

Originalarbeiten — Original Articles — Travaux Originaux

A Description of Clot Retraction as a Visual Experience^{*)}

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Clot retraction is a phenomenon which every biologist and physician looks for more or less carefully in all blood samples. After the blood is drawn it may take 30 minutes or even longer before retraction is complete or it may not occur at all. Inspection of the clot from time to time leaves the impression of looking at the same picture, the changes being slow. Our curiosity about the visual aspects of the phenomena led to the production of a time-lapse motion picture film in which events of about two hours duration pass in review in 15 seconds. The lively motion seen in this way provides a sensory experience quite useful for drawing conclusions not easily arrived at in any other way. We endeavored to see the motion of clot retraction under conditions that are considered quite elementary knowledge. However, there were situations where the outcome was doubtful on the basis of the literature we read. The results then established more firmly in our minds a preferred point of view which was documented just as any other "new" discovery in research. The record as a motion picture was supplemented with static photography and in this paper we present a documentation of clot retraction from that perspective.

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A. Materials

1. *Platelet-free plasma.* This was obtained by centrifugation of oxalated bovine blood, taken in silicone-treated containers.

2. *Dialyzed plasma.* 20 to 40 ml of bovine platelet-free plasma were dialyzed in an efficient dialyzer (1) for 6 hours against physiological saline solution changed every 2 hours.

3. *Plasma dialysate.* To obtain the material that passes through a dialysis membrane, 20 to 40 ml of bovine platelet-free plasma were dialyzed against 100—200 ml of distilled water for 3 hours. During dialysis the dialysate was stirred with a magnetic stirrer. The dialysate was then dried from the frozen state and stored. For clot retraction experiments it was dissolved in distilled water and the pH was controlled.

4. *Adsorbed plasma.* Bovine platelet-free plasma was mixed with barium carbonate (100 mg/ml of plasma) for 20 minutes at room temperature. The barium carbonate was removed by centrifugation and the adsorption was done 2 more times.

5. *Barium carbonate eluate.* The method described by Seegers and associates (2) was used. In principle, stored oxalated bovine plasma is thawed and clotted with calcium and lung thromboplastin; the serum is mixed with barium carbonate (100 mg/ml), the barium carbonate is separated, washed twice with saline and eluted in 5% sodium citrate solution. The eluate is fractionated by precipitation with ammonium sulfate; the single fractions are dialyzed against distilled water and dried from the frozen state. Before using, the protein was dissolved in physiological saline solution.

6. *Bovine platelet suspension.* These were obtained from bovine blood as described previously (2). The packed platelets were suspended in 4 volumes of physiological saline solution. Platelet suspensions of the day were used, and kept at 4° C till the moment of use.

7. *Dialyzed platelets.* 20 ml of platelet suspensions were dialyzed in an efficient dialyzer (1) for 6 hours against physiological saline solution, changed every 2 hours.

8. *Platelet dialysate.* To obtain the dialyzable materials from platelets 20 ml of platelet suspension were dialyzed against 100 ml of distilled water, by stirring with a magnetic stirrer. The dialysate was dried from the frozen state, stored and dissolved in distilled water at the moment of use, the volume being equal to that of the original platelet suspension.

9. *Commercial fibrinogen.* This was purchased from Armour & Co., Chicago. The fibrinogen was dissolved in buffered saline (9 vol. saline + 1 vol. Imidazole buffer pH 7.2).

10. *Purified fibrinogen.* This was bovine fibrinogen prepared by the method of Ware, Guest and Seegers (3).

11. *Plasma dialysate fractionation.* For the separation of phosphorylated intermediates in the plasma dialysate, the method of Umbreit (4) was used: after extraction with 5% trichloroacetic acid, three fractions are obtained: a) Barium-insoluble, b) Barium-soluble, alcohol-insoluble, c) Barium-soluble, alcohol soluble fraction. These fractions were analyzed for the phosphorylated compounds through paper bidimensional chromatographic method of Bandurski (5) (acid solvent: methanol-formic acid 88% — distilled water 80 : 15 : 5; alkalin solvent: methanol-ammonia 28% — distilled water 60 : 8.5 : 31.5); the dried paper was sprayed with the following liquid: HClO₄ 60% ml 10, 1 N HCl ml 20, (NH₄)₂MoO₄ 4% ml 50, H₂O ml 120. The development of the characteristic spots is completed after exposure at ultraviolet light for a short time.

12. *Resin thrombin.* Prepared as described by Seegers, Levine and Shepard (6).

13. *Clot retraction.* We used a simple semi-quantitative method, based upon the direct observation of speed extent of clot retraction in siliconed test tubes (16 × 150 mm). If not differently indicated the reagents were mixed in the following order:

- a) bovine whole oxalated blood, or bovine platelet-free plasma, or fibrinogen solution, 1 ml.
- b) platelet suspension or platelet substitutes, 0.5 ml.
- c) physiological saline solution or substances to be tested in physiological saline, 0.5 ml.
- d) calcium chloride 0.04 M 0.9 ml.
- e) thrombin (100 U/ml), 0.1 ml.

Each time at least three tests were made for the same conditions. Clotting appeared to be complete within about 15 seconds, and the test tubes were then left in a water bath at 37° C. Retraction, if present, usually began after about 10 minutes, and was complete within about 1 hour. Results of the clot retraction were recorded photographically at the end of 2 hours. For a quantitative evaluation of the results the following scale was adopted.

- = Absence of clot retraction (quantity of expressed fluid after 2 hours, 0—30% of the total volume)
- + = very little clot retraction (quantity of expressed fluid after 2 hours, 30—50% of the total volume)
- ++ = medium clot retraction (quantity of expressed fluid after 2 hours, 50—60% of the total volume)
- +++ = extensive clot retraction (quantity of expressed fluid after 2 hours, 60—70% of the total volume)
- ++++ = maximal clot retraction (quantity of expressed fluid after 2 hours, 70—95% of the total volume).

