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Fibrinolysis Regulation by the Spleen

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In this work, the possible effect of the spleen on coagulation was studied. White Wistar rats were used as experimental animals, divided in three lots: normal, control and de-spleened animals.

The classic chronometrical tests of coagulation were done, and some special techniques, as the coagulography and the thromboelastography. According to the results, a hypocoagulability was observed. By other hand, the fibrinogen increased. By this, the presence of the circulating anticoagulants was deduced. By the neutralization test of protamine-sulphate, the heparin is not present in abnormally amounts. By immunology, products of the fibrinogen degradation were detected in the blood, and they have a anticoagulant role.

In spite of the efforts by a great number of researchers for year, the physiology of the spleen is still not completely known. In this work we have studied the possible effect of this organ on coagulation in rats, after the spleenectomy.

Materials and Methods

White Wistar rats were used as experimental animals, of 4-5 months of age. The animals upon having their spleens removed, suffer two traumas, one due to the laparotomy and the other as a consequence of the removal of the viscera. The latter is that which is of interest to us. It is necessary to have a control animal, separate from the integral or normal animal, that can indicate the alteration due to the first trauma, in order to know the consequence derived exclusively from the second.

Therefore, the animals have been divided into three lots: *a*) normal animals, that indicate the normal data or value of each of the determination performed. Ten animals were used for each; *b*) control animals, that suffer only the laparotomy. We have used, according to the deviation observed, five or ten animals for each determination and point studied, these being performed on the 2nd, 4th, 6th, 8th and 10th days after the operation; *c*) de-spleened animals, the object of our research. Ten animals were used for each determination and point of study, which were also performed on the 2nd,

4th, 6th, 8th, 10th and 25th days after the operation.

Time of recalcification. This was done following the Raby and Dubois method. In this test, as in the other chronometrical determinations of coagulation, we have used a Fibrometer (B-D Merieux) (3). *Time of thrombine.* We used the STEFANINI technique (4). *Time of prothrombine.* It was done using Ca-thrombokinase from the Geigy Laboratories, whose instructions have been followed. *Coagulography.* We have studied the parameters T_1, T_2, T_3, b, c, d and H, according to the RABY method usign a Unicam 800 thermostatted spectrophotometer (3). *Thromboelastography.* The constants «r» and «k» were determinated according to the ELÓSEGUI method and using a Hellige thromboelastograph (1). *Fibrinolysis determination.* We used the VON KAULLA method as modified by RABY (3). *Preparation and measurement of the antifibrine serum.* The FERREIRA and MURAT (2) norms were followed.

Results

In all chronometrical tests of coagulation (time of prothrombine, time of thrombine, time of fibrine) a slight hypocoagulability is observed, that reaches its maximum on the 4th day after the operation, and is appreciably more apparent in the de-spleened animals. The values are found to be normalized in the control animals at 6 days and in the de-spleened animals at 25 days after the operation (Figs. 1 and 2).

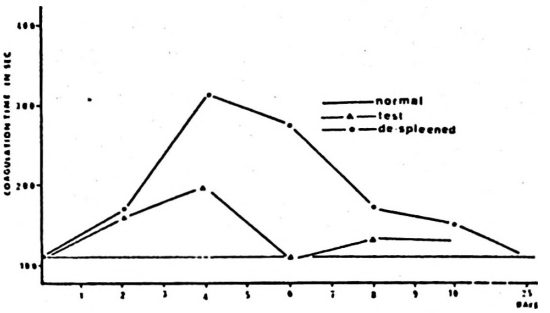

Fig. 2. *Recalcification times.* 0.2 ml of M/40 $CaCl_2$ were added to 0.2 ml of decalcified plasma. The coagulation times were determined in the B-D Merieux fibrometer.

For all the constants of the coagulogram, we found normality in the control animals and a slight hypocoagulability in the de-spleened ones. With the coagulogram we deduced the value of plasmatic fibrinogen at the same time, which we found to be increased, and to present a curve totally analogous to the previous ones, with a maximum at 4 days and nor-

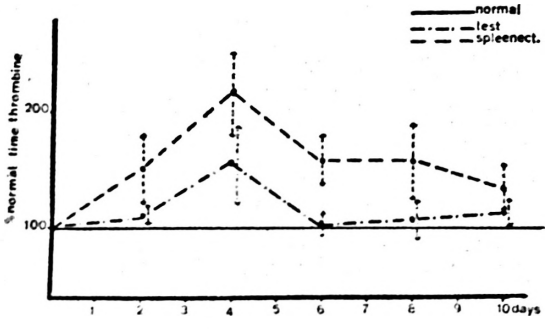

Fig. 1. *Thrombine time.* 2.5 V.I. of thrombine, were added to 0.20 ml of plasma, to eliminate the possible disturbs dued to the thromboplastine and/or thrombine formation. The coagulation times were determined in the B-D Merieux fibrometer.

