shown that the power of hastening coagulation belongs not to the pigment but to the stromata.

or only present in minute traces in the stromata of the red corpuscles.

4. **Do the stromata contain nuclein or nucleo-albumin?** The red corpuscles are in mammalian animals devoid of a nucleus, and the question whether they contain the characteristic constituent (nuclein) of nuclei is not devoid of interest.

Lauder Brunton[1] was the first to demonstrate the mucin-like characters of the substance of the nuclei contained in the red corpuscles of birds, and Plósz[2] showed that this substance contains a high percentage of phosphorus, and is identical with the nuclein obtained from the nuclei of pus cells by Miescher[3].

Worm-Müller[4] appears to have been one of the first to advance the suggestion that nuclein is not confined to the nuclei but is present in the cell protoplasm also. This has been confirmed by other observers; the nuclein in the cell protoplasm is however combined with a proteid, and the compound so formed is called a nucleo-albumin[5].

Nucleo-albumin has been described in the liver cells by Plósz[6], in the cells of the submaxillary gland by Hammarsten[7], in cells from lymphatic glands and in the cells of the thymus glands by one of us[8].

In lymph cells, the nucleo-albumin[9] is the most abundant proteid present, and it is owing to this substance that the cells form a mucinoid slimy mass when treated with solutions of sodium-chloride, or magnesium sulphate.

When the stromata of red corpuscles are treated with solutions of either of these salts, there is not the faintest indication of the formation of any such slimy material: part of the stromata goes into solution, namely, the cell-globulin; part remains undissolved; the unsoluble

[1] Lauder-Brunton, *Journ. of Anat. and Physiol.*, 2nd series, Vol. III. p. 91.

[2] Plósz, *Hoppe-Seyler's Med. Chem. Unters.*, Heft iv. p. 460.

[3] Miescher, *ibid.* p. 441.

[4] Worm-Müller, *Pflüger's Archiv*, Vol. VIII. p. 190.

[5] Nucleo-albumin appears to be probably identical with the substance called plastin which has been described by Reinke and Rodewald (*Unters. aus d. botan. Lab. Univ. Göttingen*, 1881) and by E. Zacharias (*Botan. Zeitung*, p. 281, 1887). These observers have relied chiefly upon microchemical reactions for its detection in the cells. The term plastin is, however, used in a different sense by another botanical chemist, Schwartz (*Die Morph. u. chem. Zusammensetzung d. Protop.*, Breslau, 1887): he applies the name plastin to the whole of the proteid substance of cells, not to any particular constituent of it. Nuclein is apparently identical with the chromatin of microscopists.

[6] Plósz, *Pflüger's Archiv*, Vol. VII. p. 371.

[7] Hammarsten, *Zeit. physiol. Chem.*, Vol. XII. p. 163.

[8] Halliburton, *Brit. Assoc. Reports*, 1888.

[9] Nucleo-globulin would be a more correct designation.

residue is especially large if the preparation has been allowed to stand under water, or a dilute solution of acid sodium sulphate for some hours. It might be said that this insoluble residue may contain a nucleo-albumin which differs from that derived from white corpuscles, in not forming a stringy mass. It however appears to us, that the cell-globulin is practically the only proteid present, and that the insoluble residue which increases with the lapse of time after the preparation of the stromata, does not contain a different proteid, but simply the same proteid which has been rendered insoluble by the action of reagents.

The question however is not wholly settled by experiments with the stromata prepared in the way we have described; for it is possible that the reagents used may have some action upon the nucleo-albumin so that it no longer shows its characteristic properties.

Accordingly by the use of the centrifugal machine we obtained a supply of red corpuscles wholly free from serum.

These were divided into several parts; to one portion a five per cent., to another a ten per cent. solution of sodium chloride were added; to a third, fourth, and fifth portions, a five per cent. solution of magnesium sulphate, a ten per cent. solution of the same salt, and a five per cent. solution of ammonium sulphate were respectively added; but in no case was there any formation of a gelatinous mass, which when poured into water extends in cohesive strings bearing a superficial resemblance to fibrin threads. According to this experiment then, the unaltered red corpuscles do not contain the nucleo-albumin so characteristic of the white corpuscles.

The experiment was repeated twice, using other preparations of the corpuscles similarly made, with the same result.

In two other cases, however, there was a small formation, or what we took to be a small formation of this stringy substance. In one case it was fairly well marked; but in the other case one of us felt somewhat uncertain as to whether the result was to be regarded as a positive one or not. We will therefore describe the one clearly positive result we obtained.

We used defibrinated sheep's blood as in all these experiments; by centrifugalising with a one per cent. solution of sodium chloride (repeating the operation three times) we obtained the corpuscles free from serum. The volume of the mass of corpuscles so obtained was about 50 c.c.; to this we added five grammes of sodium chloride[1],

[1] This is a method we often adopted instead of adding an already prepared solution of the salt.

grinding it up with the corpuscles in a mortar till it was all dissolved. There was however no general jellying throughout the mass. On leaving this to stand for a few hours, the upper surface appeared a little clearer than the lower portions; this was skimmed off, and the lower portions of the liquid were found to be thick, but there was no coherent gelatinous appearance. The portion skimmed off from the surface was however distinctly gelatinous; on pouring it into a beaker full of distilled water, there was a formation of fibrin-like strings: these at first coloured became quite white in a few minutes and then shrank up, forming little particles floating on the surface of the water.

