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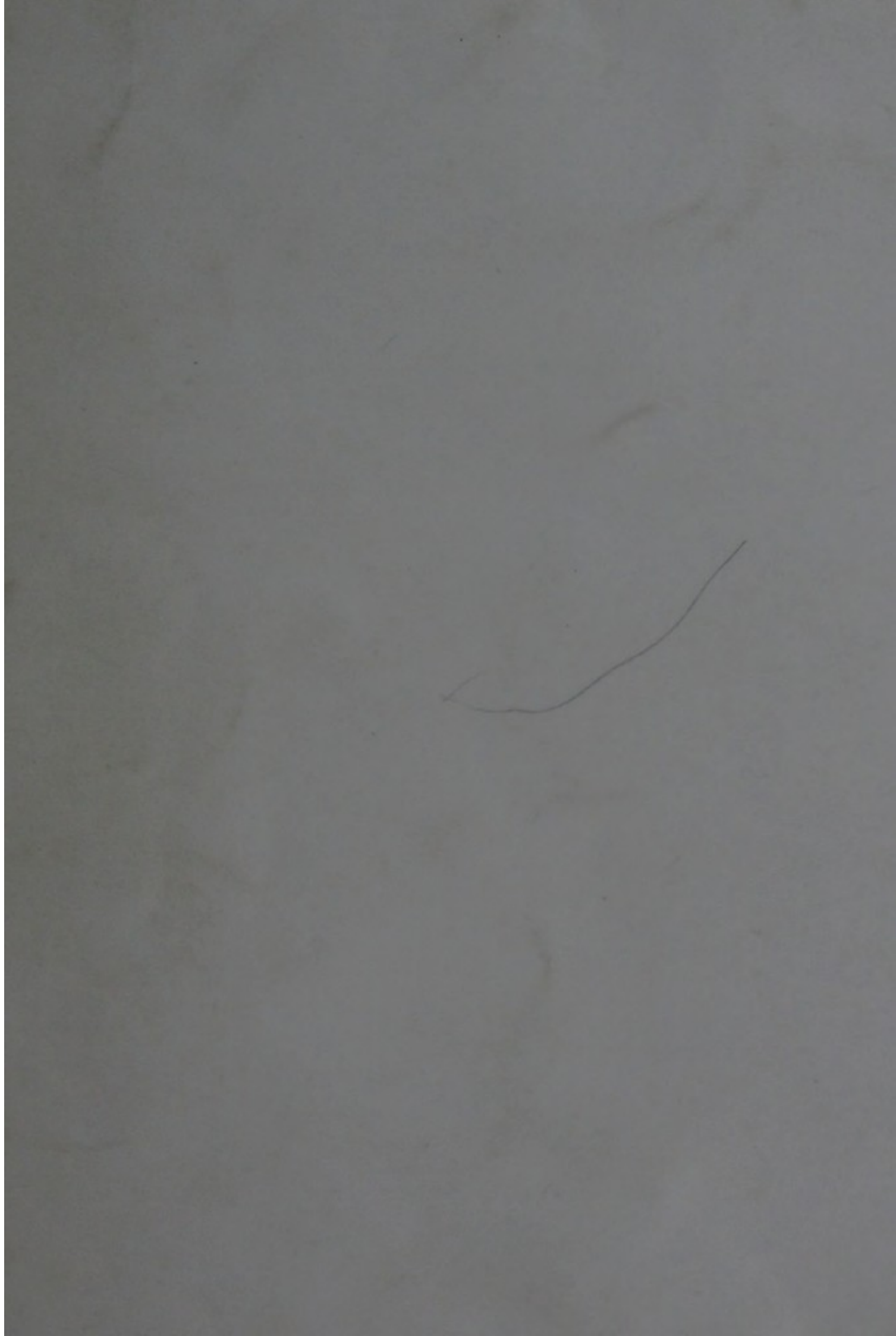
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Wooldridge. Blood plasma as protoplasm.

" Intravascular clotting.

" Coagulation of the blood.

" Chemical protection

Lingard. Infectious diseases.

Luff. Stomachies.

Ruffer. Prevention of hydrophobia.

" Bacillus pyocyaneus.

" Destruction of micro-organisms.

Hartley. Atmospheric life germs.

