C L O T R E T R A C T I O N.

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AT THE UNIVERSITY OF CAPE TOWN, SOUTH AFRICA,

by

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P R E F A C E.

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Mr. R.E. Duffett kindly made the sections of the clots, and the microphotography and the pictures of the "suspended clot method" were taken in the Department of Pathology.

My wife did some of the more complicated drawings; she did much more by smilingly accepting the meagre lot of a research-worker's mate. The somewhat amateurish appearance of the rest of the drawings and photography was due to my desire to initiate myself into these accessory, but essentialsarts of scientific work.

The literature, with the exception of the few Russian and Japanese articles, I have translated myself, and although much of the original elegance may have been lost in the quotations, I believe that the sense has been retained.

Financially, the work was made possible by the generous grants from the Council of Scientific and Industrial Research of South Africa, and I have great pleasure in acknowledging this obligation.

My gratitude is also due to innumerable people who have so liberally given their knowledge, experience and time; although not individually mentioned here for lack of space, their contributions are not forgotten. They include the bountiful donors who supplied the gallons of blood that flowed during these investigations.

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I N T R O D U C T I O N.

The haemorrhagic disorders form only a small, although often most alarming part of the diseases inflicted upon mankind, and in spite of the enthusiasm of the disproportionally great number of workers investigating the problem of the coagulation of the blood, this problem until quite recently could only be looked upon as being mainly of academic physiological interest. With the advent and daily increasing use of the highly dangerous anti-coagulant therapy for thrombosis and embolism, demanding at least a general understanding of the mechanism of blood clotting, every aspect of this mechanism has become of practical clinical importance, and no serious effort to clarify the question can be considered wasted.

It is peculiar that the last phase of coagulation, the contraction of the clot, which is by no means the least spectacular, has received rather scanty attention compared to the enormous amount of work that has been expended on the

earlier stages. No agreement exists with regard to the mechanism or purpose of clot retraction; the factors controlling it are to a large extent unknown, and even the fundamental question of the part played by the platelets is undecided.

The first aim of this work was to collect and review the whole literature dealing with clot retraction. This has never been attempted before, and as the work progressed it became very apparent that a real purpose would be served by such a survey. Unnecessary repetition and the presentation of old fact and fiction as new have occurred before in the history of Medicine, but rarely to the extent as in this small branch of the subject. It is hoped that this review will lighten the task for future workers interested in clot retraction.

The literature on other aspects of coagulation has naturally been consulted, but as the bibliography on these questions would fill a volume of its own, reference has only been made to works of essential importance for the problem under discussion. The same applies to publications on platelets and colloidal chemistry, which played a considerable role in the elucidation of many of the problems attacked. Due to the complex and contradictory nature of the literature it was found unsuitable to deal with it in one historical introduction, and it has therefore been discussed under the appropriate sections. This has introduced some unavoidable

repetition and cross-references, but it is ventured that the arrangements have led to greater ease of reading.

The second aim of the work was to confirm those very few observations on which general agreement had been reached, and to re-investigate the numerous unsettled problems of the retraction of the clot. In many instances satisfactory explanations for previous disagreement were found, in a few the temptation to speculation was irresistible. An endeavour was made to keep the investigations within the framework of human physiology, pathology and therapeutics, as it was felt that occasionally research on coagulation has become so remote from the natural state, that any inference drawn would be hazardous and unwarranted. The possibility remains that further information may be gained by such procedures.

After the demonstration of the simple external factors influencing clot retraction, the effect of the red and white cells, the role played by the platelets, the fibrinogen, and other plasma fractions, the underlying mechanism of the retraction is explained. The physiological function of clot retraction is then discussed, and its behaviour and importance in clinical medicine pointed out. It was, perhaps, a slight disappointment that at the end of the work the conclusion was reached, that clot retraction serves no purpose in human life, but is only a manifestation of a haemostatic mechanism

carried down from some prehistoric ancestors of all mammals. However, even negative conclusions may have some value as long as they help to bring the right perspective into more positive thought. It is the humble hope that these investigations may add something to the intricate problem of haemostasis.

CHAPTER 1.

METHODS FOR THE ESTIMATION OF CLOT RETRACTION.

Much of the confusion and most of the conflicting opinions with regard to the physiological and clinical significance of clot retraction can be ascribed to the unsatisfactory methods which have been used for its estimation. This fact has been known and repeatedly pointed out for more than 150 years, but has nevertheless, wittingly or not, been neglected in much of the work on the problem.

In the days of the Phlebotomists great attention was naturally paid to the behaviour of the blood from the unfortunate patients subjected to blood letting. William Hewson (1772) wrote: "When fresh blood is received into a basin, and suffered to rest, in a few minutes it jellies, or coagulates, and soon after seperates into two parts, distinguished by the names of Crassamentum and Serum. These two parts differ in their proportions in different constitutions, in a strong person, the crassamentum is in greater proportion to the serum than in a weak one; and the

same difference is found to take place in diseases; thence is deduced the general conclusion, that the less quantity of serum is in proportion to the crassamantum, bleeding, diluting liquors, and a low diet, are the more necessary; whilst in some dropsies, and other diseases where the serum is great, and the crassamentum is small in proportion, bleeding and dilution would be highly improper".

It would be of historical interest to attach a name to the first method of clot retraction estimation. It is described in detail by Thackrah (1819), but it is clear from the context of his work that the procedure was commonly used at the time. The method had the virtue of simplicity and was quantitatively as exact as any since proposed. The blood was run directly from the arm vein into a vessel and allowed to stand until no further contraction took place, i.e. from three to twenty-four hours. The vessel with the total blood was weighed, the serum was poured off, and the vessel with the clot weighed again; the difference in the weights gave the amount of serum expressed. Thackrah found the average amount of serum squeezed out in normal blood to be about 42 per cent which agrees with most figures since published. He was fully aware, as was Hewson, that the temperature must be controlled during the test.

Palmer in a comment in his edition of the Works of John Hunter(1837) gave more explicit times for coagulation and contraction:- "Coagulation commences in about 3 to 4 minutes on an average, and is completed in about 10. In about 15 or 20 minutes the coagulum assumes a pretty thick consistency;

but it continues still further to contract and to express the serum for at least 3 to 4 hours, and sometimes much longer". He then compares the behaviour of the clot to that of gelatine.

As blood letting fell into disrepute, so the interest in blood coagulation, and especially clot retraction, waned. Schklarewsky (1868) watched the phenomenon in capillary tubes under the microscope which led him to the use of the word "retraction", because the clot retracted from the glass wall. This has remained the general description, although "contraction" would be preferable.

A new era begins with Hayem's presentation of his paper on the examination of the serum of the blood at the Congress at Grenoble in 1878. From then onwards a great number of new or allegedly new methods has been published.

Methods using test-tubes.

Hayem's own method for the estimation of clot retraction was undoubtedly a regression as compared with the earlier one of Thackrah. Two ccs. of blood were run directly from a prick in the finger into the bottom of a small test-tube which was left at room temperature for 4 to 6 hours. The degree of retraction was roughly estimated by the appearance of the clot.

Le Sourd and Pagniez (1906), whose classical work on the relationship between clot retraction and platelets will be discussed later, used much the same method, and Glanzmann (1918) in his investigation on familial forms of purpura employed it too. The unreliable results which he obtained with this method

evoked a confusion in the study of haemorrhagic disturbances which has not yet been cleared. The test-tubes he used were of 8 mm. internal diameter, and it was very soon pointed out by Finkelstein (1920), and demonstrated experimentally by Opitz and Matzdorf (1921) that in such narrow tubes retraction, even when using the same blood, may be absent in some of the tubes, whereas it is normal in others.

A method that has been widely used in clinical work is the "Retraktilometer" of Fonio (1921). The procedure is shown in figure 1. The tube is smeared with liquid paraffin before use. It is filled with 1 cc. of blood which is allowed to clot with the tube in the upright position. The clot is then loosened from the sides with a fine needle, the tube is inverted and left for 24 hours. The degree of retraction is the distance the clot has sunk from the original level.

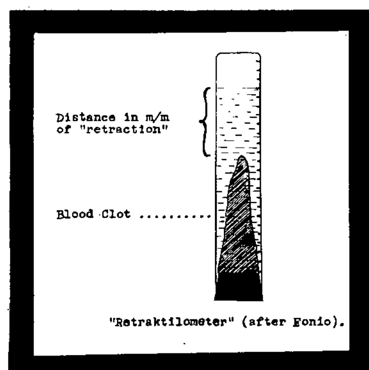


Figure 1.

Somewhat surprisingly Levy-Solal and Tzanck (1923) stated that the measurement of clot retraction until then had been purely qualitative. Instead their method should be employed: Collect 10 ccs. of blood in a test-tube; next day pour the expressed serum in to a graduated test-tube and measure the amount. A simple calculation gives the percentage of serum squeezed out. "This precise method demonstrates well, how much the qualitative estimation is subject to errors".

Mas y Magro (1924) used small haemolysis test-tubes and diluted the blood, taken from the finger, with saline. The test was carried out at room temperature and the retraction judged by the shape of the clot. A cylindrical clot indicated a poor retraction, conical was fair, and a clot like a nail suggested strong retraction.

Katrakis (1931) ran 30 ccs. of blood into a tube of known weight, placed it in an ice-chest, and watched the progressive retraction every 15 minutes or half hour. After 24 hours the amounts of serum and clot were determined by weight. He noticed that the time when the first signs of retraction were seen did not run parallel with the extent of the subsequent retraction.

Tocantins (1934) reverted to the use of small tubes in the manner of Hayem's. He estimated the extent of contraction by measuring the size of the clot in three dimensions from the outside of the tube.

A "serum volume test" was described by Boyce and McFetridge (1937). Three ccs. of blood were collected in graduated test-tubes and allowed to stand for 4 hours at room temperature; the clot was then lifted out of the tube and the volume of serum read off. They suggested an index, i.e. the serum volume over half the volume of the total blood, which in normal cases was near 1.

Another graduated test-tube method was introduced by Macfarlane (1938) and is illustrated in figure 2. The tube was kept at 37°C. for one hour; the glass rod with the adherent clot was lifted out, and the serum measured on the

scale of the tube. Aggeler and Lucia (1942) replaced the glass rod in this method by a cork-screw shaped wire, presumably to prevent the clot from slipping off in lifting it.

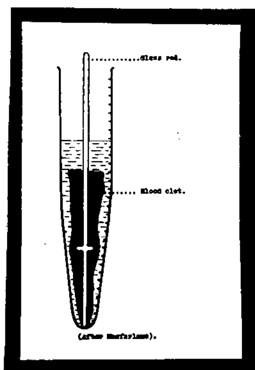


Figure 2.

In the estimation of the clot retraction time Hirschboeck and Coffey (1942) used test-tubes 1 cm. in diameter. They added 2 ccs. of blood to the tubes, which were closed with a rubber stopper and shaken to create a certain amount of foam. The coagulation time was first estimated by inverting the tubes, and after clotting they were left at room temperature. The time of the first sign of retraction of the clots from the sides of the tubes was taken as the retraction time.

Werner (1943) returned to a simpler method; he kept the blood in ordinary test-tubes at room temperature and pipetted off and estimated the serum 24 and 48 hours after clotting.

Slightly more complicated was the procedure of Zahn (1944). A graduated glass cylinder, washed out with liquid paraffin, was filled with 5 ccs. of blood and a layer of paraffin placed on top; the tube was kept for 15 minutes at 38°C. and the clot then loosened with a fine needle. The tube was then left for 6 hours after which the serum was poured into another graduated tube and the clot removed with a hook. This method with very slight modifications was also employed by Gleiss(1947).

Methods using watch-glasses.

Morawitz and Bierich (1907) found the use of test-tubes unreliable and substituted large watch-glasses. Five ccs. of blood in each of two carefully dried watch-glasses were left in a humid chamber at known temperature until contraction was complete. No quantitative estimation was attempted. Much the same method was used by Frank (1915) in his work on essential thrombopenia.

Opitz and Matzdorf (1921), who as already mentioned criticised Glanzmann's use of small tubes also found watch-glasses more reliable; they concluded that all previous work on clot retraction using test-tubes for its estimation should either be repeated or ignored. This criticism was confirmed by Krömeke (1922), who also found the use of capillary tubes unreliable; he therefore introduced a refinement in the watch-glass technique: one cc. of blood was run into spectacle lenses of -5 diopters and left at room temperature in a humid chamber. A quantitative estimation was not attempted.

Methods using capillary tubes.

Kaznelson (1919) introduced U-shaped capillary tubes of 1 mm. internal diameter, kept at room temperature. The reason why the tubes must have this particular shape is not clear, but he pointed out that even if retraction was absent in ordinary small tubes it might frequently be present in the U-tubes.

The capillary method was reproposed by Sooy and Moise (1927)

Like Schklarewsky 60 years earlier they watched the retraction under the microscope.

Evans (1928) discovered accidentally a modification of the capillary method. After estimating the coagulation time in such tubes with a lead shot according to the procedure of Dale and Laidlaw, he immediately washed out the clot with water. In the case of clots which by other methods showed poor retraction, the tubes and shots could be washed completely clean. With normal blood some part of the clot invariably remained adherent to the sides of the tubes and to the shot. This does appear a round-about way of estimating clot retraction.

Finally, Mackay (1931) modified the capillary tube method by suggesting that the tubes should be flamed to red heat before use, and that they should be tapped against a glass slab to ensure loosening of the clot if no retraction had occured. The clot retraction was expressed as nil, poor, fair and good.

More complicated methods.

An ingenious method was suggested in Italy in 1907 by Cesana. A specially constructed tube, illustrated in figure 3, was suspended from a machine registering the changes in weight occuring as the serum dropped out of the tube; the whole was enclosed in a humid glass box kept at a fixed temperature. A retraction curve was obtained showing progression of the contraction of the clot.

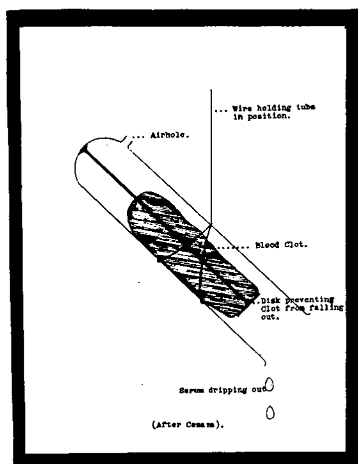


Figure 3.

A real advance was the methods of Van Allen (1927). In the one method the blood was placed under a clear bland oil, as shown in figure 4. In the other the serum was centrifuged away from the clot in a specially constructed centrifuge tube, a procedure, however, which must produce faulty results as even gravity alone under many circumstances is sufficient to cause an artificial seperation of the serum from the clot, as will presently be proved.

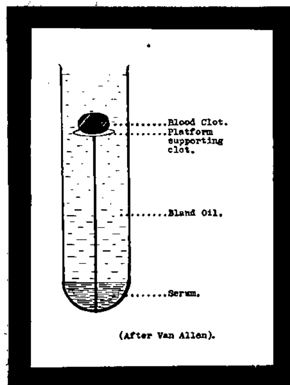


Figure 4.

Instead of letting the blood rest on a platform Roskam(1926 and 1927) dropped it into liquid paraffin in wide-bore test-tubes, which he continuously inverted until coagulation had taken place. The clot was then allowed to sink to the bottom and the retraction observed.

A retractometer was constructed by Mochkovski (1931). This method is illustrated in figure 5. The blood was allowed to clot and the clot loosened from the sides before the tilting of the tube. It will be shown that such loosening of the clot introduces a source of error which must be avoided to obtain reliable results.

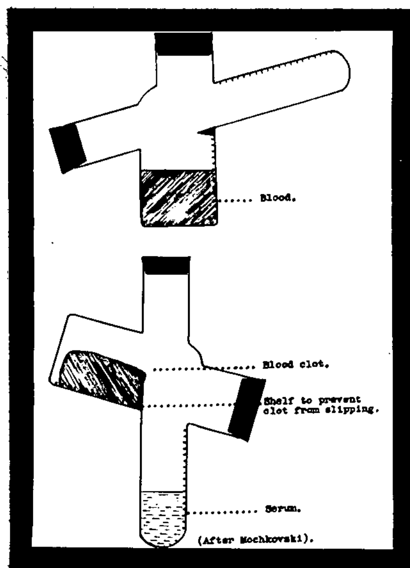


Figure 5.

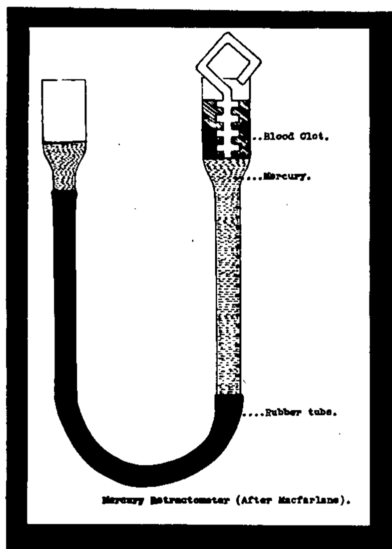


Figure 6.

The principles of a mercury retractometer as suggested by Macfarlane (1938) are shown in figure 6. After coagulation of the blood the mercury level was raised slightly to loosen the clot, then lowered; as the serum dripped down into the graduated tube the amount could be read off at desired intervals.

Apparantly unaware of the numerous procedures already described, Andreassen (1943) stated that the previously known methods of estimating clot retraction determined the retraction from the ability of the clot to express serum, but none were able to follow the progressive retraction process. He therefore constructed an apparatus as shown in figure 7. The tube was kept at room temperature.

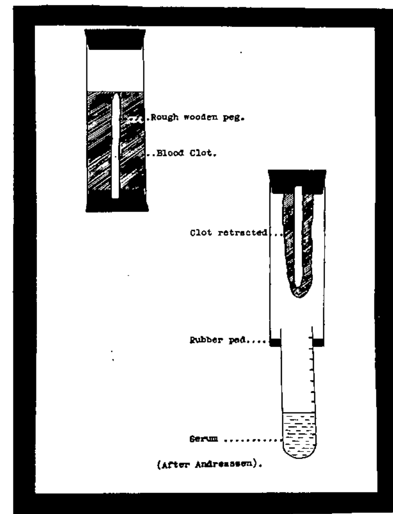


Figure 7.

Hirschboeck (1947, 1948), still persuing his investigations on the coagulation - retraction time, modified his previous method. In the new procedure a drop of blood from the finger is sucked into a blood counting pipette and 20 cmm. deposited on castor oil in a test-tube, where it is kept floating by surface tension. The test-tube is corked to avoid evaporation

and the test is done at room temperature. The time when the first serum is expressed from the clot indicates the coagulation-retraction time.

Methods using plasma instead of whole blood.

In an attempt to avoid the variable factor of the volume of red cells Czoniczer and Weber (1931) used citrated plasma in their method of clot retraction estimation. The plasma was obtained by spinning the blood for 5 minutes at 3000 r.p.m. The coagulation took place in a special tube, illustrated in figure 8. Thirty minutes after coagulation the clot was loosened from the sides of the tube. When contraction was complete the tube was weighed, the clot lifted out with forceps and the tube with the serum weighed again. A modification of this method was suggested by Tamura; it simply consisted in measuring instead of weighing the volume of serum expressed after lifting the clot out.

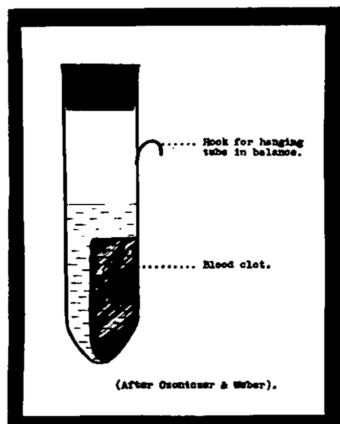


Figure 8.

Lundsteen (1942) found that the clot was torn on elevating the glass rod in Mac - farlane's test-tube method, and even coating the tube with paraffin did not prevent this destruction of the clot. He therefore proposed a different procedure: Citrated blood is left standing for hours

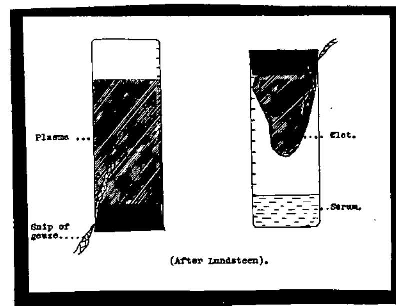


Figure 9.

until sedimentation of the red cells has taken place. Two ccs. of the plasma is pipetted into a special graduated tube coated with paraffin, and the plasma is recalcified. The further manipulation is seen in figure 9.

Tedious as this survey may be, it illustrates well the unnecessary overlap and repetition that has occured in the evolution of these methods. Only in exceptional cases was reference made to previous work. As usual in cases where no single procedure has found universal acceptance one may also assume that no method was entirely satisfactory.

Simple physical and mechanical causes are the reasons why none of the methods described has proved ideal. Some of these factors have been known from the earliest days, but the importance of others has never been realized, and unless they are constantly borne in mind during work on clot retraction, acceptable results will not be achieved. It is therefore necessary to consider them in some detail before the description of the method employed in this work, which is an attempt to overcome the errors caused by these external disturbances.

As early as 1830 Babington, using the simple method described by Thackrah (1819), pointed out that the shape of the vessel in which the separation into crassamentum and serum took place, and the amount of blood used, influenced the final retraction of the clot. To illustrate the first point he gives this table:-

Disease.	Percentage of serum exuded.	
	In Pearshaped Bottle.	In Basin.
Purpura.	40%	30%
Vertigo during pregnancy.	51%	40%
Phthisis.	51%	50%
Diabetis Melitus.	45%	40%

Table 1. The difference in clot retraction in different vessels as found by Babington(1830).

Two explanations were suggested for this phenomenon:- "This difference is owing to the greater or less distance of the coagulating particles of fibrin from a common centre, which causes a more or less powerful adhesion or contraction of these particles". And ".... in proportion as the form of the vessel departs from that of a sphere, the blood is subjected to a more extensive contact with dead matter". The same observations are mentioned in the German literature of the time (Handwörterbuch der Physiologie, 1832).

In the second edition of Thackrah's Treatise on the blood (1834) another cause of error is given. Due to the different "electric states" of different metals, contraction of the blood clot is dependent on the composition of the vessel in which it takes place. The same blood expressed 54 per cent of its serum when kept in a pewter vessel, against only 35 per cent in a copper vessel. Thackrah therefore advocates the use of glazed earthenware or glass vessels only.

Hayem's school, from which numerous works on retraction were published during the 20 years following Hayem's first paper in 1878, insisted for rather obscure reasons on the use

of capillary blood. One of his pupils, Lenoble (1898), maintained that using a syringe and venous blood might completely mask clot retraction. Another pupil, Bensaude(1904), in reply to a publication by Grenet (1903), again insisted on the use of finger blood without any real experimental backing for this contention. Grenet had observed that capillary blood run into 3 similar test-tubes, cleaned under the same conditions, showed normal retraction in the one tube, but none in the other two, and suggested that the quality of the glass might explain this difference, and also that venous blood might give more reliable results than capillary. Bensaude's reply was curt and blunt: capillary blood must be used. One is left with the impression that the Master's word is beyond petty criticism.

Le Sourd and Pagniez (1907) repeatedly pointed out the importance, in their opinion, of flaming the tubes before use. The simple procedure of washing the tubes in distilled water after flaming was sufficient to impair the retraction.

In the following year Arthus and Chapiro (1908) made the observation that if the tubes were coated with paraffin wax the retraction was inhibited. In only one of many tubes did they obtain any serum from normal blood.

Entering the French discussion on the question of venous versus capillary blood, Émile-Weil (1920) observed that "complete non-retraction is demonstrable equally well with venous blood and with blood from the finger; diminished retraction is only clearly seen in peripheral blood, venous

blood just showing less than normal contraction; if there is only slight diminution in the retraction, finger blood may exude a few drops of serum from its clot".

During a series of immunization experiments against diphtheria in children Opitz and Matzdorf (1921) noticed that about 50 per cent of their blood specimens in small tubes showed no retraction in spite of the platelet number being between 139.000 and 445.000 per cmm. These tubes were of about the same size as those used in Glanzmann's method for clot retraction estimation, and they found that blood from the same child would at one time retract and on another occasion not at all. This apparent change in the blood bore no relationship to the number of platelets, the time of day, or the time after a meal. They considered the possibility that roughness of the glass surface might account for the irregularity, but the use of new smooth tubes did not improve the results. For this reason they introduced the use of watchglasses in which retration was more constant.

By a very similar observation Gordon (1926) came to the conclusion that only slight importance should be attached to the clinical estimation of clot retraction. Over a long period he paid particular attention to the phenomenon in blood specimens sent to his laboratory for agglutination tests. In 40 per cent of cases no retraction had taken place in spite of the fact that signs of purpura were absent in all the patients.

Fuchs (1931) reinvestigated the influence of the glass surface on the retraction of the clot. Tubes treated in different ways gave different results as shown in figure 10.

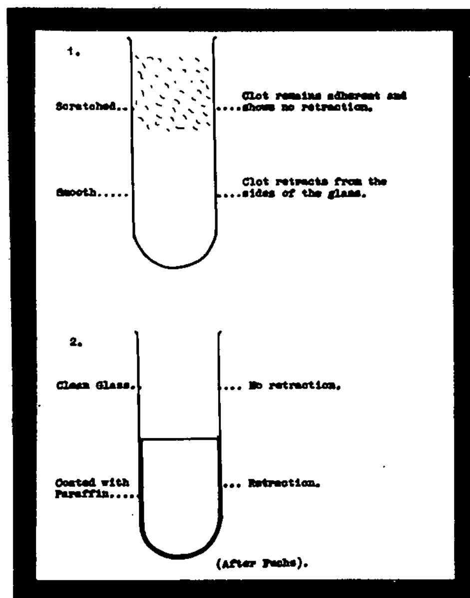


Figure 10.

By applying a weight to the surface of the clot Brocq-Rousseau and Roussel(1929) increased the yield of serum from 26.55 to 54,33 per cent. By loosening the clot at different intervals after clotting Czoniczer and Weber (1931) could change the subsequent retraction, but they nevertheless considered those methods in which no loosening was done as "wholly illusionary". Even in platelet-free plasma their manipulation produced a retraction of 10 to 30 per cent. Macfarlane (1938) found that the retraction as estimated by the "mercury retractometer" was about 15 per cent greater than by the simple graduated test-tube method, and he suggested that the loosening of the clot in the first method was the reason for this difference.

A further investigation of the influence of the glass surface was made by Ornstein (1932). He estimated the degree of retraction in differently treated tubes:- dirty tubes, tubes rinsed in water, washed in soap water, cleaned with concentrated acids, or sterilized at temperatures up to 120°C; all gave the same results; but if the tubes were sterilized

at temperatures between 180 and 200°C. no spontaneous retraction took place. He suggested that the heating changed the glass surface from a crystalline to a colloid state, and as the adhesion between colloid and colloid was greater than between crystal and colloid, the clot remained adherent. The same is seen in tubes coated with collodion in which the clot also adheres firmly to the walls.(Hirschboeck (1940).

Lampert (1932) found that the amount of serum expressed in tubes of amber, gold or silver was small compared to that in glass or quartz tubes. If adhesion was prevented by loosening the clot or by paraffin or other oil all containers gave the same results.

Émile-Weil and Perlès(1934) compared the results obtained by using wide- and narrow-bore tubes. In normal cases the retraction was much greater in the wide-bore tubes. In bloods with impaired retraction, as in purpura or hepatic cases, they found some retraction in the large tubes, but none in 18 out of 23 cases in the small tubes, the results in these being completely erratic. They concluded that non-retraction as observed in small tubes in haemorrhagic cases was simply an "artifice de préparation". Tocantins (1934) expressed the same observation in different words: the amount of retraction depends on the area of clot surface touching glass.

Summarising this survey of the literature the impression is therefore gained that clot retraction is influenced by the shape, size and internal surface of the vessel in which it takes place. This is not surprising as, after all, the clot to

act effectively in haemostasis must adhere firmly to the damaged vessel. It will be shown elsewhere that the force of contraction is extremely small, and any adhesion to a firm surface will therefore considerably impair retraction, if not completely inhibit it. The investigations bearing on these external factors will now be described in detail.

The influence of the shape, size and internal surface of the container on retraction.

Blood from one donor was distributed into a series of different containers, treated in different ways. The containers were placed in an incubator at 37°C. for 24 hours after which the amounts of serum expressed, including red cells, were estimated by carefully pouring them into graduated test-tubes. The results are tabulated in table 2 (Experiment 1).

Although the results are extremely erratic and at variance with some of the previously published findings, and would be even more unreliable with blood of less vigorous retraction, it does not necessarily mean that every method using glass vessels is useless in obtaining comparable results. Czoniczer and Weber (1931) found the standard error of their method to be $\pm$ 5 per cent, and Macfarlane (1938) by painstaking attention to detail of cleaning etc. was able to reduce the error of his simple graduated test-tube method to $\pm$3.5 per cent. These are the only statistical estimations of error published.

Type of container.	Treatment of container.	Internal Diameter	Blood ccs.	Clot retraction. (% Serum exuded)
Glass tube	Routine laboratory cleaning	40 mm	20	65
Test-tube	Routine laboratory cleaning	14 mm	5	36
Test-tube	Heated to red heat and cooled	14 mm	5	64
Test-tube	Coated with collodion	14 mm	5	38
Test-tube	Coated with paraffin wax	14 mm	5	64
Centrifuge tube	Routine laboratory cleaning	14 to 6 mm	5	48
Test-tube	Routine laboratory cleaning	9 mm	2 2	50 60
Test-tube	Boiled in HNO_3 then in distilled Water	9 mm	2 2	60 73
Test-tube	Routine cleaning. Clot loosened 10 minutes after clotting.	9 mm	2 2	83 79
Test-tube	Coated with liquid paraffin	9 mm	2 2	75 74
Watch-glass	Routine laboratory cleaning	70 mm	3	50
Watch-glass	Coated with liquid paraffin	70 mm	3	66
Aluminium tube	Routine laboratory cleaning	16 mm	5	60

Table 2. The influence of the shape, size and internal surface of the container on clot retraction.

The effect of gravity on clot retraction.

This effect has never been considered and this omission explains many of the controversies existing with regard to the whole problem, such as the apparent contraction observed in platelet-free plasma and the enhancing effect on retraction of the simple procedure of loosening the clot.

The blood clot consists of a three-dimensional network of fibrin threads. The branching and fusing of the fibrin fibres may be seen under the ordinary microscope (figure 11), but is particularly well demonstrated by the electron microscope (Wolpers and Ruska, 1936, Scmitt, 1945, Hahn, v.Zandt and Porter, 1947). .

Figure 11. Fibrin clot showing branching and fusing of the fibres

When the clot forms under physiological conditions the individual fibres are from 0 to 3 μ thick, and the mesh width from 8 to 12 μ. If the fibrinogem concentration of the plasma is low the fibres are thinner and the meshes become very wide (Bucher, 1936 and 1943).

The serum is held in this network by capillary forces. When the serum artificially is pressed out of the meshes the network collapses and does not again expand when immerged in serum, due to adhesion between the individual fibres. This can be seen by manipulation of the network under the microscope.

The capillary force holding the serum in the clot might conveniently be expressed by this formula:-

$$\text{Force} = \frac{2\,\gamma \cos\theta}{d\; g\; r}$$

where γ = the surface tension of the serum.
θ = the angle of contact of the serum with the fibrin which is an expression of the wettability of fibrin.
d = the density of the serum.
g = the force of gravity.
r = the width of the mesh.

which is simply the formula for any fluid in any capillary tube. If gravity exerts its full force on the clot and the width of the meshes is very wide, the force holding the serum inside the clot will be small and the serum will tent to run out of the clot. As long as the clot is completely adherent to the wall of the vessel in which it was formed this escape of serum will not be seen, but as soon as the clot is loosened from the sides the serum will pour out and an artificial retraction will be observed. The pressure during the loosening will in itself squeeze out some serum, however carefully it be done.