This specimen of blood was found on microscopic examination to contain a large excess of colourless corpuscles, and we are inclined to attribute the presence of nucleo-albumin to these, and not to the stromata of the red corpuscles.

Among previous observations as to the presence of nuclein in red corpuscles may be mentioned those of Hoppe-Seyler and Wooldridge.

Hoppe-Seyler[1] states that the nucleated red blood corpuscles of birds, reptiles, etc. contain nuclein, and a greater quantity of proteid than the non-nucleated red blood corpuscles of mammals, and that nuclein is absent from the latter.

Wooldridge[2] in one of his earliest papers states that a nuclein-like proteid (nucleo-albumin) is present in the stroma of mammalian[3] red blood corpuscles, but in so small a quantity that it was impossible to be positive; later researches however showed him that lecithin is the only phosphorus-containing substance in the stroma[4].

There thus appears to be a general consensus of opinion that nuclein and nucleo-albumin are not present in the stromata of the red blood corpuscles.

5. **Do the stromata contain albumoses or peptone?** After the separation of the proteid coagulable by heat, the saline extracts of the stromata which we prepared did not give the typical albumose reaction (a precipitate with nitric acid disappearing on heating and reappearing on cooling), nor the biuret reaction (the red colour with a trace of copper sulphate, and excess of caustic potash) so characteristic of the products of proteolysis, i.e. of albumoses and peptones.

[1] Hoppe-Seyler's *Handbuch*, 5th Aufl. p. 429.

[2] Wooldridge, *Du Bois Reymond's Archiv*, p. 392, 1881.

[3] Wooldridge does not expressly state what blood he used, but from the context we judge that it was mammalian.

[4] Wooldridge, *Practitioner*, p. 187, 1886.

On saturating a sodium sulphate extract of stromata with ammonium sulphate a precipitate was produced; this was filtered off, and the filtrate was preserved (A).

On saturating a sodium chloride extract of the stromata with ammonium sulphate a precipitate was produced; this was filtered off and the filtrate preserved (B).

On saturating a magnesium sulphate extract of the stromata with ammonium sulphate a precipitate was produced; this was filtered off and the filtrate preserved (C).

The filtrates A and B were completely free from proteid, and thus peptone (which is the only proteid not precipitable by saturation with ammonium sulphate) was absent. The filtrate C however contained proteid, but it was not peptone: the proteid present was a globulin as it was precipitable by heat, and by dialysing out the salt from the solution. This leads us to mention what one of us has confirmed by experiments with serum that the presence of magnesium sulphate partially hinders the precipitation that would otherwise be produced by saturation with ammonium sulphate. This is a somewhat important practical point in view of the large use now made of these neutral salts for the separation of proteids. It is probably explicable on the supposition that the two salts form a double sulphate ($MgSo_4 . Am_2So_4 . 6H_2O$) which has the power of precipitating proteids to a limited extent only[1].

Our experiments then have shown that albumoses and peptones are not contained in the stromata of the red corpuscles.

General conclusions. In a recent paper on the red blood corpuscles, Hoppe-Seyler[2] expresses the opinion that the chemical structure of these corpuscles is not that very generally held, that they are composed of protoplasm infiltrated with pigment, but rather that the pigment in them replaces protoplasm to a very great extent. We have instances of the replacement of protoplasm by other substances in various parts of the body: for instance, the replacement of the protoplasm of goblet cells by mucin, or of epidermal cells by keratin; and it is in a similar sense that Hoppe-Seyler appears to regard the replacement of the protoplasm of red blood corpuscles by haemoglobin or oxyhaemoglobin[3].

[1] Compare remarks on the formation of another similar sulphate in my Paper on "Proteids of Serum": this *Journal*, Vol. v. pp. 180, 181.

[2] Hoppe-Seyler, *Zeit. physiol. Chem.*, Vol. XIII. (1889).

[3] Hoppe-Seyler calls the pigment in the red corpuscles of arterial blood *arterin*, of venous blood *phlebin*. He points out that these pigments differ from the substances oxyhaemoglobin and haemoglobin that can be separated out from the corpuscles. Arterin

Our own experiments go far to support Hoppe-Seyler's views. The mammalian red blood corpuscle is not a cell in the strict morphological sense of the word; it has no nucleus. It is also not a cell in the chemical sense, for not only is nuclein absent, but the only proteid present of the four normally existing in typical cells is cell-globulin, and this exists in the stroma in small quantities only.

Previous observations on this proteid are as follows:—

Hoppe-Seyler[1] contents himself with saying that the proteid is a globulin; that it coagulates at 75° C. and is precipitable by saturation with sodium chloride.