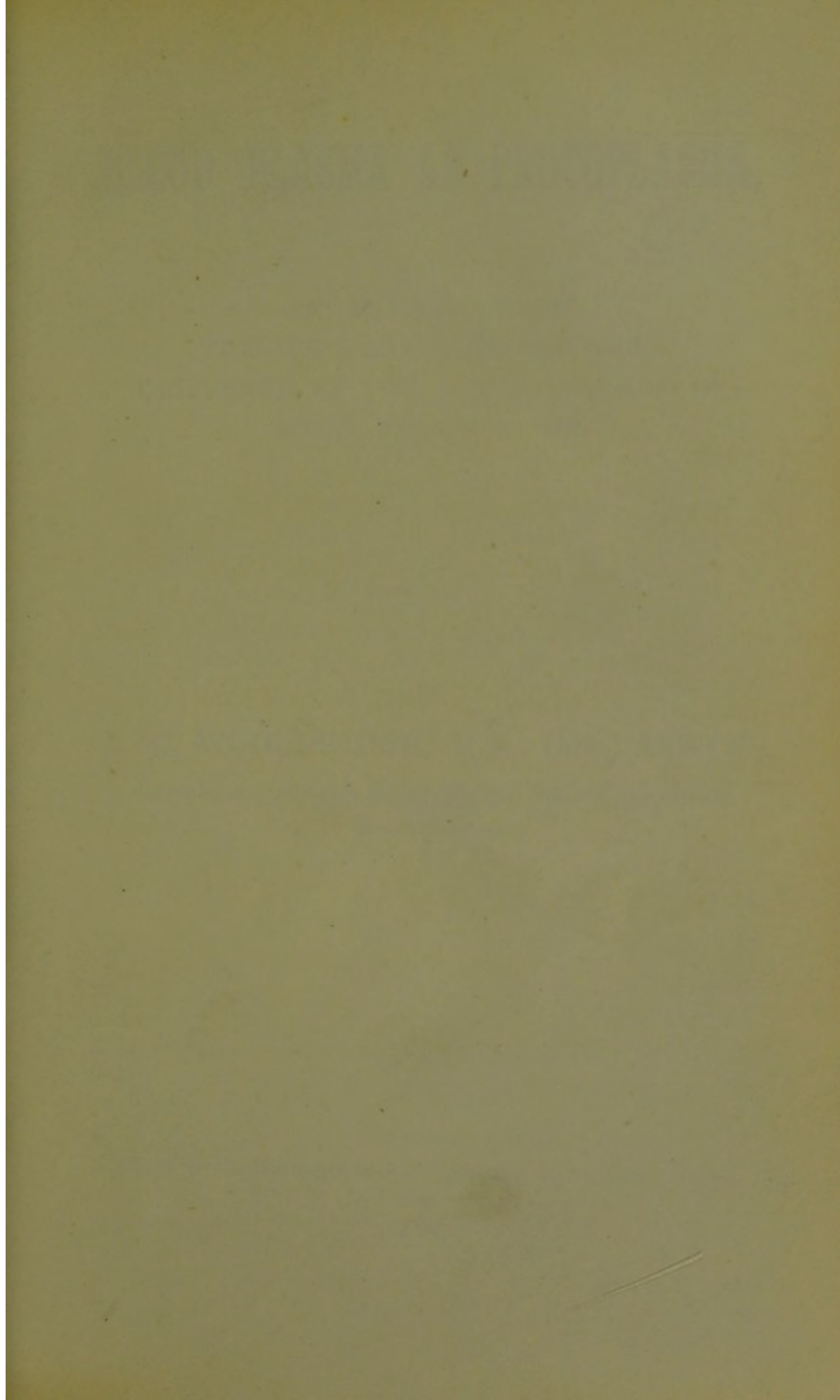
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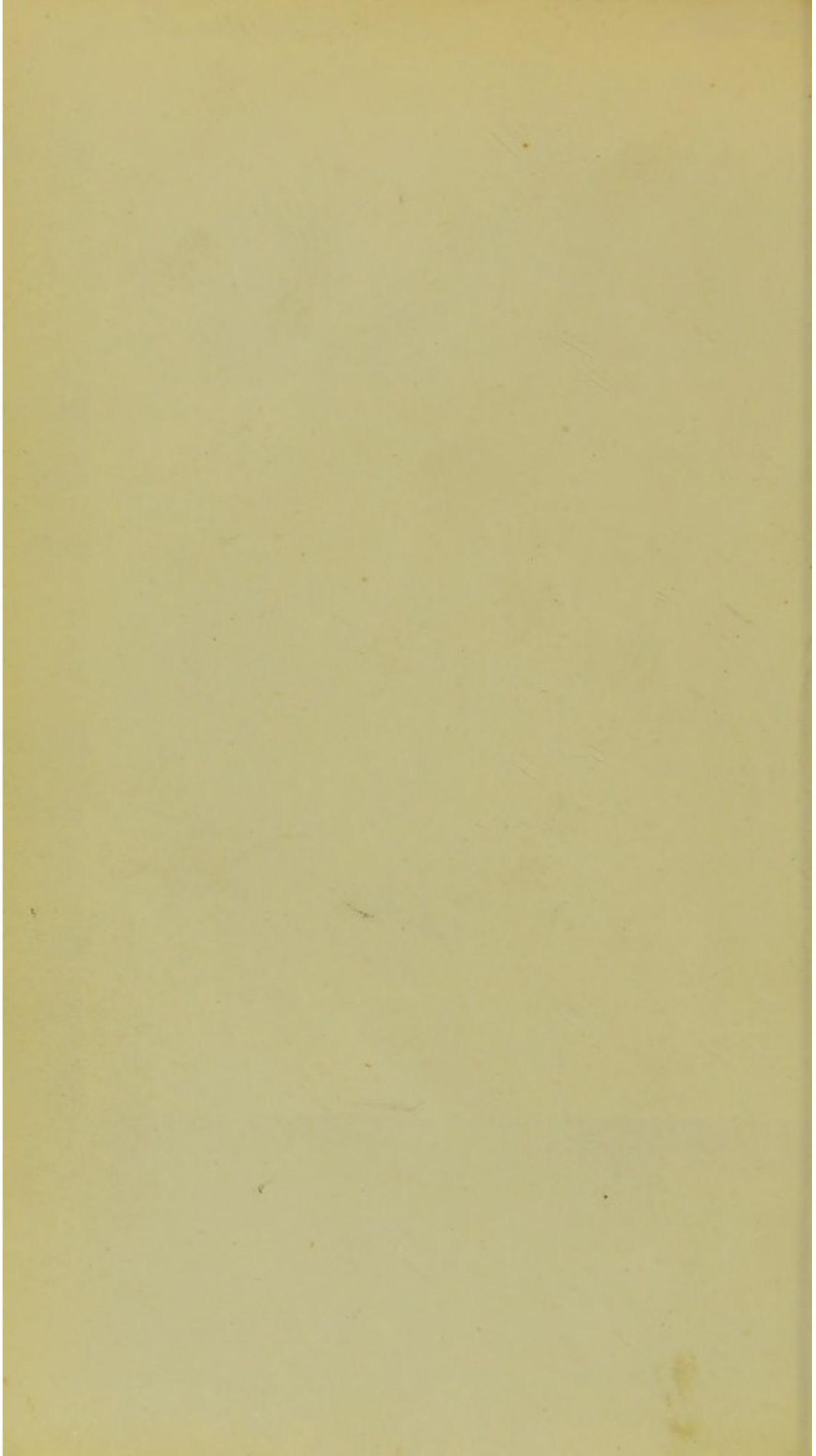
McKendrick Nervous system.

