REVISTA ESPAÑOLA DE FISIOLOGIA, 28, 33-38. 1972

Action of the Contact Factor on the Aggregation of the Platelets

J. Aznar, V. Alberola, J. A. Aznar and A. Fernández-Pavón

Laboratory of Haemostasis and Thrombosis
Service of Clinical Pathology
Ciudad Sanitaria «La Fe»
Valencia (Spain)

(Received on September 3, 1971)

J. AZNAR, V. ALBEROLA, J. A. AZNAR and A. FERNANDEZ-PAVON. *Action of the Contact Factor on the Aggregation of the Platelets.* R. esp. Fisiol., 28, 33-38. 1972.

The authors study the action of the Contact Factor on the platelet aggregation. For this purpose, they use an elution of Contact Factor obtained as per Nossel's technique. Such elution it has been proved, possesses a biolocal activity of Contact Factor.

The action of the said elution is valued on the aggregation of the platelets in a series of normal people, and the fact that it brings about a significant reduction of the thrombocyte aggregation is duly checked.

For a valuation of the platelet aggregation, the continuous photocolorimetric measurement has been used, the platelets having been activated with collagen.

Finally, a series of considerations are carried out concerning the likeable connection of this fact with the tendency to thromboembolic accidents previously described in patients suffering from Hegeman disease.

In the initiation of haemostasis, the collagen tissue plays a fundamental part by favouring on one hand the thrombocitary aggregation (13, 14, 37) and on the other the activation of the contact phase (19, 20, 36).

It has been speculated on the possible action of the CF on the thrombocitary aggregation, although in this respect there are contradictory opinions (2, 12, 16, 18, 26, 28, 31, 35). Likewise, there is not a unanimous opinion about the thrombocitary function in those cases of Hageman trait (3, 5, 6, 9, 15, 26, 30, 32, 35).

On the other hand, in the clinical field, we must point out the fact that the first patient with a congenital shortage of factor XII, HAGEMAN, died from a pulmonary thromboembolic process (29), and that the incidence of infarcts of myocardium is very high in those patients suffering from HAGEMAN trait (29).

Taking as a starting point the facts previously mentioned in the present report, we value the possible action of an elution of contact factor (CFE) on the thrombocitary aggregation, considering that the greater incidence of thromboembolic accidents in cases of Hageman trait is precisely due to lack of factor XII.

Materials and Methods

In order to obtain CFE, we use the technique described by NOSSEL (24).

CFE is distributed in 1 ml ampoules, lamp-closed, and is frozen at —20° C and kept in these condition till the moment when it is going to be used. Such elution contains the biological activity of the CF, but not that others factors of coagulation (1, 24). The biological activity of the CFE is valued with a PTGT (partial thromboplastic generation test), carried out as follows: 0.1 ml of native plasma (plasma extracted on silicon material), plus 0.1 ml of cephalin (platelin), plus 0.1 ml of CFE or 0.1 ml of saline solution if the witness test is being carried out. Incubation at 37° C for 3 minutes and a later addition of 0.1 ml of Cl_2Ca M/40. With this technique, 17 experiments are performed with as many plasmas from healthy donors, thus obtaining an average PTGT of 63 seconds in the witness test and of 31.7 where the CFE ($P < 0.01$) has been added.

As an aggregation technique, we have used the photocolorimetric one of continuous reading, with the following methodical technique: Normal blood extracted with sodium citrate at 3.8 % (1 ml of the said solution and 9 ml of blood) and on plastic material. It is then left to rest at ambient temperature (22-24° C) for 2/4 hours. It is centrifugated at 1,000 r.p.m. for ten minutes and then by using plasma lacking platelets from the same donor, the number of thrombocites is adjusted at approximately 275,000 per mm^3. Out of this plasma, we take 0.6 ml and add 0.1 ml of CFE in the problem test, or 0.1 ml of saline solution in the witness test. Then, after shaking it very slightly, it is incubated at 37° C for 10 minutes and the plate aggregation is valued by adding to each sample 0.2 ml of collagen solution (Stago) to a final concentration of 40 μg/ml.

Results

In this preliminary report, the action of the Contact Factor on the aggregation of the platelets is studied. For that purpose, the problem plasma is distributed in two fractions, valuing on them the action of CFE. It can be noticed (Fig. 1, 2 and 3), in those experiments where CFE has been used, the thrombocyte aggregation has diminished considerably. This valuation is repeated in 15 different normal plasmas and similar results are obtained in all of them.