A demonstration of these features is given in figures 12 A and B.

Figure 12 A shows a platelet-free clot on which the force of gravity has been neutralized by keeping it floating in an inert oil for 24 hours during which time it showed no signs of contraction. It was lifted out of the oil and photographed immediately afterwards. The test-tube in this picture contains a similar platelet-free plasma clot which was left undisturbed and adherent to the glass wall for 24 hours. Directly after the photograph was taken this

Figure 12 A.

clot was carefully loosened from the sides of the tube with a fine glass rod.

Figure 12 B shows the appearance of the clots 20 minutes later. Most of the serum has run out of the "suspended" clot which has flattened to less than half its original size. Immediately before this photograph was taken the exuded serum in the tube was poured into another graduated test-tube, and as is clearly demonstrated in the picture, this clot has "retracted" to about 5o per cent of its original size.

Figure 12 B.
(For explanation see text).

It will be shown in Chapter 2 that temperature has a profound influence on true clot retraction, and further to demonstrate the difference between the artificial loss of serum due to gravity and actual clot retraction

Two tubes with platelet-free clots and low fibrin contents of 81 mgs.per 100 ccs and 162 mgs.per 100 ccs respectively were placed in the refrigerator at 4°C; two similar tubes were kept at 37°C in the incubator. After one hour the clots were carefully loosened and 5 minutes later the serum run out was estimated. As controls clots were suspended in inert oil as will be explained in the following pages. The results are shown in table 3. (Experiment 2).

Type of clot.	Tempe-rature. °C.	Fibrin content. mgs.%	Serum exuded. %
Platelet-containing, suspended	4	324	28
Platelet-containing, suspended	37	324	72
Platelet-free, suspended	37	81	0
Platelet-free, in test-tube	4	162	48
Platelet-free, in test-tube	37	162	52
Platelet-free, in test-tube	4	81	66
Platelet-free, in test-tube	37	81	70

Table 3. The independence of temperature of the artificial "retraction" produced by gravity.

The running out of serum due to the force of gravity is thus independent of the temperature, and its degree is determined mainly by the amount of fibrinogen in the plasma which again controls the width of the meshes of the clot, or r in the formula. If the fibrin content is high, all the serum may be held in the clot even after the disturbance of loosening the clot. Thus a fibrinogen solution of approximately 1000 mgs. per 100 ccs was clotted with thrombin and the clot placed on a glass surface; no serum ran out and the shrinkage of the clot after many hours was entirely due to evaporation (Experiment 3).

To obtain some idea of the wettability of fibrin the inside of a fine-bore glass pipette was coated with a thin layer of fibrin, and the height to which distilled water rose in it measured 11 mms. Repeating the experiment with the tube chemically clean the water now rose to 28 mms. (Experiment 4) Although not a very satisfactory procedure, the experiment does show that the wettability of fibrin is much less than that of glass, which would be expected as otherwise the force necessary to press fluid out of a clot would be enormous.

The size of the meshes in the fibrin network, and therefore the clot retraction, can be altered by less physiological procedures than the simple increase in fibrinogen concentration. Ferry and Morrison (1947) investigated the clotting time, opacity, rigidity, friability and syneresis of fibrin formed under different conditions of hydrogen ion concentration and concentrations of other ions, and under the influence of certain polyhydroxyl compounds.

They estimated the "syneresis" by placing the clot on blotting paper, and remarked that the syneresis was not the same as true spontaneous retraction. The degree of this syneresis depended on whether the clots were fine or coarse. Their experiments were so removed from physiological conditions that further discussion of their results is unwarranted in this work, but they are further confirmation of the observations just described.

It must therefore be accepted that whenever a clot rests on a firm surface, whether it be inside or outside a tube, an artificial seperation of serum may take place which may simulate true clot retraction closely.

Fibrinolysis and putrefaction.

Both these factors will by their destruction of the fibrin network allow the escape of serum from the clot according to the previous discussion. Fibrinolysis is so closely bound up with the history of clot retraction that a special chapter will be devoted to this subject at a later stage. The same applies to the frequent comparison between clot retraction and the syneresis of gels, which will be shown to be entirely different processes.

The conclusion to be drawn from this review of the literature and the recorded experiments is, that the ideal method for the estimation of clot retraction would be one that:

1. Presents an identical surface to the clot each time the

test is carried out, or even better, prevents the clot from making contact with any solid surface whatever.

2. Excludes air contact and other external influences on the clot, and prevents evaporation.

3. Always estimates the retraction in clots of the same size and shape.

4. Prevents any touching or disturbance of the clot during the contraction.

5. Counteracts the influence of gravity.

6. In an easy manner controls the temperature.

7. Inhibits bacterial growth and thus prevents putrefaction.

8. Gives a quantitative as well as qualitative estimation of both the time and the amount of clot retraction.

The "Suspended clot method" employed in this work fulfils most of these criteria, and it has the further advantage of being so simple to perform that it can be carried out as a routine method without special apparatus, cleaning or other precautions.

THE SUSPENDED CLOT METHOD.x)

(Figures 13, 14 and 15.)

Materials employed in the method:

1. Suspension Fluid. Liquid paraffin (specific gravity 0.9) and trichlorethylene (specific gravity 1.47) are mixed in

x) The method was demonstrated at the University of Cape Town in May,1946, and at the International Physiology Conference at Oxford in July,1947, but as it is really an extension of the procedures of Van Allen(1927) and Roskam(1927) no special priority is claimed.

such proportions that the specific gravity is that of blood (normal average: 1.052 to 1.063). The specific gravity need not be very accurate and may be measured with an ordinary urometer; in cases of anaemia it must be lowered by addition of liquid paraffin; in polycythaemia trichlorethylene is added. If it is remembered that the specific gravity of plasma is about 1.030 no difficulty is encountered in roughly estimating it in any particular blood, and adjusting the suspension fluid accordingly.

These substances are physiologically inert as far as coagulation is concerned. If blood is collected without liberation of thromboplastin, as is possible by coating needle and syringe with silicone (Jaques, Fidlar, Feldsted and Macdonald,1946), it does not coagulate when suspended in a mixture of the two. This is not due to an anti-coagulant action as blood collected by ordinary syringe clots in the normal time on suspension.

Other inert fluids such as benzyl benzoate and olive or peanut oil may be used. Their only advantage is in experiments concerned with the expression of red cells from the clot as these tend to lyse after long periods in the fat-solvent trichlorethylene; otherwise they are less satisfactory as the separation of the serum from them is difficult.

2. Container for the suspension fluid.

Any beaker will serve this purpose. The taller it is, the less important is the specific gravity of the suspension fluid.

3. Five ccs.pipette to run the blood into the suspension fluid.

4. Pasteur pipette to suck off the serum squeezed out.

5. Graduated test-tube to measure the amount of serum.

6. Spoon to lift the contracted clot out of the fluid.

7. Dish with ether to wash the contracted clot.

8. Twenty-five ccs. Measuring cylinder to estimate the volume of the clot by the volume of water it displaces.

9. Water-bath or Incubator to control the temperature.

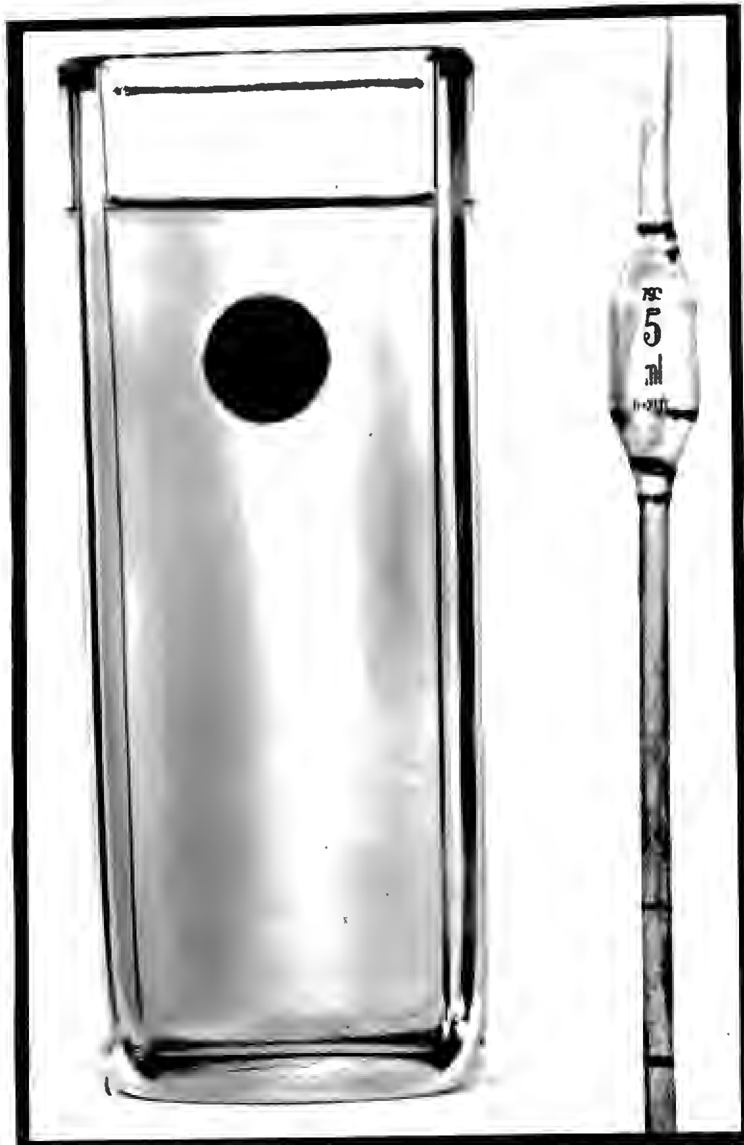


Figure 13. Five ccs. of blood suspended in the Trichlorethylene-paraffin mixture.

The test is carried out in the following way:- Collect venous blood and run 5 ccs. into the suspension fluid with the pipette. The high viscocity of the fluid makes this procedure easy. The blood forms a suspended sphere in the medium. (Figure 13).

The contraction of the clot formed is seen to occur as a

continuation of the coagulation. A small bubble of serum

Fig. 14. The clot is contracting and bubbles of serum rise to the surface.

is squeezed out of the sphere, usually on its upper surface. In normal blood, red cells are invariably expressed with the serum. When the bubble reaches a certain size it separates from the clot and rises to the surface of the medium due to its lower specific gravity. As the contraction proceeds serum collects in the upper part of the suspension fluid, whilst the clot sinks lower.(Figure 14).

At any desired time the amount of serum expressed may be estimated by pipetting it off with the Pasteur pipette and measuring it in the graduated test-tube. If some of the suspension fluid comes off at the same time addition of a little ether and shaking will separate the two without measurable loss of serum volume.

After the completed contraction of the clot, easily determined as no new bubbles of serum form, all the serum is pipetted off and measured; the clot is then lifted out of the suspension fluid with the spoon, washed quickly in the porcelain dish with ether, and its volume is determined by dropping it into the measuring cylinder containing a

a known quantity of water. This further check on the result enhances the quantitative value of the test, as the necessity of running exactly 5 ccs. of whole blood in at the beginning is obviated. (Figure 15).

Figure 15. The test is completed. 1.7 ccs. of serum including red cells have been expressed during the contraction of the clot formed from 5 ccs. of blood.

During the whole process the beaker in which the clot is suspended is kept at 37°C. in the water-bath or incubator.

The result is expressed as the percentage of serum of the original volume of blood, e.g. if 5 ccs of blood are run into the suspension fluid, 2 ccs. of serum pipetted off after complete contraction, and the clot volume is 3 ccs., then the clot retraction is 40 per cent.

Exudation of red cells always occurs in normal clot retraction and appears to be an integral part of the process.

The separate estimation of the red cells expressed would therefore introduce an artificial factor in the reading of the result. This point will be discussed in more detail in Chapter 3.

To determine the inherent variability of the suspended clot method 10 clots from the same sample of blood were put up (Experiment 5). Eight of these gave a clot retraction of 44 per cent, one of 45.1 per cent, and one of 43 per cent. The error is therefore statistically negligible and was in this experiment found to be due to faults in the graduations of the graduated test-tubes.

The method is unsuitable for the estimation of the coagulation time for two reasons: firstly, the extent of contact with foreign surfaces before suspension in the medium greatly influences the time. If, for example, after a venipuncture with little admixture of tissue fluid the blood is run directly from the syringe into the medium, the first signs of coagulation may only be seen in 15 to 20 minutes, whereas the same blood collected from the syringe in a test-tube and run into the medium with the pipette will show signs of coagulation in 4 to 5 minutes. It is not possible to control these external factors. Secondly, the coagulation is a markedly prolonged process; it begins at the outside of the suspended sphere as the formation of a thin skin and slowly progresses inwards through the clot. The exact times of onset and completion cannot be accurately determined.

These external factors also influence the speed of clot

retraction. In a case with thrombosis of the femoral vein one sample of blood was run directly into the suspension fluid from the syringe and showed the first bubble of serum 24 minutes after the blood was taken. Another sample was run from the syringe into a porcelain dish and then with the pipette into the medium; in this case contraction began 14 minutes after the blood was taken and proceeded much more rapidly. But the final clot retraction in both samples was the same (Experiment 6).

This experiment offers a serious criticism to the retraction time test of Katrakis (1931) and the coagulation-retraction tests of Hirschboeck and Coffey (1942,1943, 1947 and 1948). The latest improvement of Hirschboeck's by dropping finger blood into castor oil hardly overcomes the difficulty as variable amounts of tissue juice must be present in these drops. As far as the suspended clot method is concerned it was apparent from the beginning that the estimation of the time of onset of retraction was unreliable and showed great variations in the same samples of blood. Little attention has therefore been paid to this aspect of the problem, but it may be stated that in normal blood the usual time of onset is 20 to 30 minutes after taking the blood, and that the retraction is completed in 2 to 3 hours. This only applies if the temperature is kept at 37°C. during the whole test.

CHAPTER 2.

THE INFLUENCE OF TEMPERATURE ON CLOT RETRACTION.

The effect of the temperature on clot retraction has long been established. Hewson (1772) showed experimentally that both coagulation and retraction were dependent on the temperature at which they took place. He considered body temperature the optimum for both, but Thackrah (1819) found that retraction was increased in speed and degree, in "a regular gradation", up to 120°F. Above this temperature the process was inhibited.

Hayem's school, although they knew that temperature to some extent controlled retraction, paid peculiarly little attention to this feature. In Lenoble's (1898) work on the semeiological characters of the serum and the clot, in which he attached an unreasonable diagnostic and prognostic importance to this form of examination, no attempts were made to control the temperature in his numerous tests.

Comparing the retraction of blood clots and coagulated milk Arthus (1893) pointed out that both contracted more or less rapidly according to the temperature. Later Arthus and

Chapiro(1908), in an attempt to prove that retraction was due to living platelets, strangely enough used the influence of temperature as a strong argument in favour of this theory.

Morawitz and Bierich (1907) included control of the temperature in their method for estimation of clot retraction, but the first convincingly quantitative demonstration of the relationship between the two was given by Cesana (1909). Retraction was maximal at 37°C., slower and less at 15°C., and absent at 4°C.

In the final review of their prolonged work on many aspects of retraction Le Sourd and Pagniez (1913) stated that the optimum temperature was 37°C. At 0°C. no retraction occured, but when the clots were later brought from 0°C to a higher temperature contraction again took place.

Van Allen (1927) surprisingly found that only the speed of retraction depended on the temperature. It should be remembered, however, that in most of his tests he centrifuged the serum away from the clot, thus producing a gross error. He maintained that at low temperature the rate was slow; few red cells but plenty of serum was squeezed out. At higher temperatures, with correspondingly faster speed of contraction many red cells and less serum was expressed; the final size of the clot was the same at different temperatures.

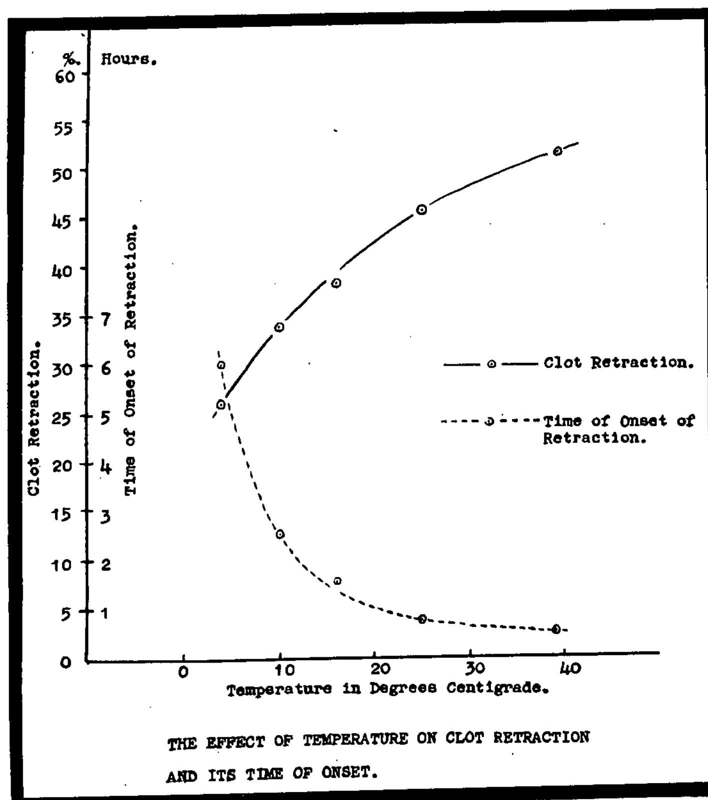
The test of Katrakis (1931) was carried out in the ice-chest, but as he does not give any figures, the results of this unusual method cannot be judged.

Lampert and Ott (1934) to a certain extent confirmed the findings of Van Allen. In ox-blood the speed of

contraction ran parallel to the temperature, but the final extent of contraction was independent of the temperature if the clots were kept for 4 days. No special precaution was taken in their experiments to guard against bacterial growth and the likely explanation of their observation is, that the clots were simply destroyed during this long period.

Macfarlane (1938) found both speed and extent of retraction dependent on the temperature, and subsequent work has been in agreement with his findings (Tamura, 1940, Lundsteen, 1942, Zahn, 1944).

With the suspended clot method a definite relationship between clot retraction and temperature can be established (Experiment 7). The correlation is shown in figure 16.



THE EFFECT OF TEMPERATURE ON CLOT RETRACTION AND ITS TIME OF ONSET.

Figure 16.

It was found in the same experiment that alterations in the temperature during the process affected the total retraction. A clot which had been kept at 39°C. for 40 minutes and at that time had expressed 32 per cent serum was transferred to 4°C; after 4 days its retraction was only

41 per cent against 52 per cent in the control kept at 39°C. the whole time. Conversely, another clot from the same blood kept at 4°C. for 6 hours and expressing 6 per cent serum during that period, gave a retraction of 40 per cent in 4 days against 26 per cent in the control kept all the time at 4°C.

Exposure of the blood to different temperatures for a short time before clotting, a feature of some importance in both clinical and experimental work, does not affect the subsequent retraction. In experiment 8 the blood was subjected to varying temperatures for 10 minutes before coagulation, this being possible by only using silicone covered glass ware during the experiment; The amount of retraction was the same in all the clots.

Higher temperatures has an inhibitory effect on retraction. Le Sourd and Pagniez (1906) found that 46°C. prevented the process. Macfarlane (1938) observed that 45°C. diminished the degree of retraction, and this effect occured both if the temperature was applied during the actual retraction, or for some time before recalcification of citrated blood, even if the subsequent coagulation and retraction took place at 37°C. Zahn (1944) obtained slight retraction at 50°C. The question is closely bound up with the platelet problem, and will be discussed later with appropriate experimental data.

The conclusion is, to repeat the neglected statement of Le Sourd and Pagniez (1913), that unless the temperature is

carefully controlled, nobody is authorized to speak of retraction or non-retraction. The most natural temperature to employ is $37^{\circ}C.$, and this has been done throughout this work.

CHAPTER 3.

THE INFLUENCE OF THE VOLUME OF RED AND WHITE BLOOD CORPUSCLES ON CLOT RETRACTION.

Retraction is generally inversely proportional to the mass of cells in the blood. This conclusion was reached by Hayem and his school (1896), but they did not experimentally show the quantitive relationship between retraction and cell volume.

Opitz and Schober (1923) confirmed the observation and explained how the red cells are caught in the fibrin network, thus impeding the squeezing out of the serum when the clot contracts.

Van Allen (1927) defined clot retraction in two ways:-

(a) The extent of retraction = $\dfrac{\text{(Serum exuded)} - \text{(Volume of red cells Expressed)}}{\text{(Total volume of blood)} - \text{(Haematocrit)}}$

(b) The rate of retraction = $\dfrac{\text{(Red cells expressed)}}{\text{(Haematocrit)}}$

as he believed that the faster the retraction the more red

cells came out of the clot. This feature had actually been suggested earlier by Schklarewsky (1868) who observed the squeezing out of white and red cells from the clot under the microscope; Pagniez (1922) also put forward similar views to Van Allen, but exactly the opposite observation was made by Tezner (1929). He slowed down the retraction artificially by adding excess of calcium and found greater expulsion of red cells in the slowly contracting clots. Glanzmann (1918) considered the exudation of many red cells as the earliest sign of poor retraction.

Czoniczer and Weber (1931), Tamura (1940), and Lundsteen (1943) tried to overcome this variable factor of the cells by estimating the retraction on plasma obtained either by short spinning or sedimentation of the whole blood. This procedure in no way overcomes the difficulty, as microscopic examination of the deposit of red cells shows an inconsistent number of platelets, depending on the sedimentation rate of the blood and the prevention or non-prevention of agglutination of the platelets. A great proportion will also be lost by adhesion to the glass wall.

A correction for anaemia was given by Boyce and McFetridge (1939) which for its application assumes that the normal red cell count is 5 million/cu.mm, and the normal haematocrit 50 per cent; no data are given to explain how the formula is derived, and it is unnecessarily involved without being

accurate.

Macfarlane (1938) found a roughly linear relationship between the haematocrit and clot retraction. An increase in the volume of cells decreased the retraction by about $\frac{3}{4}$ of the volume added. He suggests as a suitable, although not completely accurate correction for anaemia :-

$$\frac{(\text{Percentage of serum expressed}) + (\text{Haematocrit})}{2}$$

which is the same as the Clot retraction Index of Mettier and Witts. Macfarlane found, however, that this relationship only held good in blood with normal retraction. If the retraction of the plasma was deficient it was not affected by the addition of cells until the volume of cells added was greater than the volume of cell-free retracted clot. The explanation given for this phenomenon is, that if retraction of plasma is much reduced the amount of fluid retained in the clot is great. If cells were added to this clot no effect would be produced, because the volume of cells added will not occupy the whole volume of the non-retracted clot. But the diminished volume of plasma available for contraction with increasing volume of red cells is not considered in this explanation.

Macfarlane's tables show, contrary to the views of Van Allen, that the amount of red cells expelled with the serum depends on the volume of red cells in the blood.

Aggeler and Lucia and Hamlin (1942) suggest a more

complicated correction for anaemia:-

$$\frac{(\text{Total volume of blood}) - (\text{Serum} - \text{r.b.c.'s exuded})}{(\text{Total volume of blood})} \times 100 - (\text{Haematocrit})$$

This is the "extracorpuscular or fluid volume of the clot". The formula was not supported by experimental data. If it is applied to actual estimations, varying the volume of red cells in one plasma and using their modified Macfarlane method, results like these are obtained (Experiment 9):-

Haematocrit. %	Fluid volume index.	Macfarlane's formula as comparison. $\frac{(\text{Haematocrit})+(\text{Retraction})}{2}$
0	13	43.5
15	10	45.0
25	9	45.5
50	5	47.5
60	15	42.5

Table 4. Calculation of the "fluid volume of the Clot" by the formula of Aggeler, Lucia and Hamlin.

The index of the "fluid volume of the clot" thus introduces an undesirable error, high in proportion to the numerical value of the index, without giving more information than the much simpler suggestion of Macfarlane.

With the suspended clot method, the results obtained by artificially varying the volume of red cells in one plasma are shown in figure 17 (Experiment 10). In this type of experiment it is essential that the red cells added to the plasma are completely free of platelets. This precaution has not been observed by other workers and explains much of the disagreement.

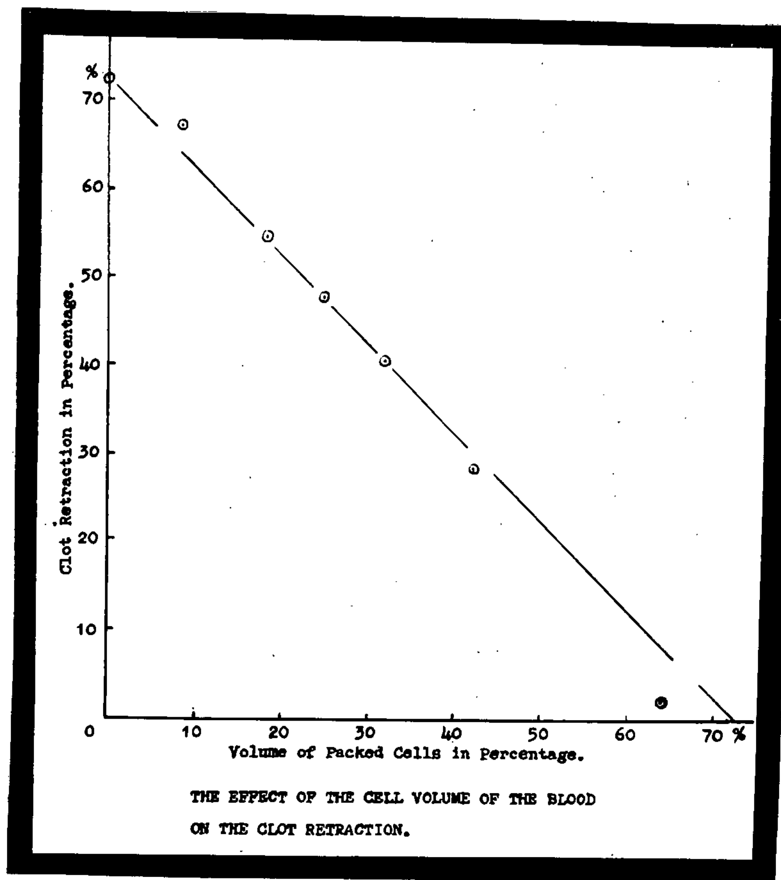


Figure 17.

The curve in Figure 17 demonstrates that within the range of haematocrits usuallly met with clinically, in the curve from 18 to 42 per cent, a rise in the volume of red cells produces a drop in the clot retraction of the same amount:-

Haematocrit. %	Observed retraction. %	Fall in clot retraction %
0	72.5	0
18	53.6	18.9
25	47.9	24.6
32	40.0	32.5
42	28.0	44.5

Table 5. The fall in clot retraction as the haematocrit rises from 0 to 42 per cent.

At very low haematocrits the decrease in clot retraction is less; thus a volume of packed cells of 8 per cent reduced the

retraction by only 5.9 per cent, in spite of the fact that no red cells were expressed with the serum. At high haematocrits the opposite holds: a volume of packed cells of 64 per cent reduced the retraction by 70.5 per cent, although a high extrusion of red dells with the serum took place.

In blood of abnormally low retraction the inhibition produced by the red cells is slightly less, as seen in figure 18. (Experiment 11).

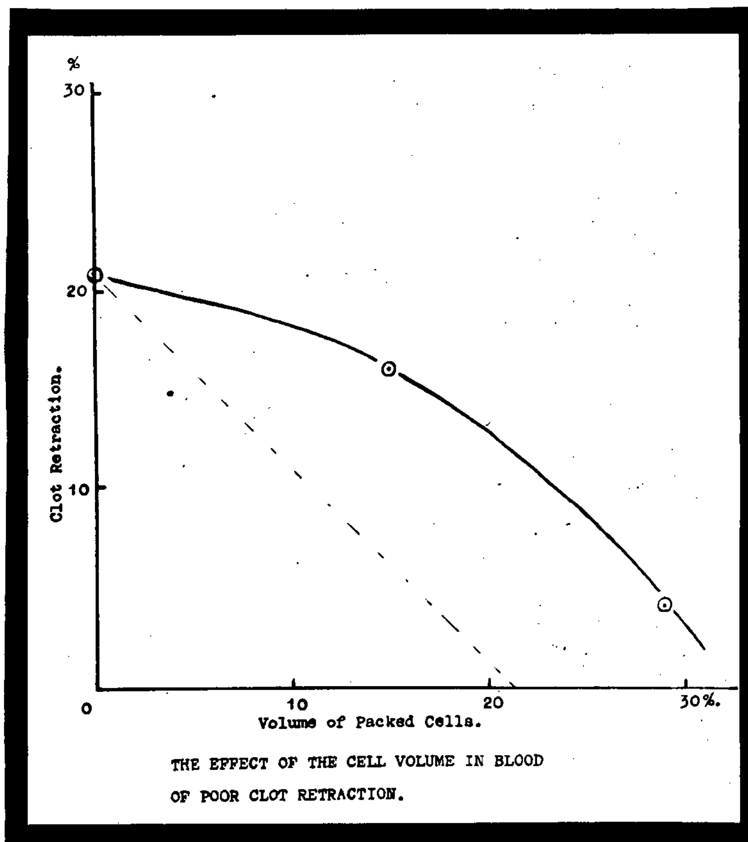
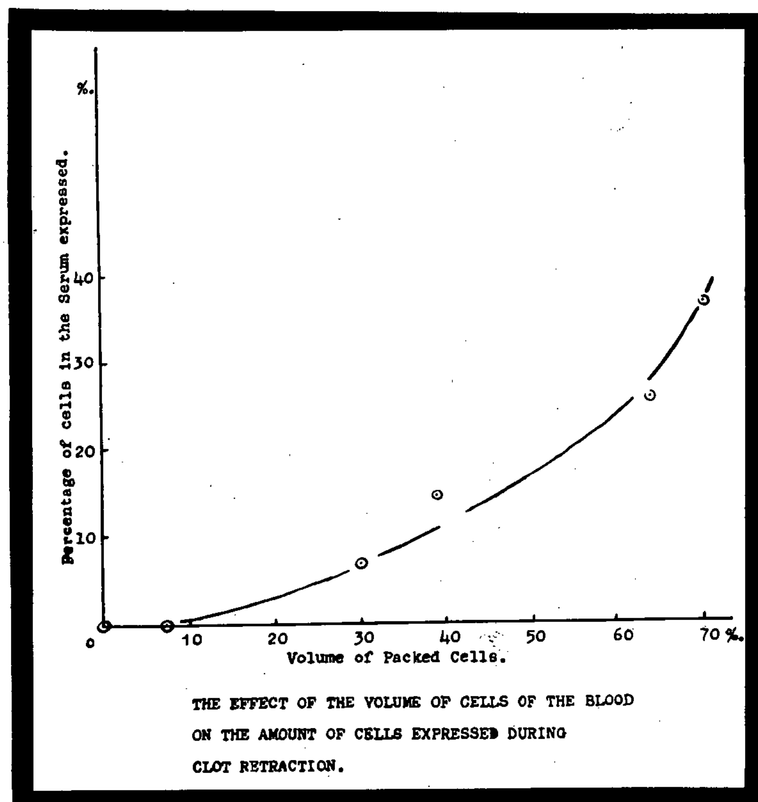


Figure 18.

In this case a haematocrit of 15 percent caused a fall in retraction of only 4 per cent, and 29 per cent haematocrit brought the retraction down by 19 per cent.

The volume of cells expressed with the serum, when estimated by the suspended clot method, depends on the



THE EFFECT OF THE VOLUME OF CELLS OF THE BLOOD ON THE AMOUNT OF CELLS EXPRESSED DURING CLOT RETRACTION.

Figure 19.

volume of cells in the blood and on the total clot retraction. (Figure 19, Table 6, Experimment 12).

