

Passive Hemagglutination

Mixture of a pathological blood (cirrhosis of the liver) with a normal blood:

Pathological serum* incubated during 2 h at 37°C in contact with:		Normal serum* incubated during 2 h at 37°C in contact with:	
Pathological red cells	–	Normal red cells	–
Normal red cells	–	Pathological red cells	–
Pathological platelets	–	Normal platelets	–
Normal platelets	–	Pathological platelets	–
Clot of the pathological plasma (without anticoagulant)	+ + ¹ / ₄	Clot of the normal plasma (without anticoagulant)	–
Clot of the normal plasma (without anticoagulant)	–	Clot of the pathological plasma (without anticoagulant)	+ + ¹ / ₂
Without any contact	–	Without any contact	–
Physiological saline incubated during 2 h at 37°C in contact with:		Physiological saline incubated during 2 h at 37°C in contact with:	
Pathological clot	+ + ¹ / ₁₆	Normal clot	–

* Sera immediately separated after clotting by the use of mechanical agitation with glass beads, incubated at 37°C with and without contact, and then heated, at 56°C for 30 min. All reactions are negative without heating at 56°C.

fraction I coating the tanned red cells. This substance agglutinates these red cells only when it has been heated previously for 30 min at 56°C.

The immunological nature of this substance is doubtful. Experiments have shown at least two different kinds of behavior in relation to rabbit antibodies against human platelets: (1) the rabbit antibody is formed in the serum and is not liberated by the incubated clot; (2) it does not require heating at 56°C to promote hemagglutination.

The relation between hemagglutination and fibrinolysis has been demonstrated by two different approaches:

(a) Clots of patients, in which the reaction was found strongly positive, have marked tendency to fibrinolysis. This was seen after incubation of blood, diluted with equal volume of physiological saline, at pH 7.2, under sterile conditions, during 24 h at 37°C. In all cases complete lysis has been observed.

(b) 1 cm³ of normal blood, clotted at 37°C in the presence of 15 mg of purified bovine fibrinolysin, gives off, after 2 h of incubation at 37°C, a serum capable also to promote the reaction. Complete lysis of the clot is observed after 24 h under the same conditions.

Conclusions. In the course of fibrinolysis of the clot, which is an especially rapid process in liver cirrhosis, this clot liberates a substance which is capable, after heating of 30 min at 56°C, to react with tanned erythrocytes coated with a plasmatic substance found in fraction I. It is probable that this phenomenon is not limited to liver cirrhosis, but can be found also in other syndromes associated with fibrinolysis. It is believed this is an exacerbation of a normal physiological process.

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Résumé

Au cours de la fibrinolyse, particulièrement précoce au cours des cirrhoses du foie, le caillot libère une substance capable après chauffage de 30 min à 56°C de réagir avec

des hématies recouvertes d'une substance plasmatique présente dans la fraction I.

Il est probable que le phénomène n'est pas limité aux seules cirrhoses hépatiques, mais s'étend à d'autres syndromes hémorragiques (fibrinolytiques) et qu'il s'agirait de l'exagération d'un processus physiologique normal.

A Dialyzable Factor from Plasma Responsible for the "Viscous Metamorphosis" of the Blood Platelets. Its Role in Clot Retraction and Haemostasis*

During coagulation of normal blood, the thrombocytes undergo a series of morphological changes which lead finally to their total disintegration. This phenomenon is generally called "viscous metamorphosis" of the platelets, and its most easily observed manifestation consists in agglutination and clumping of these cells. Since it is known that in most cases thrombus formation in venous thrombosis starts out from agglutinated and fused platelets adhering to the wall of a blood vessel, attention in recent years has been focused on the factors involved in the thrombocyte agglutination¹. A new dialyzable factor was discovered in the course of the following experiments.

Some authors still believe *clumping* of the thrombocytes to be a secondary phenomenon due to the conversion of small amounts of fibrinogen to fibrin or pro-fibrin², in spite of the fact that platelets in afibrinogenaemic plasma agglutinate normally³, and that elec-

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¹ Throughout this paper agglutination is understood in the sense of describing the first manifestation of viscous metamorphosis, thus excluding nonspecific aggregation or serological reaction of intact cells with antiplatelet antibodies.

² K. APITZ, *Erg. inn. Med.* 61, 54 (1942). – K. LENGGENHAGER, *Weitere Fortschritte in der Blutgerinnungslehre* (Georg Thieme, Stuttgart, 1949), p. 7 and 177.