Rutherford. Introductory lecture.

Johnson. Muscular arterioles

Taylor & Wilks. The human body.





7. 218

BLOOD PLASMA AS PROTOPLASMA.

(1)

AN EXTRACT FROM
THE ARRIS AND GALE LECTURES,
DELIVERED AT THE ROYAL COLLEGE OF
SURGEONS, JUNE, 1886,

BY

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Grocers Company.*



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BLOOD PLASMA AS PROTOPHYLLIN

AN EXPERIMENTAL STUDY
ON THE ACTION OF PROTOPHYLLIN
ON THE BLOOD PLASMA
AND THE EFFECT OF
SUGAR AND SALT

L. C. WOODRUFF, JR., D.D.S., M.D.

PH.D. THESIS, UNIVERSITY OF CALIFORNIA, 1914

BLOOD PLASMA AS PROTOPLASMA.

THERE can be no question that there exists a very great analogy between the coagulation of the blood and rigor mortis. Rigor mortis can hardly be looked upon otherwise than as the last vital manifestation of muscle, and hence there are sound reasons for looking on coagulation as the last act of the living blood. No one doubts for an instant that muscle plasma is living protoplasm, and the only very strong reason which would prevent us from adopting blood plasma as protoplasm, is the supposition which exists relative to the participation of recognised form elements of the blood in the process of coagulation.

Admitting that coagulation is a "vital" act, so long as it is apparently necessary that form-elements should intervene to effect this process, so long are we justified in doubting the right of the plasma to the epithet, living. But the idea that form elements do participate in coagulation, and indeed are necessary factors in the process, has taken very deep root amidst physiological doctrines, and if we are successfully to uphold the claims of the plasma to be fluid protoplasm, we must first shew that this doctrine is unfounded.

