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The Splenectomy Effects on Blood Coagulation. II. Alteration of the Coagulation-Fibrinolysis System

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Splenectomy cause a delay in the coagulation phenomenon, due to the presence of circulating anticoagulants. The purpose of this study is to elucidate the anticoagulant nature. Thromboelastography indicates that the resulting coagula from a splenectomy do not possess an adequate structural formation. The use of immunochemical techniques shows a rise of the noncoagulant fibrinogen degradation products in the serum. At the same time an intense fibrinolytic activity is observed. The conclusion is reached that after a splenectomy both fibrinogen and fibrine degrade very rapidly, and that degradation products (antithrombin VI), on account of their anticoagulant nature, cause a delay in fibrine formation. *X* and *Y* fragments are enclosed in the coagulum, whereas *C*, *D* and *E* are retained in the serum. The latter are the ones detected by immunology.

Splenectomy alters the blood coagulation process, producing hypocoagulability attributed to the presence of various heparin circulating anticoagulants (1, 2). This paper studies mainly the coagulolytic process by thromboelastography, which also shows the coagulum mechanical traits. Its objetive is to know the circulating anticoagulant nature, made manifest in earlier studies. All this leads to a deeper knowledge of the already discovered alterations. It is very dificult to obtain valid concessions on the coagulation problem with partial studies. Only a confrontation of a series of results may lead to a proper hypothesis. The results obtained in this paper and those in earlier ones can help in the interpretation of the observed phenomena.

Materials and Methods

Experimentation animals. White Wistar rats, 4 months old, weighing from 180

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to 200 g have been used. They were divided into three lots: normal, witness and problem (2).

Plasma problem. To obtain comparative results with those obtained in Part I of this study, platelet-free plasma (following the technique described there) has been used.

Thromboelastography. ELOSEGUI's technique has been followed using a Hellige thromboelastography (3). From the graph obtained by this technique (fig. 1), the values for the *r* constant, representing the thromboplastin generation speed according to LEROUX (8, 9) and for the *K* constant (which indicates the coagulum mechanical quality and its evolution in function of the thrombinoformation), have been deduced in seconds. Since the *am* constant in the graph is decisively affected by the thrombocytes and since platelet-free plasma has been used, the resulting values have been eliminated.

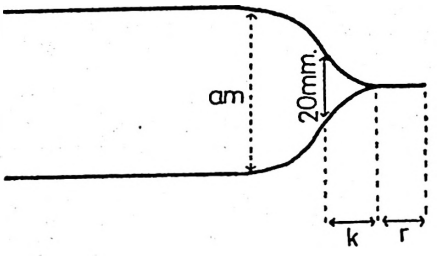

Fig. 1. *Thromboelastogram (type).* Constants single out: *r* (thromboplastin formation time); *k* (mechanical quality of fibrine and its evolution).

Prothrombin times. There were determined by annotating the coagulation times of the platelet-free plasmas, to which Geigy calcium thromboplastin (following the accompaying directions) had been added. The times have been determinated using the Merieux B-D fibrometer, capable of registering differences in tenths of a second during the coagulation process.

Identification of the fibrinogen degradation products. Of the fragments resulting from the plasmin or fibrinolysin action, some have a high MW, which partly coagulate with the fibrinogen, and are absorbed by the fibrin coagulum. Others non-coagulant, remain in the serum. The techniques used to identify the latter ones are based on the certain fibrinogen and fibrin antigenic determinants in these fragments, which permit their identification through antigen-antibody reactions (4). The first thing needed is an appropiate antiserum.

Antiserum obtention. Fibrinogen or fibrin may be used as antigen. The antiserums obtained from fibrin possess a higher tritation than those obtained from fibrinogen. For this reason, several rabbits were inoculated with a fibrin suspension, obtained by recalcification of a citrated plasma. The fibrin thus obtained, whased with a physiological solution and suspended in the same solution, is tested by the Lowry's method and diluted until 950 mg protein/100 ml. Three rabbits are inoculated every other day during 9 days with 0.5 ml of the said suspension. Eight days after the last inoculation, the blood is drawn from the animals through intracardiac puncture. The serum thus obtained is preserved in a cold room with sodium azide (4).

Purifying the antiserum. The antifibrin thus obtained contains antibodies againts other plasmatic proteins drawn from the fibrin during the flocculation (5, 6), which unpurify the antigen suspension administered to the rabbit. The foreign antibodies are eliminated then by adding an excess of its antigens, obtained by mixing the antiserum with 20 % of its volume of a normal rat serum.