Haematocrit %	Retraction. %	Cells exuded as % of total volume of blood. %	Cells exuded as % of total fluid squeezed out. %
0	86	0	0
7	82	0	0
30	60	4	6.7
39	50	7	14
64	18	4.6	25.6
70	8	3	37.5

Table 6. The amount of red cells expressed with the serum with increasing haematocrit.

In the previous discussion the stress has been laid on the red cells, but as might be expected the white cells produce the same effect (Table 7, Experiment 13).

Haematocrit, i.e. the volume of packed white cells. %	Clot retraction, i.e. Serum + Leucocytes exuded, as percentage of total volume of blood. %
0	74
21	60
43	33

Table 7. The effect of the volume of leucocytes on the clot retraction.

The most logical explanations of this effect of the cells on clot retraction appear to be the following:-

(a). The red and white cells do not actively influence the retraction of the clot, but are present as inert bodies suspended in the serum. The large majority are held back in the fine meshes of the fibrin network when contraction takes place.

(b). The most marked effect of this mass of inert cells will be to diminish the percentage of plasma, including platelets, available for contraction. This effect is illustrated in figure 20, where for convenience sake clots of 100 ccs. are considered.

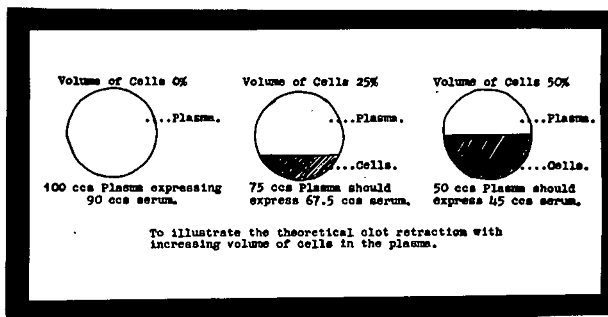


Figure 20.

If, however, this theoretical diminution of clot retraction with rising volume of cells is plotted on the graph showing the actual diminution taking place, it is seen that the inhibition is much greater than expected, and that the difference between the two curves increases with rising volume of cells. (Figure 21)

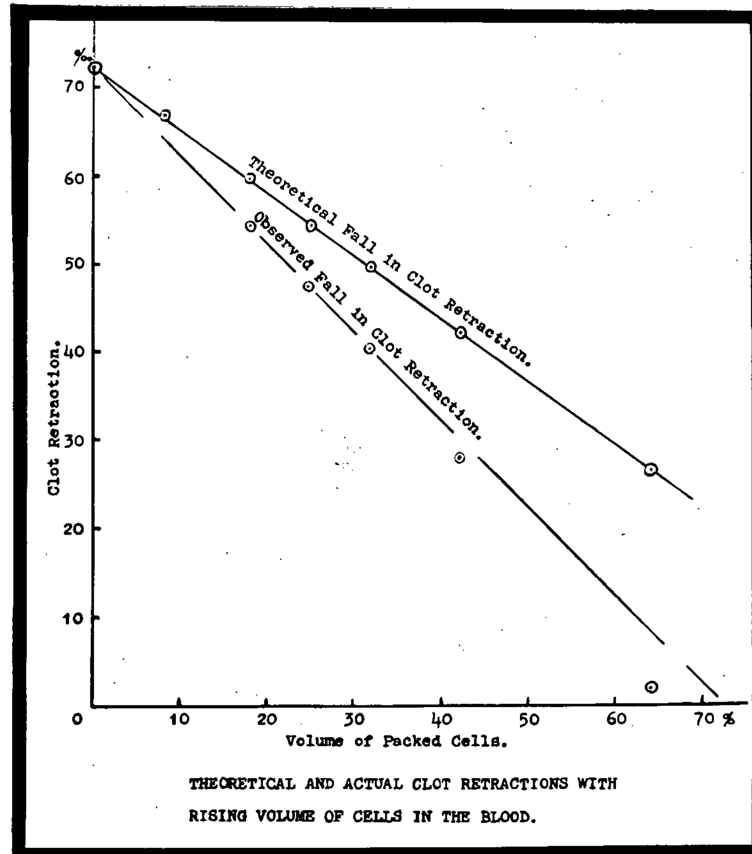


Figure 21.

(c). This discrepancy may be explained by the increasing viscosity of the blood with rising haematocrit. The force necessary to squeeze a fluid out of a sponge or capillary network in a given time depends partly on the viscosity of the fluid. It is interesting to note from the curve that in the range of haematocrit from 18 to 42 per cent the

correlation between retraction and volume of packed cells is linear. This would correspond with Nygaard, Wilder and Berkson's (1935) finding of a linear relationship between the viscosity of the blood and the volume of packed cells in the range of haematocrits from 15 to 50 per cent.

(d). Lastly the issue is complicated by the amount of cells squeezed out with the serum which to a slight extent counteracts the two previous inhibitory influences.

In the case of blood with a very fast sedimentation rate the red cells may settle to a great extent before coagulation takes place in the suspension mixture. In such cases, as the upper part of the clot is free of cells, the retraction will proceed rapidly to begin with, but due to the inhibited contraction in the lower part of the clot the total retraction remains unaltered. In blood from a case of Hodgkin's disease with an extremely fast sedimentation rate, the sedimentation was varied by letting the blood clot at different speeds in the suspension mixture. The subsequent retraction of the clot was not significantly altered. (Experiment 14). Aggeler, Howard and Lucia (1946) suggested that some cases of diminished clot retraction with fast sedimentation rate or slow coagulation were due to the large amount of fluid occluded within the part of the clot free of cells. Their argument is difficult to follow and not consistent with experimental observation. The diminished clot retraction in such cases as myelomatosis or liver disease with

a fast sedimentation rate is due to the increased fibrinogen concentration in these patients' blood as will be shown later.

The increased speed at the onset of the retraction in the cases of increased sedimentation rate, due to the cell-free part of the clot, also explains Hirschboeck and Coffey's (1943) finding that the coagulation-retraction time was to some extent porportional to the sedimentation rate. It introduces another uncontrollable factor into their test which, however, does not completely invalidate its use. It might be mentioned that Van Allen (1927) found high sedimentation rates associated with slow clot formation.

It is understandable that the complex nature of the influence of the cells on clot retraction must make the construction of a formula for its correction a mathematical problem of some magnitude. As, in practice, the estimation of the factors involved are not of mathematical precision no attempt at this formulation has been made in this work.

It appears most logical to bring the clot retraction for any particular blood back to the degree of contracting that would occur if no erythrocytes and leucocytes were present in the plasma. For practical purposes this may be done simply by adding the volume of packed cells to the clot retraction. In the experiment which has been used as illustration in this discussion the addition gives the following results:-(table 8)

Haematocrit %	Clot Retraction %	Haematocrit + Retraction. %
0	72.5	72.5
8	66.6	74.6
18	53.6	71.6
25	47.9	72.9
32	40.8	72.5
42	28.0	70.0
64	2.0	66.0

Table 8. The simple correction of the clot retraction by addition of the observed retraction and the volume of packed cells.

It should then be remembered that the error introduced is great in blood with poor retraction.Applying the results of experiment 11 to this formula the following correction is obtained:(Table 9),

Haematocrit %	Clot Retraction %	Haematocrit + Retraction. %
0	20.8	20.8
15	16.0	31.0
29	4.0	33.0

Table 9. Correction for blood with poor retraction.

and this experiment shows the greatest fault of this simple method of correction. In the particular case all haematocrits higher than 30 per cent will completely prevent clot retraction and the formula is therefore inapplicable. In such cases it would be necessary to resort to an estimation of clot retraction on the plasma after centrifugation or sedimentation, with the unavoidable loss of platelset.

The conclusion is therefore, that no fully satisfactory correction can be made for the amount of cells present in the blood. For practical purposes the simplest is to add the haematocrit reading to the observed clot retraction.

CHAPTER 4.

PLATELETS AND CLOT RETRACTION.

Are platelets essential for clot retraction to take place? This fundamental question has exercised the mind of most workers in this field and has often led to embittered controversy since the day when Hayem (1878) first postulated the hypothesis, that the contraction of the clot was dependent on the presence of haematoblasts, so called, because he believed that the platelets originated from the red cells.

It is not the purpose of this work to give a complete review of the literature on platelets on which 2000 papers were published between the years 1917 and 1937 alone (Schäffer, 1938). Only the publications with a direct bearing on clot retraction will be considered, and in this chapter mainly those dealing with the experimental aspect of the question, leaving to a later stage the inferences to be drawn from clinical material.

Donné (1842) first described the platelets which he called globulins. Zimmermann (1846) studied horse blood after addition of $MgSO_4$ and observed the platelets, the

action of water and acetic acid of them, and that they were either isolated or clumped together. The behaviour of the platelets on the warm stage was described by Schultze (1865) who attributed to them the property of auto-agglutination. Ranvier (1873) demonstrated the coagulation phenomenon under the microscope and observed the change of shape and refraction in the "granulations" before the fibrin threads appeared, and described in detail the network of fibrin with the "granulations" as the cornerstones of the meshes. This work was confirmed by Vulpian (1873) who also noticed the adhesion of the platelets to the glass slides.

Considerable doubt was thrown on the independent existence of these bodies, but Osler (1874) demonstrated their presence in the venules of the rat immediately after death, and Zahn (1875) showed their accumulation in injured living capillaries.

Bizzozero (1882) demonstrated the platelets in the circulating blood. He actually coined the word "Platelet" which after much opposition has become generally accepted except in Scandinavian literature. He again described how the platelets formed the "knots" in the fibrin network and noticed the blobs of clear fluid given off from them during their degeneration. This work was completely confirmed by Lavdowski (1883) and Ebert and Schimmelbusch (1885), who also demonstrated that fibrin is laid down independently of platelets. Mosen (1893) gave a further impetus to the experimental work by publishing a method for the separation

of platelets by differential centrifugation.

For simplicity's sake the discussion of the literature will be divided into two parts, on presenting the evidence for the platelets being essential for clot retraction, and the other dealing with the opposite view.

1. <u>Evidence from the literature that platelets are essential for clot retraction to take place.</u>

Hayem (1878, 1889) in two classical experiments demonstrated the close relationship between clot retraction and platelets:- By tying off the external jugular vein of a horse in two places far apart and excising the vein, he obtained a tall column of blood, which, when kept at $0^{o}C.$ for some hours, separated into three layers. The intermediate zone, which was full of "haematoblasts", clotted in about ½ hour after removal from the vein, and the clot contracted vigorously and completely. The upper layer coagulated in two hours and retracted slowly and incompletely, whereas the lower zone with the red cells clotted slowly and unsatisfactorily in more than 3 hours and exuded only a few drops of serum.

The second experiment consisted in filtering a portion of the plasma in the cold and showing absent clot retraction in this filtered part, whereas the non-filtered plasma contracted strongly.

It is fitting at this place to give verbatim his conclusions:- " The spontaneous retraction is thus dependent, not on the fibrinous filaments, but on the haematoblasts, and

it is a manifestation which springs naturally from the modifications these elements undergo outside the organism. It must be remembered, in fact, that the alterations of the haematoblasts, in spite of the suddenness of their onset, do not reach their full degree instantaneously. The alterations continue for a variable time, usually extending beyond 24 hours and are accompanied by a very notable diminution in the volume of the heaps of haematoblasts."

Hayem's school (Bensaude, 1897, Lenoble, 1898) later became worried by the fact that blood from some patients suffering from different diseases, especially pneumonia, diphtheria, severe jaundice, typhoid fever and rheumatoid arthritis, showed no retraction in spite of normal platelet numbers. They tried to explain away the awkward observations by invoking a chemical substance capable of influencing the quality of the fibrin, or a "vital action directly on the platelets" without producing any experimental evidence for these statements.

Schmidt (1892) agreed that retraction did not take place in cell free plasma.

The production of non-retractile blood by intravenous injection of peptone, diphtheria toxin or blood of another species was shown by Gley (1896), but he did not consider whether this were due to the disappearance of the platelets or not. He did find that clots from the peptone treated animals underwent rapid dissolution, i.e. fibrinolysis.

Delezenne (1897), working with birds' blood, found that

if the blood was collected without admixture of tissue fluid it did not clot for many hours or even days during which time complete sedimentation of all cells occured. After coagulation under these circumstances, and if the tubes were left at rest, no retraction took place.

Fuld (1902) working on much the same lines showed that the cell-free bird plasma did not contract even after the addition of tissue fluid which speeded up the coagulation enormously.

The explanation of Gley's work was given by Pratt (1903) who demonstrated the complete disappearence of platelets from the blodd stream after the injection of peptone. The production of anti-platelet serum by injection of rabbit platelets into guinea-pigs, first suggested by Marino (1905), had a profound influence on research on the whole problem.

Perhaps the greatest protagonists for the theory, that platelets were essential for clot retraction, were Le Sourd and Pagniez (1906a, 1906b, 1907, 1908, 1911a, 1911b, 1912, 1913 and Pagniez, 1909 and 1913). Only a short summary of their brilliant experiments can be given here. All aimed at the same purpose: the demonstration that without intact platelets no clot retraction will occur.

(a). They added washed platelets to cell-free plasma and to hydrocele fluid which did not spontaneously contract after clotting; the more platelets added the greater retraction occured.

(b). Heating the platelets to 55°C. for 10 minutes destroyed

their ability to promote retraction, suggesting that in those clinical cases with normal platelet number and no clot retraction, the platelets might be defective in a similar way.

(c). Anti-platelet serum added to rabbit's blood produced non-retraction in proportional amount to the volume of serum added. Anti-red cell serum did not produce this result. Intravenous injection of the anti-platelet serum produced disappearance of the platelets from the blood-stream and non-retractile clots. Normal and haemolytic sera did not have this effect.

(d). Ageing of the platelets for 24, 36 and 48 hours diminished their ability to promote retraction, but addition to the plasma of minute amounts HCl, H_2SO_4 or CH_3COOH brought this function back. This restoring effect was somehow produced through the plasma, as aged or heated platelets treated with the acid and then added to fresh plasma did not produce retraction.

(e). Addition of extracts of different organs only led to retraction in the case of the spleen and the bone-marrow due to the high content of platelets of these organs.

(f). Addition of platelets to non-retractile purpuric blood restored normal retraction.

(g). Grinding or drying of the platelets and deprivation of calcium prevented retraction. Other colloid substances such as gelatine, gum arabic and egg-white did not produce retraction unless platelets were present.

Arthus and Chapiro (1908) endeavoured to prove that clot retraction only took place in the presence of "living" platelets. Substances lethal to cells such as fluoride inhibited retraction; addition of water to the plasma in proportions above 1:1 produced the same result. The effect of ageing of the blood and the marked influence of temperature on retraction were pointed out in favour of their theory. Tchastny (1908) found that haemolytic serum alone caused a diminution in the number of platelets by their agglutination but not their destruction, and that the haemolytic serum removed from the platelets their ability to retract the clot.

Vinci and Chistoni (1909) maintained that bird's blood is non-retractile and contained no platelets or thrombocytes. Lymph from the thoracic duct of dog, cat and rabbit was also platelet-free and non-retractile, but addition of blood or isolated platelets to the lymph produced normal retraction.

Cesana (1909) with his special method found an absolute correlation between the number of platelets and retraction, and Bordet and Delange (1912) working on much the same lines as Le Sourd and Pagniez confirmed their work and added, that extracts of platelets did not produce contraction. Exactly the same conclusion was reached by Lee and Vincent (1914) and Lee and Robertson (1916). An important observation of theirs was that if blood was run into leech-extract (Hirudin), the platelets quickly agglutinated into large lumps, but these lumps after being washed free of the hirudin still produced clot retraction like normal platelets, but solutions

of extracts of platelets did not promote retraction.

Ledingham (1914) produced purpura in animals by injection of anti-platelet sera and found the purpuric blood irretractile.

Ducceschi (1915) without producing much new evidence supported the findings of Le Sourd and Pagniez.

Kaznelson (1919) tried to find the actual number of platelets necessary for retraction. Only numbers below 47,000 per cmm. produced a real inhibition and he concluded:- "In the hundreds of tests which we performed we have not once found absent retraction at room temperature when the platelet count was high and the blood was run into U-tubes of 1 mm. diameter. Only in ordinary test-tubes did it occur that no retraction took place in spite of high platelet numbers".

Fonio (1923) with his particular "Retraktilometer" again demonstrated the necessity of platelets for contraction of the clot, and Opitz and Schober (1923) tried to replace the platelets by glass dust of about the same size without result. They found that about 70,000 platelets per cmm. were essential for retraction to occur. The greater the number of platelets the faster did the retraction proceed. They did find, however, that after X-ray treatment the platelet count might fall to very low figures and retraction still occur after many hours.

Mackuth (1933) could not obtain retraction in oxalated platelet-containing plasma on recalcification and believed that the precipitate of calcium oxalate interfered. Otherwise he found an absolute relationship between platelets and

retraction.

Macfarlane (1938) by centrifuging plasma at increasing periods found a progressive diminution of retraction in proportion to the diminution in the platelet count. He, however, like Hayem's school, found some instances with normal platelet number but deficient retraction, as for instance in cases of pneumonia, and concluded that other factors than variation in the platelet count might play a part in clot retraction. In no instance did he find the opposite, i.e. a normal contraction with a platelet count below 100,000 per cmm.

Tamura (1940) observed the same close relationship between platelets and clot retraction, and so did Lundsteen (1942) who showed that both the speed and the extent of retraction depended on the number of platelets.

Aggeler and his co-workers (1942a, 1946) tried statistically to show a correlation between the number of platelets and clot retraction. They did find a significant correlation although they did not take into account the profound influence of the fibrinogen content of the blood, which, as will be shown in the next chapter, is of the same magnitude as that of the platelets.

By repetition of previous experiments Werner (1943) again demonstrated that platelets were essential for clot retraction. He tried unsuccessfully to replace the platelets by glass particles of the same size, or by talcum, charcoal, and kieselguhr. Heating, freezing, crushing, prolonged

spinning, exposure to shortwaves, ultra-violet light, galvanic current and X-rays in high doses of the platelets all led to inhibition of retraction. He repeated Mackuth's experiment with oxalated plasma and obtained quite normal retraction in such plasma. He finally clearly demonstrated that in the experiemtns, in which the clot is loosened from the glass wall, some artificial contraction may be produced, without, however, explaining this phenomenon. His conclusion was that the platelets fulfil a special vital function as far as clot retraction is concerned.

The evidence thus appears strongly in favour of the platelets playing an essential role in the retraction of the clot, but the opposite view is supported by experiments which on their face value would appear equally convincing.

2. Evidence from the literature that the platelets are not essential for clot retraction.

Sacerdotti (1908, 1909) repeated the injection of anti-platelet serum and noticed retraction of the blood before the platelets returned to the peripheral blood.

Complete denial of any relationship between platelets and clot retraction came from Achard and Aynaud (1908b, 1909a, 1909b) and Aynaud (1913). Injections of "Electrargol", colloidal arsenic and silver sulphide, egg albumin, lecithin, gum arabic, animal and vegetable fats, were followed by disappearance of the platelets from the blood stream, but retraction occured after the coagulation of the blood. In animal Trypanosomiasis

they found very low platelet counts but no correlation between the number of platelets and the clot retraction. They also found that high concentrations of NaCl inhibited retraction, which they strangely enough used as evidence for their contention that platelets bear no relationship to this phenomenon. Retraction was supposed to be a manifestation of "cadaverisation".

In a much quoted paper Howell (1916), without giving details of the methods used, stated that cell-free oxalated plasma or solutions of pure fibrinogen clotted by thrombin retracted to a marked degree after loosening the clots from the sides of the vessels. Clots which artificially by addition of alkalies etc. were made to form structureless gels did not contract but were soft and transparent. "Crystalline" clots always retracted. A clue to the reason for these observations may be found in this sentence: "Even in very dilute solutions in which the fibrin is deposited as a delicate membrane contraction is shown distinctly when the membrane is detached from the wall or is shaken gently. It shrivels up to a much smaller membrane-like structure". It has already been shown that such "contraction" is not active and spontaneous, but purely the mechanical inability of the wide-meshed fibrin network to hold the contained fluid within it.

Pickering and Hewitt (1923) spun oxalated plasma at 4000 r.p.m. for 30 minutes and recalcified it in uncoated wide- mouthed glass vessels containing 20 ccs of diluted plasma; they always, but usually only after loosening of the

clots, obtained "typical syneresis" and concluded that platelets were not necessary for contraction of the clot.

Leschke (1925) "killed" the platelets by KCN or deprivation of O_2 and still got retraction and considered the clot an ordinary colloidal gel with syneresis.

One of the pitfalls of this type of investigation is demonstrated by the work of Roskam (1926, 1927a, 1927b). He recalcified cell-free plasma and added serum treated with chloroform. After coagulation the clots retracted in an apparently normal manner, both in glass tubes and when suspended in liquid paraffin. He therefore concluded that clot retraction is entirely a function of the fibrin and bears no relationship to the platelets at all. What he really showed was that plasma treated with chloroform contains large amounts of fibrinolysin. Naturally, if the fibrin of a clot is digested away only serum will be left, and one may thus obtain an appearance of retraction which may be misleading. Further proof that Roskam was observing fibrinolysis is provided by his own experiments:-

(a). If he removed the serum as it formed he got a greater "retraction" than if the clot was left swimming in the serum. By taking away the serum he actually removed anti-fibrinolysin present in the albumin fraction, and increased fibrinolysis consequently followed.

(b). In plasma 10 days old he could not produce the "retraction" with the chloroform serum. Fibrinolysin is destroyed on storage.

When he presented this work to the Belgian Medical Academy (Roskam, 1927b) it was in fact pointed out that

chloroform might produce all kinds of unknown changes and that control experiments using other ways of coagulating the blood ought to be done. But no further communication on the subject appeared.

The following year Mills and Ling (1928) showed that clots formed by coagulating fibrinogen solutions with thrombin appeared to retract after 2 to 3 days. They, however, demonstrated that the apparent retraction was really lysis, as weighing the dried fibrin of the clots immediately after coagulation, and 2 or 3 days later, proved that a marked loss in weight had occured.

Shindoh (1930) by a series of experiments, which have not been reproducible in this laboratory, endeavoured to discount the clot retraction-platelet relationship. Addition of calcium increased retraction, whereas addition of glucose did not; Retraction was greatest at pH 5 to 6: the formed elements of the blood did not influence the retraction in any way. He unwarrantably draws the conclusion that retraction occurs at the isoelectric point of fibrinogen, in fact at pH 5.5, a state of acidity which is never found in the blood.

Fuchs (1931), as opposed to Mackuth, obtained retraction in recalcified cell-free oxalated plasma, but not in citrated plasma. He believed that calcium oxalate crystals and any other rough surfaces gave rise to "coagulation centres" and that such centres alone were responsible for the contraction of the clot.

Mackay (1931) found no proportionality between platelets and retraction and came to the conclusion that the platelets were of secondary importance and served to augment the retractile properties of the plasma. This, however, was pure speculation.

By loosening the clot from the sides of their special test-tubes Czoniczer and Weber (1931) obtained 10 to 30 per cent retraction in cell-free plasma; otherwise they did little experimental work on the problem.

Tocantins (1934, 1936a, 1936b) showed that if the area of clot touching glass was small, platelet-free clots would "retract", but addition of platelets greatly increased the retraction. In a U-tube addition of platelets to one limb increased the retraction in that limb as compared to the other. Aged platelets, dried platelets, platelets treated with water or saponin, and platelets shaken for some time, did not increase the retraction. He therefore concluded that blood or plasma, even when free of platelets, possessed all the elements needed for syneresis, but that the syneresis was greatly helped by an adequate number of intact platelets.

Hirose (1934), like Mills and Ling, found considerable retraction after 3 days in clots formed by thrombin acting on pure fibrinogen solution, and also demonstrated the marked loss of weight. She therefore comes to the conclusion that "the syneresis of clots is in reality a resolution ending in complete liquification". The simple control experiment of adding a few platelets and producing retraction

in an hour or less would have shown that there is no loss of weight of the solid part of the clot during spontaneous clot retraction (Experiment 15).

Ferguson and Erikson (1939) came to much the same conclusion as Hirose, except that another complicating factor, trypsin, was brought into the picture. "Thrombin activated without trypsin practically never induces lysis in the subsequent clots, whereas clots obtained with trypsin-thrombin show syneresis (clot retraction) and fibrinolysis within a few minutes". Later Firguson (1943) suggested that some independent proteolytic agent is responsible for conversion of prothrombin into thrombin by activating thromboplastin from an inactive form. Excess of this enzyme will account for clot retraction and dissolution of the clot.

There is no doubt that the majority of these experiments, intending to discount the essential part played by the platelets in the retraction of the clot, can be explained either by the unavoidable occurence or direct production of the type of artificial "retraction" which was amply demonstrated in chapter 1 as the inability of the fibrin network to hold the serum in the clot against the force of gravity. The rest is due to fibrinolysis destroying the clot, and to the difficulty, when using glass tubes, of distinguishing this destruction from true retraction, a difficulty which does not occur with the suspended clot method, as will be shown later.

The experimmnts carried out with the suspended clot technique all support the hypothesis that the platelets play the fundamental role in the occurence of true clot retraction.

Clot retraction has never occured without platelets being present in the clot. In the many hundreds of experiments made during the two years since this work was undertaken this rule has been observed in every instance.

In the cases of great fibrinolytic activity a layer of serum may form round the clot, quite unlike the bubbles squeezed out in true retraction, and in such cases the loss of fibrin substance is obvious to the naked eye, and the clot is friable, non-elastic, with the retained serum pouring away from it immediately after lifting it out of the suspension mixture. This difference is masked when clot retraction takes place in ordinary glass tubes.

To find the true correlation between the number of platelets and the clot retraction, an extensive series of experiments was undertaken to find a method of platelet counting that would give reliable results. Most of the previously published methods were tried, and several new techniques evolved, including the measurement of the volume or the weight of the platelets, but the errors of all the methods were so great that for this particular purpose platelet counts were unsuitable.

It has been shown in this laboratory (Biggs and Macmillan, in press) that such widely used procedures for platelet

counting as the indirect one of Dameshek (1932) and the direct one of Lempert (1935) when used as routine methods, carry a standard error of 41.3 per cent and 22.8 per cent respectively. It is admittedly true, as also pointed out by Aggeler, Howard and Lucia (1946), that one skilled person, specially trained, and using his own finesses and criteria, may obtain better results. But the fact remains that any experiment, based purely on platelet counts, must always be subject to grave suspicion.

To overcome this difficulty the following procedure was adopted (Experiment 16):-

Cell-free plasma obtained by high speed spinning (15,000 r.p.m.) was mixed in carefully controlled proportions with plasma containing platelets and the clot retraction estimated in the usual way. Anti-coagulant and other artifices were avoided by the silicone technique. An exact decrease of the platelet number in identical plasma was thus obtained, and a definite correlation between platelets and clot retraction was established. This correlation is illustrated graphically in figure 22.

To get some idea of the actual number of platelets a count was made on the original plasma using Dameshek's fluid for the dilution and doing the count in an ordinary counting chamber. The average of 3 counts was 168,000 per cmm. which would correspond to about 84,000 per cmm. in whole blood. It should be remembered that counting in a counting chamber gives much lower results than the indirect

method of counting the platelets against the red cells under an ordinary coverslip. The fibrin content of the plasma was 300 mgs. per 100 ccs.

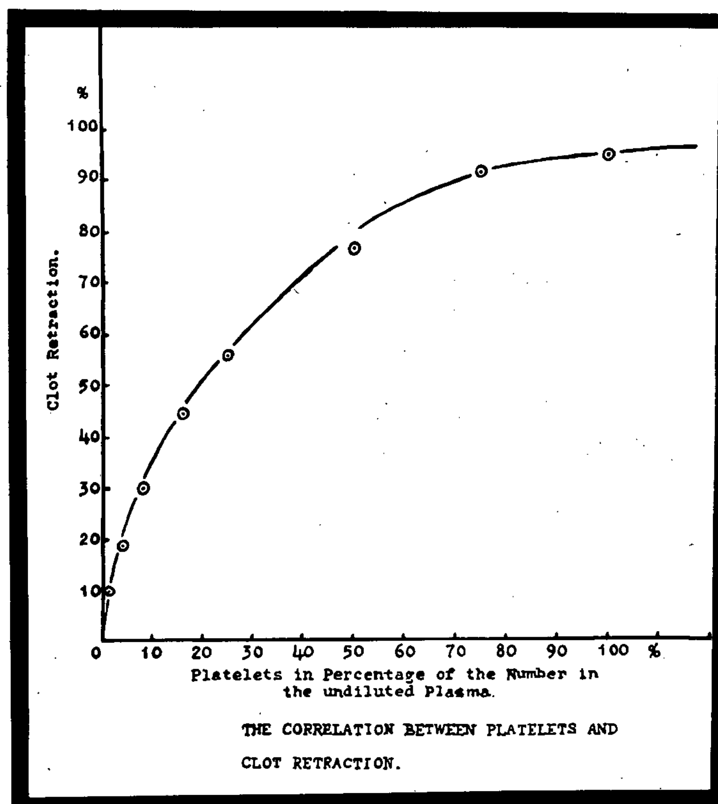


Figure 22.

Several important facts emerge from this curve. Above a certain number of platelets (about 126,000 per cmm. whole blood in this case) the influence on the retraction is immeasurably small as it is already maximal. As the number of platelets gradually falls the decrease in clot retraction becomes disproportionally greater, in fact the curve approximates closely a logarithmic function in its lower part:-

CLOT RETRACTION = K x log NUMBER OF PLATELETS.

There is no absolute lower limit to the number of

platelets producing clot retraction. In whole blood the lower limit will be determined by the volume of red and white cells and, as will be demonstrated in the next chapter, by the concentration of fibrinogen in the blood.

At this stage the conclusion is therefore reached that platelets are essential for the occurence of true clot retraction, which does not take place in their absence. The degree of retraction is directly dependent on the number of platelets present in the clot. It will now be demonstrated that those cases which have been described with a deficient clot retraction in spite of a normal number of platelets can be explained by the influence of the fibrinogen concentration on clot retraction.

C H A P T E R 5.

FIBRINOGEN AND CLOT RETRACTION.

In spite of the fact that it has been generally assumed that the contraction of the fibrin fibres in the clot is the primary cause of clot retraction, somehow influenced by the presence or absence of platelets, remarkably few investigations have been made on the relationship between the fibrinogen content of the blood and its retraction after coagulation.

Thackrah (1819) noticed that in inflammatory conditions, the fibrin of the blood was considerably augmented which resulted in the clot being larger than normal in proportion to the serum.

The first experimental attempt to show a definite influence of the fibrinogen concentration on retraction was made by Opitz and Schober (1925). They came to this conclusion:- "When the fibrinogen content remains constant, the degree of retraction depends on the amount of serum added; when the serum content remains constant the retraction depends on the

degree of dilution of the fibrinogen". By the context of their paper it is clear that this involved statement simply means that the less fibrinogen in the plasma, the more retraction occurs.

Mackay (1931) suggested that syneresis depended on the state of the plasma, particularly with regard to its fibrin content, but did not determine whether the relationship was direct or inverse. Kilduffe (1931) believed that non-retraction, as found for instance in jaundice,was due to diminished fibrin. Jepsen (1932) maintained that he could find no correlation between clot retraction and fibrinogen concentration, but actually if his figures for platelets, fibrinogen and clot retraction are combined, quite a good correlation can be established.

Lampert (1932) who believed that the fibrin fibres shortened during retraction tried to show that increased fibrinogen content led to stronger retraction. Boyce and McFetridge (1937, 1939) on purely speculative evidence, as they estimated neither the prothrombin nor the fibrinogen, believed like Kilduffe that the non-retractile clots sometimes found in jaundice were due to a defect in fibrin, caused by an iadequate conversion of fibrinogen, which again was due to a diminution in prothrombin. The contention that less prothrombin leads to the formation of less fibrin is certainly not true, although it may delay the conversion of the fibrinogen.

Tamura (1940), on the other hand, found that the retraction was inversely proportional to the ampunt of fibrinogen. Aggeler and his collaborators (1942a & b, 1946) did not investigate this correlation which was a pity, as their statistical work has lost much of its value by this omission. They apparently believed that more fibrin caused more retraction and speak vaguely of "fibrinasthenia" to explain cases with high fibrinogen and poor retraction.