A very prevalent belief is that the white blood corpuscles are important agents in the process. The acceptance of this view is chiefly due to the experiments of Alexander Schmidt, and in the main his experiments are as follows:— By allowing blood from the vein of a horse to flow into long vessels, surrounded by ice, the coagulation of the blood is greatly delayed and the corpuscles are able to sink. The red corpuscles sink more readily than the white, the latter partly forming a layer on the surface of the column of red, and partly being scattered through the lower layers of the plasma. Ultimately, in spite of the cold, coagulation takes place, and it is then observed that clotting begins earlier and is most complete in those strata of the plasma where there are most white corpuscles. Again, by filtering the plasma at a low temperature, before coagulation has had time to occur, the complete removal of the white corpuscles can be effected, and the plasma so obtained is found to clot very slowly and scantily, even at ordinary temperatures.

Relying chiefly on these experiments, Schmidt was led to the conclusion that white blood corpuscles were essentially concerned in coagulation. But as I shall now point out, this conclusion is totally unjustified. In another form of plasma, and more suited to experiments of this nature, to which I shall afterwards frequently have to allude, I have found that simply cooling down the plasma suffices to give rise to a marked precipitate. A substance separates from the plasma on cooling, and it can be proved by the most exact experimentation that this very substance is of the greatest importance in coagulation. In fact it plays the part attributed by Schmidt to the white corpuscles. And in Schmidt's experiments there can be no doubt, since all the operations were carried out at a low temperature, that in removing the white corpuscles this new substance was at the same time removed. Indeed, Schmidt himself describes the existence, side by side with white corpuscles, of a large quantity of granular matter. In the one case this sinks with the white corpuscles, in the other it is removed by filtration. Schmidt supposes that the cold prevents the white blood corpuscles from breaking up, but not entirely so, and he looks upon this granular matter as being *débris* of white corpuscles which have gone to pieces in spite of the cold. If this were so his conclusions might be justifiable, but it is a pure assumption on his part; and in face of the fact that in other forms of plasma, perfectly free from any corpuscles, a granular precipitate arises on cooling, it is obvious that Schmidt's conclusions as to the participation of the white corpuscles were not justified.

Since the publication of these fundamental researches of Schmidt, in which he endeavoured to show that the absence of white corpuscles renders the coagulation very slow and very imperfect, an apparent confirmation of his views has been afforded by experiments of a more direct nature.

I first showed that by adding to plasma, free from cells, isolated lymph corpuscles, obtained from lymphatic glands, coagulation at once ensues. I myself at this time looked upon this as a confirmation of Schmidt's views. But it is obviously only a proof if lymph corpuscles are identical with white blood corpuscles, and this I believe not to be the case. No doubt the white blood corpuscles are to a great extent recruited from the lymph corpuscles; but I have described experiments which shew that when once a lymph corpuscle has got into

the circulating blood plasma, it loses all its power of inducing coagulation. It is, then, extremely doubtful whether white blood corpuscles have anything to do with coagulation; and on the other hand, it is certain that plasma free from all corpuscular elements will clot spontaneously. This latter statement must now be considered in greater detail.

The Coagulation of Plasma without the participation of any Cellular Elements.

This can be most advantageously studied in a form of plasma to which I shall allude in the future as peptone plasma. It is obtained by the injection of a solution of peptone into the circulation of a dog. A few minutes later the animal is bled to death; the blood does not clot, and by means of the centrifugal machine plasma can be obtained perfectly free from all form elements. This plasma is spontaneously coagulable, or more exactly, it will clot, yielding normal fibrin without the addition of anything which can be in any way regarded as a fibrin factor. The injection of peptone prevents the normal coagulation from taking place. The plasma is however coagulable. The process only requires to be started by some simple chemical or mechanical stimulus; just as muscle is contractile, but requires to be set going by a stimulus before it actually contracts. Among the means by which the plasma can be made to coagulate are neutralization with acetic acid or carbonic acid, dilution with water, or even salt solution. Mechanical means, as filtering through a clay cell, or even in some cases through filter paper, are also effectual.

Now, filtration through a clay cell is not a "fibrin factor;" acetic acid is not a "fibrin factor;" they are stimulants applied to the blood plasma, which answers by clotting. Peptone plasma is not the only instance in which mechanical stimulation produces clotting, for I think it must be admitted that the plasma in the vessels clots on mechanical stimulation. It is well known that if a thread be drawn through a vessel it will become covered with clot. This cannot be attributed to the presence of a foreign body, for Zahn has shewn conclusively that a foreign body only produces coagulation when it incompletely blocks the lumen of a vessel. The blood current must continue. Around a globule of mercury, completely blocking the lumen of a vessel, clotting does not occur; but if it only partly obstructs the blood current it gives rise to a thrombus.