B. General Experimental Results

1. *Surface Contact.* The solutions were mixed as follows: platelet-poor plasma 1 ml, platelet suspension 0.5 ml, 0.9% NaCl 0.5 ml, CaCl₂ 0.04 M 0.9 ml, and thrombin 0.1 ml (Fig. 1). In the plain glass tube there was no clot retraction. Merely forcing a thin glass rod between the clot and the test tube wall — entire circumference — was followed by clot retraction. At the bottom there were usually a few strands of fibrin which were the last to let go as the clot pulled itself upward through its buoyancy. In paraffin or silicone lined tubes there was retraction without mechanical separation of the clot from the

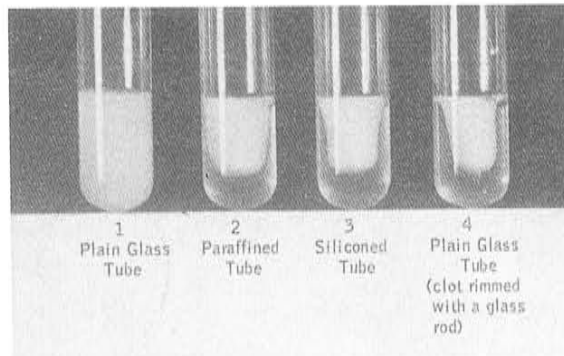


Fig. 1: Clot retraction in test tubes with different surfaces. Temperature, 37° C. Time, 2 hours. Plasma, platelet, calcium and thrombin mixture.

wall. The shape of the two clots was, however, never exactly the same. There must be very subtle forces in operation that are not analyzed in this way of study.

2. *pH of Solutions.* Mixtures consisting of 1.0 ml platelet-poor plasma and 0.5 ml platelet suspension were taken to a volume of 2 ml with NaOH 0.1 M and 0.9% NaCl or HCl 0.1 M and 0.9% NaCl so that the pH varied from 5.5 to 9. After addition of 0.1 ml thrombin 100 U/ml and 0.9 ml CaCl₂ 0.04 M and incubation for 2 hours at 37° C clot retraction was recorded. Optimum clot retraction was found at pH 7, whereas at both lower or higher pH values the process was not so vigorous (Fig. 2).

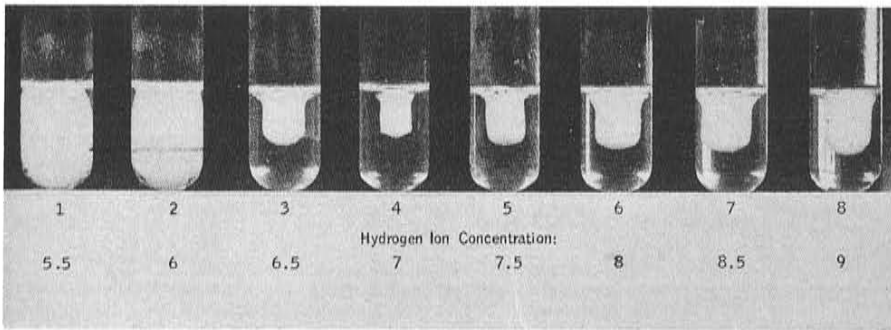


Fig. 2: Retraction of platelet-poor plasma adjusted to several pH values. Temperature 37° C. Time, 2 hours. Plasma, platelet, calcium and thrombin mixture.

3. *Thrombin Concentration.* Mixtures were composed of the following: 1.0 ml purified fibrinogen (600 mg^{0/0}), 0.5 ml platelet suspension, 0.5 ml normal plasma, 0.9 ml calcium chloride 0.04 M. To this 0.1 ml of thrombin solutions of different concentrations were added. After standing for 2 hours at 37° C, the photograph of Fig. 3 was taken. Clot retraction was extensive even at very low thrombin concentrations, but was always greater with the higher thrombin concentration. In a further discussion below, conditions are described where thrombin interferes with clot retraction.

4. *Calcium Ion Concentration.* The mixture was composed of 1 ml purified fibrinogen (600 mg^{0/0}), 0.5 ml platelet suspension, 0.5 ml normal plasma, 0.1 ml thrombin 100 U/ml. To this 0.9 ml of calcium chloride solution of different concentrations was added. An optimal clot retraction was obtained with the addition of 0.04 M calcium chloride concentration (Fig. 4). This gave a final concentration of 0.013 M; calcium ion concentrations, up to 0.1 M inhibit completely the retraction. It was interesting that NaCl, at the same molar concentration, does not inhibit. There is also an optimum with strontium salts.

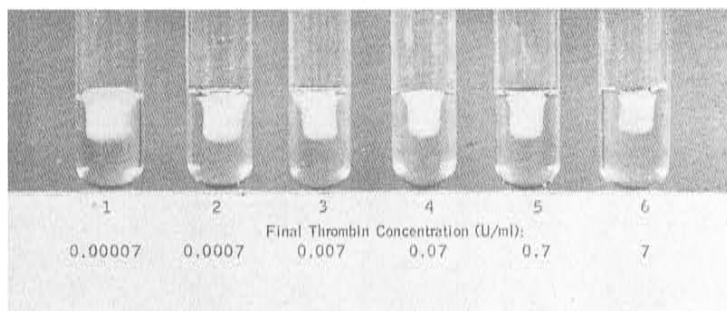


Fig. 3: Over a wide range of thrombin concentrations retraction of purified fibrinogen is associated with a noticeable difference only in the dilute solutions. Please compare also Fig. 14. Mixture of purified fibrinogen, platelets, plasma, calcium, and thrombin.