After keeping the mixture at 4° C for 24 hours, the resulting precipitate of gamma globulin-antigamma globulin is eli-

minated by centrifugation. Flocculation tests are performed then to ascertain the antifibrin serum purity, by double diffusion against normal plasma and serum, using noble agar plates at 1.5 % in phosphate buffer to *p*H 8.2. If the result is positive keep adding small amounts of normal serum to the antifibrin until the reaction is negative.

Tritation of the fibrinogen degradation products. The tritation of the fibrinogen degradation products was performed after the obtention of the antifibrin serum title, using the precipitation technique in a liquid medium (4). To this end 0.05 ml of antifibrin serum was mixed with 0.05 ml of the problem serum from 10 different dilutions (F = 0.5).

The precipitate obtained from the fibrinogen degradation products is more difficult to observe than the one obtained from the native fibrinogen (7). For this reason the readding technique was slightly changed. After shaking the mixture for ten minutes, the plates were left in a stove at 37° C during 20 minutes. The presence of precipitates are observed with indirect light in a dark room, adding the number of obtained precipitations to the ten different dilutions of the problem serum. Thus, the expression of the equivalent fibrinogen results is avoided (10, 11, 12, 13).

Fibrinolytic activity. It was measured by the proteolysis rate of the platelet-free plasma euglobulins, following the Von Kaulla's method, modified by RABY (9).

Results

In Table I the mean values of the prothrombin times are measured in seconds. In figure 2 the mean values of the *r* constant of the thromboelastogram from operation time are measured in seconds. In figure 3, the mean values of the *K* constant are also accordingly measured. In the

Table I. *Variations of the prothrombin time (sec.) after the operation.*
The prothrombin times have been determined on platelet-free plasma, added with Ca^{++} thromboplastin Geigy, in the B-D Merieux Fibrometer.

Time experimentation (days)	ANIMALS* Laparot.	Splenect.
2	9.2±1.7	9.8±1.2
4	10.3±0.4	11.4±1.4
6	10.1±0.4	10.6±1.2
8	9.3±0.5	10.1±0.9
10	9.6±0.9	10.2±1.5

* Control animals = 9.5 ± 0.8.

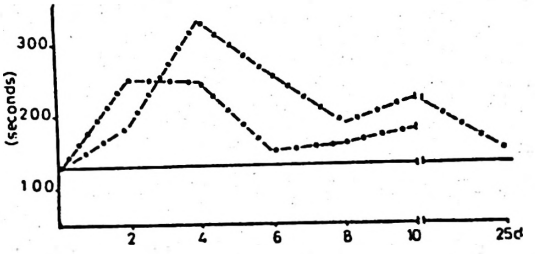

Fig. 2. *Variations of the* r *constant (in seconds) according to the values obtained in the thromboelastogram, versus the time elapsed after the operation (in days).*
--- normal; -o-o- laparotomized; -●-●- splenectomized animals.

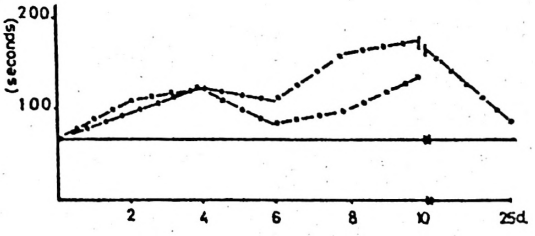

Fig. 3. *Variations of the* k *constant (in seconds) according to the values obtained in the thromboelastogram, versus the time elapsed after the operation (in days).*
--- normal; -o-o- laparotomized; -●-●- splenectomized animals.

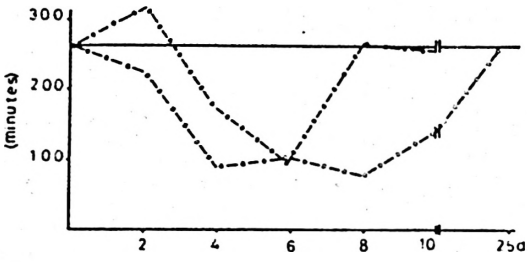

Fig. 4. *Variations of the fibrinolytic activity at different times of the experimentation (in days).*
--- normal; -o-o- laparotomized; -●-●- splenectomized animals. Plasmin activity has been determined by precipitation of plasmatic euglobulines with 0.1 % $H^+CH_3COO^-$, *p*H 5.2 and their proteolysis with $CaCl_2$ and Owren-Koller's buffer. The values in minutes measure the time elapsed until the total fibrinolysis.

