Lecture on tissue- or cell-fibrinogen in its relation to the pathology of blood / by A.E. Wright.

12

LECTURE

ON

TISSUE- OR CELL-FIBRINOGEN

IN ITS RELATION TO

THE PATHOLOGY OF BLOOD.

Delivered at the Theatre of the Laboratories of the Conjoint Colleges of Surgeons and Physicians, on Feb. 4th, 1892,

BY

A. E. WRIGHT, M.D. Dub.,

LATE GROCERS' RESEARCH SCHOLAR.

Reprinted from "THE LANCET," February 27 and March 5, 1892.

LECTURE

ON

TISSUE- OR CELL-FIBRINOGEN

IN ITS RELATION TO

THE PATHOLOGY OF BLOOD.

THE first step in the direction of the acquirement of a control over the processes of coagulation in the living body was made by Wooldridge when he succeeded in demonstrating that intra-vascular coagulation could be obtained by the injection of solutions of tissue-fibrinogen. I do not think that I am over-estimating the importance of this discovery; for when it had been confessed by Schmidt's own pupils[1] that the injection of practically unlimited quantities of fibrin ferment into the vessels made the blood more liquid instead of producing coagulation in it, it became evident that there was no possibility of influencing the coagulability of the blood by following the lines of the fibrin-ferment theory. Now Wooldridge's work was a departure on new lines, and he succeeded in the most brilliant manner in demonstrating that it was possible by working upon these new lines to obtain a practical control over the phenomena of coagulation in the living body. It is to the facts that Wooldridge discovered, and to such others as I have been able to add to them or to bring into relation with them, that I would wish to direct your attention this afternoon. And to begin with, I would like to say that in ascribing the credit of the method of producing intra-vascular coagulation to Wooldridge I believe that I am only giving him what is strictly his due; for, although Groth,[2] one of Schmidt's pupils, had described the occur-

[1] Jabowicki, Birk, Köhler: Disserts., Dorpat, 1875, 1877, 1880. Edelberg's contradictory results (Archiv f. Exp. Path., 1880) may be neglected. They were obtained with extraordinarily impure fibrin-ferment solutions.

[2] Dissert., Dorpat, 1884.

rence of intra-vascular coagulation in connexion with the injection of leucocytes in 1884—i.e., before the date of Wooldridge's publication—he had elicited the phenomena in a much less distinct manner, and he had not determined to what constituent of the leucocyte the intra-vascular coagulations were due. Instead of this he ascribed these coagulations to a production of fibrin ferment in the blood—that is to say, Groth ascribed his coagulation to a cause which we know to be quite ineffective. I think we need not therefore consider a question of priority here; but, on the other hand, we shall do well to keep in view the fact that Groth was successful in obtaining intra-vascular coagulations by the injection of the expressed juice of lymph-glands, and we have to note also that Groth regarded his experiments as conclusive as to the participation of leucocytes in the processes of coagulation. Groth s work thus opens up a number of important issues, which we shall have to consider in connexion with the production of intra-vascular coagulation by tissue-fibrinogen.

First, with regard to the relation of white corpuscles to coagulation, we are met by the fact that Wooldridge not only did not consider that Groth's experiments proved the existence of any such relation, but he went further than this, and expressed it as his opinion that the leucocytes of the blood had not been shown to stand in any relation whatever to coagulation. Wooldridge based this view, for the one part, on certain defects in conclusiveness in the evidence which had been adduced by Schmidt and his pupils to implicate the leucocytes in the processes of coagulation. Schmidt had, for instance, described the superior coagulability of the so-called granular layer which is seen above the corpuscular layer in horse blood which has been kept from coagulation by cooling; and Schmidt had asserted the granules of that layer to be with certainty derivatives of disintegration of white blood-corpuscles, and upon this assumption he had concluded that the superior coagulability of the granular layer was evidence of the participation of the leucocytes in coagulation. Wooldridge, however, pointed out that there was a flaw in this evidence, inasmuch as Schmidt had not excluded the possibility of these granules being derivatives of the plasma—i.e., the possibility of their being simply a precipitate produced in the plasma by cooling. In this connexion Wooldridge showed that peptone plasma gives such a precipitate on cooling, and I may say that I have observed that a similar precipitate can invariably be produced by the action of cold upon decalcified plasma. It follows, I think, that the

argument from the superior coagulability of the granular layer must be finally abandoned. Again, with respect to the experiments of Groth and afterwards of Krüger,[3] in which an increased coagulability was observed after leucocyte injections, Wooldridge drew attention to the fact that the experiments were conducted with insufficiently isolated leucocytes, and pointed out that a fallacy was, in his opinion, introduced into them by the fact that the lymph plasma which was injected along with the lymph cells contained a coagulative substance—namely, tissue fibrinogen. Further, Wooldridge repeated the experiments of Groth and Krüger with washed leucocytes, and found that he obtained a diminution instead of an increase of coagulability in the blood. These appear at first sight to be very grave, if not fatal, objections to Schmidt's view of the important *rôle* of the white corpuscles in coagulation; still I hope to be able to show that it is possible to settle satisfactorily with them.