Lundsteen (1942, 1943), by diluting plasma with serum and adding platelet suspension, showed a decrease in clot retraction with increasing fibrinogen concentration, but did not believe that high fibrinogen content could more than half the retraction.

Zahn (1944) considered the increased retraction found in such conditions as thrombosis and varices to be mainly due to increased fibrinogen content, in much the same way as Hirschboeck (1943, 1948), who suggested that the rate of contraction was directly proportional to the fibrin concentration.

Pinniger and Prunty (1946), investigating a case of congenital afibrinogenaemia, showed that a fibrinogen concentration of 50 mgs.per 100 ccs. was the minimum for a contiguous clot to form and therefore the minimum for clot retraction. Whitby and Britton (1946) stated that defective retraction in some cases was due to diminished fibrinogen concentration.

Most of the scanty experimental evidence is thus in

favour of an inverse relationship between fibrinogen concentration and clot retraction; it is mainly when speculation has taken the place of experiment that the opposite view is porpounded.

To demonstrate the true correlation between fibrinogen concentration and clot retraction the following experiment was carried out:-(Experiment 17)

Equal amounts of pure fibrinogen solutions of varying concentrations were added to platelet-containing plasma and the retraction estimated in the usual way. By using the silicone technique a minimum of artificial factors were introduced into the system; the results are given graphically in figure 23.

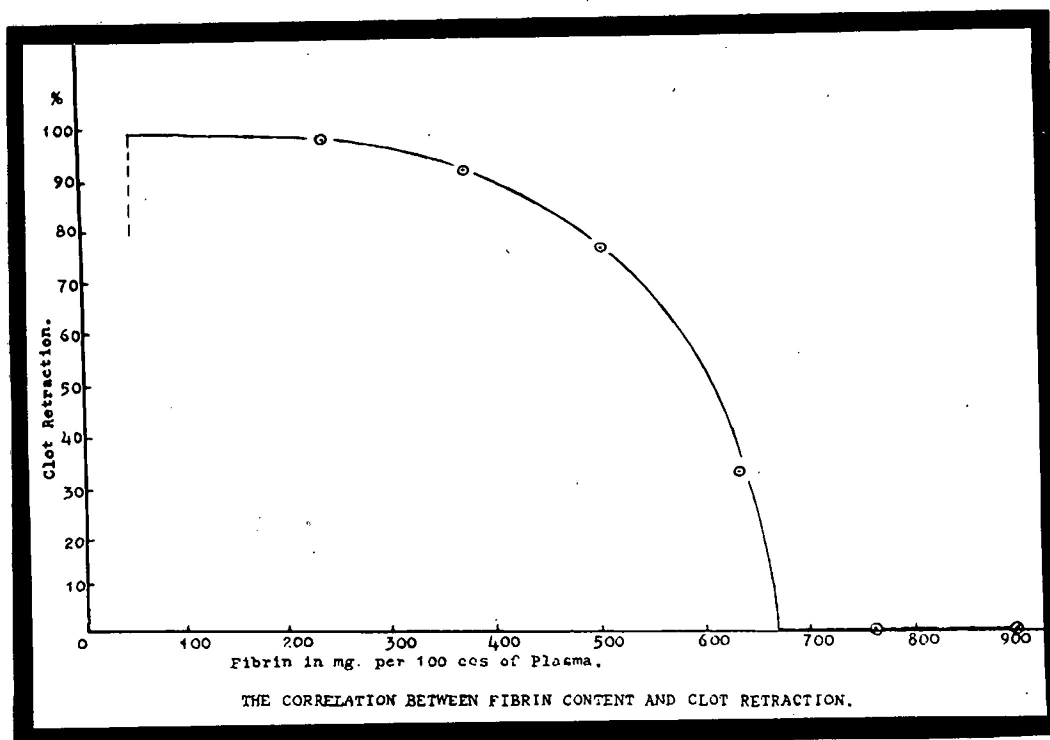


Figure 23.

Repeating the experiment with whole blood gave similar results (Experiment 18).

It will be noticed that the curve only covers such values of fibrinogen as are met with in physiological and pathological human blood. It demonstrates in a definite manner that the higher concentrations of fibrinogen can completely inhibit clot retraction, an important observation to keep in mind before one draws conclusions from deficient retraction in clinical work. It will also be seen that the curve is approximately a mirror image of the graph giving the correlation between platelets and clot retraction. This close similarity between the two curves explains the old observation by Arthus and Chepiro (1908), Mas y Magro (1924), Lampert (1932) and Macfarlane (1938), that dilution of the platelet containing plasma with physiological saline does not alter the subsequent retraction. This finding was confirmed in experiment 19, in which it is shown that even marked dilution of the plasma produced no change in clot retraction.

With the experimental evidence here presented, which explains the independence of retraction of dilution over vast ranges as an interplay between diminishing platelet number decreasing the retraction, and decreasing fibrinogen concentration correspondingly increasing the retraction, the two balancing each other, this finding becomes of the highest importance. It immediately indicates that all other factors

present in the plasma play only a minute role in the phenomenon of clot retraction, a suggestion which is fully born out by subsequent investigations.

The same observation permits the use of citrated and subsequently recalcified plasma for experiments on clot retraction. It will be remembered that Czoniczer and Weber (1931) and others after them, made use of citrated plasma for their methods for the estimation of retraction. It had been pointed out earlier that recalcified blood and plasma retracted normally (Gichner, 1927), and the procedure would be perfectly legitimate, if it were not for the unavoidable loss of platelets. Even the recalcification need not be particularly exact, as wide variations in calcium concentration within the range at which normal coagulation takes place, does not influence the retraction.

As this fibrinogen factor which so profoundly influences clot retraction has been neglected in most of the work on the problem, it is no surprise that inconsistent results have been obtained, and conflicting views expressed. This applies particularly to clinical observations, and it will be shown that the fibrinogen factor will explain the otherwise puzzling differences.

Apart from the influence of the fibrin concentration on the capillary forces holding the serum in the clot, another factor unquestionably plays a part. A dense heavy network, as formed when the fibrin content is high, is more difficult to pull together than a delicate open-meshed net,

which is formed at low fibrin concentrations. This presupposes that the fibrin only plays a passive part in the contraction of the clot, a presumption which will be proved later.

CHAPTER 6.

OTHER PLASMA COMPONENTS AND THEIR INFLUENCE ON CLOT RETRACTION.

The whole problem of the coagulation of the blood is at the present moment in a flux, and new factors partaking in its mechanism are suggested with confusing rapidity. The final elucidation of the exact processes controlling clot retraction must probably therefore await such times, until it becomes possible to fractionate the plasma into its ultimate components.

Little information can be gleaned from the literature on clot retraction. The unreliability of the current methods for the estimation of retraction has already been sufficiently stressed, and most of the experimental work on the plasma components in this connection deals with conditions so far removed from physiology and even pathology, that they bear little relation to the problem. In dealing with a colloidal

system like the plasma and with delicate bodies like the platelets, the limits within which one can manipulate are very narrow.

Hydrogen ion concentration.

The pH of the plasma was changed beyond reasonable limits by Howell (1916) who in that way produced structureless gels which did not show syneresis. It was mentioned earlier that as he was working with platelet-free plasma, it was not true clot retraction that he was estimating. The same criticism holds for the observations of Lampert (1932) who added up to 10 per cent HCl and 20 per cent lactic acid to the plasma without altering its syneresis. Macfarlane (1938) found that changes in the pH from 8.2 to 7.3 did not influence true retraction as estimated by his graduated test-tube method.

With the suspended clot technique variations in the hydrogen ion concentration from pH 7.0 to 7.8 did not change the retraction in one plasma (Experiment 20). This, naturally, is of only slight physiological importance, the blood being an excellently buffered system, and life being incompatible outside this range; but it serves to distinguish clot retraction from the syneresis of colloidal gels, which is markedly influenced by changes in the pH.

Sodium Chloride.

Aynaud (1913) found that concentrations of more than 5 per cent of NaCl inhibited coagulation, whereas a concentration of $2\frac{1}{2}$ per cent prevented contraction. He used

this observation as an argument against the part played by the platelets in clot retraction, although the connection is difficult to see. Tezner (1929) found exactly the opposite; both NaCl and KCl speeded up the retraction of the clot, but it is quite clear from his experiments that he was simply diluting whole blood and therefore decreasing the volume of red cells. Ebbeke and Knüchel (1939) found that very high and very low concentrations of NaCl inhibited contraction, but they themselves and their collaborator Hauch (1939, 1941) frequently used platelet-free plasma, and it is doubtful what they were really investigating. Aynaud's experiments were later repeated by Doladilhe (1938) and Placide and Pontiers (1939).

In the present investigation no significant change was found by alterations in the concentration of NaCl within reasonable limits (Experiment 21) which again distinguishes clot retraction from the syneresis of gels.

Calcium Chloride.

The influence of this ion was first determined by Opitz and Schober (1923) who showed that excess calcium prevented retraction in the same way as it delayed coagulation. Tezner (1929) found the same for $CaCl_2$, $SrCl_2$ and $BaCl_2$; only concentrations that interfered with coagulation would inhibit contraction. Similar results were obtained by Mas y Magro (1937).

Macfarlane (1938) found no difference in retraction of

of citrated plasma when calcified so that the $CaCl_2$ varied from 0.09 to 0.48 per cent. Lundsteen (1942, 1943) in a similar type of experiment showed that maximum retraction occured between 0.3 and 0.4 per cent final calcium concentration in the previously citrated plasma. Both higher and lower concentrations inhibited the retraction, but the differences were very small. Due to the presence of the citrate in these experiments it is difficult to work out the real calcium concentration present in the plasma.

As the calcium ion plays a specific role in the coagulation of the blood and can only be partly replaced by ions of its own group, such as strontium, it must naturally be present in normal clot retraction which must be preceded by coagulation. But even if the blood is clotted by means of pre-prepared thrombin, calcium is still essential for retraction. If blood is collected by careful silicone technique and the calcium rapidly removed by precipitation with oxalate in the cold, thus preventing completely the initiation of the normal coagulation process, and the blood is then clotted with thrombin, no contraction of the clot takes place. (Experiment 22).

It has repeatedly been shown that citrate, unless it is added in vast excess, does not completely inhibit clotting (Tzanak, Sureau and Pittaluga, 1940, Quick, 1942), and if the same experiment is performed using citrate instead of oxalate retraction occurs. If the silicone technique is not observed and ordinary needles and glass are used, even oxalate does not

prevent clot retraction (Experiment 22). These experiments indicate that although calcium ions are necessary for retraction the amounts essential are much smaller than those indispensable for normal coagulation.

To demonstrate the effect of excess calcium $CaCl_2$ was added to normal blood and the retraction estimated. Only concentrations considerably greater than the normal physiological levels impaired the contraction of the clot as seen in table 10 (Experiment 23):-

Ca concentration in mgs. per 100 ccs	Observed clot retraction. %
10	47
17.2	48
28.4	39
38.8	34

Table 10. The effect of excess calcium on clot retraction.

One way in which excess calcium may inhibit retraction was indicated by Ferry and Morrison (1947). Addition of calcium to their system of thrombin and fibrinogen led to the production of fine-meshed, friable and non-syneresing clots, but for clinical purposes, and also within wide limits in experimental work, the effect of the calcium concentration may be disregarded.

Thromboplastin and Prothrombin.

Tissue juice was found by Cesana (1909) to increase retraction in the presence of platelets, but did not produce retraction in their absence. Bordet and Delange (1912), and

and Opitz and Schober (1923) showed that extract of platelets did not promote retraction. Contrary to this Mills (1927 a & b), without actual experimental evidence, stated that "in the last several years we have repeatedly noticed that plasma clotted with tissue fibrinogen showed much stronger tendency to give rapidly contracting clots. It is apparent that it is the tissue fibrinogen content of the platelets that accounts for their ability to cause clot retraction". McKhan and Edsall (1939), again without giving data, methods or references, expressed the opinion that the presence of tissue globulins impeded the contraction of the clot. Even minute amounts of tissue extract not only accelerated the rate of coagulation, but resulted in the formation of larger, firmer, less retractile, i.e. more "hydrophilic" clots. Lundsteen (1942) demonstrated that thrombokinase had no influence on the retraction, and this was confirmed in the following year by Werner (1943).

The correlation between prothrombin concentration and clot retraction in clinical material was established statistically by Aggeler and Lucia (1939), but later the same authors (Aggeler, Howard and Lucia, 1946) were unable to find such statistical relationship. Lundsteen (1942) observed no correlation between retraction and prothrombin. Soulier and Gueguen (1947) produced hypoprothrombinaemia by phenyl-indone-dione and noticed that even with only 16 per cent prothrombin the blood contracted normally. Owren's (1947) patient lacking "Factor V" and Quick's (1947)

two cases of congenital hypoprothombinaemia and pseudo-hypoprothrombinaemia all had normal clot retraction. A diminished retraction in hypo-prothrombinaemia induced by dicoumarin was found by Koller and Pedrazzini (1947) and Jürgens and Studer (1948), but as the fibrinogen concentration was not mentioned in these cases the observations are of no value. In the cases of dicoumarin poisoning examined during the present work the fibrinogen content of the blood has invariably been high, thus accounting for a low retraction.

Recently new evidence has been published which indicates that "thromboplastin" is a complex consisting of at least two components, one present in the platelets and the other in the plasma (Quick, 1947, Brinkhaus, 1947). Whether the plasma factor is a single substance identical with Factor V of Owren (1947), prothrombin A of Quick,(1947), or prothrombokinase of Milstone (1948), or consists of several components cannot be answered with certainty yet. In haemophilia these fractions are apparently present in normal amounts, and some other plasma factor might therefore be lacking, but in this condition the possibility of an inhibitor delaying the coagulation must be remembered,

The platelet factor is in the nature of things essential for clot retraction, but if the plasma component is the missing factor in haemophilia then its influence on retraction is small as the clot contracts normally in this disease.

In an attempt to get a clearer picture as far as clot retraction is concerned experiments were carried out with

simplified coagulation systems. If a system is set up consisting of washed platelets, calcium chloride, prothrombin and fibrinogen solution, the clot formed retracts in an apparently normal manner (Experiment 24). The "Prothrombin" solution, however, contains other globulin fractions, and their complete separation has not yet been achieved. If in the experiment the prothrombin is replaced by thrombin, no retraction takes place. This would indicate that it is in some way the initial part of the coagulation process, either the conversion of"prothrombokinase" into "thrombokinase" or the subsequent conversion of "prothrombin" into thrombin, which is essential to stimulate the platelets into activity. Minute quantities of the factors are sufficient for this stimulation, for addition of 0.1 cc. of fresh serum, in which the conversion presumably still goes on, to 5 ccs. of the last mixture will restore retraction (Experiment 24).

To decide whether the thromboplastic component or the prothrombin conversion is the indispensable stimulus, another system was set up:- Brain extract and $CaCl_2$ were added to a mixture of platelets, thrombin and fibrinogen, which in itself produced non-retracting clot. The brain extract did not restore retraction. Prothrombin, $CaCl_2$, platelets and fibrinogen, on the other hand, produced a normally retracting clot, and the degree of retraction was unaffected by the addition of Factor V and brain extract (Experiment 25).

This experiment strongly suggests, although it does not

completely prove, that it is during the conversion of prothrombin into thrombin that the platelets are activated to produce retraction of the clot formed. It was felt that at the present stage of knowledge with regard to the factors involved in the first steps of coagulation, further research on these lines would fall outside the specific purpose of this work.

As a final and simple demonstration of the fact, that the actual concentration of prothrombin in the plasma above a small minimum is without effect on retraction, blood was obtained from two patients; one was on treatment with dicoumarin and had a prothrombin content of 17 per cent according to Quick's one stage method and a coagulation time of 13 minutes. The corrected retraction of this blood was 60 per cent, rather low because of its high fibrinogen concentration, i.e. 630 mgs. per 100 ccs. of plasma. Addition of prothrombin sufficient to shorten the coagulation time to 3½ minutes did not significantly alter the retraction. The other case was a baby, aged 12 days, suffering from Haemolytic Disease of the Newborn, whose blood contained only a trace of prothrombin as estimated by Quick's method. The coagulation time of recalcified plasma was 25½ minutes against the normal time of 90 to 125 seconds. This blood also contracted normally after the delayed coagulation had taken place (Experiment 26).

It has been suggested (Barratt, 1920, Tocantins, 1936) that the thickness and length of the fibrin needles are inversely proportional to their speed of development, and it

might therefore be expected that excess thromboplastin would interfere with clot retraction as the meshes in the fibrin network should be narrower; but Ferry and Morrison (1947) found that the fineness or coarseness of the clot, and therefore its ability to hold the serum, was only slightly dependent on the thrombin concentration in the coagulation mixture; the fibrinogen concentration was the determining factor. This is born out by the observation that addition of thromboplastin in the form of brain extract to haemophilic blood does not alter the subsequent retraction, in spite of the enormous speeding up of the fibrin formation(Experiment 27).

Thrombin.

This substance itself did not produce retraction in Tocantins' (1934) experiments. Jürgens and Studer (1948) showed that addition of thrombin to the blood speeded up the retraction, but did not change the final degree.

The previous experiments have already demonstrated that even in the presence of active platelets and calcium ions thrombin is not sufficient for retraction to take place. Addition of thrombin to normal blood does not enhance the retraction (Experiment 28).

Albumin Fractions.

An experiment to demonstrate the absolute independence of clot retraction on the albumin fractions of the plasma, which contains most of the inhibitors and anti-enzymes of the blood, is difficult to arrange. The fact that saline dilution

as already shown, and particularly dilution with serum, do not change the retraction of the clot is overwhelming evidence for this independence (Experiment 29).

Heparin.

The influence of heparin was examined by Zahn (1944). Two mgs. to 100 ccs. of blood led to only partial clot formation and partial retraction, but amounts below 1.5 mgs. per cent did not disturb the retraction. As opposed to this Jürgens and Studer (1948) stated that heparin added in vitro diminished the retraction, an effect which could be counteracted by thrombin.

With the suspended clot method it was found that as long as coagulation was not prevented by heparin, this substance did not interfere with the retraction of the clot(Experiment 30).

Other factors.

Addition of bile salts to the blood was the first recorded experimental attempt to demonstrate the influence of a plasma factor on retraction. Morawitz and Bierich (1907), working on the pathogenesis of cholaemic bleeding, showed that these salts in concentrations met with in jaundice did not disturb either coagulation or retraction. Inaba (1935) found that small amounts of bile acids promoted clotting and contraction, whilst larger amounts inhibited both; this was confirmed by Leschke and Wittkower (1926). Rabinowitz (1941) tried to demonstrate that cysteine inhibited contraction. He used quite unreasonably high concentrations of the substance, and it was

later pointed out by Aggeler and Lucia (1944) that the inhibition was due to acidification of the blood, coagulation of the plasma proteins, and complete inhibition of fibrin formation.

Clinically, a high blood urea was associated with diminished clot retraction in Lundsteen's (1943) cases, but these patients had low platelet number and high fibrinogen concentration which would account for the observation.

In the present investigation patients with jaundice and uraemia showed the clot retraction to be expected from the number of platelets, the fibrinogen concentration and the haematocrit (vide chapter 9). As bile salts frequently disappear rapidly from the blood of jaundiced patients, sodium desoxycholine was added to normal plasma in such amounts as might occur in jaundice without change in the retraction. (Experiment 31).

It is finally necessary to mention complement in this discussion. In the processes of agglutination and the development of adhesiveness and movement of cells complement probably plays a part. Lee and Robertson (1916) showed that the agglutination and lysis of platelets by anti-serum took place only in the presence of complement, using it up during the process. Fuchs (1933) suggested that prothrombin was the same as "middle-piece" of the complement complex, and Quick (1935) also demonstrated a certain relationship between the two substances. Wising (1938) demonstrated that middle-piece of complement is not identical with prothrombin, but quite

recently the close connection between these substances has again been suggested (Milla, Morpungo and Cominetti, 1941, Mann and Hurn, 1948). This type of investigation is fraught with difficulties, and the whole question needs further probing.

In the previous experiments it was tacitly assumed that the substance added in fresh serum or in "prothrombin" solution was actually prothrombin itself, but there is no absolute proof that the factors activating the platelets were not part of the complement complex. This fact must be born in mind in considering the actual mechanism of clot retraction which will be shown in chapter 8 to be due to an activity on the part of the platelets closely akin to the movements and sticking together of leucocytes, phenomena which are generally looked upon as being under the influence of complement.

The final conclusion appears to be, that of the plasma components only fibrinogen plays an easily measurable role in clot retraction. An activation of the platelets, which is sufficient to bring about clot retraction, takes place in the very first stages of coagulation, and the retraction proceeds afterwards independently of other plasma components. The activation is initiated by minute amounts of the essential substance or substances, and is not appreciably increased by their excess.

CHAPTER 7.

FIBRINOLYSIS AND CLOT RETRACTION.

As a last plasma component that might influence retraction fibrinolysis will now be considered.

In malignant petechial fevers the clot is "so broken as to deposit a sooty powder at the bottom of the vessel, the upper part being either a livid gore, or a dark-green and exceedingly soft jelly". These observations were made in the early part of the 19th century (Thackrah, 1819). The same type of clot was noticed post mortem in cases of death from lightning, canine madness, blows on the stomach, and mental emotions. In 1947 Biggs, Macfarlane and Pilling suggested that the occurence of fibrinolysis was a component of the initial phase of the alarm reaction of Selye, thus confirming these century old observations.

Johnston (1822) and Duncan (1822) noticed in the same case of purpura haemorrhagica that the blood clot possessed little consistency or tenacity. Gley (1896) found that peptone, blood from different animals, or diphtheria toxin,

p 93 is between pp. 95 & 96.

Figure 24.
A platelet-containing (A) and a platelet-free clot (B).

clots are shown of which the one (A) is full of platelets and the other (B) is completely free of them. There is no difference between the firmness or rigidity of the two clots, and the clot strength as measured by the"clot-cutter" illustrated diagramatically in figure 25 was also the same. By this machine the clot strength is estimated by the time taken for a given weight - throughout this work 250 grams - to pull 3 wires through the clot formed from a certain quantity of blood or plasma (5ccs. in this work). The estimation is not very accurate, but from the clinical

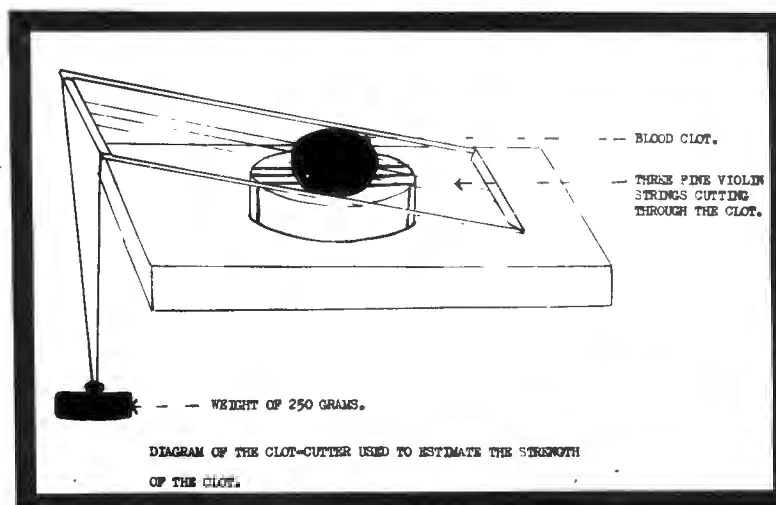


Figure 25.

material recorded in chapter 9 it emerged that the strength of the clot depends firstly on the fibrin concentration in the clot, as would be expected from first principles, and secondly on destruction of the clot by fibrinolysis. A platelet containing clot certainly becomes firmer as the serum is squeezed out during retraction, but the strength is not altered. On the other hand, the destruction of the clot by fibrinolysis is clearly demonstrated in the photograph in figure 26 which shows a clot from a patient with marked fibrinolytic activity. Other non-physiological factors affecting the character of the clot were demonstrated in the work of Ferry and Morrison (1947).

The experiment during which the clot shown in figure 26 was obtained demonstrated conclusively that clot retraction is in no way affected by the process of fibrinolysis. A patient was given a transfusion of 500 ccs. of blood and the clot retraction estimated at different intervals after the transfusion. During this time the patient developed very marked fibrinolytic activity; the clot taken 60 minutes after the transfusion, at which time the patient felt faint and was pale and cold, was digested completely in 3½ hours. This fibrinolytic activity rapidly passed off and the clot

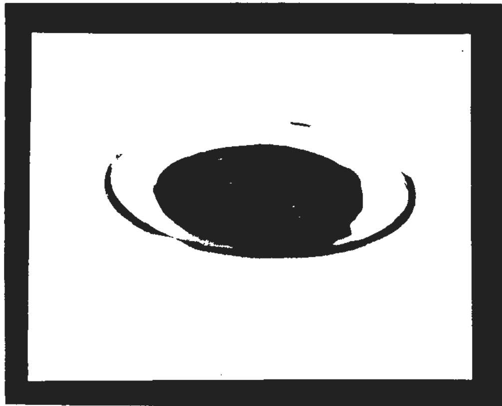


Figure 26. Clot from a patient with marked fibrinolysis. The picture was taken 3 hours after the clot had formed.

when injected intravenously led to absent clot retraction and lysis of the clot. Lenoble (1896) remarked on the dissolution of the clot in "marsh cachexia" with purpura and in severe jaundice; this type of clot was observed in generalised purpura (Grenet, 1903), thyrotoxicosis (Kottmann and Lidsky, 1910), cirrhosis of the liver with haemorrhagic tendency (Goodpasture, 1914), in purpura and hepatic cases (Émile-Weil, Bocage and Isch-Wall, 1922), in obstructive jaundice (Carr and Foote, 1934), in cases with haemorrhagic diathesis (Émile-Weil and Perlès, 1934) and in jaundice by Boyce and McFetridge(1937, 1939). Duke (1912) and Fonio (1923), on the other hand, found the clot very strong, although non-retractile, in cases of purpura. Brill and Rosenthal (1923b) believed that the platelets caused a firmer and more fibrinous coagulum, but Kristenson (1932) showed experimentally that platelet-free clots are stronger than clots with platelets.

Tocantins (1936a & b) produced thrombocytopenic purpura in dogs by injection of anti-platelet serum and found the clots very poor and fragile. He therefore concluded that platelet-rich clots are firmer, more rigid and elastic than platelet-free ones, a statement which has since been repeated in most textbooks (Quick, 1942, Wintrobe, 1947). The possibility of fibrinolysis to account for these poor clots was not considered in the experiments.

A very simple control experiment demonstrates the independence of the character of the clot on the presence or absence of platelets.(Experiment 32) . In figure 24 two

taken 240 minutes after the transfusion showed no lysis even after 24 hours. But the retraction in all the 5 clots taken during the experiment remained the same (Experiment 33). There is thus no doubt that clot retraction and fibrinolysis are different and unrelated processes.

It is not the purpose of this work to investigate the problem of fibrinolysis, but it was hoped that the presence or absence of fibrinolytic activity might in some way differentiate between the "idiopathic or essential" and the "allergic or hypersensitive" types of purpura. This hope was not fulfilled by the small number of cases - nine - examined since the investigation was begun. Although fibrinolysis was less common in the typical essential purpuras it did occur in one such case, and it was absent in two cases occuring in relation to the taking of drugs. As fibrinolysis to such a marked degree depends on the mental state of the patient during a very short period before the blood is taken for examination and may be only a passing event, the negative result is perhaps not surprising. A much greater number of cases must be investigated before any conclusions can be reached.

In these experiments it became apparent that the suspended clot method completely overcomes the possible error of mistaking fibrinolysis for true clot retraction, especially when using whole blood for the test. During true retraction the serum is squeezed out in bubbles and rises to

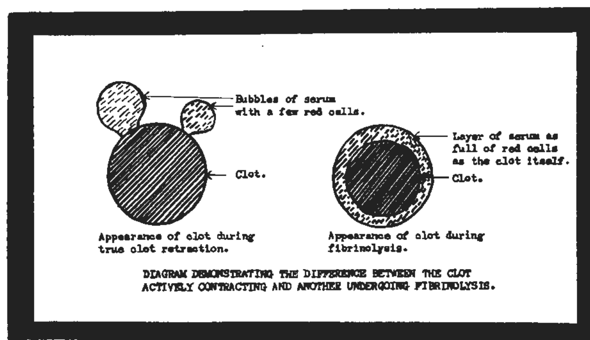


Figure 27.

the surface of the suspension fluid. The serum liberated by fibrinolysis is so intimately mixed with the cells of the blood that the mixture retains the specific gravity of the clot and remains as a layer on the outside. This difference is illustrated diagrammatically in figure 27. The experiment quoted (Experiment 33) showed the fastest lysis that has been encountered during the two years the phenomenon has been looked for, but even in this case the retraction was completed before the lysis became effective, and as mentioned the fibrinolysis did not affect the final retraction.

In conclusion it may therefore be stated that the strength and firmness of the clot is not dependent on the presence of platelets in the blood. It depends on the fibrinogen concentration and on fibrinolytic activity. Some cases of thrombocytopenic purpura have fibrinolytic activity and therefore show a poor clot, but so do some cases of purpura not associated with few platelets. The same type of poor, fragile clot may be present in many other morbid conditions, and may be found in otherwise normal persons after anxiety, shock, or excessive exercise. Fibrinolysis by itself does not influence clot retraction, and the two processes are probably entirely unrelated.

CHAPTER 8.

THE MECHANISM OF CLOT RETRACTION.

The outward similarity between clot retraction and the syneresis of gels would quite naturally lead to the belief that the two processes were of the same nature. As early as 1837 Palmer compared the behaviour of blood to that of gelatine.

The earlier workers in the period after Hayem speculated only vaguely on the mechanism of the retraction. Le Sourd and Pagniez (1907) thought it to be an expression of regular, perfectly achieved coagulation. Arthus (1893) and later Bräuler (1910) compared the coagulation of milk to the clotting of blood. The influence of temperature and salt concentration on the retraction of both types of clot was demonstrated, but a definite difference was found in the microscopic appearance of the two clots; the fibrin clot showed a network from the moment coagulation set in which remained unchanged; in milk the network was at first indistinct, but became increasingly clear and well-defined during a period of an hour or more. As the contraction of

milk clots is not explained this type of comparison is of little value.

Howell (1916) apparently considered syneresis and retraction much the same. He brought into the argument several electrophoretic observations, suggesting that fibrinogen might be of two types, one negatively and one positively charged, but no real explanation emerged. Leschke (1926) called retraction deswelling ("Entquellung") and thought the non-retraction was due to a disturbance of ion-concentration.

A unique view was expressed by Lampert (1932) who believed that the retraction was partly due to shortening of the fibrin fibres, but was mainly caused by the drawing out of the serum from the clot by attraction between serum and the wall of the container in which the clotting took place; hence the marked dependence of clot retraction on the material of which the vessels were made. In spite of this, he also considered the syneresis of gels, such as silica, the same as the retraction of the blood clot.

Ebbeke(1939, 1940) after an involved physico-chemical discussion considers the retraction of silica gels and blood clots as an "after-coagulation" ("Nachgerinnung"), which is supposed to be due to a continued polymerisation of the fibrin fibres after the real coagulation has occured, in fact much the same as the suggestion of Hawn, v.Zandt and Porter (1947), who from their studies of fibrin formation under

the electron microscope hinted that the tendency to form compound fibres might in part explain the syneresis of coarse clots observable in the gross.