Zahn attributes the clotting which occurs under these circumstances to the white corpuscles. The latter, he thinks, attach themselves to the foreign body, and by their disintegration yield fibrin. If the blood current is completely stopped, enough white blood corpuscles will not present themselves, so that no thrombus will be formed; but if a certain amount of flow can go on, enough corpuscles will be brought to the spot and a thrombus formed.

But in view of the fact that mechanical stimulation, *e.g.*, sucking through narrow pores, suffices to induce the coagulation of pepton plasma, entirely free from form elements, Zahn's explanation is open to considerable doubt. Moreover, the microscopical observations on which he relied admit of another explanation. Rindfleisch also remarks, as the result of his anatomical observations, that thrombi are especially liable to be formed, under otherwise favourable circumstances, at those parts of the vascular system which from their configuration are prone to give rise to eddies and sudden changes in the flow of the blood, and that pathological changes in the vascular wall are not constantly associated with thrombosis, provided no projecting processes or marked unevenness of the diseased surface be present.

To return to peptone plasma. I have stated and explained its spontaneous coagulability. This power is, however, entirely lost if we cool down the plasma to a temperature of about 0° and maintain it at this temperature for some time. As the result of the cooling a precipitate is formed, and with the removal of this precipitate the power of spontaneous coagulation is lost. The plasma will still clot, will still yield large quantities of fibrin, but only on the addition of bodies which must be looked upon as fibrin factors. I must call special attention to this precipitate. The most important point in connection with it is its peculiar microscopic appearance. Chemically it is a very complex proteid substance, but when examined microscopically it appears, not in the form of irregular granular masses, as is generally the case with proteid precipitates, but in the form of perfectly regular round discs. These discs are three or four times smaller than red corpuscles. Their discoidal character is readily seen when they roll over. After they have been separated for some time they run together into masses and finally form granular heaps. When first separated they will redissolve on warming, and if

this process be observed under the microscope interesting observations may be made. In a preparation examined immediately after the removal of plasma from the ice we see the discs, some isolated, others collected in groups of four or five hanging together, so as to form rosettes. If one of these rosettes be kept in view for a few minutes, whilst the temperature of the plasma becomes raised to that of the warm room, it will be seen that its outlines are changing, that an irregular angular lump is forming, and that this lump is gradually becoming round. Finally, a round disc is formed, differing only from the smaller ones in being larger. It may be as large as a red blood corpuscle. These discs are distinctly biconcave, the larger ones being, so far as form is concerned, quite indistinguishable from a red blood corpuscle. In the smaller ones it is not quite so easy to make out the central depression, high powers being necessary.

These changes can only be observed in the case of a plasma thoroughly under the influence of peptone and shortly after the separation of the substance. In other cases the discs rapidly run together into irregular granular masses.

There is no doubt that this body separable by cold is identical with the so-called blut-plättchen of Bizzozero. The latter are, according to all observers, plentifully present in peptone blood, and these unquestioned blut-plättchen are absolutely indistinguishable in every respect from the discs which separate from the plasma on cooling.

Blut-plättchen are regarded by all observers who have attacked the problem as definite form elements, by which I understand them to mean organised bodies; and it is difficult to know how else to regard them, for they have just as much right to be regarded as a form element as a red corpuscle has. Many attribute to these bodies, in regard to coagulation, the powers which Schmidt has referred to the white corpuscles. I think they are perfectly right, but they leave the question practically where it was before. Whether this or that form element must necessarily step in to help on or to initiate the powers of coagulation is, for the problem we are concerned with, practically without importance. It is obvious that if the blood plasma cannot clot without the intervention of form elements, be they white corpuscles or be they the structures called blut-plättchen, our contention that the plasma is "living" has but little to support it. But we

have seen that form elements are not necessary; that blut-plättchen have a fluid representative in the plasma. Blut-plättchen are only form elements at a certain temperature.

The substance, separable by cold, the microscopical characters of which have just been described, is dissolved in the alkali of the plasma. The lower the temperature the less quantity of the substance can remain in solution. A very slight cooling suffices to precipitate a certain quantity. The substance can be also precipitated by acids, and by certain strengths of neutral salts.*

The Fibrin Ferment.

It has already been pointed out that peptone plasma is spontaneously coagulable only so long as the peculiar body we have just described is present. But this coagulation presents a very special and important feature, for when it occurs not only is fibrin formed, but at the same time a large amount of fibrin ferment makes its appearance.

The fibrin ferment is a substance capable of converting solutions of fibrinogen into fibrin. We know it is not a proteid, and we know nothing more about its chemical nature except that it is destroyed by boiling.