First, with regard to the defective isolation of the cells which was insisted upon by Wooldridge, it must be conceded that Groth's leucocytes were not at all isolated. They were simply injected along with the inter-cellular fluid of the lymph gland. With regard, further, to Krüger's attempt at isolation by means of filtering off the lymph plasma, Wooldridge is probably also correct in stating that this method is entirely ineffective, inasmuch as any attempt to filter off leucocytes from a solution of tissue fibrinogen would be rendered futile by the fact that a considerable amount of tissue fibrinogen would invariably be retained on the filter. It is evident, therefore, that we have to concede to Wooldridge that neither Groth's nor Krüger's experiments were performed with isolated leucocytes.

When we come to the second heading of the objection, which is based upon the fact that the lymph plasma contains tissue fibrinogen, and would thus introduce a fallacy if not effectually separated from the leucocytes before injection, we must, however, pause for consideration. It is not at all necessary to meet this objection by asserting that there is no tissue fibrinogen in the lymph plasma. Wooldridge was a very careful observer, and we may well accept the fact upon his statement; but we may, on the other hand, with advantage inquire how the tissue fibrinogen got into the lymph plasma; and when this question is once formulated, it is easy to see that the answer must be that the tissue-fibrinogen is almost certainly

[3] Zeitschrift f. Biolog., 24, p. 189.

derived from the disintegration of leucocytes, inasmuch as Wooldridge himself has shown that the lymph cells contain this substance. We thus see that if the coagulative substance of the lymph plasma was derived from the leucocytes, the contamination of the leucocytes with this plasma in the experiments of Groth and Krüger cannot be considered to vitiate their results with reference to the connexion between leucocytes and coagulation. Lastly, as to Wooldridge's objection that the injection of washed leucocytes from lymphatic glands produces increased fluidity instead of increased coagulability of the blood, it will be evident among other things that the extraction of the contained tissue-fibrinogen in the processes of washing would easily account for this result, the injection of washed leucocytes being in point of fact equivalent to an injection of attenuated leucocytes.

Having thus cleared our way from the obstacles which seemed at first to forbid us to contemplate the possibility of there being an essential unity in the results obtained by Wooldridge with tissue-fibrinogen injections and the results obtained by Groth and Krüger by their injections of leucocytes, we may proceed to inquire whether there are any positive grounds for assuming the fundamental identity of the phenomena. And here we have, first of all, to note the fact that tissue-fibrinogen—or, as I might perhaps venture to call it, "cell-fibrinogen,"[4]—though found also in the cells of the testicle, and I believe also of yeast—is yet pre-eminently a constituent of the leucocytes of the thymus and of the lymphatic system generally, and we must regard these leucocytes as at least potential blood leucocytes. Here, then, we have immediately an important link between leucocytes and tissue-fibrinogen. With reference to this, however, we again find that Wooldridge was not satisfied of the conclusiveness of the argument. There might well be, argued Wooldridge, all the difference in the world between the leucocyte of the lymphatic system and the leucocyte of the blood; and further than this there was, in his opinion, distinct reason to believe that the lymph cell puts off its old nature and assumes an altogether new one when it once obtains access to the blood stream. This was Wooldridge's view; and here again, since I wish to push my argument beyond the leucocyte of

[4] This term would appear to be a more appropriate one than tissue-fibrinogen, since this proteid appears to occur not in the inter-cellular substance of tissue, but in the cells themselves. It appears also to be more widely distributed in free cells than in cells which are found in tissues.

the lymph to the leucocyte of the blood, I am compelled to dwell a little on the question as to whether a lymph cell is in any way demonstrably different in its chemical character from a blood leucocyte. If I understand Wooldridge aright, the experiment he relied upon as establishing a difference between the leucocyte in the gland and the leucocyte in the blood was the following. He took lymph cells and added them to "strong peptone plasma," which is not coagulable by the addition of leucocytes. He then collected his leucocytes again from this strong peptone plasma and proceeded to add them to a weak peptone plasma, which is invariably coagulable by the addition of leucocytes. The leucocytes which he now added, however, failed to coagulate it, and the conclusion which was drawn by him from this fact was that the leucocytes had undergone some fundamental alteration in their chemical constitution during the period of their stay in the strong peptone plasma. Such a change of nature Wooldridge considered to be associated with the passage of the lymph cell over the threshold of the blood. The experiment in question is, however, hardly sufficient to establish this, and it is not at all difficult to put a different construction upon it than that which Wooldridge attributed to it. I think we are, in fact, constrained to do so in view of the facts which have been elicited by Rauschenbach in connexion with the chemical and physiological properties of the leucocytes. The researches in question I believe conclusively establish that though there is an undoubted line of distinction which can be drawn between leucocyte and leucocyte, that line cannot be drawn so as to include all the white blood-corpuscles in one category and all the lymph cells in another. Rather the distinctions can be more naturally explained with reference to differences in maturation in the leucocytes whether these occur in the blood stream or without it. The physiological differences between leucocytes are apparently only quantitative differences, but they are at least very appreciable quantitative differences, and they permit of our sorting the leucocytes into two classes. The basis of this classification would be the greater or less stability of the leucocyte as shown by its resistance to disintegration when it is introduced into a spontaneously coagulable plasma. It is, however, to be noted that such a coagulable plasma has the power of disintegrating any leucocyte [5]