There can be little doubt that the formation of the fibrin network during the coagulation takes place in some way like this:- The large fibrinogen molecules, with a molecular weight of about 500,000 (Nanninga, 1946), join together to form fine chains or fibrils, These in turn aggregate laterally and longitudinally to form thicker fibres which eventually become visible even under the ordinary microscope. The electron microscope clearly shows how the fibres give off branches which in turn run into other fibres and form the network. (Figure 28)

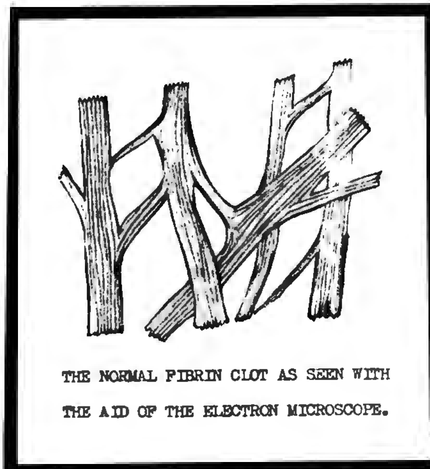

THE NORMAL FIBRIN CLOT AS SEEN WITH THE AID OF THE ELECTRON MICROSCOPE.

Figure 28.
(After Wolpers & Ruska, 1939, Schmitt, 1945 and Hawn, v.Zandt & Porter, 1947)

One observation of some importance in this connection is that the structure of the network, apart from the platelets themselves, is independent of the presence or absence of platelets during the coagulation (Wolpers and Ruska, 1939, and figures 29 and 31). The fibres may show a distinct cross-striation very similar to that seen in muscles fibres, and it is interesting that Bailey, Astbury and Rudall(1943) and Astbury(1943) found that according to its X-ray diffraction pattern the

fibrin molecule belongs to the same group as myosin and keratin.

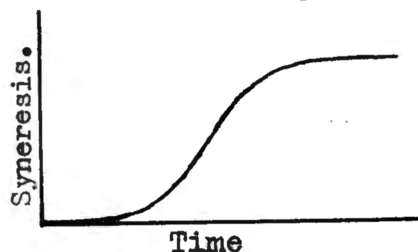
This particular mode of formation of the fibrin network has been observed and confirmed for many years (Mayer, 1907, Hekma, 1916, Wolpers and Ruska, 1939, Mommaerts , 1945, Schmitt, 1945, Hawn, v.Zandt and Porter, 1947).

Two tempting explanations of clot retraction arise from the process of fibrin formation. The first has been mentioned:- the lateral aggregation of the fibrin fibres continues after the coagulation has taken place, and the serum is thus squeezed out from the spaces between the fibres. The other explanation would be, that as fibrin is so closely related to myosin it might contract in a similar manner. Bailey, Astbury and Rudall (1943) actually showed that an unstretched α form and a stretched β form exists both of fibrinogen and fibrin, but they have never seen the one form change into the other without artificial stretching(personal communication). However, both these explanations presuppose that the clot contracts in the absence of platelets, a phenomenon which never takes place. Furthermore, it might be expected that the aggregation or the shortening of the fibres could be observed under the microscope, but that has not been seen.

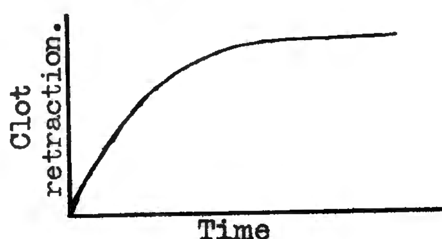
Comparatively little experimental work has been carried out on the syneresis of gels, and its exact mechanism is by no means clear. (Graham, 1864, Kuhn, 1928, Kunitz,1929, Liepatov, 1929, Ferguson and Appleby, 1930, Prakash and Dhar, 1930). There are, however, important differences

between syneresis and clot retraction, which definitely excludes the possibility of their being the same phenomenon.

Syneresis follows an S-shaped curve:-



whereas the curve of clot retraction is of this shape:-



Syneresis is never as extensive as retraction and is much slower. Cellulose xanthate, which apparently shows the most complete syneresis of all gels, only squeezes out 50 per cent of the dispersion medium and takes 200 hours to do it, whereas a clot formed from plasma will expel 95 per cent of its serum within 1½ hours. The velocity of syneresis and the extent to which liquid is pressed out during the process generally increase with the concentration of the gel, while in clot retraction both are decreased with increasing fibrin concentration. There is no general rule for the influence of temperature on syneresis, whereas clot retraction is markedly controlled by changes in the temperature. The electrolyte concentration and the presence of other colloids profoundly influences syneresis; clot retraction is largely independent of these factors as long as coagulation takes

independent of these bodies.

As mentioned, the French school did not attempt an explanation of the action of the platelets in clot retraction. They did believe that they were living cells and observed the movements and changes in them (Arthus and Chapiro, 1900, Achard and Aynaud, 1908). Duke (1912) suggested that absent clot retraction might be due to the fact that platelets were not present to anchor together the filaments of fibrin, and in their contraction the fibres would therefore simply slip between each other, but the clot as a whole would not contract. Frank (1915) stated that "the masses of platelets sitting in the fibrin network bring about retraction by their shrinkage". Tait and Green (1926) observing the phenomenon in frog's blood came to the conclusion that "the contraction of the net was due to a pull exerted by the agglutinated thrombocytes upon the threads".

Roskam (1926) believed that retraction essentially depended on the properties of the fibrin, which could be altered in the direction of greater contractibility by the presence of numerous platelets in the fluid during its coagulation. This was much the same view as that of Mackay (1931). A slightly different opinion was expressed by Fuchs (1931):- the retraction was supposed to depend on the non-homogeneous structure of the fibrin network which was due to the occurence of coagulation-centres in the plasma. Platelets, rough glass walls, calcium oxalate etc. could all give rise to such centres and therefore to retraction.

Much the same idea of "coagulation-centres" was expressed by Tazkam (1937).

Tocantins (1934) stated that "mammalian blood or plasma, even when free of platelets, possesses all the elements needed for syneresis. Its promptness, degree, or any manifestations at all of its presence under certain conditions, are greatly helped by an adequate number of intact platelets". He again observed under the microscope how the platelet in the interior of the clot converged towards the fibrin needles, adhered to them and formed large "knots" at their intersections, which were supposed to strengthen the fibrin framework and render the clot more rigid, firm and elastic. As the knots were formed, the fibrin fibres became bent, twisted and shortened, and it was perhaps while this was going on that the clot underwent the visible reduction in volume, or syneresis (Tocantins,1936).

The suggestion that clot retraction and fibrinolysis are much the same manifestation (Hirose, 1934, Ferguson and Erickson, 1939, Ferguson, 1943) has already been discussed and disproved.

Finally, it should be mentioned that Glanzmann (1918), after quoting the observation of Sacerdotti (1908) that following the injection of anti-platelet serum the retraction recurred before the return of platelets to the blood stream, came to the conclusion that the retraction depended on a ferment, "Retraktozym", which in some cases of "thrombasthenia" was absent, or present in a less active form. This would

presumably be much the same as the "tissue fibrinogen" of Mills (1927) which was mentioned earlier.

Some of these theories are directly opposed to the observed facts of clot retraction, others are mere suggestions which have passed through the authors' minds while working on the problem. The forces at work and the way they are applied during the retraction have remained obscure.

The greatest obstacle to the final elucidation of the mechanism of clot retraction is the difficulty or impossibility of watching the process under the microscope. Platelets and particularly fibrin adhere to glass surfaces, and with the small amounts of plasma with which one is compelled to work under these circumstances the forces of retraction are too small to overcome the adhesion, and no retraction takes place. Even in hanging-drop preparations watched under the phase-contrast microscope, no convincing observations could be made. Coating the slides and coverslips with silicone or ferric stereate, which has the same non-wettable properties; letting the clot float in liquid paraffin, or coating the glass with gelatine, did not improve the conditions. The only important facts that emerged from these time-consuming efforts were, that although threads of fibrin might occasionally be seen waving freely in the serum they were never observed to contract. On the other hand, two or more platelets were seen to merge together even when originally far apart, the distance sometimes being 12 to 15 times their diameter. But as a fine strand invariably stretched between these platelet

groups it was not possible to determine what factor actually pulled them together.

To get more definite information of the changes occuring during retraction, plasma clots were suspended in the usual way. At different stages of their retraction they were fixed, sectioned, and stained. A silver impregnation method was used for the photography, but other stains gave the same pictures.

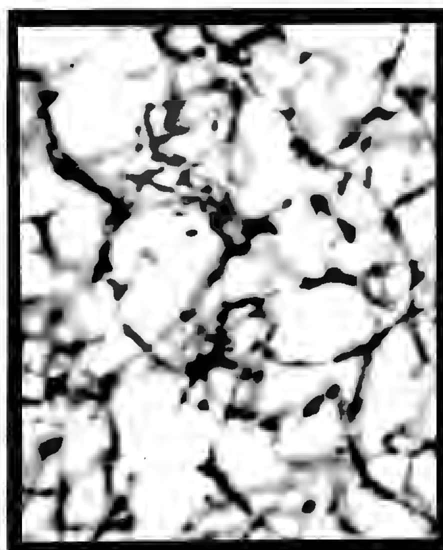


Fig.29. Platelet-free clot.

Fig.30. Same platelet-free clot 4 hours later.

The photograph in figure 29 shows first the section of a clot formed in the absence of platelets. It should be compared to the one shown in figure 31 which was formed from plasma full of platelets and fixed immediately after coagulation, before retraction had commenced. There is no difference between the network in the two. The platelet-free clot is closely knit together and forms a continuous whole, and the fibrin fibres in no way lie freely among each other.

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and have lost completely their distinct outlines and original characters. This merging of the platelets is

This only confirms what is seen under the electron microscope. After 4 hours the platelet-free clot has remained entirely unchanged (Figure 30). There is no sign of shortening or lengthwise merging (coacervation) of the fibres to form compounds.

The photographs in figure 31, 32 and 33 show the gradual contraction of the platelet-containing clot. The clots were fixed in osmic acid at 20 minutes interval.

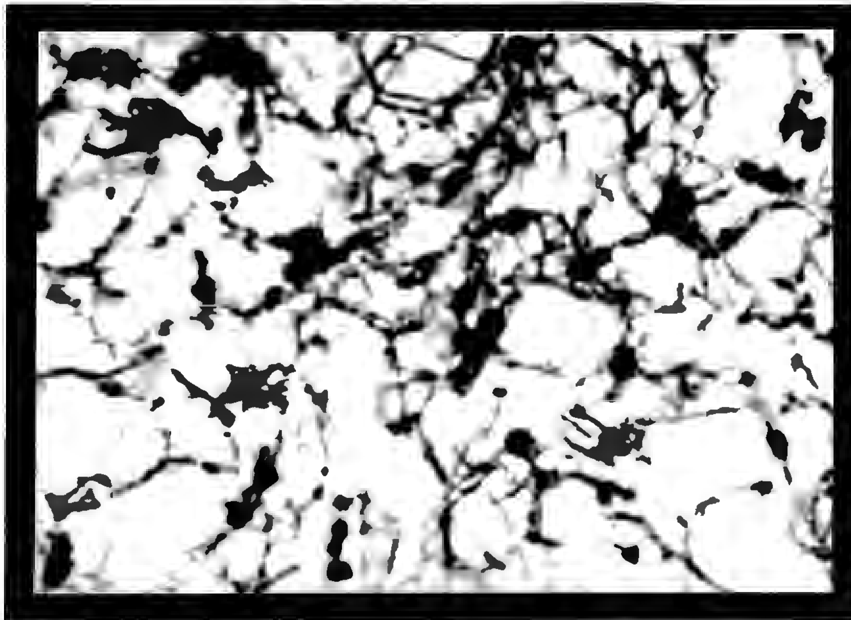

Figure 31. Platelet-containing clot immediately after coagulation, before contraction has commenced.

Immediately after coagulation the platelets are small and well defined, lying in the corners of the fibrin network. They soon begin to swell and to move towards each other, and as the contraction continues they form greater and greater masses in which the individual platelets have merged together and have lost completely their distinct outlines and original characters. This merging of the platelets is

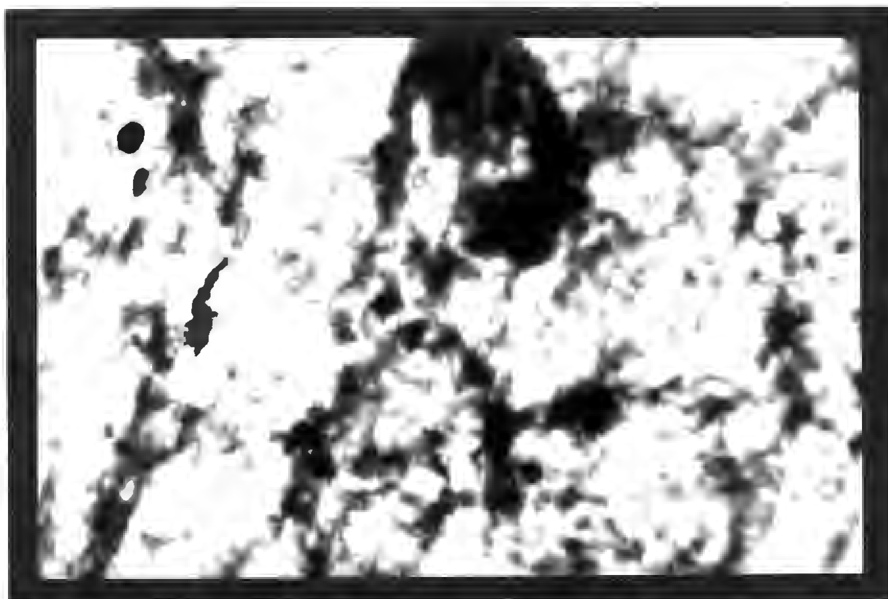

Figure 32. Same clot as figure 31,
20 minutes later after
a clot retraction of about
35 per cent. (The fibrin network
is out of focus, but is in reality
fundamentally unaltered)

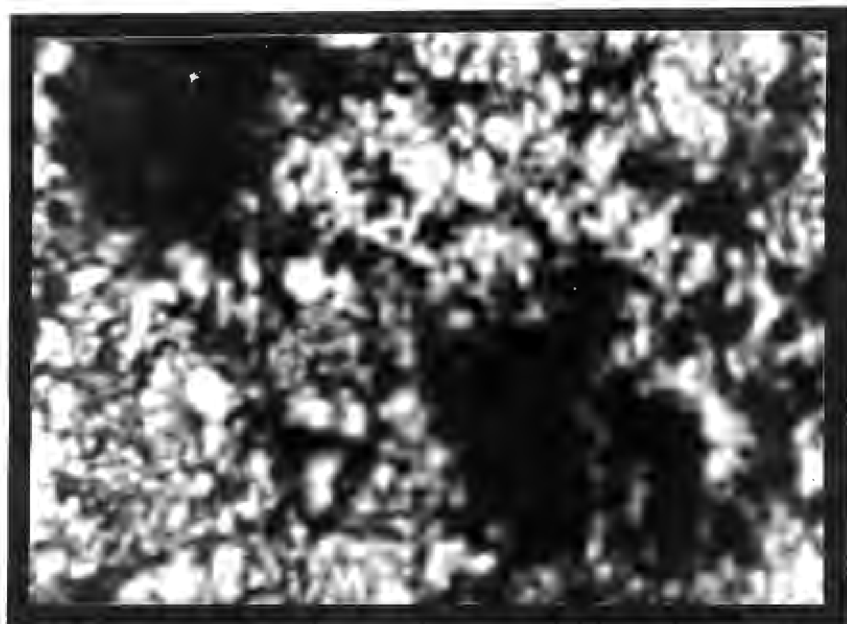

Figure 33. Same clot as figure 31,
40 minutes later after
a clot retraction of about
80 per cent.

peculiarly patchy throughout the clot, a fact that would suggest that it is not a generalised shortening of the fibrin fibres which produces the contraction, a suggestion which is also supported by the observation that the fibrin fibres have not become thicker or in other ways changed. But it cannot be determined from these pictures whether the fibrin fibres draw the platelets together, or whether the platelets pull the fibrin network with them as they merge.

Many attempts were made to stain the sections differentially in an effort to demonstrate some linkage, other than fibrin, between the platelets, but no suitable stain was found. The same difficulty was encountered by Pinniger and Prunty (1946).

In view of the fact that birds' blood produces a much more finely meshed fibrin network, and that the cells corresponding to the mammalian platelets are large nucleated thrombocytes, it was expected that more information could be derived from this type of blood. The previous procedure was therefore repeated on the plasma from a hen, whose whole blood showed a clot retraction of 28 per cent with the usual suspended clot method.

The photographs in figures 34, 35 and 36 show the sections of the clots at different stages of their contraction. Again the nucleated thrombocytes are seen lying discretely in the clot in the pre-contracted stage. Later, as retraction takes place, they come together in clumps and in this case it is quite clear that it is the

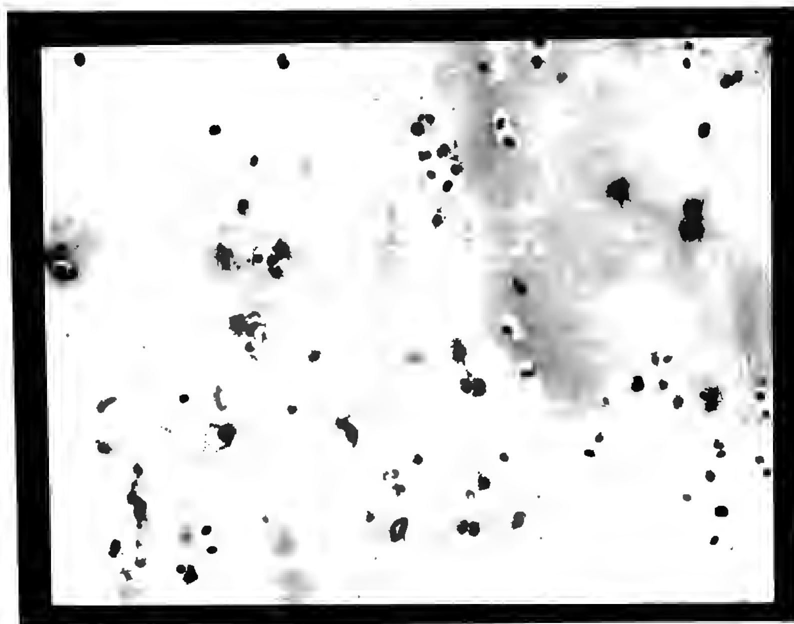

Figure 34. Clot of chicken plasma immediately after coagulation. The fibrin network is so fine that is appears in the picture as a homogeneous sheet.

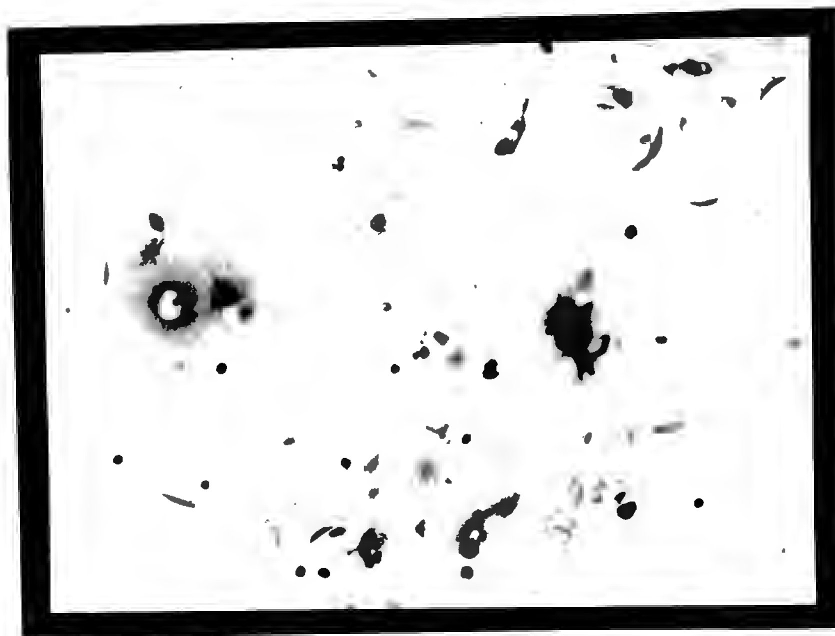

Figure 35. The same clot 20 minutes later. Two small masses of merged thrombocytes can be seen.

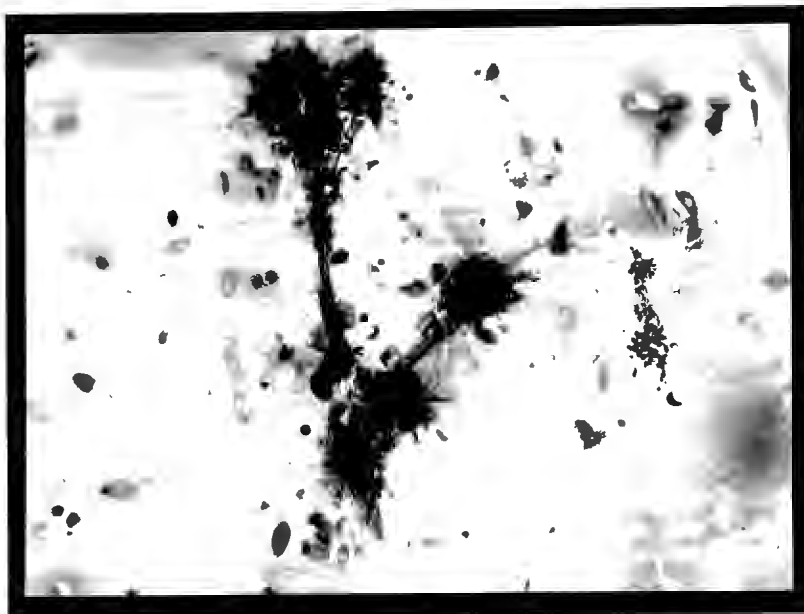

Figure 36. The same chicken clot as in figure 34, 40 minutes later. The great masses of thrombocytes with bridges of trombocyte-material between them, and drawing them together, are clearly seen. The scattered, pale nucleated cells are a few red cells which remained after the spinning of the plasma.

the thrombocytes which pull the network with them, and not the opposite. What is more, even with the simple stain used (haematoxylin and eosin) definite strands can be seen linking the different masses of thrombocytes, and these strands have not the appearance of fibrin. It is difficult to reproduce this effect in a black and white photograph, but in the stained sections the thrombocytes and the strands between them are a brilliant, transparent red, whereas the fibrin network has a dull greyish-pink appearance.

As trypsin does not attack living tissues a final experiment was done to demonstrate the same phenomenon in human blood. (Experiment 34). By digesting the fibrin, and probably other proteins, before and during the process of coagulation it was possible to overcome the adhesion of the platelets to the glass slides; the phenomenon illustrated in figure 37 could then be observed. As staining would inhibit the activity of the platelets photography was unsatisfactory and a drawing is therefore presented.

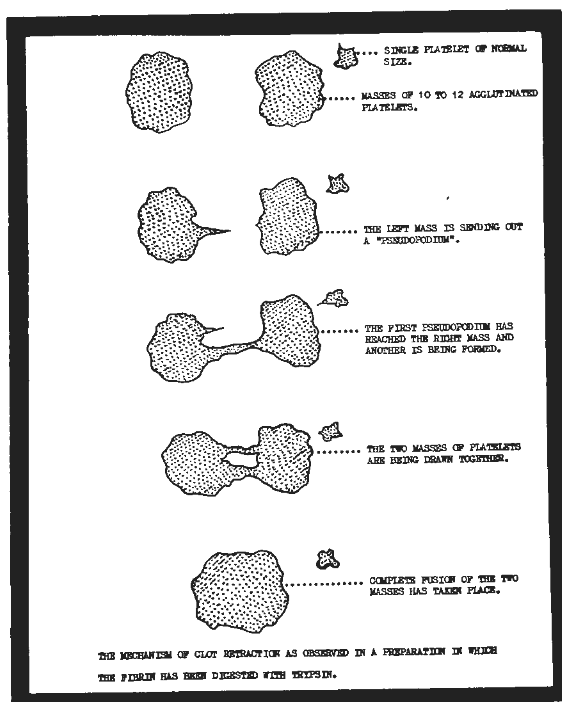

Figure 37.

The whole process shown in the picture took 20 minutes to be accomplished. No fibrin was seen anywhere in the preparation and the "pseudopodia" were considerably thicker and less sharply defined than ordinary fibrin fibres.

This observation thus explains what takes place during clot retraction, an explanation which was already suggested by the photographs of the ordinary human and the chicken clots during their contraction. The platelets send out fine strands of cytoplasm which join with the cytoplasm of other platelets. Subsequent shortening of these strands draws the platelets together, and as they are firmly

attached to the fibrin network the fibrin fibres are also pulled together and the whole clot contracts with a squeezing out of the serum. Strong support for this explanation is found in the coagulation process of lower animals. Although a certain caution is essential in drawing conclusions from comparative physiology, the analogy in this instance is so close that it appears safe to accept it.

Deckhuyzen (19o1) showed that the clot in the lower forms of animal life was made up of a network of cells. Loeb (1903, 1906, 1907, 1909, 1910, 1927, 1928), working on the coagulation and haemostasis in crustacea has for many years stressed the primary importance of the cells in the clotting mechanism of these animals.

In the blood of the crab, Limulus polyphemus, which does not contain fibrinogen at all, the coagulation is entirely a function of blood cells. In the gross the clot contracts in the same way and with the same speed as mammalian blood, and under the microscope the process can be followed closely. In the first few minutes the blood cells become connected in some way by submicroscopic threads, even when far apart and without having previously touched each other. During the next 5 minutes the cells begin to come together. They merge into clumps or strings, between which fluid-filled spaces become visible. In the end a network of cells is formed and the pseudopodia-like processes of the cells are all connected to each other, and this cell-network then retracts exactly as the mammalian clot, although no fibrin is present.

In the lobster two forms of coagulation occur ; one is the process just described which takes place first; the other is a superimposition of fibrin formation of an extremely fine meshed type.

In the mammalian blood the two processes take place at nearly the same time, and this would appear to be the reason why the platelets have received rather scanty attention, and that their importance for some decades has been minimised in the mechanism of haemostasis.

Loeb found that cold, high concentration of sodium chloride, hydrochloric acid and other acids, and heating to 43°C. hardened and stiffened the cells and thus prevented the coagulative changes in them. Calcium was essential to the process, but in excess it "stiffened" the cells in the same way as the treatments just mentioned. These are the very same things which have been found to inhibit clot retraction.

Alsberg and Clark (1908) confirmed Loeb's early observations and showed that the cell fibres making up the clot in Limulus had quite different properties as compared to fibrin, but was related to elastin and collagen. Tait and his collaborators (Tait, 1918, Tait and Burke, 1926, Tait and Green, 1926, Tait and Gunn, 1918), also working with lower animals, came to much the same conclusions as Loeb, and, as already mentioned, found that in the frog's blood the contraction of the clot was due to the pull of the agglutinated thrombocytes. This coagulating function of the crustacean cells had been suggested by Hardy (1892) who described "explosive

cells" which during their bursting formed the clot by the protoplasm liberated; Tait and Burke (1926) showed by dark ground microscopy that mammalian platelets give off blebs of fluid which float away from them in straight lines, after which strands of "fibrin" form where they have passed. Bizzozero (1883) demonstrated that the composition of the thrombi formed in the vessels of the frog was essentially the same as in the rabbit, in both cases consisting entirely of cell accumulations. Apitz (1930) produced thrombosis in the veins of the rabbit by painting the walls with alcohol; The thrombi formed long before fibrin was produced, or even without fibrin ever being laid down, and they were seen to consist of a network of platelets with red cells entangled in the net. Later this network was completely contracted and the red cells squeezed out. Hirudin prevented this network formation of the platelets. Again the analogy is close to the Limulus clotting.

The comparison between the nucleated cells of lower animals and the platelets of mammals is old. Dekhuyzen (1901) discussed this problem at length, and Schridde (1911) came to the conclusion that the thrombocytes of lower animals and the mammalian megakaryocytes were structurally and genetically the homologues of one and the same cell which circulated in the blood of extinct animals; this conclusion was repeated nearly word for word by Hartmann (1925). The whole question has been summarised by Fuchs (1933) and in the admirable review by Silberberg (1938)

In the phylogenetic evolution two processes on the part of the blood, and quite apart from the important vascular mechanism, have developed against blood loss:- the cellular mechanism and the conversion of the colloid plasma constituent fibrinogen into fibrin. The first was the earliest and is the one existing in the more primitive organisms. The second has been added in the higher animals, but the primitive cell mechanism persists and precedes the coagulation. In invertebrates the "amoebocytes", in the lower vertebrates the "spindle cells" or "thrombocytes", and in the mammals the "platelets" react in a similar manner to certain environmental changes. It may be added that one of these reactions is the contraction of the network that forms the clot.

Care has been exercised in the previous discussion not to use the word "agglutination" as descriptive of this retractile function of the platelets. Wright and Minot (1917) pointed out that "viscous metamorphosis" must not be confused with agglutination and lysis as produced, for example, by anti-platelet serum. From their minute description of the changes during viscous metamorphosis it is abundantly clear that these changes are identical to the changes in the platelets during retraction described above. Good pictures of viscous metamorphosis have been published by Stubel (1914), Ferguson (1934), and Best, Dowan and Maclean (1938). Photographs taken with the electron microscope, showing beautifully the long cytoplasmic pseudopodia sent out from the platelets,are presented in the papers by Wolpert and

Ruska (1939) and Bessis and Burstein (1948). The "adhesiveness" of the platelets investigated by Wright (1941) is undoubtedly the same prosess.

Simple agglutination of platelets frequently takes place without the occurence of viscous metamorphosis, and confusion as to the function of the platelets will persist as long as the distinction between the two phenomena is disregarded.

In Wright and Minot's experiments viscous metamorphosis was caused by fresh serum and by oxalated plasma on addition of calcium, but not before; a mixture of oxalated plasma and thrombin did not cause metamorphosis until calcium was added. The serozyme of Bordet and Delange (1912) which is in reality impure prothrombin, produced marked metamorphosis when calcium was added. Whether or not free thrombin was present in the mixture made no difference. The substance causing the platelet metamorphosis was partly used up during the process, which presumably must mean that the prothrombin was converted into thrombin while the metamorphosis was going on. Serum globulin plus calcium caused a very rapid and typical change in the platelets. Heated serum and serum treated with barium sulphate for two hours did not bring about this change. If large amounts of cephalin or platelets were added to the serum and then spun away, the serum lost its power to cause metamorphosis although it would clot fibrinogen, presumably again because all the prothrombin had been converted into thrombin. Pure thrombin, anti-thrombin, thromboplastin alone or mixed with thrombin, anti-thrombin and calcium, did not

cause the specific change in the platelets, and finally fibrinogen solution had no effect. Most of these substances agglutinated the platelets, which was obviously an entirely different effect. Agglutinated platelets, when given the right stimulus, would still undergo metamorphosis in the same way as it has been mentioned that they will produce clot retraction and that an injection of peptone or colloidal substances will cause an agglutination of the platelets in the organs which is rapidly reversible; these previously agglutinated platelets afterwards produce normal retraction.

If these observations are compared with the findings presented in the previous chapter, the similarity is striking. Clot retraction and viscous metamorphosis of the platelets will only take place under the stimulus of the conversion of what is at the present moment spoken of as prothrombin into thrombin, and the effect is in the form of a "trigger-action" and only minute amounts of the substance or substances need to be present.

In this argument, in which clot retraction is considered a biological phenomenon and not a simple physico-chemical process like syneresis, it becomes unavoidable to consider also the old controversial question whether platelets are living cells or simply dead bits of protoplasm floating round in the blood stream. Amoeboid or very similar movements of the platelets have repeatedly been observed(Deetjen, 1901, 1909, Kopsch ,1901, Achard and Aynaud, 1908, Stubel, 1914, Van Herwerden, 1920). This movement can be inhibited by

"killing" the platelets by many different means, which also prevent viscous metamorphosis and clot retraction:- Heating to 42°C. apparently changes the surface layer of the platelets. They become rounded and fixed (Achard and Aynaud, 1908a) and will not produce retraction (Experiment 35). This effect and the effect of ageing can be reversed by addition of small amounts of acids (Le Sourd and Pagniez, 1910). Øllgaard (1945) who rediscovered the phenomenon showed that mercuric chloride and saponin in minute amounts had the same restoring action. These substances must act in the presence of plasma to reverse the heating effect, and the exact mechanism is not completely clear.