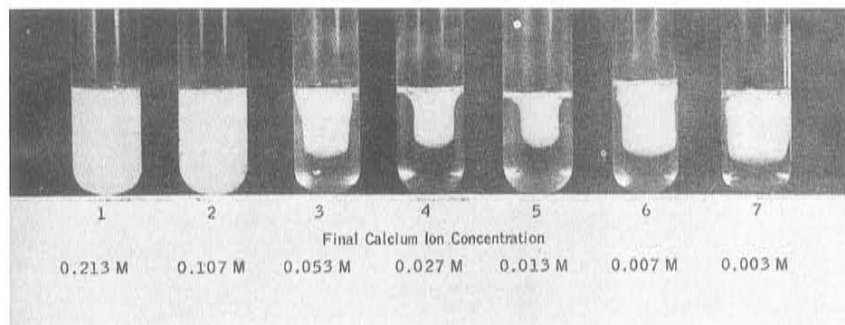


Fig. 4: Purified fibrinogen was clotted in presence of various calcium ion concentrations. Temperature, 37° C. Time, 2 hours. Mixture of purified fibrinogen, platelets, plasma, calcium, and thrombin.

5. *Storage of Platelets.* The platelet suspensions (packed platelets diluted 1:5 with physiological saline solution) were placed in a refrigerator at a temperature of 4° C. Tests for clot retraction were with the following: 1 ml platelet-poor plasma, 1 ml platelet suspension (not stored or stored for X days), 0.9 ml CaCl₂ 0.04 M, and 0.1 ml thrombin 100 U/ml.

On the fourth day of storage the clot retracting power was distinctly present, but on the fifth day it could just be detected. More or less five days is thus the maximum time for retaining clot retracting function of the platelets under these conditions (Fig. 5).

6. *Dialysis, Ultrasonic Treatment, and Freezing.* There is great sensitivity of the retracting function of the platelet to various damaging agents such as high and low temperatures, freezing, aging, irradiation with ultraviolet light or X-rays, ultrasonic treatment with a frequency above 175 kilocycles, homogenization, suspension in hypotonic solutions, or dialysis.

Dialyzed platelets were obtained by dialysis of whole platelet suspension against physiological saline at 4° C for 3 hours. Platelet dialysate was obtained

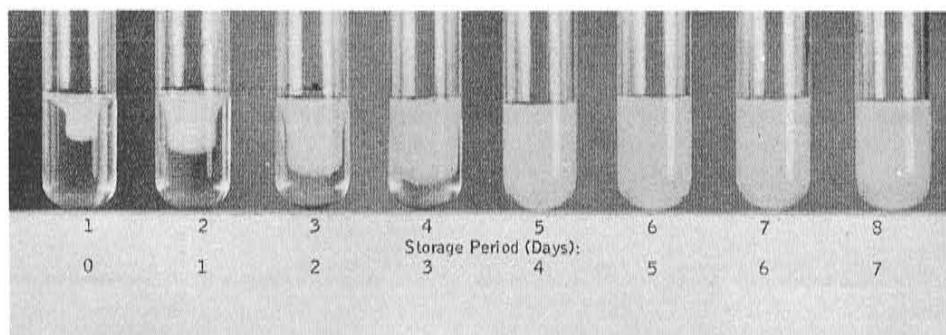


Fig. 5: A 1:5 suspension of platelets in physiological saline solution was stored at 4° C. In 5 days clot retraction function was lost. Mixture of plasma, stored platelets, calcium ions and thrombin.

from whole platelet dialysis against distilled water at 4° C for 3 hours; the dialysate was dried from the frozen state and dissolved in physiological saline up to the original platelet suspension volume. Then the following solutions were combined:

Tube No.	1	2	3	4	5
Whole platelet suspension	0.5	—	—	—	—
Dialyzed platelets	—	0.5	—	0.5	—
Platelet dialysate	—	—	0.5	0.5	—
Physiological saline	0.5	0.5	0.5	—	1.0
Platelet-poor plasma	1.0	1.0	1.0	1.0	1.0
Thrombin 100 U/ml	0.1	0.1	0.1	0.1	0.1
Calcium chloride 0.04 M	0.9	0.9	0.9	0.9	0.9
Total volume (ml)	3.0	3.0	3.0	3.0	3.0

The dialysis procedure abolished the platelet retractile property (Fig. 6); this property was not found in the platelet dialysate alone, nor was it found when the dialyzed platelets were combined with the platelet dialysate.

Ultrasonic frequencies are damaging to the clot retraction quality of platelets. Suspensions were placed in an ultrasonic generator (600 kc) for different periods of time. The test mixture consisted of platelet-poor plasma 1 ml, platelet suspension 1 ml, 0.04 M CaCl₂ 0.9 ml and 0.1 ml thrombin

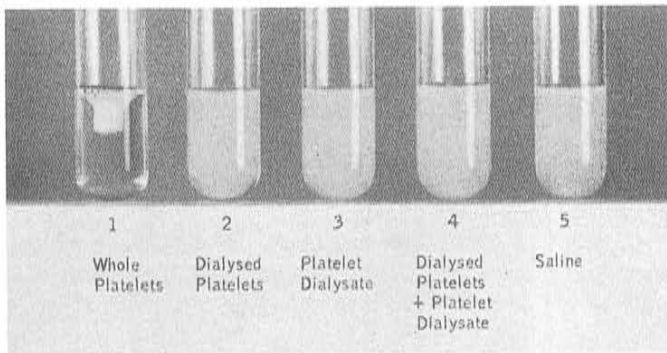


Fig. 6 : Platelet-poor plasma does not retract in presence of dialysed platelets, platelet dialysate, or the two combined. Temperature 37° C. Time 2 hours. Calcium and thrombin added.

100 U/ml. Even one minute in the ultrasonic apparatus was sufficient to make a great difference (Fig. 7). Eight minutes of treatment was associated with a complete loss of clot retracting function.