[5] According to Rauschenbach's researches the plasma has the power of disintegrating not only leucocytes, but many other varieties of animal and vegetable cells.

whatever which is added to it, and of using up one of its constituents, not, it must be noted, its paraglobulin, but its tissue- or cell-fibrinogen in the formation of fibrin. When, however, it comes to be a matter of choice, the plasma naturally employs for its purposes the more easily disintegrated leucocytes which have been described by Rauschenbach as the α-leucocytes. These α-leucocytes are distinguished from the β-leucocytes, also by certain more strictly chemical characters. They, for instance, stain in carmine only with extreme difficulty, whereas the β-leucocytes take on the stain almost instantaneously. Again, the α leucocytes further show their defect in stability by undergoing a slimy metamorphosis when they are treated either with NaCl solutions of moderate strengths, or with dilute caustic alkalies. These characters are, I think, possibly due to the presence of considerable quantities of tissue-fibrinogen in the α-leucocyte.

When these differences between leucocytes had been established Rauschenbach proceeded to the study of what we may call the geographical distribution of the α- and β leucocytes, but it is only possible to glance at the main results of his most interesting work so far as they affect the questions at present more directly under discussion. It is, for instance, of direct importance to us to note that both α- and β-leucocytes are found to be present in the lymph glands, and, further, that the same two varieties of leucocytes are found also in the blood. We thus see that Rauschenbach's observations do not support the theory of the essential duality of the blood leucocytes and the lymph leucocytes. Rauschenbach's work further indicates the lines upon which the explanation of Wooldridge's experiment is to be sought, for we can now see how it is almost certain that Wooldridge must have added to his "strong peptone plasma" a mixture of α- and β-leucocytes, and it is probable that he would, under the circumstances, only have recovered the less easily disintegrated β-leucocytes from it—i.e., leucocytes whose coagulative power may have been inadequate to bring about coagulation in his weak peptone plasma. The result obtained would then easily be accounted for without the hypothesis of the leucocyte putting on a new nature when it entered the blood. Similarly we could on these lines account for Wooldridge's observation that the inter-cellular fluid of the lymph glands was much more potent in inaugurating coagulation than the isolated lymph cells. The α-leucocytes, which disintegrate easily, would naturally pass into solution in the lymph plasma and impart their coagulative efficacy to it, while the more resistant

cells, which survive the chemical processes involved in isolation, probably consist of β leucocytes in which the coagulative power is at its minimum. This may in part account for the relative coagulative inefficacy of the leucocytes employed by Wooldridge in his injection experiments. We shall, however, have to return to this subject at a later stage of this lecture.

We have in this manner, if my arguments are correct, disposed of the objections which Wooldridge raised to admitting the coagulative influence of leucocytes, and we have now only to establish that the phenomena observed after the injection of tissue-fibrinogen are identical with the phenomena seen after the injection of leucocytes, before we proceed to vindicate that wider importance for tissue-fibrinogen coagulations which would naturally accrue to them if they were shown to stand in relation to any increased disintegration of leucocytes in the blood.

I shall therefore proceed to describe to you from Wooldridge's work on the subject and from work of my own, which was directed to follow up Wooldridge's researches, the phenomena observed after the injection into the blood of solutions of tissue-fibrinogen, and I shall then bring these into connexion with the results obtained by Groth by injections of leucocytes into the blood. I may say that I am only concerned here in putting before you the main facts of my work on the subject. The actual experiments and the general details I have already communicated to the Royal Irish Academy, and the paper has just appeared in their Proceedings.[6]

The injection of a solution of tissue-fibrinogen into the vessels is followed by an increase of the coagulability of the blood, which is at its height immediately subsequent to the injection. If the quantity of tissue-fibrinogen injected is large enough, the increase of coagulability leads to the immediate occurrence of intra-vascular coagulation. The coagulation which occurs under these circumstances in the case of the dog occurs only in the portal vascular area; but I have been able to show that we can determine it to any predetermined vascular area by increasing the venosity of the blood in the veins of that particular district, or we can generalise the coagulation and obtain it all over the vascular system, both in the arteries and the veins, by increasing the amount of CO_2 in the blood generally. These alterations in the direction of the

[6] Proc. Roy. Irish Acad., third series, vol. ii., No. 2.

"positive phase of coagulation," to use Wooldridge's convenient expression, form, however, only a part of the effects which result from the injection of a solution of tissue-fibrinogen.