In the aggregation curve carried out with the CFE, there is a complete diminution of platelet activity. That is to say, we obtain every depressed curves after the action of the Contact Factor, though they approximately keep their original structure. In our opinion, this speaks in favour of a physico-chemical action of the factor.

Discussion

As it has been previously mentioned, the action of the CF on the thrombocitary aggregation is not well defined. Some authors think that the CFE has no influence on the platelet aggregation (2). Others belive that by putting the platelets in contact with strange surfaces, it helps to the aggregation of thrombocites (2) or their viscous metamorphosis is accelerated, increasing the size of the aggregates (34). NORDOY and CLANDLER (23) suggest that the Hageman factor activated could increase the adhesiveness of the platelets when a thrombus induced by ADP is formed. OKONKWO (26), however, sustain that factor XII does nor interfere with the thrombocitary aggregation. Finally, for others (2, 12, 16, 18) the action of the contact factor would be carried out through the activation of factor XII, absorbed on the surface of the platelets.

There is not a unanimous opinion about the function of the platelets in patients

suffering from Hageman disease, for although there is a number of authors that and it somewhay diminished (3, 5, 9, 30), others think that is normally carried out (15). Some find differences is their own patients, describing some of them with alterations of the aggregation and others without then. Is this respect, SINAKOS (31), in a case of Hageman trait, finds the aggregation of the platelets quite normal when stimulated with ADP or adrenalin, while it is not normal in another case where a rapid disaggregation is also produced after the initial aggregation. In both patients, the aggregation with collagen was normal. OKONKWO (26) finds a decrease of the thrombocitary aggregation in patients affected with a shortage of factor XII, but thinks that such alteration is not due to lack of the said factor, but to the absence of a different protein which can be located in fraction 1-0. CAEN (5) points out that there is a defective adhesiveness of the platelets as regards the glass balls in those patients suffering from Hageman disease, and yet the adhesiveness and aggregation in these patients is normal when stimulated with collagen (6, 32). WALSH (35), in several patients, finds normal aggregation with ADP, thrombin and collagen, and a diminshed adhesiveness to glass, while on the other han the adhesiveness is normal when being *in vivo*.

On the other hand and in the clinical field, it is some how incongruent that patients lacking a coagulation factor and consequently showing a more or less noticeable tendency to plasmatic hypocoagulability, should present a high incidence of infarcts of myocardium (10, 11, 33) or thrombophlebitis (4). If we consider that just when these cases were described there were about 115 patients with Hageman disease, it can be concluded that the incidence of thromboembolic accidents in them is higher than the average of the normal population. If we add to it the death through pulmonary emboly of HA-

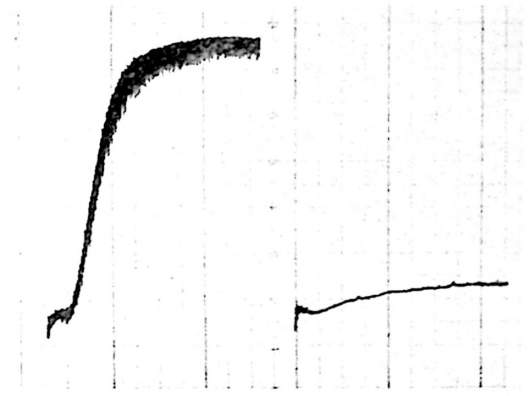


Fig. 1

Fig. 2

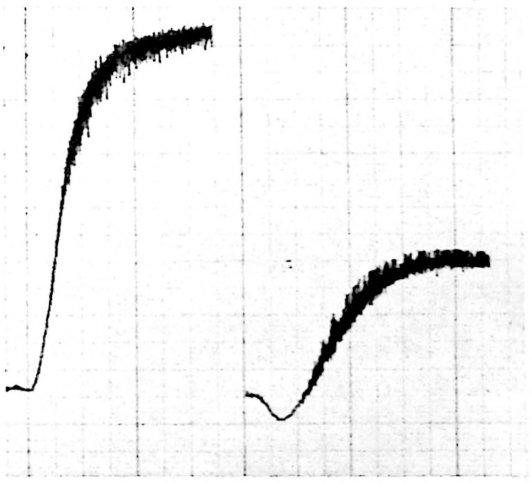
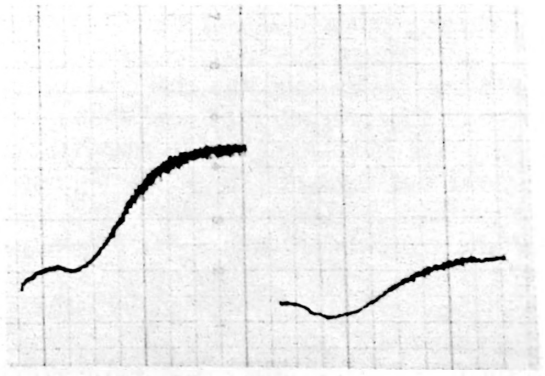