Poisons of many sorts "kill" the platelets. As early as 1819 Thackrah demonstrated that opium prevented clot retraction, and since then it has been found that ether, chloroform vapour, cocaine, many stains like methylene blue, aniline blue, brilliant cresyl blue, methyl violet and trypan red has the same effect (Achard and Aynaud, 1908e, 1909 b). It is interesting to note that neutral red in low concentration does not interfere with retraction (Experiment 36). Neutral red stains the granules of the platelets if they are "alive" but not when inactive, an easy test for their functional integrity. It is possible that penicillin in large amounts has the same action although it also in some way affects the coagulation mechanism, shortening it in vivo and prolonging it in vitro. (Moldavsky, Hasselbrock, Cateno and Goodwin, 1945, Fleming and Fish, 1947). It should be noted that platelets

"killed" by these diverse methods will still agglutinate among themselves and will also cause agglutination of bacteria (Achard and Aynaud, 1909, Roskam, 1923).

Other evidence of the vital activity of the platelets is suggested by such observations that they, like other living cells, reduce methylene blue (Achard and Aynaud, 1908c), and that they consume oxygen and produce anaerobic glycolysis (Loeber, 1911, Endres and Kubowitz, 1927). Since Wright (1910) demonstrated their origin from the megakaryocytes it has been shown convincingly many times that the platelets contain nuclear substance (Van Herwerden, 1920, Willi, 1935, Rohr and Koller, 1936). In this connection it must be remembered that even after the nucleus has been artificially removed from an amoeba the cytoplasm will still continue its amoeboid movements, and a common observation is to see bits of cytoplasm torn off from leucocytes move in the same way as a whole cell in vital staining preparations. The borderline between "life" and "death" is difficult to determine in these comparatively simple structures in which life closely approaches physical chemistry. The platelets have much the same chemical composition as leucocytes (Erickson, Williams, Avrin and Lee, 1939), and, as demonstrated in experiment 34, they are not attacked by such strong proteolytic enzymes as trypsin. Most of the phylogenetic and experimental evidence, therefore, appears to indicate that the platelets are living structures.

To summarise, the conclusion is reached that in the lower forms of life the cells - amoebocytes or spindle cells - are

the only clot-forming elements in the blood. On injury to the vessels of the animal, or exposure of the blood to foreign surfaces, these cells undergo changes that conveniently may be called viscous metamorphosis which leads to the formation of a network; this network later contracts and becomes firmer. Higher in the scale, as in the lobster, this clotting still takes place, but it is after a short time followed by the change of the plasma component fibrinogen into fibrin, which presumably further strengthens the clot. The next step is seen in birds in which the beginning of the metamorphosis of the cells, now more specialised structures called thrombocytes, and the laying down of the fibrin take place nearly simultaneously and where the contraction of the cell-network pulls the fibrin-network together, squeezing out the serum and producing a firmer and more compact clot. Finally, in the mammals the cells have lost their definite nucleus and have become the highly specialised platelets, but the mechanism is fundamentally the same; the onset of the metamorphosis is the primary change in the blood after injury to the vessels or exposure of the blood to foreign (wettable) surfaces, and it is immediately followed by the next step, the formation of a fibrin network. After the fibrin is laid down the metamorphosis of the platelets continues and, as before, causes that final shrinkage of the clot with exudation of serum which is called clot retraction. The degree of retraction is therefore primarily dependent on the number of platelets.

CHAPTER 9.

THE FUNCTION OF CLOT RETRACTION AND ITS BEHAVIOUR IN DISEASE.

Three suggestions have been made for the function of clot retraction. The generally accepted one is that of Fonio (1921, 1928) who believed that retraction acted as a "physiological ligature" of the vessels. The clot becomes adherent to the injured or torn vessel wall and by its contraction diminishes the aperture in the vessel, thus promoting haemostasis. No proof for this contention has ever been provided. The second and less comprehensible suggestion is that of Lampert (1932, 1934, 1938). He thought that retraction was the forerunner of embolism:- "By the strong surface forces of the vessel wall the serum is drawn out of the fibrin meshes, and the adhesion between wall and clot is overcome". The clot thus becomes loose in the lumen of the vessel and embolism follows. This peculiar conception needs no comment.

Both these suggestions presuppose a considerable force exerted during the contraction of the clot. All the available

evidence points in the other direction. The slightest adhesion of the clot to the walls of the glass tubes impair the retraction; stronger adhesion as to collodion completely impairs it.

The third suggestion therefore came from Kristenson (1932) who in a very indirect way tried to measure the force of retraction. He let plasma clot in long glass tubes, and after the retraction of the clots had taken place, considerably shortening the clots, he measured the weight necessary to pull the clots out to their original length. This force he took to be the same as that of the previous retraction. The force was very small indeed, and would only suffice to bring the walls of capillaries together. His suggestion for the function of retraction was therefore that after thrombosis the contraction of the clot restored the patency of the vessels, although the clot remained adherent to the wall in some parts; the very opposite of Fonio's idea.

As Kristenson's way of measuring the force of clot retraction is obviously not acceptable an attempt was made to estimate it in a more direct manner (Experiment 37). A thin-walled bag of collodion with a volume of about 20 ccs. was constructed and attached to a Westergren sedimentation tube. The bag was filled with platelet-rich plasma with a clot retraction of 88 per cent, and the retraction was allowed to proceed with the bag immersed in the trichlor-ethylene-paraffin mixture. The force of the retraction was

spent in drawing the bag together and lifting the column of serum in the Westergren tube. Figure 38 illustrates the procedure. In several experiments the highest the serum rose in the tube was 19 mms. Although the exact force necessary to pull the collodien bag together was not calculated it must be very small, and the total force of the clot retraction must be correspondingly weak. In consideration of its mechanism as explained in the previous chapter, depending on the contraction of extremely delicate strands of platelet cytoplasm, this result was not unexpected.

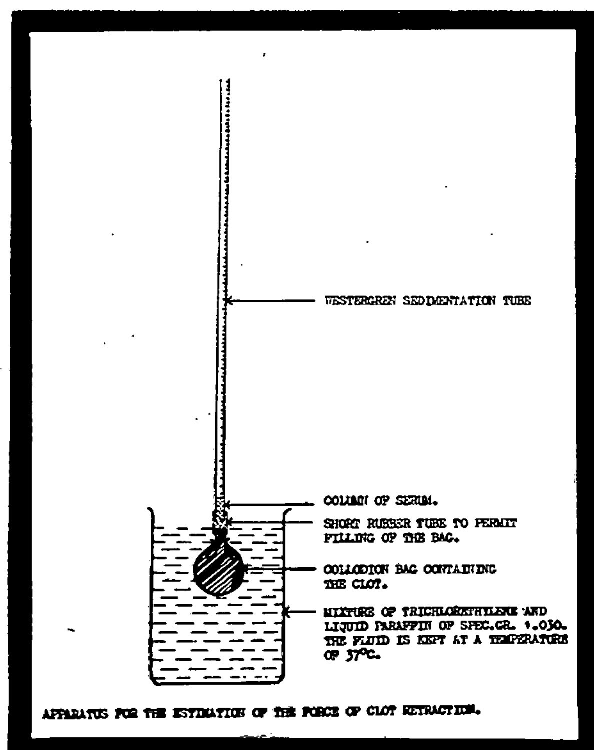

Figure 38.

The most reliable direct measurements of the pressure inside the capillaries have shown that the pressure in the arteriolar end of a capillary is 43.5 cms. and in the venous end 16.5 cms of water (Landis, 1934). Both these pressures are therefore much higher that the force exerted by clot retraction, and it appears most unlikely that the retraction in any way influences the lumen of even the smallest vessels; that it should act as a physiological

ligature is out of the question. Further, when it is remembered that both platelets and fibrin have a marked tendency to adhere firmly to foreign surfaces as presented by an injured vessel wall, an adhesion which, at least in vitro, is only broken down by the clot undergoing fibrinolysis, it becomes unlikely that retraction occurs with any frequency in vivo.

In cases of thrombosis the most likely sequence of events seems to be that platelets accumulate at a point where the surface of a vessel is temporarily or permanently altered. The loose masses of platelets become more compact by their contraction and therefore less easily swept away. The accumulation is followed by an obstruction to the free flow of blood with a subsequent laying down of a fibrin clot. This clot will lie in a vessel with intact walls and will therefore not be adherent, and it would presumably be of advantage if this loose bulky clot shrunk to a smaller and more compact size, and one function of clot retraction might thus be stipulated. It would be a very insignificant function, and in any case highly speculative; from the reasoning presented in the last chapter a more acceptable hypothesis is proposed:-

The peculiar change occuring in the amoebocytes of the earliest and lowest animals, i.e. viscous metamorphosis, which apparently in these animals is the main haemostatic process acting in conjunction with the more important tissue and vessel contraction, has been retained through the development of the nucleated thrombocytes to the highly specialised

platelets. Clot retraction may therefore be looked upon as a phylogenetic relic which has been retained in spite of the development of more complicated and more efficient haemostatic mechanisms; in the higher organism it no longer serves a purpose, but is simply by chance superimposed on the coagulation system of these animals. It should be understood that in this hypothesis the role of the platelets themselves in haemostasis is in no way minimised. They serve an important purpose by temporarily blocking breaches in the vascular system as pointed out by Macfarlane (1938), and they are presumably a ready source of thromboplastin for the coagulation of the blood. It is only their function as promotors of clot retraction which has become redundant with the development of the more perfect haemostatic mechanism of higher animals.

This idea is supported by clinical observations. It has never convincingly been demonstrated that a defect in clot retraction alone in any way reduces the efficiency of haemostasis. Only when it indicates a diminution in the number of circulating platelets, as in some cases of purpura, is it of significance in the investigation of haemorrhagic diatheses. Cases of pneumonia, myelomatosis, liver disease etc., with abnormally high fibrinogen concentration and therefore impaired or even absent clot retraction in spite of normal numbers of platelets, show no bleeding tendency, the tendency in liver disease being related to low prothrombin or vascular changes (palmar erythema and spider telangiectasis) The same applies to polycythaemia. The position of Glanzmann's

disease will presently be dealt with in detail.

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There can be no doubt that the reports in the literature on clot retraction in different diseases must be accepted with caution and scepticism. As discussed in chapter 1 a multitude of unreliable methods have been employed for its estimation, and in the majority of the reported cases the particular method employed is not even indicated, thus adding to the uncertainty. As the true significance of clot retraction has been obscure or confused in the mind of most clinical observers, its estimation has quite rightly been relegated to the less important investigations of a case. When great disproportion is found between the number of platelets and the retraction in a particular case, even with normal fibrinogen content, rapid and often considerable changes which occur in the number of platelets in the peripheral blood should be remembered. Ivanitsky-Vasilenko and Klinova (1937, 1938) reviewed the literature on this question, and in their own experiments they obtained remarkable results. They found the average normal platelet count to be about 300,000 per cmm by Lempert's method. During the night the count dropped to 200,000 per cmm. Dipping the hand in hot water raised the number to 480,000. Strong emotions and heavy work caused a rise of 200,000. Injection of atropine, compression of the carotid sinus, or holding the breath raised the number by 130,000 to 200,000. Lowering of the count by 100,000 to 200,000 per cmm. was produced by such procedures as standing

on the head for 3 minutes, hypnotic sleep, looking at pleasant food, sham feeding, chewing gum, eating an ice cream, forced breathing, pressure on eyeballs, taking morphine, bromides or quinine, or injection of pilocarpine. Another source of error was pointed out by Birch (1930) who demonstrated the budding off of separate pieces of cytoplasm from white cells which looked exactly like platelets and might explain high "platelet" counts in spite of bleeding in such conditions as leukaemia. Unless the platelet count and the clot retraction estimation are done at the same time, or even better on the same blood, no correlation between the two will be found. Of all haematological investigations the platelet count is also the most unreliable.

Purpura.

It is not possible to trace the description of the first case of the classical combination of purpura and deficient clot retraction. Thackrah(1819) mentioned that it was known at his time, and Duncan (1822) who bled a case of purpura haemorrhagica, both as a means of possible cure and as he was anxious to see the state of the blood in this disease, found that no serum separated from the clot. The same observation on the same patient was made by Johnston (1822).

The diminished number of platelets in the condition was described by Brohm (1881) and by Denys (1887) who also described a case of phthisis with purpura in whom the platelets were normal. It was Hayem (1895) who first correlated the

poor clot retraction with the low platelet number, and since then they have been accepted as the two characteristic blood abnormalities in essential or idiopathic purpura haemorrhagica. The name "essential thrombopenia" was suggested by Frank (1915) and has come into general use. That purpura could occur without deficient clot retraction was pointed out by Grenet(1903) but he did not count the platelets in his cases which were of inflammatory nature. Kaznelson (1919) thought that the platelets must fall below 47,000 per cmm. before retraction became impaired, and other figures have been published: 80,000 by Brill and Rosenthal (1923a), 120,000 by Clapton(1925). A few cases have been described in which retraction occured in spite of low platelet numbers (Whipple, 1926, Rosenthal, 1928) but from the information given these can be explained as due to severe anaemia and low fibrinogen concentration in the same patients. Loosening of the clot would naturally produce this result as it would then "retract" even in the complete absence of platelets. It would serve no purpose to tabulate here the complete literature consulted on the question of purpura. All other accounts agree with the finding of deficient clot retraction in cases with a diminished number of platelets.

Haemophilia.

With a few exceptions it has been generally accepted that clot retraction proceeds normally in haemophilia (Morawitz and Lossen, 1908, Minot and Lee, 1916, Émile-Weil and Perlès, 1934,

Macfarlane, 1938, Aggeler, Howard and Lucia, 1946, Gleiss,1947). Lenoble (1898) remarked on the extensive sedimentation that took place before clotting, which, however, did not alter the retraction. Fonio (1936) noticed the curious behaviour of the clot in some cases of haemophilia. The fibrinogen coagulated in stages and some of the fibrin clot started retracting before all the fibrin was formed. This phenomenon is frequently seen with the suspended clot method. The outer layer of the sphere coagulates first, starts contracting and squeezes out some of the blood from the inside of the clot, which in turn clots and subsequently contracts. This phenomenon does not alter the final retraction because a clot from the same haemophilic patient made to coagulate completely in less than a minute shows the same retraction. (Experiment 27).

McKhan and Edsall (1939) called the haemophilic clot hydrophobic, because they thought it was thready and webby, very rapidly retractile, and permitted the esaape of much of the contained fluid with many of the formed elements. They brought no experimental evidence in support of this statement. Andreassen (1943), on the other hand, stated that in haemophilia the retraction begins later that usually and proceeds slower, but the final result is normal. Jürgens and Studer (1948) found the retraction impaired, the defiency being corrected by the addition of thrombin.

Liver disease.

As early as 1898 Lenoble found absent clot retraction in patients with liver disease. This defect could not be

correlated with the severity of the disease or the intensity of the jaundice, and other cases had normal contraction. This discrepancy has been noticed many times since (Morawitz and Bierich, 1907, Kappis and Mackuth, 1930, Émile-Weil and Perlès, 1934, Carr and Foote, 1934, Boyce and McFetridge, 1937, Macfarlane, 1938). Émile-Weil, Bocage and Isch-Wall (1922) found a lowering of the number of platelets in hepatic cases; most of the counts were under 100,000 per cmm. and some right down to 25,000 per cmm. This would naturally explain the impaired clot retraction, but many cases of liver disease have a normal count and still the retraction is defective.

Aggeler and Lucia (1941) tried to correlate the poor retraction with the lowered prothrombin concentration, an attempt which they abandoned later (Aggeler, Howard and Lucia, 1946).

The obvious explanation of the discrepancy is the change which occurs in the fibrinogen concentration in liver disease. Only in very severe cases of liver atrophy is the fibrinogen diminished, otherwise the tendency is to a marked increase (Whipple and Hurwitz, 1911, Foster, 1924). Very high values may be reached, such as 1,200 mgs. per cent (McLester, Davidson and Frazier, 1925), or 1,150 mgs. per cent (Linton, 1932) which will completely inhibit retraction.

Thrombosis and Embolism.

Katrakis (1931) found that the onset of clot retraction after the blood was taken, i.e. the retraction time, was very constant in each individual, but that the occurence of

thrombosis in the patient shortened the time considerably. The degree of retraction was not affected. As mentioned, Lampert (1932) believed that retraction was the forerunner of embolism. The shortening of the retraction time was also found by Hirschboeck and Coffey(1943)and Hirschboeck(1948). Zahn (1944) believed that the degree of retraction was increased in thrombosis due to the increased fibrinogen concentration and the changes in acid/base balance of the blood in this condition.

It has already been intimated that the onset of retraction is dependent on external factors which are extremely difficult to control. All the evidence points to the fact that the stimulus to the platelets, setting off retraction, is given in the very earliest stages of the coagulation process, and the logical method to detect alterations in this mechanism would be to use some three-stage analysis of coagulation as suggested by Milstone (1948), by which the autocatalytic conversion of "prothrombokinase, and the subsequent change of prothrombin may be estimated.

Other conditions.

In table 11 are tabulated other conditions recorded in the literature in which deficient clot retraction could be explained by a diminished number of platelets:

Disease.	Author.
Anaemia.	
Aplastic	Aubertin, (1905 a & b) Rosenthal (1925 & 1928).
Pernicious	Hayem (1896) Lenoble (1898) Barbonnier & paisseau (1910) Rosenthal (1925 & 1928)
Refractory	Aggeler, Howard & Lucia (1946)
Splenic	Rosenthal (1925 & 1928) Evans (1929) Aggeler, Howard & Lucia (1946)
Bismarsen Sensitivity	Aggeler, Howard & Lucia (1946)
Cachexia	Hayem (1896) Bensaude (1897)
Infectious Mononucleosis	Aggeler, Howard & Lucia (1946)
Leukaemia	Aggeler, Howard & Lucia (1946)
Myelomatosis	Aggeler, Howard & Lucia (1946)
Pneumonia	Minot & Lee (1917)
Scarlet Fever	Boucin (1925)
Septicaemia	Raybaud & Scarpellini (1933a)
Smallpox	Hayem & Bensaude (1901)
Typhoid Fever	Raybaud & Scarpellini (1933a)
X-ray Therapy	Aggeler, Howard & Lucia (1946)

Table 11. Cases recorded in the literature in which deficient clot retraction could be ascribed to a diminished number of platelets in the blood.

Of more interest, because they have been a puzzle to those who observed them, are the cases of deficient retraction in spite of normal numbers of platelets. They are tabulated in table 12.

Disease.	Author.
Asthma	Thackrah (1819)
Bronchitis with emphysema	Paviot & Chevallier (1930)
Enteritis	Thackrah (1819)
Hodgkin's disease	Macfarlane (1938)
Myelomatosis	Stewart & Weber (1938) Lundsteen (1942)
Malignant disease	Lenoble (1898) Hayem (1900) Van Allen (1927)
Osteitis in Typhoid fever	Hayem (1900)
Pleurisy	Hayem (1900)
Pneumonia	Thackrah (1819) Hayem (1900) Bensaude (1897) Lenoble (1898) Macfarlane (1938)
Pregnancy	Thackrah (1819)
Rheumatoid Arthritis	Lenoble (1898)
Syphilis	Hayem (1900)
Uraemia	Lenoble (1898) Lundsteen (1943)

Table 12. Cases recorded in the literature of deficient clot retraction in spite of a normal number of platelets in the blood (liver diseases not included).

Thackrah naturally did no platelet counts in 1819, but his cases are included here as there is no reason to believe that the number of platelets was diminished in the conditions he described.

The striking feature common to all these conditions is the possibility of abnormally high fibrinogen concentrations in the blood. One need only think of the better known sedimentation rate, which depends on a disturbance of the plasma proteins including the fibrinogen, to realize the truth of this statement. Foster (1924) found high fibrinogen values in carcinoma, pregnancy, pneumonia, pyelitis and typhoid fever; in McLester, Davidson and Frazier's (1925) series values of 1400 mgs. per cent were found in carcinoma, pneumonia and tuberculosis. Nissen (1941) measured the degree of intoxication in pneumonia by the height of the fibrinogen which often went above 1250 mgs. per cent, and finally Stewart and Weber (1938) mention the enormous value of 5480 mgs. per cent in myelomatosis.

It is beyond doubt that the diminution in clot retraction in all these cases, which in many of them was not great, can be explained entirely by a raised fibrinogen concentration.

It may finally be mentioned that the few reported cases of abnormally high clot retraction have invariably been associated with anaemia (Aynaud, 1911a, Gleiss, 1947). Clot retraction in polycythaemia has only been found mentioned in one publication, and in those cases the fibrinogen concentration was extremely low and no proper clot formed (Björkman, 1948).

Glanzmann's Disease and Allied Disorders.

In 1916 Glanzmann drew attention to the low number of platelets in purpura, and in 1918 he published a series of cases under the name of Hereditary haemorrhagic thrombasthenia. This article has been misquoted repeatedly until at present the general conception is, that Glanzmann described a very rare condition of haemorrhagic diathesis in which the only demonstrable abnormality in the blood was absent clot retraction. This is very far from Glanzmann's original idea, and as it is of fundamental importance in connection with the present work whether a condition of this type exists or not, the article will be translated in some detail. This may also serve the purpose of bringing the misquotations to an end, especially by people who have obviously never read the original work. Estren, Médal and Dameshek(1946) have already drawn attention to some of this misconception.

After a short review of the status of the platelets at his time Glanzmann reports his observation that the number of platelets falls in the peripheral blood during anaphylactoid shock and in most febrile infections, the platelets agglutinating in the organs, particularly in the liver and lungs; in those conditions associated with exanthema in which the reaction mainly takes place in the skin and periphery this phenomenon is not found; in scarlet fever, measles, serum sickness and anaphylactoid purpura the platelets thus increase in the peripheral blood. He therefore suggests that the anaphylactoid purpuras are due to changes in the blood vessels comparable to

the other manifestations in the skin such as erythema, oedema and urticaria, and similar to the damage to the vessel walls due to faulty nutrition as in scurvy.

He now describes his first case, a child with acute purpura and makes the observation that as the platelets disappear from the blood the clot retraction becomes absent! He counts the platelets by the indirect method by which the normal count is about 400,000 per cmm. The clot retraction he estimates at room temperature in tubes of 8 mms.internal diameter, which as already pointed out is a most unfortunate method, as adhesion and inhibited retraction are bound to occur in an unpredictable number of the tubes.

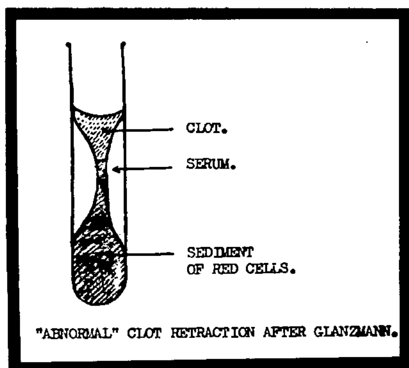
In this first patient the examination gave these results:-

Platelets 18,000 per cmm.-Clot retraction absent.
Platelets 48,000 per cmm.-Clot retraction absent.
Platelets 403,000 per cmm.-Clot retraction not certain.
Platelets 288,000 per cmm.-Clot retraction again normal.

The clinical observations and these findings were absolutely typical of Werlhof's disease, or what today is called thrombocytopenic purpura, and Glanzmann himself was certain of this diagnosis.

He goes carefully into the family history and examines several members with a history of a bleeding tendency. In an aunt of the child the clot retraction was "somewhat delayed but then strong". The clot first became loose at the bottom and was pulled strongly upwards, whilst the upper third of it did not separate from the glass wall. Plenty of serum was pressed out. The fibrin was apparently not diminished. There was no abnormal sediment of red blood corpuscles. One of her

children had 148,500 platelets per cmm. and absent retraction. The mother of the case had had "inflammation of the lungs" not less than six times. At the first examination the platelets were 342,000 per cmm. and retraction was absent, but after the patient had been treated with arsenic the clot retraction became normal, although an abnormal sedimentation of red cells took place. The father had 270,000 platelets per cmm. and normal retraction, and one brother suffering from pleurisy had 202,500 platelets, and of retraction there was only a slight indication at a small place low down at the glasswall. Another brother had 249,600 platelets per cmm., and the clot "pulled itself loose from the glass wall and formed a weak, central, narrow pillar. Below an abnormally rich sediment of red cells collected. He gives a picture of the clot which is reproduced in figure 39, and which he considers typical of abnormal retraction, a view that would certainly not be shared by others working on this problem. A third brother died of a fulminating form of "Morbus Werlhofii". He concludes this family investigation by stating that there were four cases definitely of Werlhof's disease with thrombocytopenia, two in the second and two in the third generation. In the second generation out of 12 members 6 suffered from haemorrhagic diathesis, and in the third generation three children had already shown signs of the condition.



"ABNORMAL" CLOT RETRACTION AFTER GLANZMANN.

Figure 39.

In the second family which he examined the mother had

112,500 platelets per cmm. and clot retraction "nearly absent". One daughter had 315,000 platelets and no retraction, but after treatment with Fowler's solution clot retraction recurred; another daughter had 365,000 platelets and there was evidence of retraction; lastly, one child died of typical Werlhof's disease.

In the third family the mother showed 317,000 platelets per cmm., but the clot retraction test was not performed; the daughter had 504,000 platelets per cmm. and clot retraction was present, although with an abnormally "rich sediment of red cells".

In the fourth family only one member was examined. She showed 320,000 platelets per cmm. and the clot retraction was "somewhat delayed".

In the fifth family the patient had 393,600 platelets per cmm and no retraction; her daughter had only 59,200 platelets and naturally no retraction.

In the sixth family again only one member was investigated; she had heart trouble and pain in the chest, cough and catarrh and no retraction in spite of 202,400 platelets per cmm.

The patient in family seven had urticaria, 403,000 platelets per cmm and no retraction.

In the eighth family the mother showed 216,000 platelets per cmm., but the coagulation remained "incomplete" and the clot did not retract. In the child the coagulation time was 20 minutes, but here some retraction occured with 302,000 platelets per cmm.

Finally, in the ninth family, again with only one member, the patient had 405,000 platelets per cmm., and the retraction

"was late and weak"; only in one narrow place did some serum come out.

Glanzmann now states:- "The morbus maculosus Werlhofii thus appears to be only an usually transitory, but particularly severe manifestation of a haemorrhagic diathesis for which I might suggest a name "Hereditary haemorrhagic thrombasthenia". In its lighter forms the diathesis is apparently very common". In his opinion the most characteristic finding is the large sediment of red cells outside the clot, which he calls abnormal clot retraction.

He performs some experiments which aim to show that the platelets from a case of Werlhof's disease shortens the clotting time of plasma more than normal platelets because they go more easily into solution, a contention which is not supported by other observations (Buchman, 1928, Fowler, 1937). His next conclusion from these experiments is that retraction depends on the ferment "Retraktozym" which is supposed to be lacking or be less active in "thrombasthenia".

Glanzmann considers the morphology of the platelets in Werlhof's disease or "thrombasthenia" distinctly abnormal, but the pictures submitted to support this contention are particularly unconvincing.

He then discusses the plasma, and from the increased blood flow from puncture wounds in thrombasthenia he concludes that the viscosity is diminished due to a diminished size of "molecules", which again must be due to diminished protein content and particularly fibrinogen. He compares the blood of

these cases to plasma diluted 10 to 20 times with water, and the rich sediment of red cells outside the clot might also indicate diminished fibrinogen; fibrinopenia is therefore part of the cause of the haemorrhagic tendency in Werlhof's disease.

The periods of severe manifestations of thrombasthenia which represents what other people consider to be "Werlhof's disease" are explained in this way:- the faulty platelets of the condition are destroyed faster than normal ones; as the megakaryocytes are also supposed to be abnormal, it is not surprising that they now and again become exhausted.

In the differential diagnosis the deficient clot retraction is most important. This deficiency is only found elsewhere in severe septic infections in which the platelets are functionally paralysed. It is not present in the anaphylactoid purpuras, in scurvy or in haemophilia. The coagulation in his cases was often incomplete:- "A division into a red and white plasmatic coagulum is often not apparent, and what is more, the top layer of plasma often remains quite fluid if the red cells have sedimented. This form of clotting can be imitated closely by dilution. There exists therefore a lowering of the fibrinogen content". It appears that he has observed fibrinolysis which, as mentioned, is not uncommon in purpura haemorrhagica. The bleeding time is usually normal corresponding to the number of platelets.

It is quite clear that this article is nothing more than an attempt to draw attention to the common occurence of milder forms of Werlhof's disease, or what today is called essential, primary

or idiopathic thrombocytopenic purpura, in other members of the family, an observation which had already been made by Hess (1916) and has been confirmed since (Krömeke, 1922, Witts, 1932, Wintrobe, Hanrahan and Thomas, 1937). Only very few of Glanzmann's cases had a normal platelet number.

No suggestion of a new syndrome was intended, although a new name was introduced for an old disease. The tracing of the transformation in the literature of this publication into the description of a special, exceedingly rare and unique syndrome is beyond the purpose of this work. Suffice it to say that the evidence for the functional abnormality of the platelets, and therefore their inability to produce clot retraction, is unacceptable.

Since Glanzmann's article a considerable number of cases of atypical haemorrhagic diathesis have been recorded, atypical in the sense that they do not fit into the more well-defined and more easily diagnostic groups. The pathogenesis and the cause of the bleeding in these cases have often been obscure, except when telangiectasia is obvious. Many of them show a distinct familial tendency. Most of the cases were reported before clinical tests for prothrombin were available, and with Quick's finding of families with congenital hypoprothrombinaemia and pseudo-hypoprothrombinaemia, and Owren's case of "Factor V" deficiency, and the ever increasing armamentarium for the investigation of coagulation and haemostatic defects, there is hope of more understanding of future cases of this order.

Estren, Médal and Dameshek (1946) in their most comprehensive review of these conditions under the name of "Pseudohaemophilia" list 62 cases described in some detail in the literature. In the 50 cases in which clot retraction was estimated it was normal in 43 and abnormal in only 6. In one case it was normal once and abnormal at another time. In these seven cases the method for the estimation is not stated or the abnormality is slight. Thus in Giffin's (1928) series of five cases 2 had normal retraction, in two the retraction was not estimated, and in one it was absent even after splenectomy. No indication of the method is given, but Kennedy (1928) who reported one of the same cases as Giffin and one other stated that the retraction was "none in 6 hours" and for his own case "retraction 24 hours"; the meaning is not clear. The same applies to the case of Kugelmass (1934); the findings were "absent clot retraction and Lysis 12 per cent". In Bailey and McAlpin's (1935) cases, occuring in two negro brothers, the retraction in the one did not begin until after 20 hours. The degree is not stated, and it was normal in the other patient. In Macfarlane's case the retraction was actually 34 per cent which can hardly be called abnormal, especially as the platelet count at that particular moment was not stated and the fibrinogen concentration not estimated.

A few more cases must be mentioned in this work, although the criteria for "pseudohaemophilia" as stipulated by Estren, Médal and Dameshek(1946) may not be entirely fulfilled. Krömeke (1922), discussing the question of Glanzmann's disease,

did not accept his views on the dysfunction of the platelets. In his own case which in all other respects was similar, the clot retraction was absent in the small tubes, but normal when estimated by the watch-glass technique. A case which has frequently been quoted as typical of hereditary thrombasthenia was described by Van der Zande (1923), but here the retraction was actually normal. Fonio's (1930) case of "infantile hereditory thrombasthenia of Glanzmann" had a clotting time of 35 minutes, and the clot retraction was 3 mms. instead of Fonio's normal 6 mms.

Farber (1934) described a series of cases of a familial haemorrhagic condition simulating haemophilia and purpura haemorrhagica in which the clotting time was normal; but the number of platelets was occasionally low, and when that occured the bleeding time was slightly prolonged and the clot retraction somewhat delayed.

Mathewson and Cameron (1937) quoted a case with 290,000 platelets per cmm., a bleeding time of 24 hours, and absent clot retraction. No method is indicated, but it was noticed that the clot was soft.