There is no ferment in peptone plasma, but if the plasma be made to clot by the means described above (CO_2 , &c., &c.,) the serum which exudes from the clot is found to contain large quantities of the ferment.

Both clotting and fibrin formation are dependant on the presence of the body separable by cold. The more of this substance there is present, the more easily does clotting occur and the more ferment is found. With the gradual removal of this substance, clotting becomes less and less easy, and less and less ferment is formed. I attribute very great importance to this coincident formation of fibrin ferment. It would be absurd to contend that every process of fibrin formation is of a vital character. It is on a consideration of the whole of the

* Hence there are two kinds of "salt plasma." The one obtained by using strong sulphate of magnesia solution, containing none of this substance, owing to its having been precipitated. The other by using more dilute salt solutions, still containing this substance and clotting on dilution.

phenomena, as seen in the plasma, that I base my claims of vitality for the blood plasma.

It would appear extremely probable, from what we know of the subject, that ferments make their appearance as the result of protoplasmic disintegration or death. Take, for instance, the pancreas. We here get, in the interval of rest, a storing up of definite granules, which, under the influence of activity, are discharged or disappear in the form of secretion. There is further reason to believe that these granules are eminently of a protoplasmic nature, eminently living (Ogata). Previous to the appearance of ferment these granules must undergo fundamental change, for in a fresh gland, no matter how much "zymogen," as it is called, be present, there is no free ferment. But in a gland in which post mortem changes have taken place, ferment is present, and this change can be accelerated by artificial means.

How very marked is the analogy between the case of the pancreas cell and the blood plasma. Trypsin is formed as the result of the disintegration of this cell, fibrin ferment of the blood plasma. The capability of forming a large amount of trypsin goes hand-in-hand with the presence of a large amount of granules in the inner zone of the pancreas cell. The capability of forming a large amount of fibrin ferment depends on the amount of a substance, which, as soon as it appears at all, appears in the form of the most definite "granules" conceivable. I may here call attention to the fact that fibrin ferment appears to be the post-mortem ferment of muscle, a fact which greatly strengthens the analogy between rigor mortis and coagulation of the blood.

Histologists have distinguished in the protoplasm of cells two distinct constituents, the one formed, generally appearing in granules, and designated protoplasma, in a restricted sense; the other, more fluid, unformed, and called paraplasma (Kupfer). The formed constituents appear to be more especially concerned in the functional activity of the cell, hence the denomination protoplasma (in a special sense). As instances of cases in which "granules" are obviously the functionally active part of a cell, we might instance the chlorophyll granules of the vegetable cell and the granules which are so obvious in many secreting cells. The nearly universal occurrence of such granules in cells, and the strong reasons there are for regarding

them as the most actively living part of the cell has been shown in a recent important work of Altmann (*weber die Zelle*).

Now, in the case of the blood plasma, we might regard the substance which separates from the plasma on cooling, as the more special protoplasma, the rest of the plasma being the paraplasma. The power of spontaneous coagulation, the power of giving rise to fibrin ferment, depends on a special substance, which, when it is made visible, appears in the form of the most definite "granules."

The Vascular Wall and the Blood.

Genetically considered, both blood and endothelium are differentiations of one and the same protoplasmic mass. The blood is merely the more fluid central part of the originally solid protoplasmic cord. The blood and the vascular wall may then be looked upon as a protoplasmic unit. That the vascular wall exerts a great influence on the blood, is evident from the fact that the blood undergoes changes, which finally terminate in coagulation so soon as it leaves the vascular wall. And similarly, we know that a motor nerve separated from its ganglion cell, undergoes degenerative changes and death.

The change in the blood takes place much more quickly than the change in the nerve, and its nature will become more evident from what follows. The great point on which I wish to insist, is the fact that it is the plasma which undergoes change. Previous authors have always supposed that the change took place in the form elements. Thus Schmidt supposed there was a "death" and breaking up of the white corpuscles. But the fundamental change is a change of the blood plasma itself. The blood plasma may be looked upon as the most sensitive part of the blood.

The exact nature of this change I do not know, but that it does occur may be proved in the following way:—Peptone blood clots with leucocytes from lymphatic glands, and it is the plasma of the blood which has this power of clotting with leucocytes. But blood which has not left the vessels, be it ordinary normal blood, or peptone blood, does not clot with these leucocytes, hence it is obvious that the plasma must undergo some change when it leaves the vascular wall. A small quantity of leucocytes added to peptone blood

immediately after its removal from the vessel will cause rapid clotting, but these same leucocytes injected do not cause clotting, whether a large quantity be injected into the general circulation or into an isolated vein. It is the plasma, then, which so rapidly undergoes change when the blood leaves the vascular wall. This great sensitiveness is, I contend, another argument in favour of the protoplasmic nature of the plasma.