³ B. ALEXANDER, R. GOLDSTEIN, L. RICH, A. G. LE BOLLOCH, L. K. DIAMOND, and W. BORGES, *Blood* 9, 843 (1954).

tron microscopy⁴ and staining methods disprove the presence of fibrin in the agglutinates. We have been able to show⁵ that a "serum" rich in prothrombin obtained by recalcification from a platelet-free plasma and containing no detectable amounts of fibrinogen agglutinates added thrombocytes completely in a few minutes. On the other hand, normal serum obtained from spontaneously clotted fresh blood has lost this platelet-destroying property; this suggests that one of the responsible factors is consumed during clotting in the presence of platelets. Serum from patients with haemophilia A or haemophilia B, lacking antihæmophilic factor or Christmas factor, respectively, proved fully active in our agglutination test⁶. This disproves the explanation offered by BERGSAGEL⁷ that the combination of Christmas factor, antihæmophilic globulin and calcium is responsible for platelet agglutination. The pathological sera with pronounced platelet-destroying activity, i.e. from cases of haemophilia A and B and of thrombopenia, and the serum from platelet-free plasma have one property in common, this being their high contents of prothrombin. Our work seemed therefore in agreement with the observations by WRIGHT and MINOT⁸ who found as early as 1917 that "serozyme" can cause viscous metamorphosis of the platelets. However, in our experiments incubation of washed platelets with prothrombin and calcium did not cause agglutination of the cells. (Platelets were obtained by centrifugation from citrated bovine blood, washing 5 times in NaCl-citrate solution, and suspending in physiological saline). The same negative result was found with tissue thromboplastin, Ca, and prothrombin, or when an equivalent amount of thrombin was added to washed platelets. Purified preparations of prothrombin or thrombin alone are therefore not responsible for platelet agglutination; presence or absence of a wettable surface is thereby without influence.

According to our concept of *clot-retraction*, material in the platelets—and in no case fibrin—is the retractile substance, the fibrin playing the completely passive role of being drawn together by the retracting "pseudopodia"-material of the thrombocytes. Sending out of pseudopodia by the platelets is considered to be the first sign of viscous metamorphosis, and therefore the ability of a thrombocyte-containing fibrin clot to retract was taken as a criterion for the presence of a system capable of producing viscous metamorphosis of the platelets in the fibrin network.

Such minimum systems for clot-retraction have been described by several authors. BUDTZ-OLSEN⁹ found that in a system of fibrinogen, prothrombin, CaCl₂ and platelets retraction occurs; according to ELLICOTT and CONLEY¹⁰ thrombin, fibrinogen, platelets and serum albumin are another example for such a minimum system. Working with the retraction test described by FONIO¹¹, and with material of bovine origin, we found that neither

system led to retractile clots as long as the components used were pure enough. (Fibrinogen was prepared according to WARE, GUEST, and SEEGER¹²; prothrombin was adsorbed on BaSO₄ and reprecipitated after elution. Acetone-precipitated lung thromboplastin, Armour cryst. bovine albumin, thrombin "Roche", and thoroughly washed platelets were used.) It was found, however, that a system consisting of a dialyzate from citrated or oxalated bovine plasma or serum (0.3 ml), washed bovine platelets (0.2 ml), bovine fibrinogen (0.4 ml, 0.6% in NaCl) and coagulated by addition of 0.1 ml of a 0.1% solution of thrombin "Roche", was always capable of retracting normally to one-third of the original length of the clot. (In terms of fluid expressed this corresponds to well over 90% the original volume of the unretracted clot.) It can be shown that the dialyzable substance¹³ is a limiting factor with respect to completeness of retraction, that is, the lengths of the fully retracted clots depend upon its concentration. This strongly suggests that the dialyzable factor and the thrombocytes are linked together by some stoichiometric relationship.

The same system without fibrinogen should cause viscous metamorphosis of the platelets and this was indeed the case. It can be shown that retraction begins only after viscous metamorphosis has taken place. By using a more concentrated suspension of thoroughly washed intact platelets, a much more direct proof for our view can be offered: a few minutes after addition of dialyzate and thrombin at 37°C the platelets formed a fragile coagulum, which, carefully detached from the walls of the test tube, retracted rapidly to a small knob. This occurred in a system completely free of even traces of fibrinogen. The solution expressed from this retracted platelet-clot added to a small amount of fibrinogen clotted the latter due to the presence of thrombin. This clot showed no retraction whatsoever, although it must have been rich in serotonin. Retraction also did not occur when the dialyzate in our minimum system was replaced by the same volume of a solution of 100 mg% serotonin in NaCl. This result is in obvious contrast to the findings by FENICHEL and SEEGER¹⁴. We believe, therefore, that a viscous protein contained in the thrombocytes, formerly called by us protein "S", and not the fibrin is the retractile material in thrombocyte-containing fibrin clots.

The implications of a dialyzable and thermostable factor (30 min at 100°C) in plasma which together with thrombin¹⁵ causes the viscous metamorphosis of platelets are evident: it obviously is part of the system which is essential in the mechanism of haemostasis in addition to the fibrinogen-fibrin system. It may offer in part an explanation for cases described of bleeding tendencies in spite of a completely intact clotting system, which up to now have been ascribed to defective thrombocytes (i.e. thrombasthenia GLANZMANN). It may be noted that in these conditions retraction is indeed sometimes absent.