Fig. 3



Figs. 1, 2 and 3. *Action of the Contact Factor on the Aggregation of the Platelets. The platelet aggregation has been carried out by stimulating it with collagen (see technique in the text). Right. Aggregation curve made with eluate of Contact Factor Left. Control curve.*

GEMAN (29) and the frequency on which a group of Scandinavian patients — affected with more or less intense shortage of factor XII — showed cerebro-vascular accidents, some of which in the authors opinions could not be excluded from being due to thrombotic or thromboembolic accidents (7 and 8), we can reach the conclusion that it is possible the existence of a relation between the shortage of factor XII and the tendency to thrombosis in those patients.

In this respect and as suggested in this previous report, if the CF should have some kind of inhibition on the aggregation of the platelets, in its absence (Hageman disease) the thrombocites could be more easily aggregated and in this case they could favour the thromboemolic accidents we are referring to.

Together with the clinical facts previously mentioned, there are some experimental ones full of suggestions. For instance, it is rather curious that factor XII should be more easily absorbed on the collagen when using plasma poor in platelets than when doing so with plasma rich in them (19). Authors describing this suggest that the platelets stuck on the collagen fibres could make the later adhesiveness and activation of the CF rather difficult, though this is not easily explainable bearing in mind the chemical structure of the collagen (25), and that most likely the activation of factor XII is carried out through the $-COO^-$ groups of glumatic and aspartic acids, and that of the platelets through $-NH_2^+$ groups (E-amino of lysine). On the other, it could be thought that the platelets negatively charged could adsorb the CF on their surface, protein positively charged, and in this way act quite competitively with the collagen.

It is also noticeable the fact that the hypercoagulability induced *in vivo* with CFE should only cause a shortage of the coagulation time, without parallely altering other parameters of the haemostatic system and especially without producing microthrombus (21). Something similar happens with the injection of ellagic acid to experimental animals, which reduces possible traumatic hemorrhages of same but does not cause thrombosis (23). This contrasts with the secondary hypercoagulability caused by the injection of tisular thromboplastin, wich brings about a consumption coagulopathy with a decrease in the number of platelets. That is to say, it seems as if the CFE or the ellagic acid (activator of the Hageman factor) should only potentiate the plasmatic system of coagulation, but without favouring the formation of the platelet thrombus.

Everything mentioned above gives rather a suggestive idea that a plasmactic factor, which on one hand develops its action in the initial moments of the haemostasis by favouring the formation of the intrinsic activator of prothrombin (24) and on the other has a definite action on the fibrinolytic system (1, 17, 22), could also influence on the thrombocitary aggregation, hindering it. This could somehow explain the tendency to thrombosis in patients affected by a shortage of factor XII, since by lacking a factor with antiaggregating and fibrinolytic activity the formation of the platelet thrombus would be favoured, while at the same time the later lysis of same would be hindered. In this way, we would give a unitary sense to the activity of the FC and it could be thought that it works as an accelerating element in the genesis of the intrinsic activator of the prothrombin which, once its procoagulating action had been fulfilled, would prevent the celular progression of the white thrombus, hindering the later aggregation of new platelets, while at the same time and due to its fibrinolytic action it would oppose to the excessive deposit of fibrin.

Will the contact factor, therefore, be an «equilibrium factor» in the initial moments of haemostasis?