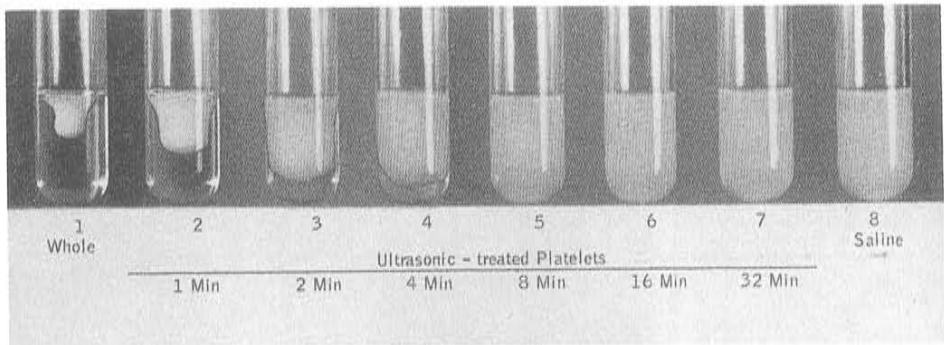


Fig. 7 : In an ultrasonic apparatus platelets lose "retracting" function very rapidly. Contraction of platelet-poor plasma at 37° C, in 2 hours, with treated platelets, calcium ions and thrombin.

The next work was begun on the basis of reading the paper by Hartmann and Conley (7), in which there is a description of the platelet retractile activity after freezing and thawing and the protective properties of glycerol.

Packed platelets were diluted 1 : 5 with various glycerol-physiological saline mixtures. These platelets were "frozen" with alcohol-dry ice and thawed in a water bath at 37° C. This was done three times. The retraction test consisted of mixing 1 ml of platelet-poor plasma, 0.1 ml of thrombin 100 U/ml, 0.9 ml of calcium chloride 0.04 M, and 1 ml of the frozen and thawed platelets. Whereas the platelet suspension in saline (tube 1) lost the retractile property,

some clot retraction activity was preserved in the presence of sufficient glycerol (Fig. 8).

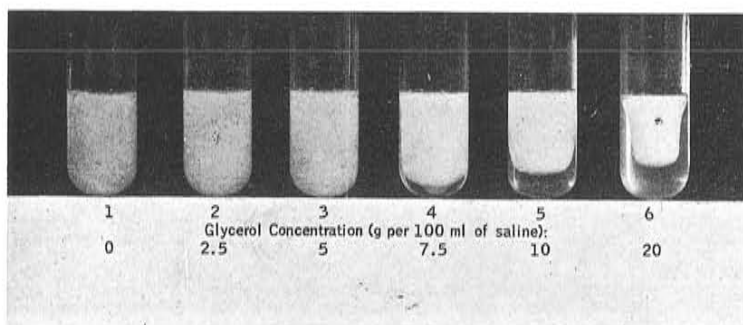


Fig. 8: Glycerol protects the platelet clot retracting function. The concentration of glycerol indicated refers to the conditions of the freezing and thawing of platelets. For the clot retraction the concentration of glycerol was one third owing to further dilution, with platelet-poor plasma, thrombin, and calcium chloride solution.

Having thus noted damage to platelets by freezing, dialysis, and ultrasonic waves, it was of interest to see that no fraction could consistently be prepared to substitute for platelets. Several fractions of platelets were tried in a series of concentrations. No retraction was seen with platelet-poor plasma plus calcium ions, plus thrombin, and the following in place of platelets: 1) alcohol extract of platelets, 2) acetone extract of platelets, 3) serotonin 0.06 M and 0.006 M^{*}), and purified platelet factor 3.

C. Mixtures of Platelets and Purified Fibrinogen

A system composed of fibrinogen, platelets, thrombin and calcium is not sufficient for complete clot retraction. However, such an "artificial system" sometimes shows a substantial clot retraction if the single components are not sufficiently purified: for example a commercial fibrinogen preparation may retract in the presence of platelets, calcium and thrombin whereas purified fibrinogen, (clottable protein content up to 98%) in the same conditions, may

^{*}) In some experiments there was some clot retraction with serotonin. To see an effect with serotonin the conditions must be arranged quite differently (8, 9, 10). To use the phrase "There is not clot retraction without platelets" we would have to say that effects seen with serotonin, and in the absence of platelets, is not clot retraction. Perhaps it could be called fibrin retraction. In any event a description of any phenomenon with platelets has nothing to do with a description of a phenomenon without platelets. In the presence of serotonin antagonists there is not clot retraction even with platelets (11, 14).

not retract. The degree of purity of thrombin is also important. We used thrombin preparations free of any clot retracting principle, that could not be ascribed to the enzyme thrombin.

For the study of fibrinogen the reagents were mixed as follows:

Tube No.	1	2	3	4
Platelet suspension	1.0	1.0	—	—
Commercial fibrinogen (600 mg ^{0/0})	1.0	—	1.0	—
Purified fibrinogen (600 mg ^{0/0})	—	1.0	—	1.0
Physiological saline	—	—	1.0	1.0
Thrombin 100 U/ml	0.1	0.1	0.1	0.1
Calcium chloride 0.04 M	0.9	0.9	0.9	0.9
Total volume in ml	3.0	3.0	3.0	3.0

Commercial fibrinogen preparations may show clot retraction in presence of platelets, thrombin and calcium ions (Fig. 9); a little clot retraction was observed also in the absence of platelets. Highly purified fibrinogen did not retract under the same conditions.

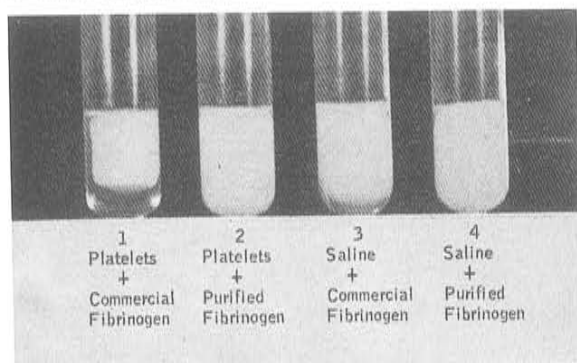


Fig. 9: Retraction of some fibrinogen preparations is seen with platelets, thrombin and calcium ions alone. Temperature, 37° C. Time, 2 hours. A commercial fibrinogen preparation retracted a little with only thrombin and calcium ions.