Kühne[2] also speaks of it as a globulin; he states it is precipitable by a stream of carbonic acid; that it acts fibrinoplastically, and that it is paraglobulin.

Hammarsten[3] showed that pure paraglobulin has no fibrinoplastic action, and that A. Schmidt's name "fibrinoplastic substance" is therefore a misnomer. Fibrin is formed from fibrinogen by the activity of the fibrin-ferment, and any fibrinoplastic activity the globulin of serum may have is due to admixture with fibrin-ferment, or to adopt the nomenclature one of us has introduced, with cell-globulin, derived from the disintegration of the white blood corpuscles.

Wooldridge[4] also speaks of the proteid as paraglobulin, and states that it coagulates in a 4—5 per cent. solution of sodium chloride at about 66° C.; a fact which we have fully confirmed.

As already stated, fibrin ferment has been prepared from the stromata by Schmidt's method[5] by several of Schmidt's pupils (Nauck, Rauschenbach, Krüger).

Two observers (Krüger and Wooldridge) have found that injection of the stromata into the circulation causes extensive intravascular clotting. Krüger[6] attributes this action to the fibrin-ferment[7], Wooldridge[8] on the other hand to the lecithin the stromata contain.

is probably a compound of oxyhaemoglobin with lecithin, phlebin of haemoglobin with lecithin.

[1] *Physiol. Chemie*, p. 391. [2] *Lehrbuch der physiol. Chemie*, p. 193.

[3] *Pflüger's Arch.*, Vol. XVII. p. 447; Vol. XVIII. p. 39; Vol. XIX. p. 363; Vol. XXII. p. 431.

[4] *Du Bois Reymond's Archiv*, p. 390, 1881.

[5] That is, extracting the dried precipitate produced by alcohol, with water.

[6] Krüger, *Zeitschr. f. Biol.*, Vol. XXIV. p. 189.

[7] Birk (*Inaug. Diss. Dorpat*, 1880; Maly's *Jahresb.*, 1881), Edelberg, and Köhler have all shown that injection of fibrin ferment into the circulation generally causes intravascular clotting; but not invariably, as the normal body has the ability of destroying or resisting the action of the ferment to a very great extent.

[8] *Practitioner*, p. 187, 1886.

There have thus been two sets of previous researches; one which deals with the nature of the proteid, the other with the presence of fibrin-ferment in the stromata of the red corpuscles.

Our own work dealing with both these questions has consisted to a very great extent in confirming the results of previous observers; and in putting them together to show that the two questions are in reality one.

We have found that the proteid, though it resembles paraglobulin (or serum-globulin), is really cell-globulin: and the fibrinoplastic action of this proteid is in reality the action of fibrin-ferment, with which substance cell-globulin is probably identical.

One further question still remains to be asked. It is this;—Do the red corpuscles contribute to the formation of fibrin in coagulation as it usually occurs in shed blood? This is a question that cannot be readily answered. The red corpuscles of clotted blood are apparently normal in appearance, and do not undergo disintegrative changes, as do the white corpuscles and blood-tablets. There is therefore no necessity to suppose that the red corpuscles shed out any fibrin-ferment, at any rate, in the earlier stages of the formation of a blood clot. It is however quite possible that the ferment they contain may assist in the formation of fibrin in later stages, and thus partially account for the increase in firmness that the clot undergoes when it is allowed to remain untouched for some time. It is also possible that the existence of fibrin-ferment in the stromata may account for what Landois calls "stroma-fibrin."

This question, among others, has been taken up from the stand-point of the pathologist and therapeutist by Bonne[1]. In a pamphlet full of interesting suggestions, the probable rôle of fibrin-ferment in causing intravascular clotting in certain morbid conditions of the blood is pointed out. The normal body has the power of destroying or resisting the action of fibrin-ferment to a great extent; but in debilitated conditions this power is lessened, and the absorption of fluid exuded from wounds may produce thrombosis. An increased disintegration of white blood corpuscles within the vessels may produce a similar effect. If, however, the quantity of ferment is small, the effect is not thrombosis, but a febrile condition. Lastly, there are certain forms of disease in which the red corpuscles are destroyed within the vessels; the plasma of the blood becomes stained with haemoglobin (haemoglobinaemia); and this may pass through into the urine (haemoglobinuria or methaemoglobin-

[1] Ueber das Fibrinferment und seine Beziehungen zum Organismus von Dr G. Bonne, Würzburg (G. Hertz) 1889.

uria). In these conditions there are febrile attacks corresponding to the periods when the corpuscles are destroyed, and there appears every reason to believe that the fever is due to the presence of fibrin-ferment in the circulating blood derived from the red corpuscles. This cannot however be considered absolutely certain; we are not aware that thrombosis has ever been described in these cases, nor do any attempts appear to have been made as yet to estimate quantitatively the amount of fibrin-ferment present in the blood in this condition. The question we have investigated from the physiological point of view is thus full of interest to the pathologist also.

The expenses involved in the foregoing research have been partially defrayed from a grant made by the British Association to a committee appointed for the purpose of investigating the physiology of the lymphatic system.