REVISTA ESPAÑOLA DE FISIOLOGIA, 30, 217-222. 1974

The Splenectomy Effects on Blood Coagulation. I. Enzymatic Process Alterations in Coagulation

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(Received on June 6, 1973)

D. CARRIAZO, M. NIETO and A. GALARZA. *The Splenectomy Effects on Blood Coagulation. I. Enzymatic Process Alterations in Coagulation.* Rev. esp. Fisiol., 30, 217-222. 1974.

As an amplification of an already published paper on the splenectomy influence in the coagulation-fibrinolysis process, the various coagulation stages in splenectomized animals are studied, using a special technique, coagulography, which together with other tests, makes possible to classify the alterations produced during the hemostat enzymatic process. The conclusion is reached about the existance of an anticoagulant factor that affects all the coagulation stages and that it is not identifiable with heparin. Its appearance is produced also in laparotomized animals used as controls, but its effects are noticeably less pronounced.

In previous published papers (3, 5, 9), the effect that splenectomy produces on blood composition and on the coagulation-fibrinolysis process was studied. Among others effects, it was seen that the extirpation of the spleen produces a coagulation time lengthening. The inhibition of the heparin by protamine sulphate produces a very little variation of the values obtained in the splenectomized animals. The present paper is a continuation of the previous one, and in it each of the coagulation stages in splenectomized animals, specially by means of the coagulography technique (which allows us to learn the altered enzymatic phases and the plasmatic fibrinogen levels), is studied. At the same time, it sustantiates the possible existence of circulating anticoagulants, since it is a coagulation-dilution technique (6, 8). The presence of anticoagulants by means of correction and transference test, is also investigated, as well as the mastocyte variations in the bone-marrow.

Materials and Methods

Animals. White Wistar rats of both sexes, four months old and weighing

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between 180-200 g, were used in these experiments. The animals were divided in three groups: *a*) normal animals; *b*) laparotomized animals; *c*) splenectomized animals.

Sample taking. Blood was extracted from ether anesthetized animals through intracardiac puncture. Sodium citrate was used as the decalcifying agent.

Coagulography. Raby's technique (10) has been followed. Plasma free of platelets through centrifugation in a freezer at 3,000 r.p.m. during 30 min. and diluted to 1/10 with physiological saline solution (1, 3, 10) has been used. The progresive increase of O.D. that takes place after recalcification with $CaCl_2$ (10) was determinated at 400 nm by an UNICAM 800 spectrophotometer thermoregulated at 37° C provided with a chronometer.

Coagulography is a true kinetic study of coagulation that serves at the same time to discover masked hypo- and hipercoagulabilities (10).

Correction and transference tests. The technique described by STEFANINI has been followed (12, 13).

Mastocyte recount in the bone marrow. It was accomplished by dyeing with Giemsa areas of the femur bone-marrow. A thousand cells were counted and the results were expressed in mastocytes per cent (2, 15).

Fibrinogen. According to writings pending publication and other authors, the mg of fibrinogen/100 ml of plasma were deduced from the maximum amplitude of the coagulogram [mg fibrinogen $= (H \times 0.908 \times 10^3) - 4.240$] (4, 7, 11). For practical purpuses the fibrinogen mg are obtained by multiplying O.D. $\times 10^3$. Since coagulography takes place in plasma free of platelets, the H values are not affected by the platelet values (14).

Results

In coagulography a sigmoid curve is obtained. Figure 1 shows a coagulogram type, in which some chronometical constants can be singled out. The values of the chronometical constants can be singled out. The values of the chronometric constants are represented on various graphs, placing as abscises the days elapsed after the operation, and as ordinates the mean value of the constants obtained with ten experimental animals. The values of these constants are expressed in the number of seconds required by the diluted and re-

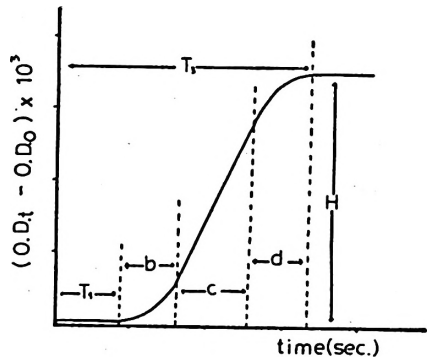

Fig. 1. *Coagulogram tipe.* Constants singled out: T1 (= time thromboplastin formation); T3 (coagulation total time); T2 (T3-T1); *b* (time of thrombin activation); *c* (time of fibrine formation); *d* (exhaustion of the thrombin activity); *H* (levels of plasmatic fibrinogen).