1. *Dialyzable Plasma Factor(s)*. The thrombelastographic pattern of a mixture platelets-fibrinogen-thrombin-calcium ions shows a deficient clot retraction whereas with a little serum there is clot retraction (12). This leads to the view that serum has an indispensable retraction cofactor. This cofactor is present in normal and hemophilic serum, in serum from platelet-rich as well as from platelet-poor plasma. H a r t e r t (12) found other characteristics: it is stable by

heating at 100°C for 30 min., preservable at room temperature for many days, dialyzable, water soluble, not precipitable with the protein fractions after saturation with ammonium sulfate, not adsorbable on barium salts and $\text{Al}(\text{OH})_3$. In this connection reference is also made to the publication of Lüscher (13).

Experiments were designed to study the nature of the factor(s) that can be passed through a dialysis membrane. Platelet-poor plasma was dialyzed in an efficient dialysis apparatus against physiological saline for 3 hours at room temperature. For another preparation plasma dialysate was obtained from platelet-poor plasma by dialysis against distilled water for 3 hours; the dialysate was lyophilized and dissolved in distilled water up to a tenth of the original plasma volume, and further diluted 1 : 10 or 1 : 100 in distilled water. For measuring clot retraction 0.5 ml of plasma, plasma dialysate or saline was added to 0.5 ml of platelet suspension, 1 ml of purified fibrinogen (600 mg $^{\circ}/_0$), 0.1 ml of thrombin 100 U/ml and 0.9 ml of calcium chloride 0.04 M (Fig. 10).

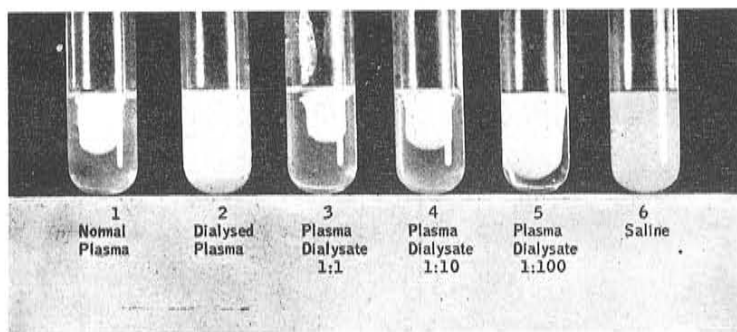


Fig. 10: The mixture consisted of purified fibrinogen + calcium ions + thrombin + platelets. To this was added some normal plasma, dialyzed plasma, plasma dialysate in 3 concentrations, and saline in the control tube. Dialyzed plasma does not promote clot retraction, it does, however, contain material that can be separated from it and promote clot retraction (Fig. 12).

With normal plasma there was clot retraction, whereas dialyzed plasma did not have this property. That property can be found in the plasma dialysate, and the retraction is proportional to the plasma dialysate concentration (Fig. 10). The dialyzable factor can be a limiting component with respect to the final retraction observed, and this factor(s) and the platelets function synergistically by some quantitative relationship. We determined that the factor(s) is resistant to heating at 100°C for 3 hours at pH 7, but destroyed completely in 7 minutes in 0.1 N HCl at 100°C and almost completely by the same treatment in 0.1 N NaOH. The retraction principle(s) is partially soluble in cold alcohol, more soluble in hot alcohol, and virtually insoluble in acetone or ether.

Clot retraction was observed in the presence of glucose and phosphate or acetate buffer whereas the addition of glucose alone inconsistently induced partial clot retraction (15). Perhaps there is an interrelationship between platelet glycolytic process and clot retraction. D e u t s c h and M a r t i n y (11) did not observe retraction in a platelet-fibrinogen-thrombin-calcium system even after addition of glucose to a concentration of 100 mg^o%, but clot firmness was increased in the thrombelastogram. Glucose alone in some way helps alter the mechanical properties of the clot.

We tried a series of combinations with purified fibrinogen (1 ml 600 mg^o%), platelet suspension (0.5 ml), thrombin (0.1 ml 100 U/ml), and calcium (0.9 ml 0.04 M). To this we added separately 0.5 ml plasma dialysate solution (equivalent to 0.06 M glucose content) or glucose (0.06 M) or phosphate buffer (0.06 M, pH 7.2) or glucose plus phosphate (both 0.06 M, pH 7.2) or adenosinetriphosphate (ATP 0.06 M) or adenosinediphosphate (ADP 0.06 M) or phosphocreatine (Ca salt 0.06 M) or physiological saline solution. The photograph taken at the end of two hours is figure 11.

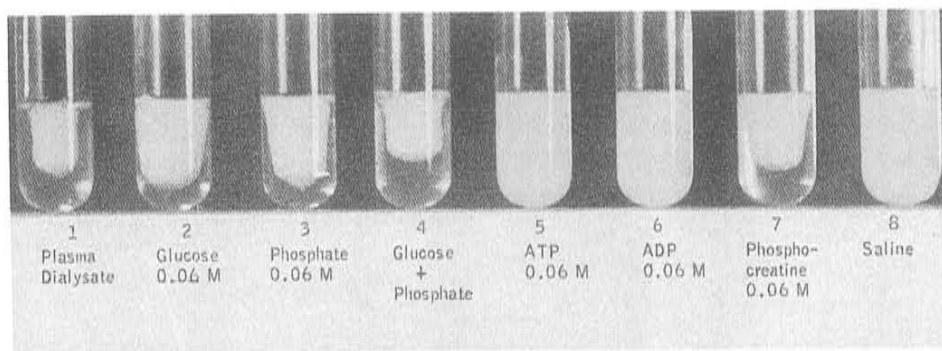


Fig. 11: Retraction of purified fibrinogen in presence of dialyzable compounds, calcium ions, platelets and thrombin.