Before leaving this division of our subject, it may be well to allude to the relation of the red corpuscles to the plasma. We have seen that the blood vessel and the plasma form what I call a protoplasmic unit, and we have evidence to show that the red corpuscles are also integral parts of this endothelium and plasma union. I must admit, however, that this point is still extremely obscure. It appears, however, pretty certain that the red corpuscles of shed blood are not quite the same things as the circulating red corpuscles. It has been shown by transfusion experiments, that the corpuscles of defibrinated blood are incapable of persisting in the organism, that they very speedily disappear, and that defibrinated blood has no more lasting effect in making up for a loss of blood than salt solution has. It would therefore appear that in the death of the blood, the red corpuscle also suffers, though in a less marked and easily demonstrable manner than does the plasma.

Some Chemical Characters of the Red Corpuscles and Plasma.

A consideration of the chemical structure of the red corpuscles is of interest in regard to the question before us. It is common to speak of the red blood corpuscles as consisting of hæmoglobin, albuminous stroma and salts. But this is only the case in the same way as chlorine and sodium are constituents of common salt. It is impossible to avoid the conclusion that the red blood corpuscle is a chemical unit, that we must speak of a red blood corpuscle substance capable, no doubt, of being split up into smaller molecules as hæmoglobin and stroma, but still as decided a chemical individual as sodium chloride, and for the following reasons:—Hæmoglobin cannot be present as such, for it is extremely soluble in plasma, and if free in the blood, becomes converted into methæmoglobin and excreted by the kidney. Under ordinary circumstances, it need hardly be said, that the hæmoglobin does not pass into the plasma; and

this cannot be explained by supposing that the stroma forms a protective envelope for the hæmoglobin, since the stroma itself, if free in the blood plasma, is a most dangerous poison, producing wide-spread intravascular clotting. I think these considerations drive us to the conclusion that the hæmoglobin and the stroma are in chemical union to form the "red blood corpuscle substance." All the evidence we possess tends to the conclusion that the red blood corpuscles are protoplasmic structures, and I think the chemical conditions I have described well illustrate the chemical relations of living matter.

Pflüger first suggested the idea of a "protoplasma molecule," and the evidence, so far as the red corpuscle goes, certainly points to the correctness of this idea. One can obtain from a red corpuscle (and from other tissues) various extremely complex bodies, and these are cited as being the chemical constituents of the tissue. But they do not exist as such, side by side; there is a chemical union.

A similar state of things prevails with regard to the plasma. Thus, a body (fibrinogen) can be isolated from the plasma, which will clot readily with fibrin ferment, yielding fibrin, and will coagulate by heat on warming to 52.55° C. But plasma itself (peptone plasma and plasma in the vessels) will not clot with fibrin ferment, neither will plasma coagulate, proper precautions being taken, till very high temperatures are reached (over 90° C.); plasma behaving quite differently in this respect to serum—a dead fluid. Plasma which has been artificially altered and changed, will clot with fibrin ferment, but the plasma in the vessels, and plasma altered as little as possible after leaving the vessels, is quite unaffected by fibrin ferment. For this, and other reasons, which I cannot adduce here, there can be no doubt that, though a definite substance, fibrinogen,* can be obtained from plasma, it is not present as such; it is a decomposition product of plasma substance, and plasma substance is not coagulable with fibrin ferment.

Now, let us consider the extreme analogy between this case and the pancreas. The pancreatic ferment trypsin arises as the result of the death of the pancreas protoplasm, and just as fibrin ferment is incapable of attacking living blood plasma, so pancreas ferment is incapable of attacking living pancreas protoplasm, and living intestinal protoplasm. Fibrin ferment

* Fibrinogen of Hammarsten.

acts on a special body--fibrinogen. The reason why it does not act on plasma, is that there is no fibrinogen there. Trypsin acts on proteids, it attacks a proteid molecule ; it cannot touch a protoplasma molecule any more than fibrin ferment can touch a plasma molecule.

The Chemical Processes in Coagulation and the Chemical Processes of Life.

I shall treat this subject very briefly, as I have discussed it at much greater detail elsewhere.* It is well-known that the existing doctrines on coagulation explain it as an essentially fermentative process. From my own researches I am entirely opposed to this explanation. I have alluded to the fact, that we may distinguish in the blood plasma, a part we may call the special protoplasma ; and another part, the paraplasma. The essential process in coagulation, is an interaction between protoplasma and paraplasma. Both protoplasma and paraplasma consist of proteid and lecithin ; that is to say, these bodies can be obtained from them. In the process of coagulation, there is a lecithin transference, the protoplasmic moiety losing, the paraplasma moiety gaining, lecithin, the result being the formation of fibrin, also a proteid lecithin mixture or compound.