We have further to deal with a negative phase of coagulation—i.e., with a condition of diminished coagulability brought about by injections of tissue-fibrinogen. This diminished coagulability was described by Wooldridge in connexion with the blood of the extra-portal vascular areas, where intra vascular coagulation had occurred in the portal system. I have, on the other hand, I believe, succeeded in establishing that the negative phase of coagulation is due to a general reaction of the system to the injection of tissue-fibrinogen, and that it is possible, by slow injection of moderate amounts of tissue-fibrinogen, to obtain a general diminution instead of a general rise of coagulability. The blood has, therefore, the power of breaking down tissue-fibrinogen into some substance or substances which condition the loss of coagulability. I will here adduce from my note-books an experiment, which seems to me to establish distinctly the sequence of events which I have just described.

EXPERIMENT 1.

Dog 119.—Cannulas inserted into the jugular vein and the carotid artery, and animal anæsthetised with a mixture of ether and chloroform. Samples of blood drawn off from the carotid to determine the condition of coagulability. Sample 1 (5.43 P.M.): Can invert tube at 5.51 P.M.; time, 8 minutes. Ran in 10 cc. of tissue-fibrinogen solution at 5.43 P.M. Sample 2 (5.44 P.M.): Still liquid 1 hour after. Ran in 10 cc. more of tissue-fibrinogen solution. Sample 3 (5.45 P.M.): Still liquid 1 hour after. Sample 4 (5.46½ P.M.): Still liquid one hour after. Sample 5 (5.48 P.M.): Still liquid 1 hour after. Ran in 10 cc. more tissue fibrinogen. Sample 6 (5.50 P.M.): Can invert tube at 5.58 P.M.; time, 8 minutes. Sample 7 (5.51 P.M.): Can invert tube at 6.4 P.M.; time, 13 minutes. Ran 10 cc. more at 5.52. P.M. Sample 8 (5.53 P.M.): Can invert tube at 6 P.M.; time, 7 minutes. Ran in 10 cc. more at 5.54 P.M. Sample 9 (5.55 P.M.): Can invert tube at 6 P.M.; time, 5 minutes. Ran in 10 cc. more. Sample 10 (5.56 P.M.): Can invert tube at 6 P.M.; time, 4 minutes. Ran in 10 cc. more. Sample 11 (5.58½ P.M.): Can invert tube at 6.2 P.M.; time, 3½ minutes. Sample 12 (6 P.M.): Can invert tube at 6.4 P.M.; time. 4 minutes. Sample 13 (6.2 P.M.): Can invert tube at 6.9 P.M.; time, 7 minutes. Sample 14

(6.7 P.M.): Can invert tube at 6.14½ P.M.; time, 7½ minutes. Ran in 20 cc. more tissue-fibrinogen solution. Sample 16 (6.14 P.M.): Can invert tube at 6.16 P.M.; time, 2 minutes. Sample 17 (6.16 P.M.): Can invert tube at 6.20 P.M.; time, 4 minutes. Sample 18 (6.19 P.M.): Can invert tube at 6.22 P.M.; time, 3 minutes. Sample 19 (6.22 P.M.): Can invert tube at 6.26 P.M.; time, 4 minutes. 6.26 P.M.: Clamped the trachea to increase the coagulability of the blood, and ran in 20 cc. more tissue-fibrinogen solution. Death occurred instantaneously, and both sides of the heart and the whole of the aorta were found occupied by a solid clot. No clot in the portal vascular area. Urine gave a very distinct biuret reaction.

We have thus proved that the positive and the negative phase are successive in time, and it was evident that the positive phase is associated with the presence of tissue-fibrinogen, as such, in the blood. It still, however, remained to be determined to what the negative phase of coagulability is due, and I have ventured in my paper referred to above to suggest that it is due to the presence in the blood of albumoses which have been split off from the tissue-fibrinogen by the action of the blood. The evidence upon which I based that suggestion is as follows: (*a*) The plasma from the negative phase areas no longer contains any of the injected tissue-fibrinogen. (This, I may remark, is an observation which was made by Wooldridge.) On the other hand, the blood of the negative phase areas was found by me to have acquired the characters of peptone or albumose blood—that is to say, it clots with CO_2, and on dilution. Coagulation can also be inaugurated in it by an addition of lime salts or of tissue-fibrinogen or leucocytes. Further, (*b*), there is a very close analogy between the distribution of the areas of the positive and the negative phase after tissue-fibrinogen injections and the distribution of coagulability after injections of peptone. For instance, the coagulability is in the case of tissue-fibrinogen injections increased up to the coagulation point in the portal area and to a lesser extent in the venous system generally. Similarly in the case of peptone injections the coagulability is retained in the venous system, and especially in the portal venous system, long after it has been lost in the arterial system. Again, the positive phase after tissue-fibrinogen injections can be extended to the arterial system by rendering the blood there venous; and I have been able to show we have something quite similar in the case of peptone injections, for it is only necessary to render the arterial blood venous in order to restore its lost coagulability to it. Lastly (*c*), I would point

out that an excretion of albumose or peptone occurs both in the dog and in the rabbit after the injection of tissue-fibrinogen into the veins or into the subcutaneous tissue. Here, again, I think we have evidence that the tissue-fibrinogen is broken down into albumose in the system.