References

1. AZNAR, J., LÓPEZ-BORRASCA, A., CALATAYUD, R. and BARATA, J. J.: *Rev. Clin. Esp.*, 24, 276, 1963.
2. BARTH, P., KOMMERELL, B. and PFLEIDERER, T.: *Thromb. Diath. Haem.*, **16**, 378, 1966.
3. BIGLS, R., SHARP, A. A., HARDISTY, R. B., STEWART, J. and DAVIDSON, W. M.: *Brit. J. Haemat.*, 4, 177, 1958.
4. BOTTI, R. E. and RATNOFF, O. D.: *J. Lab. Clin. Med.*, **64**, 385, 1964.
5. CAEN, J., LARRIEU, M. J. and SAMAMA, M.: «L'Hemostase». L'Expansion Scientific. Ed. Paris, 1968, p. 56.
6. CAEN, J., LETRAND, Y. and SULTAN, Y.: *Thromb. Diath. Haem., Suppl.*, **40**, 181, 1970.
7. EGEBERG, O.: *Thromb. Diath. Haem.*, **23**, 441, 1970.
8. EGEBERG, O.: *Thromb. Diath. Haem.*, **23**, 432, 1970.
9. FANTL, P. and SWEET, B.: *Aust. J. exp. Biol. med. Sci.*, **44**, 123, 1966.
10. GLUECK, H. I. and ROEHLL: *Ann. Int. Med.*, **64**, 390, 1966.
11. HOAK, J. C., SWANSON, L. W., WARNER, E. D. and CONNOR, W. E. *Lancet*, **2**, 884, 1966.
12. HOROWITZ, H. I. and FUJIMOTO, M. M.: *Proc. Soc. exper. Biol.*, **119**, 487, 1965.
13. HOVIG, T.: *Thromb. Diath. Haem.*, **9**, 248, 1963.
14. HUGUES, C. R.: *Soc. Biol.*, **154**, 866, 1960, Paris.
15. HUGUES, J.: *N. Rev. Franc. Hemat.*, **9**, 529, 1969.
16. IATRIDIS, P. G., FERGUSON, J. H. and IATRIDIS, S. G.: *Thromb. Diath. Haem.*, **11**, 355, 1964.
17. IATRIDIS, S. G. and FERGUSON, J. H.: *Thromb. Diath. Haem.*, **6**, 411, 1961.
18. JURGENS, J.: *Thromb. Diath. Haem.*, **7**, 48, 1962.
19. NIEWIAROWSKI, S., BANK/WSKI, E. and FIEDORUK, T.: *Experientia*, **20**, 367, 1962.
20. NIEWIAROWSKI, S., BANKOWSKI E. and ROGOWICKA, I.: *Thromb. Diath. Haem.*, **14**, 387, 1965.
21. NIEWIAROWSKI, S., ROGOWICKA-GORZELAK, I., WOROWKI, K. and MYSLIWIEC, M.: *Bull. Acad. Pol. Sci.*, **15**, 599, 1967.
22. NIEWIAROWSKI, S. and PROU-WARTELLE, O.: *Thromb. Diath. Haem.*, **3**, 539, 1959.
23. NORDÖY, A. and CHANDLER, A. B.: *Lab. Invest.*, **16**, 3, 1967.
24. NOSSEL, H. L.: «The contact phase of blood coagulation». Davis Company. Phyladelphia. Blackwel. Scientific. Publications, 1964.
25. NOSSEL, H. L., WILNER, G. D. and LEROY, E. C.: *Nature*, **221**, 75, 1969.
26. OKONKWO, P., POST, R. and SIZE, H.: *Thromb. Diath. Haem.*, **23**, 423, 1970.
27. OLLENDORFL, P.: *Thromb. Diath. Haem.*, **6**, 104, 1961.
28. RABY, C.: «Biologie des hémorrabies et des thromboses». Masson. Ed. Paris, 1970.
29. RATNOFF, O. D. BUSSE, R. J. and SHEON, R. P.: *N. Engl. J. Med.*, **279**, 760, 1968.
30. SHARP, A. A.: *Brit. J. Haemat.*, 4, 28, 1958.
31. SINAKOS, Z., SULTAN, Y., CAEN, J. and BERNARD, J.: *Press. Med.*, **75**, 269, 1967.
32. TANGEM, Y. and CAEN, J.: *N. Rev. Franc. Hemat.*, **5**, 79, 1965.
33. VROMAN, L. and ZIEVE, P.: Personal communication to RATNOFF, O. D. *N. Engl. J. Med.*, **279**, 760, 1968.
34. WAALER, B. A.: Thesis Oslo University, 1959.
35. WALSH, P. N.: *Lancet*, 2, 575, 1970.
36. WILNER, G. D., NOSSEL, H. L. and LE ROY, E. C.: *J. Clin. Invest.*, **47**, 2608, 1968.
37. ZUCKER, M. B. and BORELLI, J.: *Proc. Soc. exp. Biol. Med.*, **109**, 779, 1962.