Table II. *Determination of the products of the fibrinogen degradation by immunology.* The values are expressed adding the partial possitive immunological reactions wits antifibrin serum.

Time experimentation (days)	ANIMALS* Laparot.	Splenect.
	Total flocculations	
2	3	12
4	25	38
6	2	26
8	2	14
10	0	8

* Control animals = 0.

figure 4, the fibrinolytic activity of normal, witness and problem plasmas are shown from the time of operation. In the table II, the immunological results of the fibrinogen degradation products, present in the serum, are indicated. The values are expressed as a sum total of the positive flocculation reactions from operation time, in ten different dilutions of the same serum.

Discussion

The addition of calcium thromboplastin eliminates, in the coagulation process, those reactions that lead to the thromboplastin formation and causes only those related to thrombin and fibrin formation accompanied by antithrombin presence. It is one of the few tests that is not influenced by platelets and gives normal results in hemophilic state. Any test alteration indicates a dysfunction in the thrombin of fibrin formation, alien to those causes. The results show a lengthening of the prothrombin times in witness and problem animals, wit maximum lengthening on the fourth day after the operation. Recovery is alower in problem than in witness animals. The evolution of these values is similar to that obtained in earlier works concerning recalcification and thrombin times. They show a slight hypocoagulability, more pronounced in splenectomized animals than in laparotomized ones. If the values of the *r* constant is an index of thromboplastin formation rate, the results found in figure 2, together with those for the T1 constant (found in the first part of this paper), show that the coagulation alteration affects not only the thrombin and fibrin formation stages, but also the thromboplastin genesis. It show also the presence of a circulating anticoagulant that affects the three classic coagulation stages, acting with maximum intensity on the fourth day.

Thromboelastography is not realy akinetic coagulation study about such factors as the fibrinogen and platelets quantity and quality. Since pletelets-free plasmas have been used, the external factors to the enzymatic reaction itself, have been reduced to the fibrinogen derivates. An increase of the *k* values indicates a deficient structuration of the coagulum, i.e. a coagulum formation of poor quality. This could be attributed to a deficiency of factor XIII, a fibrin stabilizer, but since the witness animals present the same characteristics than the problem animals and since there is no sufficient reason to believe that they also suffer a deficiency of

the above mentioned factor, this hypothesis was discarded. Nevertheless the possibility that the alterations could be considered. The fibrinigen degradation products (X, Y, C, D, E protides) possess anticoagulant properties. An excess of these protides would delay the coagulolytic phenomenon. X and Y products, enclosed in the coagulum, originate abnormal coagula in their physico-chemical properties, when their formation is very high. This tipe of coagula formation might be the cause for the variations of the *k* values. Since D, C and E fragments are not coagulable and are found in the serum, they can communicate their anticoagulant property to normal serums (as it happens with splenectomized animal serums, part I of this paper).

An increase in the fibrinogen degradation products implies a previous fibrinolytic hiperactivity and an increase of C, D and E products in the serum. The study of the fibrinolysis intensity indicates that the euglobulin coagulum is digested by the splenectomized animal plasma at a remarkably higher rate than that reached by normal plasma. This confirms the hypothesis of a protide hyperformation, originated from fibrin degradation. Identification of C, D and E peptides in the serum, ratifies that confirmation. These peptides possess antigenic properties and all their groups are contained in the fibrine structure. For this reason the antifibrin serum is able to precipitate them. The antiserum treatment given to the experimentation animals, shows an increase in the fibrinogen degradation product more pronounced in the splenectomized animals. This data confirms the hypothesis presented in earlier works on the splenectomy effects in the coagulation process. From all this the conclusion is reached that splenectomy upsets the fibrinolysis-coagulation equilibrium, causing an increase of fibrinolytic activity. As a result, the fibrinogen degradation products are heightened and, since they possess anticoagulant properties, they should be responsible for the delay in the coagulation process. The said products have antigenic properties; some remain enclosed in the coagulum (X and Y) and cannot be detected by immunochemical methods, since the coagulum fibrine has their antigenic groups. They can be detected, however, by altering the coagulum structural configuration, which affects the thromboelastogram *k* values. Others derivates (C, D and E protides) remain in the serum and their anticoagulant nature delays the coagulation process in normal plasmas. They also possess antigenic properties and for this reason have been detected in problem serums by immunological reactions.

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