On the whole, therefore, I think we may perhaps regard it as pretty well made out that the occurrence of a negative phase after tissue-fibrinogen injections stands in some close relation with the liberation of albumose by the disintegration of tissue-fibrinogen in the blood.

Keeping therefore in view those phenomena which are observed after an injection of tissue-fibrinogen, we have to bring them in connexion with the phenomena observed after injections of leucocytes. These phenomona have been studied by Groth, Wooldridge, and Krüger. I have already indicated in connexion with Wooldridge's work that the method of experimentation he adopted appears to exclude from the injection all the rapidly disintegrated α-leucocytes, and such products of their disintegration as would naturally pass into solution in the lymph plasma. On the other hand, in the methods of Groth and Krüger there is either no provision (in the case of Groth's experiments) or ineffectual provision (Krüger's experiments) is made for the elimination from the injection of such elements of the lymph plasma as have not been derived from leucocytes. Having said thus much in criticism of the methods, we may pass on to the results obtained by these observers from their injections. Krüger and Groth both succeeded in obtaining intra-vascular coagulation with their injections. They, further, noticed that when the injections of leucocytes were not sufficiently large to entail such intra-vascular coagulation, the blood, when collected after the lapse of a few minutes, showed a very marked diminution of coagulability. The results Wooldridge obtained were in all cases a diminished coagulability of the blood after injections of washed leucocytes. It will be immediately evident that in the case of Groth's and Krüger's experiments we have to deal with the same sequence of positive and negative variation which we are now familiar with in connexion with tissue-fibrinogen injections. In Wooldridge's experiments we have only a negative phase of coagulation put on record. In connexion with this I would make the suggestion that a very transient positive variation may very easily have occurred here and have escaped notice. Groth has emphasised the fact that such a positive variation is sometimes so transient that it is necessary to draw off a sample of blood during the actual progress of the injection in order to satisfy oneself of the

actual occurrence of such an increase of coagulability, and we have seen confirmation of this in the case of the protocol which has been quoted (*supra*, p. 459). It is therefore probable that Wooldridge's failure to obtain evidence of the occurrence of a positive variation of coagulability after his leucocyte injections was attributable to his not withdrawing a sample of blood immediately after his injection was completed. I believe, therefore, that Wooldridge's experiments do not in the least contradict those of Groth and Krüger. And it may be accepted that the phenomena of a positive phase, succeeded by a negative phase, are to be observed in exactly the same way after a leucocyte injection as after a tissue-fibrinogen injection. The identity of an injection of leucocytes with an injection of tissue-fibrinogen is also brought out by the results of Groth's leucocyte enumerations of the white corpuscles in the blood. These enumerations yielded the result that not only are the leucocytes of the circulating blood not increased by the large injections of leucocytes, but they are, on the contrary, positively diminished even in cases where no intravascular coagulation can be detected. This will become evident on the consideration of the following figures which I have extracted from Groth's paper :—

	Before injection.	After injection.
	Number of white corpuscles in the blood.	
Experiment 5	10,483	6115
„ 6	29,265	5678
„ 7	17,908	2512

It is thus evident that the increase of coagulability after leucocyte injection is associated with a breaking down not only of the injected but also of some at least of the aboriginal leucocytes of the blood, and this breaking down of leucocytes must evidently go hand in hand with a setting free of tissue-fibrinogen in the blood.

Up to the present we have been dealing exclusively with injections of leucocytes derived from the lymphatic glands, and we have to amplify the data thus obtained by a study of the effects of leucocytes derived from other sources. Here, again, we are indebted to Schmidt's pupil, Dr. Groth. Groth determined that the addition of pus cells to the circulating blood was followed by very similar results to those which resulted from the intra-vascular injection

of lymphocytes. The phenomena of the positive phase—i.e., those of intra-vascular coagulation—were, however, much less prominent, and the negative phase was, on the other hand, perhaps rrelatively more marked than with lymphocytes. These observations have an important bearing upon the causation of the negative phase, inasmuch as the prominence of the phenomena of the negative phase after pus injections probably stands in close relation to the very considerable quantity of "peptone" or albumoses which Hoffmeister's researches have shown to be present in pus either as actually pre-formed or in such loose combination that the addition of moderate quantities of alkalies is sufficient to set them free.