I only wish to say that this is the main feature in the process—the actual chemical process is, no doubt, one of enormous complexity. It is the break up of the plasma molecule and the new formation of endless other smaller molecules (fibrin, constituents of serum). It is the passage from life to death. We cannot hope to explain all the chemical processes which accompany that tremendous change ; but it is definitely made out that, so far as the formation of fibrin is concerned, the process is a lecithin proteid interchange.

Coagulation of the blood is probably but the type of a process occurring everywhere in protoplasm, or in other words, many of the phenomena of life are fundamentally processes of the nature of coagulation. Previous investigators have regarded coagulation as essentially a fermentative process, the action of a special ferment, fibrin ferment, on a special proteid, fibrinogen, and the pre-

* Croonian Lecture, Royal Society, April 8, 1886.

vailing view as to the nature of the chemical processes of life is that they are in the main fermentative processes. But what we know of the blood, perhaps the one tissue which we can thoroughly get at experimentally, is not favourable to this the existing view.

The blood ferment is essentially a postmortem production, the result of the final explosion of the plasma molecule. No doubt fibrin ferment is capable of causing the appearance of fibrin. It will convert a dead and isolated constituent of the plasma into fibrin, but it will not make plasma itself clot. (I refer to plasma in the vessels and peptone plasma.)

The injection of a large quantity of fibrin ferment leaves the animal unharmed. It can then exert but little influence on the life of the blood, but on the other hand we can exercise a tremendous influence on the blood by the injection of a certain proteid-lecithin compound.* This is not a ferment; it does not act in the least like a ferment, but it will clot the blood in the vessels from one end of the body to the other, provided enough be injected. The amount of intravascular coagulation is entirely dependent on the quantity of substance injected. The substance is used up in the process and enters into the formation of the fibrin produced. The lecithin it contains is essential to its action.

If, then, the process of coagulation is rightly regarded as typical of many processes of life, these processes are not catalytic processes. They are the result of the union in definite proportions of complex proteid-lecithin compounds, it being remembered that lecithin and proteid are constant wherever there is life.

As in the case of the blood, ferments may arise as by-products, but they are effects, not causes, of the main processes. That lecithin is profoundly concerned in the formation of these ferments is certain so far as the blood is concerned.

Conclusion.

In the preceding pages I have endeavoured to shew what great claims the blood plasma has, to be regarded as living matter. One is accustomed to look upon life as always associated with form. The plasma is not formed, it is formable.

* This substance is very widely distributed. It can be obtained from lymph glands, chyle, brain, testis, thymus, &c.

Let me once more draw attention to the peculiar precipitate produced in the plasma by cold. When once this precipitate is visible, it is very difficult to look on it as other than a form element. Indeed, it has been seen and described as a form element, and it has just the same right to this title as a red corpuscle has. The morphological characters of the precipitate are identical with those of the red corpuscles, and to this must be added the interesting fact that, chemically, the stroma of the red blood corpuscles, that is to say, that part of the red corpuscle which has not become differentiated into hæmoglobin, is identical with the precipitate produced by cold. Moreover, this latter contains iron in an organic form. Now the development of the red corpuscles is profoundly obscure—overwhelming differences exist between the various authors who have investigated the question, and I put it, in face of what I have found and described above, as a suggestion, are red blood corpuscles deposits from the plasma?

The substance separable by cold may be regarded from two different aspects; on the one hand, the process is evidently allied to crystallization, the discs might be regarded as a crystalline precipitate. On the other hand, they may be looked upon as imperfect cells. *Omnis cellula e cellula* is a dogma. Is it true? Are blood corpuscles and plasma discs cells, or are they crystals? Surely, if ever we shall have a border land between "vital" process and ordinary physical and chemical processes, we shall find it in the blood plasma.

The first part of the paper is devoted to the general principles of the theory of the structure of the atom. It is shown that the structure of the atom is determined by the laws of quantum mechanics, and that the structure of the atom is determined by the laws of quantum mechanics. The structure of the atom is determined by the laws of quantum mechanics, and the structure of the atom is determined by the laws of quantum mechanics.

The second part of the paper is devoted to the application of the theory of the structure of the atom to the study of the properties of the atom. It is shown that the properties of the atom are determined by the laws of quantum mechanics, and that the properties of the atom are determined by the laws of quantum mechanics. The properties of the atom are determined by the laws of quantum mechanics, and the properties of the atom are determined by the laws of quantum mechanics.