Mas y Magro's (1937) cases of a "haemophiloid" condition were similar to Glanzmann's cases, but had normal retraction. Of the 11 cases recorded by Estren, Médal and Dameshek (1946) one had a delayed retraction, but the degree was not stated. Woodbury's (1946) case of "pseudohaemophilia" had normal clot retraction; and finally the case of "constitutional thrombopathy" described by Quattrin (1947) had a very vigorous retraction.

It can now be stated with some confidence that if the three

main factors controlling clot retraction: platelets, fibrinogen and haematocrit are taken into consideration, and if the method employed has been at all reliable, no clinical case has been recorded in the literature in which the clot retraction has deviated from the laws laid down in the previous chapters.

In table 13 is presented the findings in 65 different clinical cases, using the methods described in this work. Many more cases have been investigated, but as one or other of the estimations was not carried out personally they are not included in the list. In the 65 cases tabulated the different investigations were all carried out on the same sample of venous blood, obtained by venipuncture of an arm vein and collected in a paraffin coated 30 ccs. syringe.

TABLE 13.

CLOT RETRACTION IN DIFFERENT CONDITIONS.

DISEASE.	SEX.	PLATELETS per cmm.	FIBRIN mg.%	HAEMATOCRIT. %.	OBSERVED RETRACTION. %.	CORRECTED RETRACTION (H+C.R.) %.	TIME OF ONSET Minutes	SEDIMENTATION RATE. mms.	ERYTHROCYTES per cmm.	CLOT STRENGTH. Minutes.
HEALTHY (Sleeping)	M.	645.000	280	47	37	84	14	3	5.050.000	16½
HEALTHY (After exercise)	M.	1.053.000	300	47	44	91	9	2	5.100.000	6½
HEALTHY (Food + exercise)	M.	1.580.000	340	47	50	97	8	4	5.100.000	5
HEALTHY (Before menstruation)	F.	330.000	280	46	34	80	35	9	5.050.000	17
HEALTHY (After menstruation)	F.	680.000	240	47	40	87	22	1	4.920.000	6¼
HEALTHY (After menopause; age 60)	F.	350.000	300	41	44	85	28	4	4.800.000	-
HEALTHY (Age 82 years)	M.	448.000	500	50	28	78	23	8	5.600.000	-
HEALTHY (Age 9 years)	M.	668.000	280	50	38	88	35	2	5.400.000	20
THROMBOCYTOPENIC PURPURA (Werlhof's disease)	F.	20.000	360	38	0	-	-	10	4.000.000	30
THROMBOCYTOPENIC PURPURA (Werlhof's disease)	F.	44.000	340	26	6	32	38	?	3.600.000	½
THROMBOCYTOPENIC PURPURA (Werlhof's disease)	F.	98.000	320	41	14	55	60	13	4.700.000	25
THROMBOCYTOPENIC PURPURA (Associated with menstruation; ?David's disease)	F.	188.000	300	40	22	62	34	15	4.700.000	20
THROMBOCYTOPENIC PURPURA (Acute in child with Otitis media)	F.	4.600	580	33	0	-	-	35	3.600.000	¼
THROMBOCYTOPENIC PURPURA (Sulphonamide sensitivity)	F.	None	170	26	0	-	-	60	3.100.000	6¾
THROMBOCYTOPENIC PURPURA (Acute myxoedema case treated with thyroid sicca).	F.	87.000	310	31	12	43	30	11	3.400.000	7
THROMBOCYTOPENIC PURPURA (Aspirin sensitivity)	F.	6.000	420	22	0	-	-	70	2.260.000	60
ATHROMBOPENIC PURPURA (?Pseudohaemophilia)	F.	400.000	680	28	24	52	12	46	2.210.000	-
ATHROMBOPENIC PURPURA (?Pseudohaemophilia)	F.	1.100.000	380	44	44	88	12	2	5.100.000	
ATHROMBOPENIC PURPURA (?Pseudohaemophilia)	M.	470.000	320	47	28	75	25	10	5.100.000	
FAMILIAL NOSE BLEEDING (Only slight purpura)	F.	877.000	310	46	37	83	6	8	4.800.000	7½
UNDIAGNOSED SPLENOMEGALY (Very severe purpura)	F.	1.666.000	330	48	52	100	8	1	5.500.000	-
UNDIAGNOSED SPLENOMEGALY (Severe bruising)	F.	730.000	220	38	50	88	7	12	4.100.000	10
SCHÖNLEIN-HENOCH'S PURPURA	M.	230.000	260	34	38	72	-	10	3.800.000	-
MYELOID LEUKAEMIA (ACUTE) (Purpura & gum-bleeding)	F.	87.000	410	8	26	34	18	89	980.000	16½
MYELOID LEUKAEMIA (Same case after transfusion)	F.	90.000	330	17	20	37	33	78	1.870.000	9
MONOCYTIC LEUKAEMIA (Severe purpura)	F.	50.000	800	10	4	14	65	87	1.710.000	-
MYELOID LEUKAEMIA (CHRONIC) (No purpura)	F.	311.000	300	26	46	72	12	42	3.100.000	18½
SUBACUTE BACTERIAL ENDOCARDITIS (Purpuric)	M.	400.000	340	46	36	82	25	4	4.790.000	-
PUERPERAL SEPTICAEMIA (Severe purpura)	F.	1.250.000	540	36	50	86	6	52	4.100.000	24
CARCINOMA OF THYROID (Marked purpura)	F.	407.000	420	32	40	72	22	30	3.540.000	35
WEIL'S DISEASE (CURED) (Purpura began 1 month later and has persisted)	M.	1.090.000	400	48	43	91	10	4	5.100.000	31
WEIL'S DISEASE (ACTIVE) (Jaundice; no purpura)	M.	327.000	560	44	20	64	-	36	4.800.000	-
HAEMOPHILIA	M	460.000	310	25	56	81	43	28	2.700.000	31
HAEMOPHILIA	M	1.050.000	600	48	32	80	165	12	5.100.000	lysed.
HAEMOPHILIA	M	935.000	580	35	44	79	-	51	3.800.000	10½
HAEMOPHILIA	M	204.000	290	40	28	68	68	2	4.600.000	-

TABLE 13. (continued).

DISEASE.	SEX.	PLATELETS. per cmm.	FIBRIN mg.%	HAEMATOCRIT. %	OBSERVED RETRACTION. %	CORRECTED RETRACTION. (H + C.R.) %	TIME OF ONSET. Minutes.	SEDIMENTATION RATE. mm.	ERYTHROCYTES per cmm.	CLOT STRENGTH. Minutes.
HAEMOPHILIA	M.	460.000	260	46	40	86	40	4	4.800.000	16
HAEMOPHILIA	M.	760.000	270	35	56	91	-	9	3.800.000	-
SCURVY	M.	500.000	380	37	46	83	17	40	4.730.000	30
SCURVY	M.	566.000	380	24	56	80	10	68	2.530.000	45
DICOUMARIN POISONING (Bleeding from kidneys and intestines)	M.	530.000	630	26	34	60	20	36	2.700.000	60
FAMILIAL TELANGIECTASIA	F.	560.000	300	47	38	85	15	6	5.100.000	6½
CONGENITAL OBLITERATION OF BILE DUCTS. (Deep jaundice)	F.	485.000	260	33	47	80	9	54	3.400.000	35
INFECTIOUS HEPATITIS (Slight jaundice)	M.	308.000	560	41	24	65	35	40	4.600.000	-
ALCOHOLIC CIRRHOSIS (Deep jaundice)	M.	334.000	520	50	20	70	30	15	5.400.000	70
THROMBOPHLEBITIS MIGRANS	M.	244.000	300	27	43	70	30	59	2.970.000	-
CORONARY THROMBOSIS WITH FEMERAL THROMBOSIS	M.	900.000	300	48	44	92	30	26	5.100.000	11
MALARIA (before Treatment)	M.	250.000	430	40	28	68	35	20	4.100.000	19
MALARIA (after Treatment)	M.	430.000	380	33	38	71	20	28	3.500.000	29
MALARIA (Before Treatment)	F.	130.000	270	29	36	65	8	47	3.200.000	40
PNEUMOCOCCAL PNEUMONIA (Second day).	M.	1.040.000	810	43	28	71	9	29	4.800.000	45
PNEUMOCOCCAL PNEUMONIA (Fifth day).	M.	610.000	920	44	20	64	14	40	4.500.000	13
PNEUMOCOCCAL PNEUMONIA (Sixth day).	M.	840.000	400	44	46	90	15	19	4.520.000	15½
PERNICIOUS ANAEMIA (Under treatment).	F.	360.000	300	32	42	74	19	10	4.000.000	-
HYPOCHROMIC MICROCYTIC ANAEMIA	F.	815.000	330	22	58	80	10	23	3.860.000	-
POLYCYTHAEMIA	M.	321.000	200	76	14	90	50	½	7.700.000	-
TYPHOID FEVER (Severe toxaemia - died)	M.	185.000	300	33	24	57	90	13	3.500.000	-
XANTHOMATOSIS (Cholesterol 405 mg.%)	M.	370.000	430	45	28	73	21	28	4.950.000	23
XANTHOMATOSIS (Cholesterol 700 mg.%)	M.	500.000	530	36	36	72	14	49	4.200.000	72
NEPHROSIS	M.	1.210.000	460	40	50	90	15	50	4.420.000	9½
URAEMIA (Blood urea 320 mg.%)	M.	401.000	320	46	38	84	32	1	4.950.000	-
MYXOEDEMA	F.	250.000	240	35	36	71	30	48	3.800.000	-
CARCINOMA OF UTERUS	F.	720.000	240	47	40	87	14	1	5.100.000	18½
DUODENAL ULCER	F.	1.044.000	320	33	60	93	8	-	4.400.000	12
GASTRIC ULCER	F.	850.000	310	34	48	82	11	15	4.250.000	-

Without exception, in all cases investigated, the clot retraction has been directly dependent on the number of platelets, the fibrin concentration in the clot, and the volume of packed cells. This relationship is shown in the

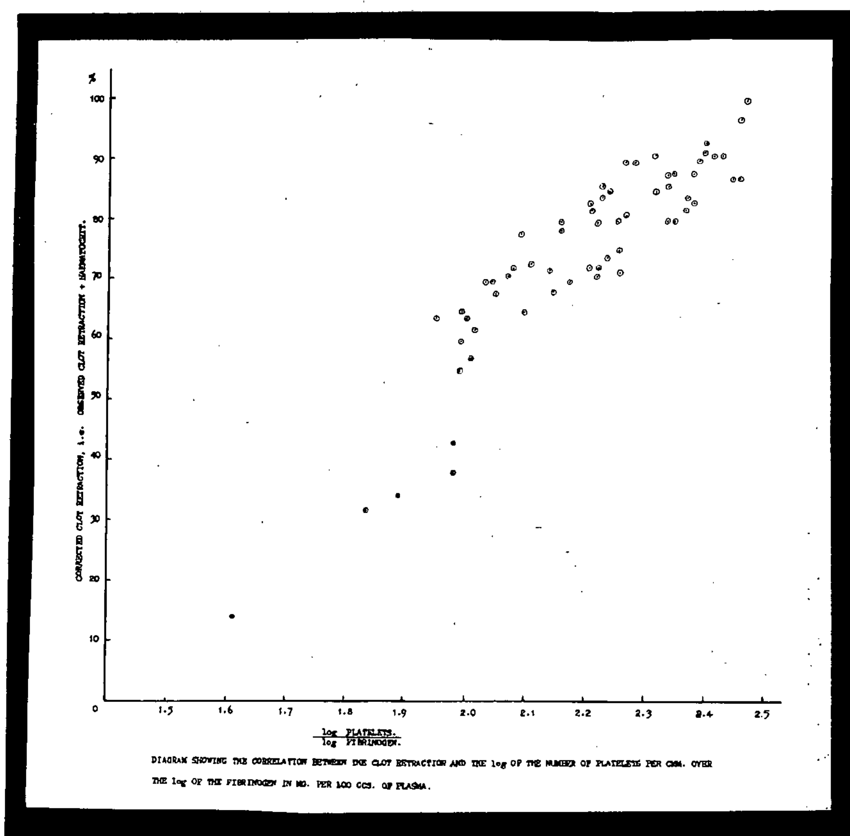


DIAGRAM SHOWING THE CORRELATION BETWEEN THE CLOT RETRACTION AND THE log OF THE NUMBER OF PLATELETS PER CMM. OVER THE log OF THE FIBRINOGEN IN MG. PER 100 CCS. OF PLASMA.

Figure 40.

scatter-diagram in figure 40 in which the corrected clot retraction, i.e. the observed clot retraction plus the volume of packed cells, has been plotted against the logarithm of the number of platelets over the logarithm of the fibrin content in mgs. per 100 ccs. of plasma, in the cases listed in table 13. In consideration of the unavoidable errors occuring in these estimations the correlation is very close and strongly supports the contention which was derived from the experimental part of the work, that clot retraction is a function of the platelets, impaired by increasing concentration of fibrin, and dependent on the volume of red and white cells in the blood.

The theoretical possibility of functionally deficient platelets still exists, but no convincing case of such a condition has ever been recorded in the literature, and none was found in this investigation.

CHAPTER 10.

GENERAL SUMMARY.

The principal facts arising from the investigations in this work are:-

1. Clot retraction is due to a special function of the platelets. During the early stages of the coagulation process, which may be initiated by the contact of the blood with foreign surfaces or the liberation of tissue-juice in the body, the platelets receive a stimulus causing them to undergo a series of morphological changes conveniently described as "viscous metamorphosis". The viscous metamorphosis leads to the formation of long strands or pseudopodia of cytoplasm connecting individual platelets or platelet-masses. These strands subsequently contract producing a patchy fusion of platelets throughout the clot. As the platelets are adherent to the fibrin network of the clot the contraction of the cytoplasmic strands and the merging of the platelets result in a contraction of the whole fibrin clot with a squeezing out of the serum and a few of the red and white cells enmeshed in the fibrin network. This is the

phenomenon observed in the gross and conventionally called clot retraction.

Experimental evidence is produced which indicates that the essential stimulus to the platelets is supplied during the conversion of the prothrombin complex into thrombin, but it is pointed out that our present knowledge of the factors concerned in this phase of the coagulation mechanism does not permit dogmatic statements with regard to the particular substance or substances starting the viscous metamorphosis and therefore the clot retraction. One fact, however, appears certain: only minute quantities are necessary to commence the process which then proceeds independent of all other plasma fractions within physiological limits.

These observations are supported by the analogy they bear to the clotting mechanism in lower animals. In the crab clot formation and retraction take place in the complete absence of fibrin and are entirely cellular functions.

2. It is demonstrated that the serum is held in the fibrin network forming the clot by capillary forces. When the clot is placed in rigid structures like glass tubes gravity tends to draw the serum out of the fibrin network against the capillary forces holding it back; an artificial "clot retraction" may thus be produced. This fact alone, which has not previously been taken into consideration, has led to most of the confusion surrounding the problem of clot retraction. It is shown that the size of the meshes in the fibrin network, and therefore the capillary forces holding the serum in the clot, depends mainly

on the concentration of fibrinogen in the plasma. The more fibrin is formed, the narrower the meshes become, and the greater is the force necessary to press out the serum from the clot in the time clot retraction takes place. It unavoidably follows that clot retraction is inversely proportional to the fibrinogen concentration of the plasma. This has been shown experimentally to be true.

3. The third factor of major influence on clot retraction is the volume of red and white cells in the blood. These cells play a passive, obstructive role, and it is demonstrated experimentally that an almost linear, inverse relationship exists between the volume of packed cells and the clot retraction. The most natural correction for this factor consists in bringing the retraction back to what it would be were no cells present in the plasma; for practical purposes this correction can be achieved simply by adding the haematocrit to the observed clot retraction.

4. As clot retraction is a biological phenomenon it is profoundly influenced by the temperature at which it takes place. Up to 42°C the retraction becomes faster and more extensive with increasing temperature. Above 42°C the retraction is inhibited. Rigid control of the temperature is therefore essential in all work on clot retraction.

5. It has been generally accepted that the character of the clot, its firmness, rigidity and elasticity, depends on the presence or absence of platelets in the fibrin network. It is demonstrated that this contention is wrong. These characters

depend nearly exclusively on the fibrin concentration and the occurence or absence of fibrinolysis. They are completely independent of the platelets. It is also demonstrated that fibrinolysis and clot retraction are independent processes. Retraction is completed before the destruction of the clot due to fibrinolysis becomes apparent, even in cases of great proteolytic activity. The possibility remains that in very severe cases the clot may be destroyed before it has retracted, in which case the estimation of retraction becomes impossible.

6. From the delicate platelet-mechanism producing clot retraction it would be expected that the force exerted by the process is very small. By a simple experiment this assumption is shown to be correct; the force is so insignificant that it becomes extremely unlikely that clot retraction serves any physiological purpose in mammalian life. It can certainly not draw together the walls of even the smallest vessels. It is suggested that the process is a reduntant phylogenetic relic of no importance in higher animal life. Haemostasis is accomplished by more efficient mechanisms in these animals.

8. A critical survey of the literature and personal investigation of a limited but representative clinical material indicate that the conclusion drawn from the experimental work are supported by clinical observations. In all recorded cases, in which reasonable investigations have been carried out and the methods employed have been at all reliable, clot retraction has followed, without exception, the rules deducted from the experimental observations. It is therefore ventured that most of the obscurities which beclouded the problem of clot retraction have been clarified,

which was the original object of this work. In the future lies the elucidation of the "viscous metamorphosis" of the platelets, the borderland between physical chemistry and biology.

APPENDIX 1.

In this part the experimental procedures are described. The aim has been to give sufficient detail to allow the repetition and checking of the results.

In an investigation of this type very many experiments are planned and carried out which later are found to be superfluous, poorly devised, or failures, and although the greater proportion of the research worker's time is thus occupied, the recording of these misfits would serve no good purpose and has therefore been omitted. The numerous unsuccessful attempts to find a method for the estimation of the number of platelets, and the days wasted in the attempts to observe the normal retraction process under the microscope, were the outstanding failures in this work.

Experiment 1.

The influence of the container on the clot retraction of normal blood.

Seventy ccs. of fresh venous blood from a normal donor

were measured off in varying amounts into different containers. The vessels were placed in an incubator at 37°C. for 24 hours after which time the amounts of serum, including red cells, expressed from the clots were estimated by carefully pouring them into graduated tubes, holding the clot back in the original vessels with a thin glass rod.

The type of container, the special treatment to which it was submitted, and the results are tabulated in Table 2.

Experiment 2.

The demonstration of the artificial "retraction" which may occur due to the force of gravity, and its independence of the temperature.

Fifty ccs. of blood were obtained from a normal donor using silicone covered needle, syringe and glass ware. The blood was centrifuged at 3000 r.p.m. for 5 minutes. Two control clots of 5 ccs. each were suspended in the trichlorethylene-liquid paraffin mixture, as described in chapter 1, and kept at 4°C. and 37°C. respectively. The remainder of the blood was spun in ice at 4000 r.p.m. for 30 minutes. The cell-free plasma was then diluted with the same amount of saline and 2 lots of 5 ccs. transferred to test-tubes. The rest of the plasma was again diluted with an equal amount of saline and 5 ccs. suspended as before as control and kept at 37°C. Another 2 lots of 5 ccs. each were transferred to test-tubes. One test-tube with the plasma

diluted 1:1 and one with the plasma diluted 1:4 were kept at 4°C., and the other two at 37°C. After 1 hour the clots in the test-tubes were carefully loosened from the sides, and the tubes were left standing for 5 minutes. The serum exuded from all the clots was then poured into graduated test-tubes and measured.

The fibrinogen concentration of the original undiluted plasma was estimated by the gravimetric method described in Appendix 2.

The results are presented below and tabulated in Table 3.

TYPE OF CLOT	SERUM EXUDED.
Platelet containing clot with 324 mgs. fibrin per 100 ccs, kept at 4°C in the suspension mixture	28%
Platelet containing clot with 324 mgs. fibrin per 100 ccs., kept at 37°C in the suspension mixture	72%
Platelet-free clot with 81 mgs. fibrin per 100 ccs., kept at 37°C in the suspension mixture	0%
Platelet-free clot with 162 mgs. fibrin per 100 ccs., Kept at 4°C in test-tube	48%
Platelet-free clot with 162 mgs. fibrin per 100 ccs., kept at 37°C in test-tube	52%
Platelet-free clot with 81 mgs. fibrin per 100 ccs.,	66%
Platelet-free clot with 81 mgs. fibrin per 100 ccs., kept at 37°C in test-tube	70%

The experiment demonstrates clearly the independence on changes in the temperature of the artificial exudation of serum from platelet-free clots. True clot retraction occurring

in platelet-containing clots is profoundly influenced by different temperatures.

Experiment 3.

The prevention of artificial "retraction" due to gravity by high fibrin concentration in the clot.

Five ccs. of pure fibrinogen solution containing 1000 mgs. fibrinogen per 100 ccs. were mixed with 3 drops of thrombin solution and the mixture rapidly transferred to the suspension mixture in which it clotted in a few seconds.

After one hour, during which time no retraction took place, the clot was lifted out of the suspension fluid and placed on a glass slab. No fluid ran out of it even after 6 hours, when the clot started drying up.

Experiment 4.

The wettability of fibrin.

A chemically clean glass tube of about 1 mm. internal diameter and graduated in millimetres was held vertically in distilled water and the height to which the water rose in the tube was measured.

The inside of the tube was now coated with fibrin by sucking platelet-containing plasma into it and letting it run out after a few seconds. This was repeated three times, leaving a thin film of fibrin on the glass. The tube was then again held vertically in distilled water and the height of the column of water measured.

HEIGHT OF COLUMN OF WATER IN CLEAN GLASS TUBE - 28 mms.

HEIGHT OF COLUMN OF WATER IN FIBRIN-COATED TUBE - 11 mms.

In spite of the bore being less in the fibrin-coated tube the water rose much less in it than in the clean glass tube. The experiment indicates that the wettability of fibrin is less than that of glass.

Experiment 5.

The error of the suspended clot method.

Sixty ccs. of fresh venous blood from a normal donor were collected in two ordinary syringes and run into porcelain dishes. 5 ccs. lots of the blood were pipetted into the suspension fluid in 10 glasses, which were kept at 37°C. in a water-bath. The retraction was estimated as usual. The results are tabulated in the table.

Glass No.	First bubble of serum seen after taking the blood. Minutes.	Total serum, including red cells. ccs.	Volume of clot. ccs.	Clot Retraction. %
1.	30	2.2	2.8	44
2.	26	2.2	2.8	44
3.	24	2.3	2.8	45.1
4.	22	2.2	2.8	44
5.	26	2.2	2.8	44
6.	32	2.2	2.8	44
7.	25	2.1	2.8	43
8.	26	2.2	2.8	44
9.	28	2.2	2.8	44
10.	25	2.2	2.8	44

It should be remembered that the reading of the volume of the clot, which is done in a measuring cylinder.

graduated with 0.2 ccs' divisions, is not a very accurate procedure.

As a further check the test-tubes used in this experiment were cleaned and 3 ccs of distilled water run into each from an accurate pipette. The readings in the test-tubes are shown below.

Graduated Test-tube No.	Reading ccs.
1	3.05
2	3.00
3	3.10
4	3.00
5	3.05
6	3.00
7	3.00
8	3.05
9	3.10
10	3.00

It is therefore obvious that with the instruments employed in the test the inherent variability of the suspended clot method is negligible, and it was deemed unnecessary to submit the results to statistical analysis.

Experiment 6.

The influence of contact of the blood with wettable or foreign surfaces before coagulation on the subsequent clot retraction.

Fifteen ccs. of blood was taken from the cubital vein of a patient with a recent thrombosis of the left femoral vein, care being taken to avoid undue admixture of tissue juice,

and using a syringe covered with a thin layer of liquid paraffin.

Five ccs. of the blood were run directly from the syringe into the suspension fluid, 1 cc. each was collected in 3 small test-tubes for the estimation of the coagulation time by the method of Lee and White, and the rest of the blood was run into a porcelain dish from which 5 ccs. were transferred to the suspension fluid with a pipette.

The results were as follows:-

Coagulation time:- 3½ mins. (Lee and White's method).

In the sample run directly from the syringe into the suspension fluid:-

Signs of clotting (stiffening of the clot): 9 mins.
First bubble of serum seen:- 24 mins.
Total retraction:- Completed in 2¾ hours.
Clot:- 1.6 ccs.
Serum:- 2.6 ccs.
R.b.c.'s expressed with the serum:- 0.2 ccs.

<u>Clot retraction:- 63%</u>

In the sample pipetted from the porcelain dish:-

Signs of clotting:- 4½ mins.
First bubble of serum seen:- 14 mins.
Total retraction:- Completed in 1⅞ hours.
Clot:- 1.8 ccs.
Serum:- 2.9 ccs.
R.b.c.'s 0.2 ccs.

<u>Clot retraction:- 63%</u>

The contact with foreign surfaces thus speeds up the onset and the completion of the retraction, but does not influence the

degree of the retraction or the exudation of red cells with the serum.

Experiment 7.

The effect of the temperature on the clot retraction.

Forty ccs. of blood were obtained from a normal donor and 7 portions of 5 ccs each were suspended in the usual way. The beakers containing the suspended clots were kept at different temperatures in the following manner.

1. Kept at 39°C. in a water-bath.
2. Kept at 25°C. in a water-bath.
3. Kept at 16°C. room temperature.
4. Kept at 10°C. in running tap water.
5. Kept at 4°C. in refrigerator.
6. Kept for 40 minutes at 39°C. in water-bath, then transferred to 4°C. in refrigerator.
7. Kept for 6 hours at 4°C. in refrigerator, then transferred to 39°C. in water-bath.

The beakers were left at these temperatures for 4 days. No dissolution or lysis of the clots took place. The retraction was then estimated. The results are given in the table below and shown in the curve in figure 16.

Clot No.	Temperature °C.	First serum seen. Hours.	Clot retraction %
1.	39	½	52
2.	25	2/3	46
3.	16	1½	38.5
4.	10	2½	34
5.	4	6	26
6.	39° for 40 m. then 4° for 4 days		32% after 40 mins 41% after 4 days
7.	4° for 6 hours then 39° for 4 days		6% after 6 hours 40% after 4 days

The experiment demonstrates that up to 39°C. the clot

retraction increases with increasing temperature. Changes in temperature during the retraction act in a similar way.

Experiment 8.

The effect of different temperatures on clot retraction, when applied before coagulation takes place.

Portions of 6 ccs. each of blood were run directly from the cubital vein of a normal donor through a silicone coated needle into 5 silicone coated test-tubes which were kept at different temperatures for 10 minutes. The samples of blood were then transferred to the suspension fluid with an ordinary pipette, and the beakers kept at 37°C. until clot retraction was complete.

Test-tube No.	Temperature of test-tube before coagulation. °C.	Clot retraction. %
1.	0	36
2.	7	36
3.	13	36
4.	22	36
5.	37	36

Exposure of the blood to different temperatures before coagulation does not influence the subsequent clot retraction.

Experiment 9.

The application of the formula of Aggeler, Lucia and Hamlin (1942) for the "extracorpuscular or fluid volume of the clot.

One hundred ccs. of blood were collected from a normal donor using the silicone technique. The blood was

run directly into a silicone coated tube through a silicone coated needle without the use of a syringe. It was centrifuged at 3000 r.p.m. for 5 minutes at 0°C and most of the plasma poured into another silicone coated tube kept at 0°C. The remaining plasma containing the red cells was now defibrinated with a wooden stick to remove any remaining platelets. The red cells were washed in saline and centrifuged again. The plasma and the washed cells were then mixed in graduated test-tubes in different proportions to a total of 5 ccs. in each, and samples pipetted off into heparin-coated Wintrobe tubes for the estimation of the haematocrits. Corks with the copper wire spirals, as used in this method, were now fitted to the graduated test-tubes which were placed in the water-bath at 37°C. for 1 hour.

The results were:-

Haematocrit %	Serum + r.b.c.'s expressed. ccs.	R.b.c.'s expressed. ccs.
0	4.35	0
14	3.80	0
24	3.40	0.05
52	2.40	0.25
62	1.55	0.40

Applying the formula for the correction to these values the following results are obtained:

$$\frac{(\text{Total volume of blood}) - (\text{Serum minus r.b.c's})}{\text{Total volume of blood.}} \times 100 - (\text{Haematocrit})$$

$$\frac{5 - (4.35 - 0)}{5} \times 100 - 0 = 13$$

$$\frac{5 - (3.8 - 0)}{5} \times 100 - 14 = 10$$

$$\frac{5 - (3.4 - 0.05)}{5} \times 100 - 24 = 9$$

$$\frac{5 - (2.4 - 0.25)}{5} \times 100 - 52 = 5$$

$$\frac{5 - (1.55 - 0.4)}{5} \times 100 - 62 = 15$$

Using Macfarlane's correction for the same values the results are:

$$\frac{(\text{Percentage of serum expressed} - \text{r.b.c.'s}) + (\text{Haematocrit})}{2}$$

$$\frac{87 + 0}{2} = 43.5\%$$

$$\frac{76 + 14}{2} = 45.0\%$$

$$\frac{67 + 24}{2} = 45.5\%$$

$$\frac{43 + 52}{2} = 47.5\%$$

$$\frac{23 + 62}{2} = 42.5\%$$

The index of the "extracorpuscular or fluid volume of the clot" varies therefore greatly when applied to samples of the same blood in which the only change is in the volume of red and white cells. The much simpler correction suggested by Macfarlane gives better results.

Experiment 10.

The correlation between the clot retraction and the volume of red and white cells in the blood.

The experiment was carried out exactly as the previous one, except that the suspended clot method was employed for the estimation of the clot retraction. The results are given in the table and shown in the form of a curve in figure 17.

Haematocrit %	Clot retraction (including exuded r.b.c.'s) %.
0	72.5
8	66.6
18	53.6
25	47.9
32	40.8
42	28.0
64	2.0

There is thus a direct correlation between the haematocrit and the clot retraction.

Experiment 11.

The effect of the volume of cells on clot retraction, when the retraction is abnormally low.

The procedure of the previous experiment was followed, but in this case the centrifugation was done at 4000 r.p.m. for 15 minutes to remove more of the platelets and thus reduce the retraction. The results are given in the table and plotted in the curve in Figure 18.

Haematocrit %	Clot retraction (including exuded r.b.c.'s) %
0	20.8
15	16.0
29	4.0

The experiment demonstrates that the inhibition of clot retraction by increasing volume of cells is less in blood with low clot retraction than in normal blood.

Experiment 12.

The relationship between the haematocrit and the amount of red cells exuded with the serum during clot retraction.

The experiment was carried out exactly as experiment 10, but after pipetting off the serum from the suspension mixture into the graduated test-tubes, these were centrifuged at 4000 r.p.m. for 10 minutes and the volume of cells exuded with the serum measured. The results are presented in the table and plotted in the graph in figure 18.

Haematocrit %	Serum + r.b.c's ccs.	R.b.c.'s ccs.	Clot. ccs.
0	4.4	0	0.7
7	4.1	0	0.9
30	3.0	0.2	2.0
39	2.5	0.35	2.5
64	0.9	0.23	4.1
70	0.4	0.15	4.6

The results are easier to evaluate when expressed slightly differently:-

Haematocrit. %	Clot retraction (including exuded red cells). %	Red cells as percentage of total volume of blood. %	Red cells as percentage of serum expressed. %
0	86	0	0
7	82	0	0
30	60	4	6.7
39	50	7	14
64	18	4.6	25.5
70	8	3	37.5

The experiment indicates that the greater the haematocrit the larger the amount of red cells exuded with the serum during clot retraction.

Experiment 13.

The effect of the volume of leucocytes on clot retraction.