The existence of a self-contained system responsible for haemostasis by clumping of the platelets followed by

⁴ R. JÜRGENS and H. BRAUNSTEINER, Schweiz. med. Wschr. 80, 1388 (1950).

⁵ E. F. LÜSCHER, Schweiz. med. Wschr. 85, 912 (1955).

⁶ E. F. LÜSCHER, V. Europ. Hämatologenkongr. Freiburg i. Br. 1955 (in press).

⁷ D. E. BERGSAGEL, V. Europ. Hämatologenkongr. Freiburg i. Br. 1955 (in press).

⁸ J. H. WRIGHT and G. R. MINOT, J. exper. Med. 26, 395 (1917).

⁹ O. E. BUDTZ-OLSEN, *Clot retraction* (Blackwell Scientific Publications, Oxford, 1951), p. 56.

¹⁰ CH. E. ELLICOTT and E. L. CONLEY, Bull. John Hopkins Hosp. 88, 321 (1951).

¹¹ A. FONIO and J. HENSELER, Schweiz. Z. allg. Path. Bakt. 15, 598 (1952).

¹² A. G. WARE, M. M. GUEST, and W. H. SEEGER, Arch. Biochem. Biophys. 13, 231 (1947).

¹³ H. HARTERT [Klin. Wschr. 32, 139 (1954)] mentions also a factor responsible for retraction which could not be obtained by methods of protein-fractionation.

¹⁴ R. L. FENICHEL and W. H. SEEGER, Amer. J. Physiol. 181, 19 (1955).

¹⁵ Until now only thrombin "Roche" has been used in our work. The possibility of a contamination of this product by other factors has to be considered, and work in this direction is in progress.

vasoconstriction due to subsequently liberated serotonin from the platelets is of interest also from a phylogenetic standpoint, since it is well known that more primitive animals, as for instance certain crabs¹⁶, have only this haemostatic mechanism. It seems, therefore, that the fibrinogen-fibrin system is a further step in the development of haemostasis, whereby the older system has kept its full importance.

Finally, it may be added that the new factor should also be considered in further studies on the mechanism and prevention of thrombus formation.

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Zusammenfassung

Die visköse Metamorphose der Blutplättchen ist ein von der Fibrinbildung unabhängiger Prozess, der durch Thrombin und einen thermostabilen, dialysierbaren Faktor aus Plasma oder Serum ausgelöst wird. Das unter diesen Bedingungen aus den zerfallenden Plättchen austretende Material kontrahiert sich spontan. Dies erklärt die Gerinnselretraktion, bei der dem Fibrin folglich eine völlig passive Rolle zukommt.

¹⁶ L. LOEB in: J. ALEXANDER, ed. *Colloid Chemistry: Theoretical and applied*, Vol. 2 (Chemical Catalog Company, New York), p. 487.

Zum Vorkommen neuraminsäurehaltiger Glykoproteide

KLENK¹ konnte erstmalig 1941 Neuraminsäure in Form ihrer stabilen Methoxylverbindung aus Gangliosiden isolieren. Während die Neuraminsäure zunächst nur als charakteristischer Bestandteil von Lipoiden angesehen wurde, gelang KLENK und LAUENSTEIN² im Jahre 1952 die Isolierung der Methoxyneuraminsäure auch aus Glykoproteiden und zwar aus Submaxillaris-mucin und Harnmucin. Wie wir³ vor kurzem mitteilten, kommt Neuraminsäure auch im Blutserum des Menschen vor. Es gelang uns die Isolierung kristallisierter analysen-reiner Methoxyneuraminsäure aus menschlichem Serum⁴. Unsere Befunde wurden inzwischen durch die Arbeiten von C. CHATAGNON und P. CHATAGNON⁵ bestätigt. Die Neuraminsäure ist im Serum nach unseren Untersuchungen⁶ nicht an Lipide, sondern an Glykoproteide gebunden. Nach WINZLER⁷ dargestellte Glykoproteide enthielten nach unseren Untersuchungen 8% Neuraminsäure⁸. In pathologischen Seren fanden wir deutliche

Erhöhungen des Neuraminsäurespiegels⁹, der bei normalen Versuchspersonen zwischen 40 und 65 mg% liegt.

Mit Hilfe der früher³ mitgeteilten Neuraminsäurebestimmungsmethode konnten wir bei 15 von uns untersuchten Tierarten im Blutserum Neuraminsäure in etwa gleicher Grössenordnung wie beim Menschen nachweisen. Die Werte schwankten zwischen 30,3 und 105,1 mg%.