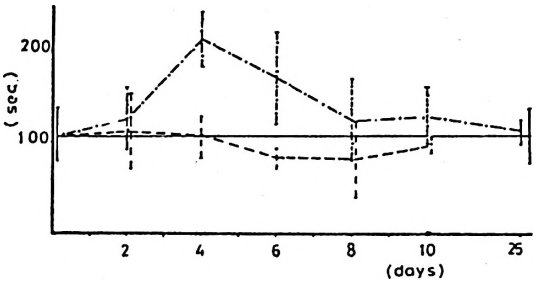

Fig. 2. *Variations of T1 according to the values obtained for this constant in the coagulogram.*
— normal; - - - laparotomized; · · · splenectomized animals.

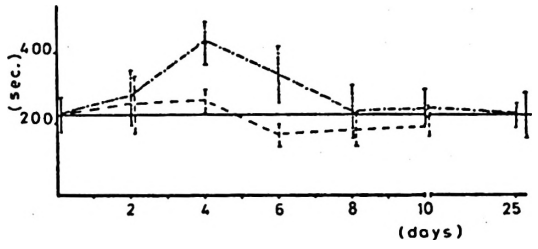

Fig. 3. *Variation of the T2 constant according to the T3-T1 values in the coagulogram, at different times of experimentation.*
— normal; - - - laparotomized; · · · splenectomized animals.

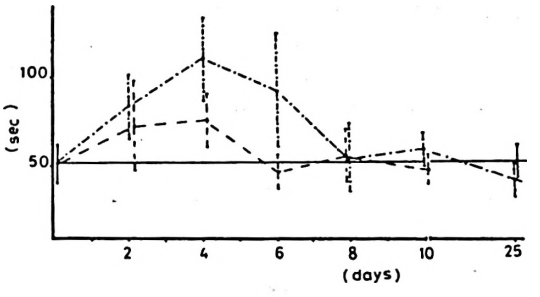

Fig. 4. *Variations of T3 values according to the results obtained in the coagulogram at different times after the operation.*
— normal; - - - laparotomized; · · · splenectomized animals.

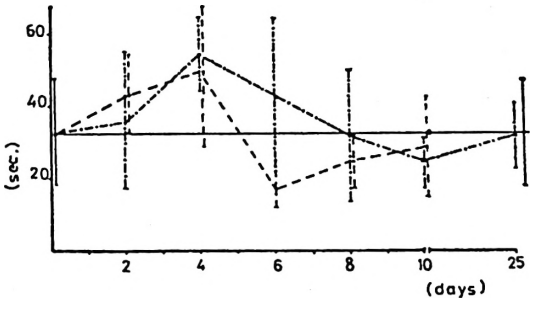

Fig. 5. *Variations of* b *constant according to the values obtained in the coagulogram at different times of the experimentation.*
— normal; - - - laparotomized; · · · splenectomized animals.

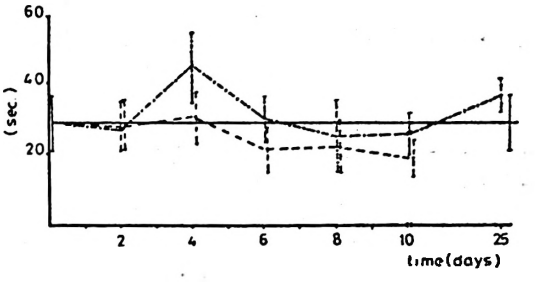

Fig. 6. *Variations of* c *constant according to the values obtained in the coagulogram at different times of the experimentation.*
— normal; - - - laparotomized; · · · splenectomized animals.

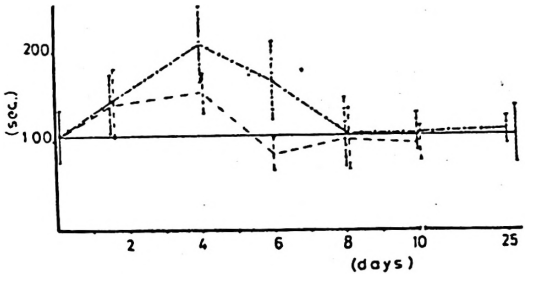

Fig. 7. *Variations of* d *constant according to the results in the coagulogram.*
— normal; - - - laparotomized; · · · splenectomized animals.

calcified plasma to acquire the O.D. in which the curve variation in the sigmoide is obtained. The variations are represented like this: T1 (fig. 2), T2 (fig. 3), T3 (fig. 4), *b* (fig. 5), *c* (fig. 6) and *d* (fig. 7), with the expressed indication of the characteristic desviation in the obtained values. The plasmatic fibrinogen, in mg/ml of plasma (O.D. of $H \times 10^4$) is found in

Table I. *Variations of the plasmatic fibrinogen at differents times of experimentation.* The fibrinogen is expressed in mg/100 ml of plasma.