I have now laid before you merely the broad facts which seem to me to point to the identity of the phenomena which are observed when, on the one hand, leucocytes, or, on the other hand, tissue-fibrinogen is injected into the blood, and if that connexion has been sufficiently established, it will necessarily follow that wherever we get a disintegration of leucocytes in the blood we have to deal with a setting free of tissue-fibrinogen. This liberation of tissue-fibrinogen in the blood will result in an immediate increase of the coagulability of the blood, which, if it does not culminate in immediate intra-vascular coagulation, will last only until such time as the organism shall have been able to accomplish the breaking down of the tissue-fibrinogen into albumoses. Further, we have to remember that when this reactive process is accomplished we are introduced to a negative phase of decreased coagulability which in its turn gradually passes off by the excretion of the albumoses in the urine. According to this view, therefore, the disintegation of leucocytes, the liberation of tissue-fibrinogen in the blood and the excretion of albumoses constitute a closely inter-related sequence of symptoms which must be constantly manifesting itself in the course of all such pathological processes as go hand in hand with an increase of leucocytes in the blood. I believe that the clinical facts do in effect bear out this view. The pathology of croupous pneumonia, for instance, supplies us with, I think, a very well-marked instance of the inter-relation between the three members of our symptom-sequence. We have first an enormous increase of the numbers of the leucocytes, which, though they are for the most part to be found outside the vessels, are yet in immediate relation to them. We have then, especially when the stage of absorption is reached, the well-marked increase of coagulability in the blood, which I take it is due to the absorption of tissue-

fibrinogen.[1] We have, further, the well-known occurrence of peptonuria, or rather, as Lea Dickinson and Fyffe have shown, of albumosuria; and in connexion with the presence of albumose in the blood we have, as Dr. Dickinson has pointed out, the frequent occurrence of diarrhœa, which, I may remark in passing, is also very frequently seen in connexion with injections of tissue-fibrinogen. The other cases of what we may call peptonuria—for the distinction between peptone and albumose is, after all, a very much over rated one—are associated with pyæmic conditions and with the defervescence period of certain fevers, such as measles or scarlatina. Here, again, we are probably dealing with leucocyte disintegrations. Further, we have the peptonuria of osteo-malacia, which, however, is apparently not an invariable accompaniment of that condition, but rather an accompaniment of any proliferation of the cells of the bone marrow, whether arising from that cause or from the presence of tumours. Lastly, we have the peptonuria of phosphorus poisoning, which is also probably due to the increase of leucocytes in the blood which is described in connexion with that condition. All these cases of what we may for convenience call peptonuria may be grouped together under the term of "hæmatogenous peptonurias."[2] I believe they are probably all due to a disintegration of tissue-fibrinogen in the blood. In contradistinction to these we have the class of what have been called "pyogenous peptonurias," because they occur wherever we have a mass of disintegrating pus in the body, provided the conditions are favourable to the absorption of the products of such decomposition. It is evident from the researches of Hoffmeister, which I have referred to above, that the further disintegration of the leucocyte, or of the tissue-fibrinogen which is liberated from it, into albumose may occur in the abscess itself, instead of in the blood. In that case we would, of course, not have any positive phase of coagulability from the absorption of tissue-fibrinogen.

We have thus seen that peptonuria is probably in all

[1] Since the date of this lecture I believe that I have definitely established this by preparing large quantities of tissue-fibrinogen from consolidated pneumonic lungs, which were very kindly obtained for me by my friends, Dr. H. S. Rolleston and Dr. E. H. Starling. A few cubic centimetres of the solution of this tissue-fibrinogen, when injected intravascularly, gave the typical universal thrombosis in a rabbit and the typical portal thrombosis in a dog.

[2] This term has been employed by v. Jaksch for peptonurias which are, upon his assumption, produced by the liberation in the blood of peptone "carried" by the leucocytes.

cases associated with an increase in the number of leucocytes in the system, and that an increase of coagulability in the blood is only to be expected where the primary product of their disintegration—i.e., the tissue-fibrinogen—obtains access to the blood. Generally speaking, this will occur when the leucocytes disintegrate in the blood stream itself. Further, the greater or less coagulability of the blood will generally depend upon the number of leucocytes present. We have an apparent exception to this last rule in the case of leucocythæmia, where, as is well known, the coagulability of the blood is decreased rather than increased. The explanation of this exception, however, here lies readily to hand, for the increased coagulability we have spoken of all along is a relation which obtains only as between leucocytes and normal plasma, and in leucocythæmia, as Jacob v. Samson-Himmelstjerna (another of Schmidt's pupils) has shown, the power which the leucocythæmic plasma has of breaking down the cellular elements of the blood for the purpose of fibrin formation is diminished, while the power possessed by the cellular elements of contributing a fibrin factor to the coagulation of healthy plasma is still retained.