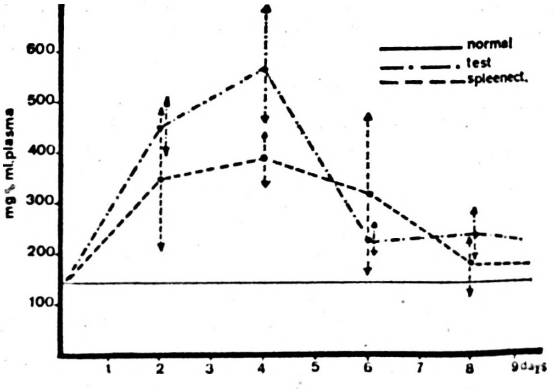

Fig. 3. *Fibrinogen determination by coagulography.*

Table I. *Heparine neutralization with protamine sulphate.*

Coagulation time/seg.	µg protamine-sulphate 0.2 ml plasma	0	1	2	3	4	5
	Normal animal	94.4	101	96.9	99.2	106.4	128.4
	Normal animal	138.1	129.2	114.9	126	138.8	154
	Test 4th day	167.4	161.3	150	149.9	146.4	148
	Test 4th day	196.8	194.2	171.9	166.8	138.6	126.9
	De-spleen. 4th day	357.9	311.4	305.4	305.9	303.4	294.3
	De-spleen. 4th day	574.4	348.9	331	318.9	248.4	236

malization paralell to the time of coagulation (Fig. 3).

From this we deduced the presence of a circulating anticoagulant in both series of animals. The count of mastocytes in the bone-marrow, and the neutralization test with protaminesulphate, allow us to deduce that the heparin is not found present, at least in physiologically amounts (Table I).

By immunology, the curves of the products of the fibrinogen degradation are also totally analogous, to the former ones in relation to the fibrinogen content and the chronometry of the coagulation (Fig. 4). The products of the degradation of the fibrinogen be have as circulating anticoagulants, active in the three classic stages of coagulation. The fibrinolysis times by the Von Kaulla method, modified by RABY (3), produce curves parallel to those mentioned above (Fig. 5).

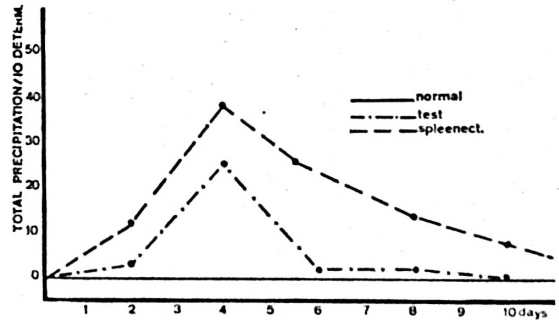

Fig. 4. *Determination of the products of the fibrinogen degradation by inmunology with antifibrine serum.*

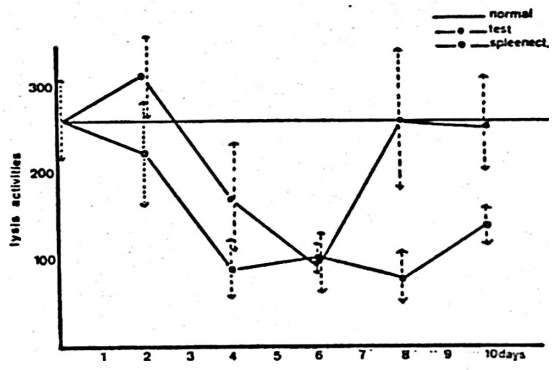

Fig. 5. *Fibrinolysis determination.*
The englobulines were obtained after precipitation with 0.1 % Cu_3-COOH, *p*H 5.2, and the fibrinolysis activities were determined with the Owren-Koller's buffer and M/80 $CaCl_2$ [according to the von Kaulla's method, modified by RABY (3)].

Discussion

From these results, we can explain the phenomena found by the following hypothesis:

Faced with a situation of stress, as that of the laparotomy, the organism increases its defenses in all its apparatus and systems, among the coagulation system, in which the concentration of all the factors increase significantly and among these factor I (fibrinogen). This state of alarm, completely physiological, in our case is mainteined for 4 days, and its mission fulfilled, is normalized, with the normalization being complete on the 6th day

after the operation. In the case of fibrinogen, the organism activates its fibrinolitic system, which proteolyzes the excess of the same, and, as a consequence, the products of the fibrinogen degradation appear simultaneously in the blood, and, acting as anticoagulants, induce a slight hypocoagulability. The coagulation and the fibrinolysis act synchronically, a dynamic equilibrium existing between the two systems oppered to each other. If the coagulolytic system prevails the lack of equilibrium may manifest itself in the form of thromboembolyc phenomena (hypercoagulability). On the other hand, from the manifestation of a predominance of the fibrinolytic system haemorrhagic tendencies would be produced (hypocoagulability). In a state of emergency, with the factors of the coagulation in concentration higher than normal, with a danger of thromboembolic accidents, which as shown are much more frequent in these postoperative states, the organism activates the opposing system, with the goal of mainteining the dynamic equilibrium between both, and the increase of the fibrinolysis, proteolyzes the excess of the coagulating factors, fibrinogen being among these, and leaves the products of degradation in the circulating blood. These, acting as inhibitors in all the stages of the coagulation, neutralize the previous hypercoagulable tendency and even produce a brief state of less dangerous slight hypocoagulability.

In the de-spleened animals, the process that take place are totally analogous to those of the control animals, which we have just explained. However, we do find two differences manifested: one, the greater intensity of these phenomena on the 4th postoperative day (fibrinolysis more intense, a greater amount of the products of degradation and therefore a more notable hypocoagulability); and two, a greater amplitude in the the time of said fenomena, the normalization not being produced on the 6th day, as occurred in the control animals, but rather, after the 10th day, which indicutes that the lack of spleen produces a transitory slowing-down in the regulation of the fibrinolytic system. This could be explained by accepting the hypothesis of the hormonal fibrinolysis regulation by the spleen. Lacking this, and while a secondary mechanism of regulation is inaugurated, the slowups are produced in the regulation, which are translated to a greater intensity of the fibrinolytic activity and a longer duration of said action.

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