Animal*	Time (days)					
	2	4	6	8	10	25
Splenectomized	452	567	226	239	217	—
Laparotomized	348	386	318	182	176	172

* Control animals: 143.1 ± 33.5.

Table II. *Correction and transference tests.*
Different proportion of normal and problem plasmas (in the 4th day after operation) are mixed and the coagulation time of the mixture determined in sec.

ml normal plasma		0.25	0.20	0.15	0.10	0.05	0.00
ml problem plasma		0.00	0.05	0.10	0.15	0.20	0.25
		Coagulation times in seconds					
Assay n.°	1	137.4	180.9	191.8	187.0	280.4	312.0
	2	126.3	283.8	287.8	247.0	351.7	430.3
	3	121.9	236.0	247.2	283.0	597.4	693.3
	4	147.2	211.4	222.8	273.4	360.9	416.1

Table III. *Mastocytes in bone-marrow.*
The mastocytes of the femur bone-marrow (treated by Giemsa) were counted and expressed in cells %.

Normal	Laparotomized	Splenectomized		
0	4 d.	2 d.	4 d.	6 d.
0.1	0.9	0.7	1.2	0.2
0.0	0.2	0.3	0.9	0.3
0.4	0.4	0.6	0.2	0.1
0.7	1.2	0.1	0.2	0.6
0.2	0.1	0.4	0.4	0.1

table I. In table II, correction and transference tests performed four days after the operation are shown, when the maximum variations in almost all the coagulographic constants occur. In table III the variations of the mastocytes in bone-marrow are expressed.

Discussion

In accordance with previous papers, splenectomy produces a lengthening in coagulation time, as derived from the recalcification time variations, thrombin and prothrombin times. This hypocoagulability is at its maximum on the four day after the operation, tending afterwards to normalization, reached from the 20th to the 25th day. A hypocoagulability occurs also in the controls animals, althougt with lesser intensity, coinciding its maximum values on the fourth day, but reaching normalization from the 6th to the 8th day.

The observation of the graphs obtained from the coagulogram for the diverse chronometic constants, corresponding to the various stages of the coagulation process, demands first of all a separation in the study of laparotomized and splenectomized animals, since their benhavior is different. In the splenectomized group, a lengthening of the constant T1, T2, T3, *b*, *c*, and *d* throughout the entire process, especially on the fourth day, is observed. The normalization of these constants occurs about the 8th day, too short a time to obtain normalization in the coagulation tests. These diferences could indicate the presence of a circulating coagulant. Inhibitors are more sensitive to dilution than activators and, whatever the importance of dilution may be, plasma with an unbalanced coagulation system in favor of activators is present. As indicated in the methodology section, a diluted plasma to 1/10 was used in this test. If an inhibitor be present, this dilution acts to lessen its action, if found in high proportion (4th day) or to inhibit it, if the concentration is weaker (days 8th-10th).

In the case of the control animals, the values are normal for all constants, except on the 4th day for constants *b* and *d*.

The explanation is analogous to the previous one. Its lesser anticoagulant concentration renders its physiological activity masked, due to dilution effect.

The results of the coagulation tests in the previously cited study and those of the coagulogram, seem to confirm the hypothesis of the presence of a circulating anticoagulant in both animal series, responsible for the hypocoagulability observed in both cases. All the stages of the coagulolitic process are affected by the activity of the supposed anticoagulant. The only non-normalized constants, *b* and *d*, may point to a more pronunced antithrombin and antipolimerase action.

The fibrinogen determination results exclude the possibility of derangement by this factor deffiency, since its levels are higher than normal, with its maximum concentration on the 4th day of the process.

A simple correction and transference test confirme the existence of a circulating anticoagulant. The neutralization test with protamin sulphate and the recount of mastocytes in the bone-marrow, seem to indicate that heparin is not found in pathological amounts. In the protamine sulphate test, only a partial neutralization is produced (in heparin and heparin-like products, the neutralization is complete, originating characteristic curves).

It is necesary to point out the importance of coagulography as an exploratory technique for the coagulation process, since it provides the plasmatic concentration of fibrinogen on one hand, and on the other it informs on the presence of inhibitors or activators. It furnishes also data on the coagulation process and on each of its stages.

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