The clinical evidence is thus apparently conclusive that the sequence of symptoms, increased production of leucocytes, increased disintegration of leucocytes in the blood, increased coagulability, subsequent peptonuria, is one which does not infrequently manifest itself; but as the importance of the subject seems to render it desirable to obtain even further experimental evidence upon this subject, I have endeavoured to make some further observations in connexion with this complex of symptoms. Two methods of experimentation suggested themselves. It was evidently possible, if a method of increasing the leucocytes in the circulating blood could be discovered, (*a*) to employ that means of increased production with a view to attaining a subsequent increased disintegration of the leucocytes in the blood by the natural destructive processes of the body, and, further, (*b*) it was possible to bring about the artificial disintegration of such leucocytes as exist at a particular moment in the circulating blood by the administration of some drug such as quinine (Binz) or atropine (Horbaczewski), which possesses this property of disintegrating the leucocytes present in the blood. I have as yet made a few preliminary experiments in each of these directions. My experiments in the first direction have been of a more or less incidental character, and were obtained in the course of experiments upon the

question of immunity from anthrax, as obtained by means of injections of Wooldridge's tissue-fibrinogen. I noticed in connexion with these that some of my rabbits which were being put through a course of preparation for anthrax inoculation, consisting of daily injections of tissue-fibrinogen solution, died before the period for that inoculation arrived; and the post-mortem examinations revealed the existence of discoloured branching coagula in the heart and great vessels, which must evidently have been formed during the lifetime of the animals. Here we had, therefore, a late coagulation as a consequence of tissue-fibrinogen injection, a phenomenon for which I was totally unprepared. Some time afterwards, in reading over Groth's work on the injection of leucocytes, his figures, which pointed to an enormous increase of leucocytes in the blood two or three days after the injection of leucocytes, arrested my attention. I have verified these observations in some twenty rabbits in the case of injections of sterilised tissue-fibrinogen solutions, and have seen the leucocytes invariably considerably increased — sometimes increased to as many as six times their normal number. The subject is one which I am following up in connexion with its evident bearing on the causation of immunity by tissue-fibrinogen injections. What interests us, however, here is only the fact that an increase of leucocytes in the blood stands in evident relation to an increase of coagulability. With regard to the second method of experimentation—viz., the administration of quinine for the purpose of artificial disintegration of the leucocytes in the blood—I am not prepared yet to make a positive statement; but in several experiments which I made upon the subject I obtained at first an enormous increase of coagulability; and though I have in no case succeeded in discovering any intra-vascular coagulations under these circumstances, still it is at any rate suggestive that it has frequently been suggested that the blindness which sometimes comes on after the administration of poisonous doses of quinine may be due to retinal thrombosis, and I find that De Schweinitz[3] of Philadelphia has recorded at least one case in which quinine blindness in a dog was associated with thrombosis of the ophthalmic and retinal veins. That thrombosis is not of frequent occurrence after quinine is probably due to the fact that the system would be thoroughly well able to cope with an amount of fibrinogen comparable to that which would be

[3] Ophthalmological Review, 1891. Reported in THE LANCET, Dec. 5th 1891.

derived from the disintegration of a normal quantity of the leucocytes present in the blood.

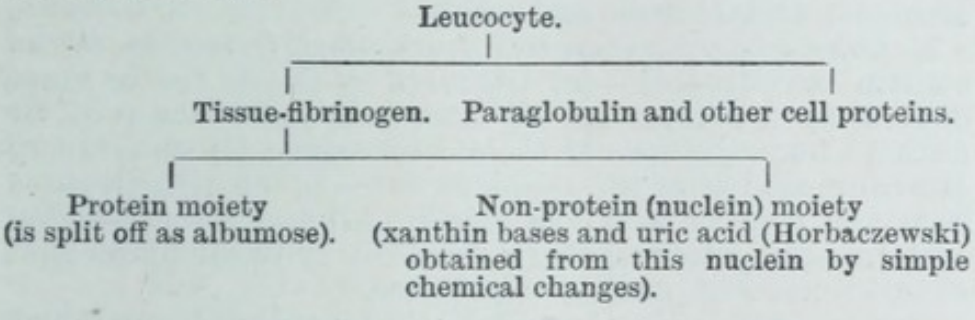
With this I have completed the survey of facts which I had prepared to lay before you on the afternoon originally fixed for this lecture. Since that time I have, however, come across a communication which has just been published by the very eminent Austrian observer, Horbaczewski, and which completes for us a whole missing chapter of our subject. I am only too conscious that I have already almost exceeded the limits of time appointed to me, so I will only ask to be allowed to put his results before you in a tabular form in their connexion with the work that has been done upon tissue fibrinogen. Before throwing this tabular synopsis upon the screen I would like to point out where the missing chapter which Horbaczewski has supplied comes into our subject. Tissue-fibrinogen, as I have previously been able to point out, is chemically a nucleo-albumen—that is to say, it is a substance which is at first sight deceptively like an ordinary protein or albuminous substance. When, however, we subject it to a peptic digestion we find that instead of passing entirely into solution it deposits a voluminous precipitate of a phosphorus-containing substance known as nuclein. Hence we conclude that tissue-fibrinogen is twi-natured, that it is by its one-half an albuminous or protein substance, and that it is by its other half a nuclein, and that these two moieties are separable from each other by the process of digestion. These properties determine its classification as a nucleo-albumen. Now we have discussed the physiological importance of the whole substance, its relation to coagulation, and the increase of leucocytes which follows its administration, and we have followed the fortunes of the protein moiety into which it disintegrates. We know that that protein moiety is excreted as an albumose. We have, however, not inquired as to what became of the nuclein moiety in the system. That is the chapter which Horbaczewski has worked out for us. Horbaczewski, I may remark, prepared his nuclein by digestion from the leucocytes of the spleen without any previous isolation of the tissue-fibrinogen.