With either glucose or phosphate alone there was only partial and delayed clot retraction; but with glucose and phosphate together there was almost the same degree as with the plasma dialysate, but the speed of the retracting process was slower. ATP and ADP were ineffective, whereas with phosphocreatine there was definitely some clot retraction. The phosphocreatine is labile and the phosphate fraction is most likely the active compound. Glucose alone did not do as well as plasma dialysate and even in combination with phosphate the retraction speed was not as great as with the plasma dialysate. We suppose other substances besides glucose and phosphate are there and account for the difference. For this reason we fractionated the dialysate.

The plasma dialysate was fractionated with the intention of separating phosphoric esters, according to the method of U m b r e i t (4). On figure 12 the clot retraction picture at the end of 2 hours is recorded. One half milligram of dialysate or the fraction derived from the equivalent was used in each clot retraction test. The active material was in the extract obtained with 5% cold trichloroacetic acid. Fractionation of this extract yielded activity in the barium insoluble and barium-soluble alcohol-soluble fraction, but not in the barium-soluble alcohol-insoluble fraction.

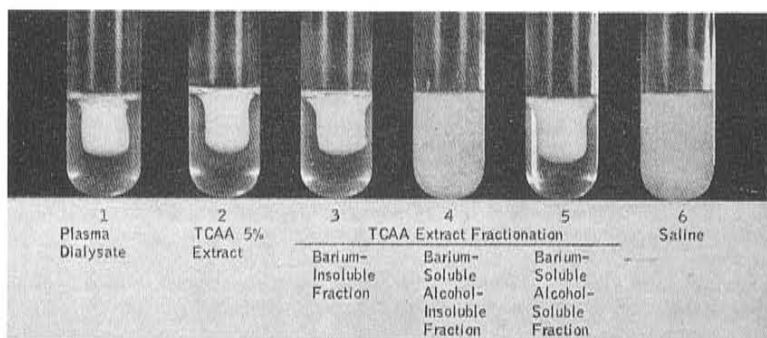


Fig. 12: Retraction of clots made from purified fibrinogen in presence of dialysate or its fractions, calcium ions, thrombin and platelets.

By paper chromatographic analysis of the three fractions some components were tentatively identified. Certain compounds were then purchased and tested. The following were not active: glucose-1-phosphate; glucose-6-phosphate, fructose-6-phosphate; fructose-1,6-phosphate, ATP, ADP, AMP, and phosphopyruvic acid. Accordingly we have not discovered the particular substances or combination of substances in plasma dialysate which gives it the strong clot retraction support.

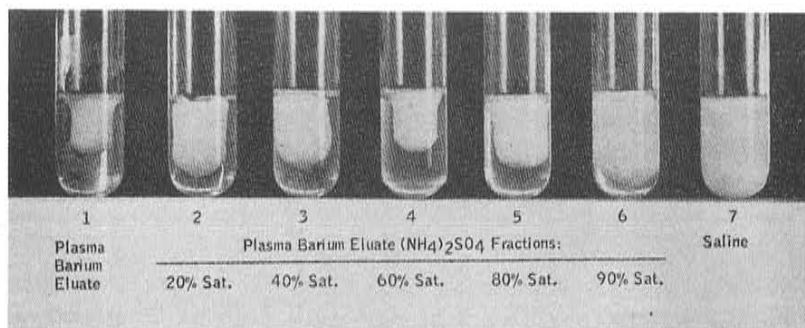


Fig. 13: Retraction of clots made from purified fibrinogen, in presence of platelets, calcium ions, thrombin, and plasma barium carbonate eluate. Temperature 37° C. Time, 2 hours.

2. *Protein Factor(s) of Plasma and Clot Retraction.* Several authors have found plasma protein fractions effective in clot retraction (10, 16, 17). The descriptions leave open the possibility that more than one substance might be of interest. In further studies we find that dialyzed plasma with platelets *does not* retract, barium adsorbed plasma with platelets *does* retract, but material adsorbed on barium and eluted *does* contribute to clot retraction when studied in relation to purified fibrinogen. Moreover, the dialyzable material discussed above is not necessary when the eluate from barium salts is used. The above statements are arranged with the intention of having the intradiction^{*)} plainly noticed.

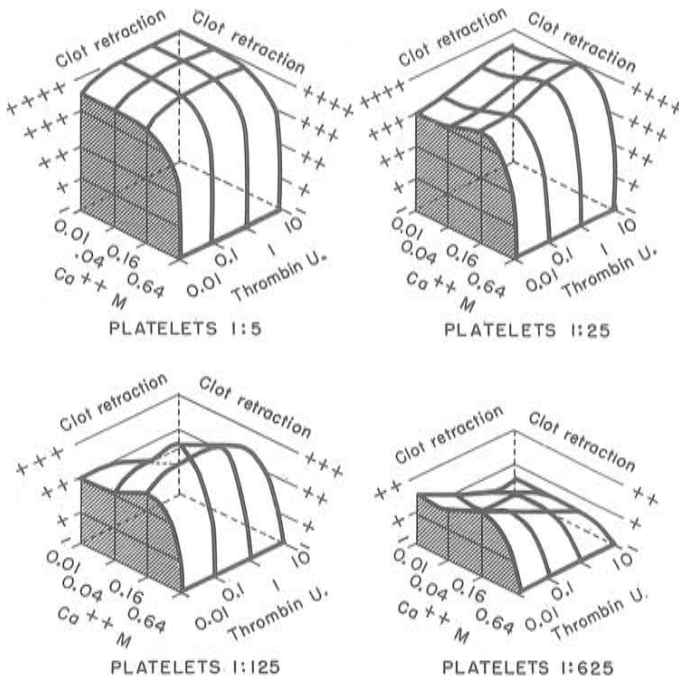


Fig. 14: Constants: fibrinogen and plasma. Variables: calcium, thrombin and platelets. Due to dilution when the test mixture was made the final concentration of the calcium ions is 3/10 of the value indicated, the final concentration of thrombin in units per ml is 1/3 that indicated and the final concentration of platelets is 1/6 of the four dilutions studied. Stated another way, the concentration given are those of the reagents before they were combined with the other reagents.