Blood was obtained from a patient suffering from myeloid leukaemia (leucocyte count: 478.000 per cmm), and the procedure described in experiment 10 was followed; but the cells added to the plasma were pipetted off the white cells layer obtained after washing and centrifuging the cells. The separation into white and red cells was not complete, but only very few red cells were transferred to the plasma. The table gives the results:-

Volume of packed leucocytes. %	Clot retraction. %
0	74
21	60
43	33

The inhibiting effect of leucocytes on clot retraction is therefore similar to that of red cells.

Experiment 14.

The influence of the sedimentation of red cells on clot retraction.

Twenty-five ccs. of blood were taken from a case of Hodgkin's disease with a sedimentation rate of 121 mms. in one hour (Westergren's method). 5 ccs. were run directly into the suspension fluid from the syringe, and the rest was transferred to a porcelain dish. Of this blood 5 ccs. were immediately pipetted into suspension fluid, and after 3 minutes another 5 ccs were transferred.

The first sample of blood clotted in about 26 minutes and showed marked sedimentation of the red cells; the clot became pearshaped due to the higher specific gravity of the red cells before it commenced contracting. The second sample clotted in 17 minutes after much less sedimentation, and the third sample clotted a few minutes after it was transferred to the suspension fluid and no sedimentation occurred. The clot retraction was estimated in the usual way:-

First clot with marked sedimentation - Clot retraction 56%
Second clot with some sedimentation - Clot retraction 54%
Third clot with no sedimentation - Clot retraction 53%

The experiment demonstrates clearly that the presence or absence of sedimentation of the red cells in the same blood does not influence the degree of subsequent clot retraction.

Experiment 15.

The influence of clot retraction on the weight of the solid part of the clot.

Twenty-five ccs. of blood were obtained from a normal donor by the silicone technique and spun for 5 minutes at 3000 r.p.m. in the cold. Two samples of 5 ccs. each of the plasma were suspended in the usual way. Five minutes after clotting and before any serum squeezed out between blotting papers. The clot was washed in saline followed by distilled water, alcohol for 10 minutes, a mixture of alcohol and ether for 10 minutes, and finally ether alone for 10 minutes. It was then dried to constant weight at 105°C. The second clot was treated in exactly the same manner after it had contracted completely during 2 hours.

The weights of the dried clots were:

1. Non-retracted clot - 19.5 mgs, or 390 mgs. per 100 ccs. plasma.
2. Retracted clot - 19.0 mgs, or 380 mgs. per 100 ccs. plasma.

There is thus no significant difference between the weights; clot retraction takes place without loss of weight of the solid part of the clot, and therefore presumably without fibrinolysis.

Experiment 16.

The relationship between the amount of platelets in the

plasma and the clot retraction.

One hundred and twenty-five ccs. of blood was obtained from a normal donor by running the blood directly from the arm vein through a silicone covered needle into a large silicone coated centrifuge tube kept on ice. The blood was dentrifuged at 3000 r.p.m. for 5 minutes and the plasma pipetted off into other silicone coated, cooled tubes. Half the plasma was now centrifuged at 15,000 r.p.m. for 10 minutes to render it cell free (no platelets were seen under the microscope in a sample taken for the purpose). The platelet-containing plasma and the platelet-free plasma were mixed in the proportions shown in the table, and the mixtures were run into the suspension fluid using ordinary clean pipettes; the retraction was estimated as usual.

The actual number of platelets in the platelet-containing plasma was estimated by pipetting 3 lots of 0.2 ccs each into samples of 2 ccs. of freshly filtered Dameshek's platelet solution in small silicone coated cups. After mixing the solutions were transferred to 3 different counting chambers and 15 minutes allowed for settling of the platelets. The counts were then done by three different people, counting all the small squares on the slides. The fibrinogen content of the platelet-free plasma was estimated by the gravimetric method. The results of the experiment are given below, and the correlation between platelets and clot retraction is shown graphically in figure 21.

Platelet-containing plasma ccs.	Platelet-free plasma. ccs.	Percentage of platelets in mixture %	Serum expressed ccs.	Clot ccs.	Clot retraction %
6.0	0.0	100	5.0	0.25	95.24
4.5	1.5	75	4.9	0.4	92.45
3.0	3.0	50	4.0	1.2	76.92
1.5	4.5	25	2.8	2.2	56.00
1.0	5.0	16.6	2.4	2.9	45.28
0.5	5.5	8.3	1.5	3.4	30.61
0.25	5.75	4.16	0.9	3.9	18.75
0.1	5.9	1.6	0.5	4.4	10.20

Platelet count; Observer 1 - 171,000
Observer 2 - 167,910
Observer 3 - 165,950

Average:- 168,280 per cmm. of plasma.

Fibrinogen:- 300 mgs. per 100 ccs. of plasma.

There is thus a direct correlation between the number of platelets in the plasma and the clot retraction.

Experiment 17.

The correlation between the fibrinogen content of the plasma and its clot retraction.

Thirty ccs. of blood were obtained from a normal donor by the usual silicone technique and centrifuged in the cold for 5 minutes at 3000 r.p.m. The plasma was mixed with saline and a 2% fibrinogen solution in the proportions shown in the table below. The clot retraction of the mixtures was estimated by the suspended clot method. The fibrinogen content of the original plasma was found by the gravimetric method. The results are presented in the table and the correlation shown in the graph in figure 22.

Fibrinogen content of undiluted plasma; 350 mgs. per 100 ccs.

Plasma ccs.	Saline ccs.	Fibrinogen solution (2.000 mgs.% ccs.	Fibrinogen content of mixture. mgs.%	Clot retraction %
2.0	1.0	0.0	233	95.8
2.0	0.8	0.2	366	90.0
2.0	0.6	0.4	500	74.2
2.0	0.4	0.6	633	31.0
2.0	0.2	0.8	766	0
2.0	0.0	1.0	900	0

The experiment demonstrates that increasing fibrinogen concentration diminishes clot retraction until the retraction is completely inhibited.

Experiment 18.

The correlation between the fibrinogen content of whole blood and its clot retraction.

The same technique as in the previous experiment was followed, except that the blood was not centrifuged, and the fibrinogen solution used was only of 1% concentration.

Fibrinogen content of the blood; 202.5 mgs. per 100ccs of plasma.

Blood ccs.	Saline ccs.	Fibrinogen solution (1.000 mgs %) ccs.	Fibrinogen content of mixture mgs.%	Corrected Clot retraction (Observed retraction + Haematocrit) %
8	2.2	0	135	93
8	1.8	0.4	195.6	87
8	1.2	1.0	286.5	73
8	0.8	1.4	347.1	71
8	0.4	1.8	407.7	60
8	0	2.2	468.3	45

Increase in the fibrinogen content of the whole blood decreases its clot retraction.

Experiment 19.

Dilution of plasma within wide limits does not influence the clot retraction.

With the usual silicone technique 30 ccs. of blood were taken from a normal donor and centrifuged in the cold for 10 minutes. The plasma was diluted with physiological saline (0.85%) in the proportions shown in the table, and the clot retraction of the mixtures estimated by the suspended clot method

Plasma ccs.	Saline ccs.	Clot retraction. %
5.0	0.0	22
4.5	1.0	22
3.5	2.0	22
2.5	3.0	22
1.5	4.0	22

The experiment demonstrates that even great dilution with saline does not alter the retraction of platelet containing plasma.

Experiment 20.

The influence of variations in the hydrogen ion concentration on clot retraction.

Forty ccs. of blood were obtained from a normal donor, and samples from the blood were mixed with N/10 Na OH and N/10 HCl in the proportions shown in the table The clot retraction of the mixtures was estimated by the suspended clot method. After the measurement of the serum it was centrifuged free of cells and the pH roughly estimated by means of universal indicator.

Blood ccs.	Acid or Alkali	Saline ccs.	pH of serum	Observed Clot retraction. %
4.0	0.4 ccs Na OH	0.6	8.4	22
4.0	0.2 ccs Na OH	0.8	7.8	48
4.0	0.1 cc Na OH	0.9	7.6	49
4.0	-	1.0	7.4	46
4.0	0.1 cc HCl	0.9	7.4	50
4.0	0.2 ccs HCl	0.8	7.3	48
4.0	0.4 ccs HCl	0.6	7.0	46

Wide variations - from a physiological point of view - in the pH of the blood do not influence the clot retraction,

Experiment 21.

The effect of the sodium chloride concentration in the blood on clot retraction.

Fresh blood mixed with different solutions of sodium chloride in the proportion stated in the table. The clot retraction of the mixtures was estimated by the suspended clot method.

Blood	Solution of NaCl.	Observed Clot retraction.
3 ccs.	1.5 ccs of 0.85%	64.4%
3 ccs.	1.5 ccs of 1.00%	66.6%
3 ccs.	1.5 ccs.of 2.00%	66.6%
3 ccs.	1.5 ccs of 3.00%	66.6%

Wide variations in sodium chloride concentration in the blood do not affect the clot retraction.

Experiment 22.

To demonstrate that calcium is essential for clot retraction.

A silicone coated needle was carefully inserted into the cubital vein of a normal donor; about 10 ccs. of blood were allowed to flow through the needle to wash away any tissue juice liberated by the puncture. About 15 ccs. of blood were then run into a silicone coated test-tube containing 50 mgs. of potassium oxalate in a drop of saline; the tube was kept on ice. After rapid mixing 3 ccs. of this oxalated blood were transferred to another tube containing 1 cc. of thrombin solution (0.3 ccs. of Lederle's "Haemostatic Globulin" in 250 ccs. saline). The blood was then run into the suspension fluid and the retraction estimated as usual. To a similar mixture 0.05 ccs. of N $CaCl_2$ solution were added and the blood suspended as before. The results were:

3 ccs. oxalated blood + 1 cc thrombin solution - Clot retraction absent.

3 ccs. oxalated blood + 1 cc thrombin solution + 0.05 ccs N aCl_2 solution - Clot retraction 52%

The whole experiment was repeated, but this time 0.2 ccs. of a 42.2% sodium citrate solution was substituted for the 50 mgs. of potasium oxalate. The results were quite different:-

3 ccs. citrated blood + 1 cc thrombin solution - Clot retraction 48%

3 ccs. citrated blood + 1 cc thrombin solution + 0.05 ccs N $CaCl_2$ solution - Clot retraction 54%

Finally, the blood was taken from the vein with an ordinary sterile needle and syringe and run into a clean test-tube containing 50 mgs. of potassium oxalate. The experiment was then continued as before:

3 ccs. oxalated blood + 1 cc thrombin solution - clot retraction 42%

3 ccs. oxalated blood + 1 cc thrombin solution + 0.05 ccs N $CaCl_2$ solution - clot retraction 48%

It should be pointed out that unless the greatest care is exercised in the manipulations in the first part of the experiment the result will be unsuccessful. It has, however, been possible to repeat the experiment on many occasions.

These findings suggest that a trace of calcium is essential for clot retraction to take place. They also indicate that if the very first stage of the coagulation process is allowed to occur, the platelets will become "activated", and subsequent removal of calcium in the system does not prevent clot retraction.

Experiment 23.

The effect of excess calcium on clot retraction.

Twenty-five ccs. of blood was obtained from a normal donor and mixed with a 1% $CaCl_2$ solution in the proportions given in the table. The retraction of the mixtures was estimated in the usual way.

Blood ccs.	$CaCl_2$ solution (1 mg $CaCl_2$ in 0.1 cc) ccs.	Saline ccs.	Approximate Concentration of Ca mgs.%	Observed clot retraction %
5.0	0	0.2	10	47
5.0	0.05	0.15	17.2	48
5.0	0.10	0.10	28.4	39
5.0	0.20	0	38.8	34

(In the calculation of the calcium concentrations the haematocrit of the blood was arbitrarily taken as 50% and the calcium concentration of the original plasma as 10 mgs. per 100 ccs. The purpose of the experiment did not warrant the trouble of actual estimation of these values.

The experiment indicates that only calcium concentrations well above physiological limits significantly inhibit clot retraction.

Experiment 24.

Clot retraction in a simplified clotting system.

Fifty ccs. of blood were taken from a normal donor through a silicone coated needle into a silicone coated tube containing 0.5 ccs. of 42.2% sodium citrate. The tube was kept on ice. The blood was centrifuged at 3000 r.p.m.

for 5 minutes. Portions of 5 ccs. each of the plasma were now pipetted into 4 smaller silicone coated test-tubes which were centrifuged at 4000 r.p.m. for 15 minutes in the cold. The plasma was poured off and the platelets resuspended in about 5 ccs of saline. The tubes were centrifuged again and the saline renewed. The washing was repeated 3 times. In the end the platelets were suspended in about 0.5 ccs of saline, and to these suspensions were added fibrinogen, prothrombin, thrombin and $CaCl_2$ solutions, and fresh serum in the proportions stated below. The retraction of the mixtures was estimated in the usual manner.

<u>Tube 1.</u>
Platelets + 2 ccs Fibrinogen sol. + 3 ccs Prothrombin sol. + 0.05 ccs. $CaCl_2$ sol. ---<u>Clot retraction 60%</u>

<u>Tube 2.</u>
Platelets + 2 ccs Fibrinogen sol. + 3 ccs Thrombin sol. ---<u>Clot retraction 0%</u>

<u>Tube 3.</u>
Platelets + 2 ccs Fibrinogen sol. + 3 ccs Thrombin sol. ---<u>Clot retraction 0%</u>

<u>Tube 4.</u>
Platelets + 2 ccs Fibrinogen sol. + 3 ccs Thrombin sol. ---<u>Clot retraction 42%</u>

(In this type of experiment no reliance can be placed on the actual degree of clot retraction. The number of platelets added and their individual damage during the process of washing are uncontrollable factors. It is also essential to repeat experiments with washed platelets several times to assure that absent retraction is not due to the use of inactive platelet suspensions.)

prothrombin into thrombin as the stimulating factor in clot retraction.

Experiment 26.

The influence of the concentration of prothrombin in the blood on its clot retraction.

A. Blood was obtained from a patient on treatment with dicoumerin. He was at that time bleeding from his kidneys and intestines. The clot retraction was estimated on one sample of the blood. To another sample 0.5 ccs prothrombin solution were added and the clot retraction found of the mixture.

The original blood had these haematological characters:-

Erythrocytes :-	3.100,000 per cmm.
Haematocrit :-	26%
Platelets :-	530,000 per cmm.
Fibrinogen :-	630 mgs. per 100 ccs of plasma.
Coagulation Time :-	13 minutes (Lee and White's method).

Quick's one stage prothrombin time:-
Patient's clotting time - 85 seconds.
Control's clotting time - 26 seconds.
Prothrombin concentration therefore about 17%

Observed clot retraction :- 34%

Corrected clot retraction = Haematocrit + observed retraction
= 60%

After addition of the prothrombin solution the findings changed:-

Haematocrit :- 22%
Coagulation time:- $3\frac{1}{2}$ minutes.
Observed clot retraction:- 40%

Corrected clot retraction = 62%

B. An even more spectacular case was a baby, aged 12 days, suffering from haemorrhagic disease of the newborn and deeply jaundiced. The experimental data are less satisfactory due to the inherent difficulty in obtaining blood from a patient of this age.

Coagulation time of recalcified plasma:- 25½ minutes.

Quick's one stage prothrombin time:-
Patient's clotting time in first test :- 960 seconds.
in second test :- 1980 seconds.
Control's clotting time :- 21 seconds.
Only a trace of prothrombin can be present in this blood.

Clot retraction of recalcified plasma:- 95 per cent.

Both these cases demonstrate that there is no correlation between the prothrombin concentration and the clot retraction; maximal retraction occurs in the presence of sufficient prothrombin to produce clotting of the blood.

Experiment 27.

The effect of "thromboplastin" in the form of brain extract on clot retraction.

Fifteen ccs. of blood were obtained from a haemophlic patient; 5 ccs. of the blood were mixed with 0.5 ccs. of saline and the clot retraction estimated as usual. To another 5 ccs. of the blood were addéd 0.5 ccs. of a brain extract suspension and the mixture was run into the suspension fluid in the usual manner.

The blood-saline mixture showed signs of coagulation in

45 minutes during which time marked sedimentation of the red cells took place. When the outer layer had clotted it began contracting, and some plasma was squeezed out from the inside of the clot. This plasma soon coagulated and later contracted in the normal way.

The blood-brain suspension mixture coagulated completely in 43 seconds. There were naturally no signs of sedimentation of red cells.

The clot retraction of the two clots were:-

1. 5 ccs haemophilic blood + 0.5 ccs saline - Observed retraction 68%
2. 5 ccs haemophilic blood + 0.5 ccs brain extract suspension - Observed retraction 68.4%

The experiment demonstrates that excess of thromboplastin in the form of brain extract does not influence the clot retraction. The extent of the retraction is not dependent on the speed of the formation of the fibrin.

The experiment incidentally confirmed the previous finding that the occurrence of sedimentation of the red cells does not affect the subsequent retraction of the clot.

Experiment 28.

The effect of thrombin on clot retraction.

Twenty ccs. of blood were taken from a normal donor. To 5 ccs were added 1 cc of saline, and the clot retraction of the mixture was determined. The rest of the blood was mixed with thrombin solution in the proportions stated in the table.

and the retraction estimated. The transfer of the mixtures to the suspension fluid had to be extremely rapid as the blood clotted within a few seconds; the experiment miscarried several times for this reason.

Blood ccs.	Saline ccs.	Thrombin solution ccs.	Observed clot retraction. %
5	1	0	46
5	0.5	0.5	48
5	0	1	49

Addition of thrombin to the blood does not significantly alter the clot retraction.

Experiment 29.

The influence of dilution with serum on clot retraction.

Platelet-containing plasma was obtained in the usual way by the silicone technique. It was mixed with fresh and with 5 days' old serum as shown in the table, and the clot retraction was estimated.

Plasma ccs.	Fresh serum ccs.	5 days' old serum ccs.	Clot retraction. %
5	0	0	82
2.5	2.5	0	80
2.5	0	2.5	84

Dilution of plasma with serum, fresh or old, does not affect the clot retraction.

Experiment 30.

The effect of heparin on clot retraction.

Twenty ccs. of blood was obtained from a normal donor and was mixed with heparin in different proportions. The clot

retraction was estimated as usual; the results are given in the table.

Blood ccs.	Heparin 0.01 mg. per cc. ccs.	Saline ccs.	Observed clot retraction. %
5	0	1	62.2
5	0.5	0.5	61.3
5	1.0	0	no clot formed.

The experiment indicates that heparin does not influence clot retraction as long as a proper clot forms.

Experiment 31.

The effect of bile salts on clot retraction.

Plasma was obtained by the silicone technique and mixed with sodium desoxycholine in different proportions. The retraction was estimated as usual.

Plasma ccs.	Sodium desoxycholine mgs. added.	Final concentration. mgs. %	Clot retraction. %
5	0	0	88
5	1	20	90
5	5	100	86

Bile salts in the form of sodium desoxycholine, even in concentrations greater than found clinically, does not influence clot retraction.

(The experiment was first tried with whole blood, but considerable haemolysis occured which interfered with the results.

Experiment 32.

To demonstrate that the character of the clot - apart from its retractility - is independent of the presence or absence of platelets in the plasma from which it is formed.

Twenty-five ccs. of blood were collected from a normal donor and spun for 5 minutes in the cold. 5 ccs. of the platelet-containing plama were suspended in the usual manner. The remaining plasma was centrifuged at 15.000 r.p.m. for 10 minutes in the cold in silicone covered tubes to remove all cells. 5 ccs. of this platelet-free plasma were now suspended. After coagulation, but before the platelet-containing clot had started to contract, the two clots were lifted out of the suspension fluids and photographed. The clots are shown in figure 24, and it is obvious that there is no difference in their character; they are equally firm and rigid.

The strength of the clots was measured in the"clot-cutter" illustrated in figure 25. The strength of the clot is expressed as the time taken for a given weight - in all the experiments recorded in this work the weight was 250 gms. - to pull the three wires through the clot formed from 5 ccs. of the blood or plasma to be tested when suspended as a sphere in the suspension fluid. In this particular experiment the times were:-

Platelet-containing clot(before retraction) - 12 minutes.
Platelet- free clot - 13 minutes.

The whole experiment indicates clearly that there is no significant difference in firmness, rigidity, or strength between platelet-free and platelet-containing clots formed from the same plasma.

Experiment 33.

The influence of fibrinolysis on clot retraction.

A patient with a slight hypochromic anaemia was for other

purposes given a transfusion of 500 ccs. of normal citrated blood. About one hour after the transfusion the patient felt faint and became pale and cold. She recovered in 10 minutes time from this untoward effect.

Some of the pertinent investigations carried out on the patient's blood are recorded in the table.

Time	Haematocrit. %	Platelets in venous blood. per cmm.	Fibrin mgs.%	Corrected retraction %	Fibrinolysis.	Clot strength minutes.
Before transfusion.	37	736,000	240	96.3	Absent	16
15 minutes after transfusion.	39	965,000	320	98.0	Absent	20
30 minutes after transfusion.	40	758,000	340	90.0	Visible	9
60 minutes after transfusion.	40	707,000	320	88.3	Complete	0
120 minutes after transfusion.		731,000	320	91.8	Strong	¼
240 minutes after transfusion.		843,000	320	90.0	Absent	15

The fibrinolysis of the clots affected became apparent about 3½ hours after the blood was taken. The clot strength was measured 6 hours after the coagulation of the blood.

This experiment demonstrates in a clear manner that clot retraction is entirely independent of fibrinolysis. It also shows the relationship between fibrinolysis and the strength of the clot. Figure 26 illustrates its fluid character.

Experiment 34.

The mechanism of clot retraction.

One cc of platelet-containing citrated plasma was mixed with 0.1 cc of a 1 per cent solution of trypsin and kept for 10 minutes at 37°C. The plasma was now recalcified, and drops of it were transferred to clean slides and covered with cover-slips. The slides were kept on the warm-stage at 37°C., and the clotting observed under oil-immersion. The rapid disappearance of the fibrin net-work could be watched; about 10 minutes after clotting all the fibrin had disappeared. The "viscous metamorphosis" of the platelets with the formation of long "pseudopodia" which eventually drew the platelets together could clearly be followed. This process, which satisfactorily explains all the phenomena of clot retraction, is illustrated in figure 37.

Experiment 35.

The inhibition of clot retraction by heating the blood to 42°C.

Fifteen ccs. of blood were obtained from a normal donor and run into 0.15 ccs. of 42.2 per cent sodium citrate solution. Half of the blood was kept at 37°C. for 2 hours and the other half at 42°C. for the same length of time. The specimens were now recalcified, and the clot retraction was estimated.

1. Blood heated to 37°C for 2 hours - Observed retraction 34%
2. Blood heated to 42°C for 2 hours - No retraction occured.

Heating to 42°C. for 2 hours thus complete inhibits clot retraction.

Experiment 36.

The effect of dyes on clot retraction.

Twenty ccs. of blood were taken from a normal donor. 5 ccs. were pipetted into the suspension fluid as a contral, 5 ccs. were mixed with 0.5 ccs. of a 1 per cent aqueous solution of crystal violet, and another 5 ccs. with a similar solution of netral red. The retraction of these mixtures was determined as usual. The results are given in the table.

Specimen.	Observed clot retraction. %
5 ccs. normal blood.	43
5 ccs.blood +0.5 ccs.crystal violet solution.	0
5 ccs.blood +0.5 ccs. neutral red solution.	38

Crystal violet will thus completely inhibit clot retraction, whereas neutral red has no significant effect.

Experiment 37.

The force of clot retraction.

A thin bag of collodion was prepared by coating the inside of a small round bottle with a layer of collodion meth.flex. After drying the bag was carefully pulled out of the bottle.

Platelet-containing plasma was obtained by the usual silicone technique. The clot retraction of this plasma was 88 per cent. Twenty ccs. of the plasma were run into the collodion bag, the bag was attached to the Westergren tube and was then submerged in the suspension mixture, kept at 37°C. Figure 38 illustrates the experimental procedure.

After clotting the serum began to rise in the Westergren

tube.

The serum rose 19 mms. above the surface of the clot in 25 minutes; it then remained stationary.

Three hours later the bag was cut open; no sèrum run out of it immediately, indicating that none had accumulated between the bag and the clot, which would obviously give a fälse result. The clot actually had to be peeled off the bag and was torn in the process.

The experiment indicates that the force exerted by normal clot retraction, even when not impaired by the presence of red cells, is very small indeed.

A P P E N D I X 2.

This part describes the technical methods used in the investigations.

1. Erythrocyte count.

The red cells were counted in a Spencer bright-line counting chamber with improved Neubauer ruling. The dilution of the blood was done with Hayem's fluid - 1:200 - in the pipette supplied with the chamber. 80 small squares were counted in each case.

2. Sedimentation rate and Haematocrit.

Heparinised blood was pipetted into Wintrobe's haematocrit tubes. The tubes were suspended in a gimbal arrangement which kept them absolutely vertical. They were left hanging for one hour at room temperature after which the sedimentation was recorded. No correction for anaemia has been made in the recorded results.

The tubes were now centrifuged for 30 minutes at 3,000 r.p.m. In reading the volume of packed cells the white cell layer was included in all cases. As these investigations were not of paramount importance in the present work, only one estimation was done in each instance.

3. Platelet count.

One drop of venous blood was run directly from the needle and syringe with which the blood was taken into 5 drops of Dameshek's platelet fluid. The fluid was kept in a small cup made of paraffin wax. The mixture was carefully shaken. One drop was transferred with a paraffin covered platinum wire loop to a clean coverslip which was then placed on a clean slide. 10 minutes were allowed for the staining of the platelets. The count was done with the oil-immersion lens, and the number of platelets seen during the counting of 2000 red cells was recorded. A simple calculation gives the number of platelets.

$$\text{Number of platelets per cmm.} = \frac{\text{Red cell count x Number of platelets recorded.}}{2000.}$$

Dameshek's fluid is made up as follows:

Brilliant cresyl blue......0.15 gms.
Sodium citrate ....0.40 gms.
Sucrose ....8.00 gms.
Distilled water ....100 ccs.

After solution of the solids the fluid is filtered.

It was found that the best spread of the cells was obtained by washing the slides and cover-slips with soap and water, and after drying polishing them with xylol and a silk cloth.

The actual counting was facilitated by cutting down the field by means of a piece of black paper with a small hole in the middle. The paper was placed in the eye-piece of the microscope.

4. Fibrinogen estimation.

Five ccs. of citrated, oxlated or heparinised blood were centrifuged for 30 minutes at 4000 r.p.m. to remove most of the cells from the plasma. Two ccs. of the plasma were mixed with

13 ccs. of a solution of thrombin (0.3 ccs. of Lederle's "Haemostatic Globulin" in 250 ccs. of physiological saline). The mixture was kept at 37°C. for 15 minutes to ensure complete clotting. A glass rod was pushed through the clot and the serum was squeezed out by twisting the rod. The clot was successively washed in saline, distilled water, alcohol, alcohol-ether mixture and finally ether alone. It was then dried for one hour at 110°C, which was found to give a constant weight.

With this method the "fibrinogen concentration" must be defined as the amount of thrombin-coagulable fibrinogen present in the plasma which is not necessarily the total amount of this protein. It might be more correct to speak of the "fibrin concentration". In the previous pages both expressions have been used at random in this particular sense.

It may suitably be pointed out here that gravimetric fibrinogen estimations carried out by recalcification instead of thrombin coagulation are unreliable, because variable amounts of the calcium compound becomes adsorbed on the fibrin. With oxalated blood the error introduced may amount to more than 300 mgs. per 100 ccs. of plasma.

5. Preparation of brain extract.

Normal human brain was stripped of membranes and large blood vessels, minced finely, washed thrice with three times its own volume of acetone, and filtered on a Büchner funnel until all acetone had evaporated. A 1 per cent emulsion of the dried brain was made in saline; the coarse particles were filtered down, and the supernatent fluid used as the source of thromboplastin.

6. Preparation of Factor V.

This plasma fraction was prepared from ox plasma according to the method of Owren (1947).

Hundred ccs. of twice Seits-filtered ox plama were mixed with 100 ccs. of distilled water and 100 ccs. of saturated $(NH_4)_2SO_4$. The mixture was centrifuged and the precipitate discarded. Another 100 ccs. of saturated $(NH_4)_2SO_4$ were added to the supernatent fluid; the precipitate was redissolved in 25 ccs. of physiological saline, and the solution was dialysed against 1000 ccs. distilled water (pH 7.0). The water was renewed after 2 hours, and the dialysis was continued for 4 hours. The solution was now centrifuged and the deposit discarded. The pH of the supernatent fluid was adjusted to 5.3 by addition of 1 per cent acetic acid, and the fluid was stored in the refrigerator for some hours. The solution was then centrifuged again; the supernatent fluid was poured off, and the residual fluid was adsorbed on filter paper. The sediment was redissolved in a few ccs. of physiological saline. The preparation was used on the same day on which it was prepared.

7. Prothrombin preparation.

The procedure of Owren (1947) was again followed.

350 ccs. of blood were collected from a normal donor and were run into a cooled, paraffined centrifuge tube containing 5 ccs. of 5 per cent potassium oxalate solution. The blood was centrifuged at 4000 r.p.m. for 30 minutes. It was left for 12 hours at 1°C, and was re-centrifuged in the cold. The supernatent fluid was collected and warmed for 5 minutes at 56°C. in thin-

walled tubes and again centrifuged. 100 ccs. of this heat coagulated plasma was diluted with 1.5 litres of cold distilled water, and 1 per cent acetic acid was added to pH 5.3. It was left for 30 minutes at 0°C., centrifuged, and the sediment was washed with distilled water. Extraction was done with about the same amount of a solution of 0.1 per cent $NaHCO_3$ and 0.1 per cent potassium oxalate. The solution was filtered in the cold to remove any undissolved material. 10 ccs. of 5 per cent $Mg(OH)_2$
in saline was added
in saline was added, and the mixture was stirred continuously for 15 minutes. The sediment was washed twice in distilled water and suspended in 25 ccs. of distilled water. The prothrombin was then liberated from the $Mg(OH)_2$ by CO_2 under 4 atmospheres of pressure. The CO_2 was released, the solution centrifuged and dialysed against water at pH 7 for 12 hours. The sediment was removed by centrifugation. After addition of NaCl to physiological concentration (0.85 per cent), and adjustment of the pH to 7.3, the solution was ready for use.

8. Preparation of fibrinogen.

The fibrinogen solution was prepared by the phosphate buffer precipitation method described by Jaques (1943), which gives nearly 100 per cent pure solutions of the protein.

Three molar phosphate buffer (Butler and Montgomery, 1932, Butler, Blatt and Southgate, 1935) was diluted to 2 molar strength before use. Equal volumes of human plasma and buffer were mixed; the precipitate was washed with molar phosphate buffer and redissolved in molar/4 buffer in slightly smaller volume than the original plasma used. The precipitation was repeated three times.

The final solution was made in 0.85 per cent saline containing 0.00765 molar sodium citrate.

The phosphate buffer is prepared by adding 817 gms. of potassium dihydrogen phosphate(anhydrous) to 1000 ccs. of distilled water. 750 ccs. of 4 N potassium hydroxide are added and the solution warmed gently to dissolve. When cool make to 2 litres and filter.

9. Silver staining of sections of fibrin clots.

For the photography of sections of human clots the following method was found most satisfactory. It was worked out by Mr.R.E.Duffett of the Department of Pathology, Radcliffe Infirmary, Oxford. The technique is:-

1. Take section - fixed in osmic acid - down with distilled water.
2. Mordant in 5 per cent tannic acid for ½ minute.
3. Rinse in distilled water for ½ minute.
4. Keep in silver bath for ½ to 1 minutes.
5. Wash in distilled water.
6. Fix in 20 per cent formalin.
7. Tone in 1:500 gold chloride for 5 to 10 minutes.
8. Keep in sodium thiosulphate for 1 minute.
9. Wash, dehydrate, clear and mount in Canada balsam.

(The silver bath is made up by adding ammonia drop by drop to 5 per cent $AgNO_3$ solution until the deposit first formed redissolves. To this mixture add more 5 per cent $AgNO_3$ until a faint opalescence develops.).

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The suspended clot method for the estimation of clot retraction, the measurement of the clot strength with the "clot-cutter", and other less important procedures have been described in detail in the main text.

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