I would now, without any further preliminaries, draw your attention to the following table.

WOOLDRIDGE'S TISSUE- OR CELL-FIBRINOGEN.

Source of tissue-fibrinogen.—The protoplasm of leucocytes, also the protoplasm of the cells of the testicle, and probably also of yeast cells.

Chemical composition of tissue-fibrinogen.—This substance is chemically a nucleo-albumen or nucleo-protein, and contains a protein (or albuminous) and a non-protein (or nuclein) moiety.



Physiological Effects of the Addition of Tissue-fibrinogen to the Blood.

1. *Immediate increase of coagulability* (Wooldridge's "positive phase of coagulability"). (*a*) Observed by Groth and Krüger after injections of leucocytes (intra-vascular coagulations produced) (*b*) Observed by Wooldridge after injection of solutions of tissue-fibrinogen (intra-vascular coagulations in portal area in the dog). (*c*) Observed by the author after injection of tissue-fibrinogen (intra-vascular coagulation in any vascular district where blood has been rendered adequately venous). (*d*) Probably comes under observation clinically in the increase of coagulability in the venous system in the resorption stage of pneumonia.

2. *Subsequent diminished coagulability* (Wooldridge's "negative phase of coagulability") —(*a*) Observed by Groth and Krüger after the injection of leucocytes; (*b*) observed by Wooldridge after tissue-fibrinogen injections, especially in the blood of the extra-portal vascular areas; (*c*) shown by author probably to depend upon the setting free of albumose or peptone by the disintegration of the tissue-fibrinogen in the blood; (*d*) no clinical record of occurrence.

3. *Excretion of albumoses or peptone in the urine* (referable to the protein constituent).—(*a*) Observed by the author after injection of tissue-fibrinogen solutions both in the dog and in the rabbit (*b*) Observed clinically in connexion with the disintegration of leucocytes in pneumonia, pus-absorption, pyæmia, and proliferating tumours of bone marrow, &c.

4. *Increased excretion of uric acid* (referable to the nuclein constituent) —(*a*) Observed by Horbaczewski (in man and animals) after administration of nuclein prepared from the

leucocytes of the spleen. (*b*) Shown by Horbaczewski to go hand in hand with every increase of leucocytes in the blood. (*c*) Comes under observation clinically in connexion with the increase of leucocytes in pneumonia (croupous) and leucocythæmia, burns, and scalds.

5. *Later occurring increased leucocytosis* (referable to the nuclein constituent).—(*a*) Observed by Groth two or three days after the injection of leucocytes; (*b*) observed by author after injections of tissue-fibrinogen; (*c*) observed by Horbaczewski after the administration of nuclein prepared from the leucocytes of the spleen; (*d*) possible connexion with immunity from anthrax obtained by tissue-fibrinogen; experiments with nuclein in progress.

I have not time here to go into the important issues which Horbaczewski's work opens up. I would merely point out that to the triad of symptoms which has been referred to in the body of this lecture we are now able to add two additional symptoms—namely, those that are enumerated on the table under headings 4 and 5. I would also point out that we find all the symptoms, 1, 2 3, and 4, making their appearance in every ordinary case of croupous pneumonia, so that we may regard this disease as affording an almost classical instance of the effects of tissue-fibrinogen on the blood. The association of an increased uric acid excretion with peptonuria and with a more coagulable condition of the blood is not, however, limited to croupous pneumonia. It occurs also in connexion with, for instance, certain "rheumatic" or "gouty" conditions, and in connexion with burns and scalds. In the case of these last, we find thromboses in the portal area (leading to gastric and duodenal ulcers) associated with peptonuria and an increased uric acid excretion.

And now, in conclusion, I would desire to be allowed to express my very sincere thanks to the Councils of the Royal Colleges of Surgeons and Physicians, and more especially to the Laboratories Committee of the Conjoint Colleges, not only for their kindness in permitting me to work in their laboratories, but also for the very liberal manner in which I have been supplied with every appliance which was necessary for my work. Lastly, I feel that I have a debt of very special gratitude to discharge to Dr. Sims Woodhead for much unselfishly afforded assistance, and, above all, for his continual gift of sympathetic interest in the work upon which I have been engaged. I believe that I am only expressing what is felt equally by every other worker in these laboratories when I say for myself that the help and sympathetic interest of such a director of

studies as Dr. Woodhead have come to me as an especial and superadded stimulus to endeavour that the researches which emanate from these Laboratories may be such as to justify the labour and care and expense which have been involved in their establishment and maintenance.