We made fractions of plasma barium carbonate eluate with the use of ammonium sulfate. The plasma barium carbonate eluate was obtained by the description given by Seegers, Johnson, Fell and Alkjaersig (2)

^{*)} This word was created and discussed elsewhere (18).

and was fractionated in the cold by adding a saturated solution of ammonium sulfate. When the mixture was at 20% of saturation the precipitate was removed by centrifugation, then more $(\text{NH}_4)_2\text{SO}_4$ was added to reach 40% of saturation and this was again centrifuged, and thus progressive increments of $(\text{NH}_4)_2\text{SO}_4$ were added. Every fraction, as well as the whole barium eluate, was dialyzed against distilled water, dried from the frozen state, and before use was dissolved in 0.9% NaCl. In this way the solution volume was one tenth of the original plasma volume from which the fractions were derived. The clots were obtained

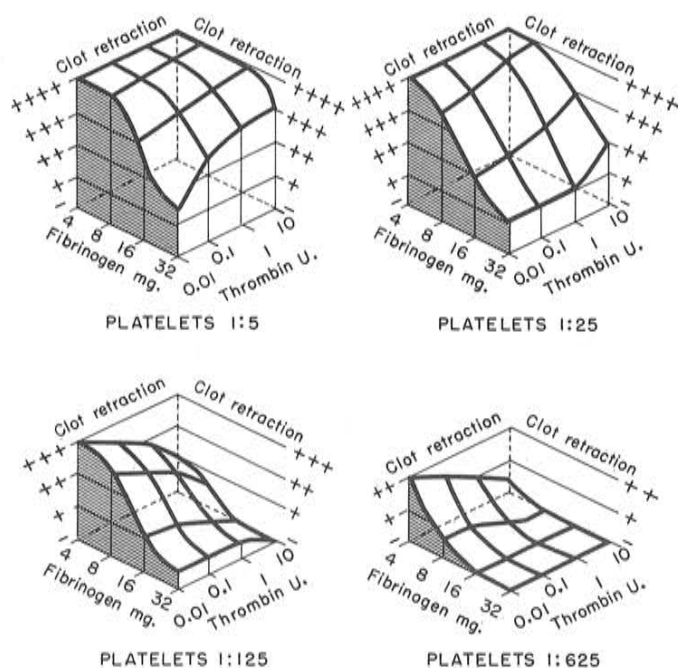


Fig. 15: Constants: plasma and calcium. Variables: fibrinogen, thrombin and platelets. The final concentration of fibrinogen is $1/3$ of the value indicated, the final concentration of thrombin in units per ml. is $1/3$ that indicated and the final concentration of platelets is $1/6$ of the four dilutions studied.

by mixing to 0.5 ml of the whole eluate or the single fractions, 0.5 ml platelet suspension, 1 ml purified fibrinogen (600 mg%), 0.9 ml CaCl_2 0.04 M, and 0.1 ml thrombin. All fractions contained activity and the one obtained at 60% of saturation contained the most (Fig. 13). If the eluate or fractions are diluted so as to be equivalent to the original volume there is no retraction under these conditions. The barium carbonate eluate contains material of concern in clot retraction. This does not pass through a dialysis membrane and can be made to function without the material in plasma which can be dialyzed. In this con-

nection we recall that the dialyzable material itself can function without the barium carbonate eluate.

D. Reconsideration of Variables in Clot Retraction

The analysis of factors required for clot retraction has emphasized the qualitative aspects and the quantitative only secondarily. The next consideration, however, is related to a more exact visualization of the quantitative interrelationships. Some interesting details were studied by means of three dimensional projection as illustrated by figures 14, 15 and 16. The arrangement of variables, and the tests performed, is described in detail by means of Table.

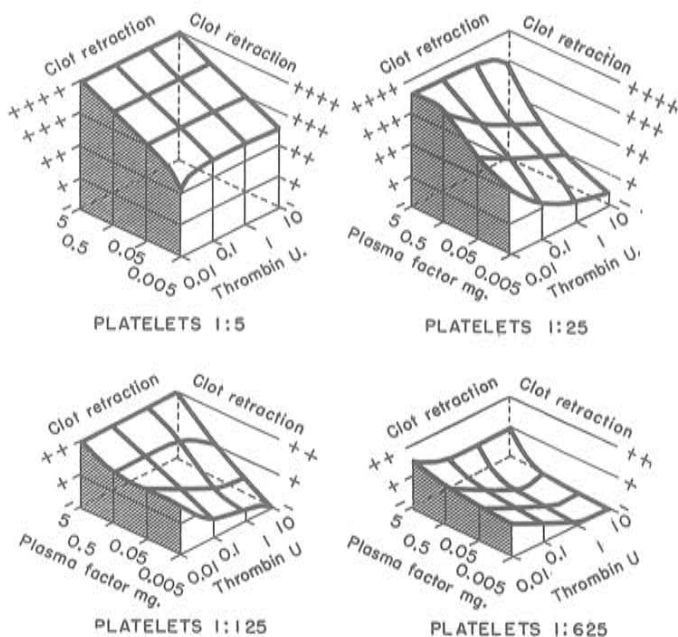


Fig. 16: Constants: fibrinogen and calcium. Variables: plasma dialyzable factor, thrombin and platelets. The final concentration of plasma dialyzable factor in mg/ml is 1/6 of the value indicated, the final concentration of thrombin in units per ml is 1/3 that indicated and the final concentration of platelets is 1/6 of the four dilutions studied.