Bei der Untersuchung von Eiweissfraktionen mit einem Reinheitsgrad von über 95% (die Substanzen wurden uns liebenswürdigerweise von Herrn Professor Dr. H. E. SCHULTZE, Behringwerke, zur Verfügung gestellt) fanden wir den höchsten Neuraminsäuregehalt im α_1 -niedermolekularen Säureprotein, das 9,14% Neuraminsäure enthielt. Abgesehen vom Albumin wurde in jeder von uns untersuchten Eiweissfraktion des Serums Neuraminsäure gefunden. Über den Neuraminsäuregehalt des Fibrinogens bzw. Fibrins haben wir bereits berichtet⁹. Hämoglobin enthält Neuraminsäure höchstens in Spuren. Da das Hämoglobin durch seine rote Farbe die Neuraminsäurebestimmung unmöglich macht, wurde es nach BÖHM und BAUMEISTER¹⁰ gespalten und die Methoxyneuraminsäure isoliert. Hierbei wurden so geringe Mengen erhalten, dass sie nicht sicher dem Hämoglobin zugesprochen werden können. Sie entstammen möglicherweise Spuren von Serum, die unserem Ausgangsprodukt angehaftet haben könnten.

Im Urin gesunder Versuchspersonen konnten wir mit unserer Methode keine Neuraminsäure nachweisen. Im eiweisshaltigen Urin jedoch fanden wir, wie auch C. CHATAGNON und P. CHATAGNON¹¹, Neuraminsäure. Der Neuraminsäuregehalt betrug bis 5,9 mg%.

Im Liquor gesunder Versuchspersonen fanden wir zwischen 0,14 und 0,42 mg% Neuraminsäure. Bei Patienten lagen die Werte zum Teil wesentlich höher; den höchsten Wert von 12,2 mg% hatte ein Patient mit einer Meningokokkenmeningitis. Im Ascites, im Pleurapunktat und in Ödemflüssigkeit liess sich ebenfalls Neuraminsäure in wechselnden Mengen nachweisen; der Neuraminsäurespiegel lag jedoch immer tiefer als der des entsprechenden Serums. Im Speichel fanden wir einen Neuraminsäuregehalt von 4,4 bis 8,2 mg%. Aus methodischen Gründen liess sich nicht in jedem Speichel Neuraminsäure bestimmen, da die Farbe nicht immer charakteristisch war. GYÖRGY und Mitarbeiter¹² gelang es erstmals, aus Frauenmilch Neuraminsäure zu isolieren. In Frauenmilch fanden wir relativ hohe Neuraminsäurewerte (33,8–146 mg%), während wir in Kuhmilch (Molkereimilch) nur etwa 13 mg% fanden.

Über die klinischen Untersuchungsergebnisse berichten wir ausführlich an anderer Stelle.

P. BÖHM und L. BAUMEISTER

Medizinische Universitätsklinik Bonn, den 2. Januar 1956.

Summary

The results are reported of investigations on the content of neuraminic acid in serum of 15 animal species, pure serum protein fractions, fibrin, haemoglobin, urine, liquor, saliva and milk.

⁹ P. BÖHM und L. BAUMEISTER, *Klin. Wschr.* 33, 712 (1955).

¹⁰ P. BÖHM und L. BAUMEISTER, *Z. physiol. Chem.* 300, 153 (1955).

¹¹ C. CHATAGNON und P. CHATAGNON, *C. r. Soc. biol.* 148, 1765 (1954); *Ann. Biol. clin.* 1955, Nr. 1–2.

¹² J. R. E. HOOVER, G. A. BRAUN und P. GYÖRGY, *Arch. Biochem. Biophys.* 47, 216 (1953). – F. ZILLIKEN, G. A. BRAUN und P. GYÖRGY, *Arch. Biochem. Biophys.* 54, 564 (1955).

¹ E. KLENK, *Z. physiol. Chem.* 268, 50 (1941).

² E. KLENK und K. LAUENSTEIN, *Z. physiol. Chem.* 291, 147 (1952).

³ P. BÖHM, St. DAUBER und L. BAUMEISTER, *Klin. Wschr.* 32, 289 (1954).

⁴ P. BÖHM und L. BAUMEISTER, *Klin. Wschr.* 32, 611 (1954); *Z. physiol. Chem.* 300, 153 (1955).

⁵ C. CHATAGNON und P. CHATAGNON, *C. r. Soc. biol.* 148, 1226 (1954).

⁶ P. BÖHM und St. DAUBER, Vortrag auf der 60. Tagung der Gesellschaft für Innere Medizin, München, 25.–29. April 1954.

⁷ H. E. WEIMER, J. W. MEHL und R. J. WINZLER, *J. biol. Chem.* 185, 561 (1950).

⁸ P. BÖHM, Vortrag auf der 7. Tagung der Deutschen Gesellschaft für Physiologische Chemie, Kiel, 20.–22. September 1954.