The extent of clot retraction depends upon platelet concentration (Fig. 14, 15, 16). If the platelet concentration is high, retraction is more extensive as the thrombin concentration increases (Fig. 14, 15), an exception being when the dialyzable plasma factor is in low concentration (Fig. 16). In the low platelet concentrations clot retraction decreases with high thrombin concentrations (Fig. 14, 15, 16). High concentrations of calcium ions inhibit completely clot retraction, and there is always an optimum calcium concentration, unless the platelet

concentration is very high (Fig. 14). The platelets themselves contain much calcium. Clot retraction is most extensive with the low fibrinogen concentrations, and there may be no visible retraction if a low platelet concentration (Fig. 15), is associated with a high fibrinogen level. If there are many platelets the plasma dialyzable factor increases clot retraction in proportion to its concentration, but this relationship does not apply when the platelets are in low concentration (Fig. 16). Evidently the force of the plasma factor is entirely dependent upon platelets and is most likely utilized by them. A high thrombin concentration helps the clot retraction process in the presence of large amounts of plasma dialyzable factor (Fig. 16).

Table of Composition for Figures 14, 15 and 16

		Composition Figure 14	Volume
Variants ^{*)}	Packed Platelets Diluted (1 : 5 or 1 : 25 or 1 : 125 or 1 : 625)		0.5 ml
	CaCl ₂ Moles/liter (0.01 or 0.04 or 0.16 or 0.64)		0.9 ml
	Thrombin Concentration in Units/ml (0.1 or 1.0 or 10 or 100)		0.1 ml
Constants	Platelet Poor Plasma		0.5 ml
	Fibrinogen (6 mg/ml)		1.0 ml
		Total volume in each of the 64 tubes	3.0 ml
		Composition Figure 15	Volume
Variants ^{*)}	Packed Platelets Diluted (1 : 5 or 1 : 25 or 1 : 125 or 1 : 625)		0.5 ml
	Fibrinogen mg/ml (4 or 8 or 16 or 32)		1.0 ml
	Thrombin Concentration in Units/ml (0.1 or 1.0 or 10 or 100)		0.1 ml
Constants	Platelet Poor Plasma		0.5 ml
	Calcium Chloride 0.04 M		0.9 ml
		Total Volume in each of the 64 tubes	3.0 ml
		Composition Figure 16	Volume
Variants ^{*)}	Packed Platelet Diluted (1 : 5 or 1 : 25 or 1 : 125 or 1 : 625)		0.5 ml
	Plasma Dialyzable Factor(s) mg/ml (5 or 0.5 or 0.05 or 0.005)		0.5 ml
	Thrombin Concentration in Units/ml (0.1 or 1.0 or 10 or 100)		0.1 ml
Constants	Fibrinogen (6 mg/ml)		1.0 ml
	Calcium Chloride 0.04 M		0.9 ml
		Total volume in each of the 64 tubes	3.0 ml

^{*)} For each variant, used in four different concentrations, all the possible combinations were made with the other variant concentrations.

Summary

Clot retraction is reviewed as a visual experience from the elementary considerations to more complicated and uncertain perspectives. The extent of retraction is dependent upon temperature, cell volume, surface contact, pH,

fibrinogen concentration, thrombin concentration, calcium ion concentration, and platelet concentration. Platelets function in their own unique way. This quality is lost by such treatment as dialysis, ultrasonic waves, storage, and freezing. In the latter case there is some protection in glycerol solutions. Plasma contains dialyzable material concerned with clot retraction. This includes glucose and phosphorous, and perhaps other substances. Moreover, plasma contains one or more proteins of interest in clot retraction, and this protein(s) can be adsorbed on barium carbonate and eluted with sodium citrate solution. There may be clot retraction without the dialyzable material of plasma if the nondialyzable protein(s) are present and vice versa. The work is illustrated extensively with photographs and variables are considered in terms of three dimensional projection. In addition the ideas reviewed were developed in terms of information obtained with time lapse motion picture photography.

Résumé

Une revue concernant la rétraction du caillot est donnée. La rétraction dépend de la température, du volume des cellules, de la surface de contact, du pH, de la concentration du fibrinogène, de celle de la thrombine, de la concentration du calcium ionisé et des plaquettes. La fonction des thrombocytes est caractéristiques. Elles perdent leur propriétés rétractives à la suite de traitements tels que: la dialyse, l'exposition aux ultrasons, la conservation et la congélation. Dans le dernier cas la présence de glycérine exerce un effet protecteur. La plasma contient des substances dialysables agissant sur la rétraction, du glucose, des dérivés contenant du phosphore et peut-être d'autres substances. En outre, le plasma contient une ou des protéines intéressées à la rétraction du caillot qui peuvent être adsorbées sur $BaCO_3$ et éluées avec une solution de citrate de sodium. On peut obtenir une rétraction du caillot sans le matériel dialysable si la ou les protéines sont présentes et vice versa. Les variables sont étudiées dans un système tridimensionnel.

Zusammenfassung

Es wird eine Übersicht über die Retraktion des Gerinnsels von den Elementarvorgängen bis zu schwierigen und nicht eindeutig gelösten Problemen gegeben. Das Ausmaß der Retraktion hängt von der Temperatur, dem Zellvolumen, der Oberfläche, dem pH, der Fibrinogen-, Thrombin- und Calciumkonzentration und der Thrombozytenzahl ab. Die Funktion der Thrombozyten ist charakteristisch. Sie geht durch Einwirkung von Ultraschall, durch Dialyse, Lagerung und

Einfrieren verloren. In Glycerinlösungen besteht ein gewisser Schutz gegen die Kälteeinwirkung. Plasma enthält ein dialysierbares Material, welches mit der Retraktion in Beziehung steht. Es sind dies Glukose und Phosphor und vielleicht andere Substanzen. Außerdem enthält das Plasma einen oder mehrere Eiweißkörper, die die Retraktion beeinflussen. Diese Eiweißkörper können an Bariumkarbonat adsorbiert und mit Natriumzitratlösung extrahiert werden. Retraktion ist ohne das dialysable Plasmamaterial möglich, wenn die nichtdialysierbaren Eiweißkörper vorhanden sind und umgekehrt. Die Ergebnisse sind mit Photographien belegt und die variablen werden in einem dreidimensionalen System dargestellt. Außerdem wurden die Ergebnisse in einem Zeitrafferfilm festgehalten.

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