Platelet Count and Thrombelastographic Maximal Amplitude in Experimental Thrombocythemias

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Thrombotic complications are often observed in thrombocythemias (2, 26, 36) and are usually interpreted as the consequence of increased platelet count and agglutinability. Hemorrhagic disorders also represent a frequent complication of thrombocythemias (5, 7, 8, 14, 16, 21, 28, 29, 38), and may be associated with the thrombosing tendency. Their explanation is not easy and a number of interpretations was suggested. A moderate increase of blood platelet was correlated with the thrombosing tendency, while massive increases of platelets were indicated as the cause of the hemorrhagic disorder. Such an explanation does not take into consideration some other possibilities, which represent the pathophysiologic basis of the disease. Experimental studies have shown that an excess of platelets *in vitro* may inhibit blood coagulation (23, 28, 33, 38). An anticoagulant activity was ascribed to platelets under such circumstances (36). A functional impairment of platelet was also taken into consideration, independently of the actual platelet count (thromboplastin generation test with platelets brought to constant concentration (16,35); subnormal serotonin content in platelets (4).

Additional defects, such as vascular fragility and hypofibrinogenemia, were also included among the possible pathogenetic factors (29, 39). The presence of hypofibrinogenemias was discussed for explaining both the thrombotic and the hemorrhagic complications. A decrease of the fibrinogen concentration should be considered as the consequence of the intravascular clotting. In polycythemias with thrombocythemia, the hypofibrinogenemia is aggravated by the relative fibrinogen deficiency (2).

Thrombelastographic studies in cases of thrombocythemia have shown that an excess of platelets may disturb the physical properties of the clot (thrombophilic patterns of the thrombelastogram; thrombelastogram "*en goulot de bouteille*", according to Marchal and Leroux [30—32]). *In vitro*, a rather *definite proportionality was observed between platelet count and maximal*

amplitude of the thrombelastogram, by varying artificially the platelet concentration (12).

It seemed, therefore, interesting to study the influence of the increased platelet count on the characteristics of the thrombelastogram, and particularly on the maximal amplitude, in subjects with experimental thrombocythemia, in order to identify the isolated effect of an excess of platelets, independently of the other factors, which may play a role in thrombocythemia. It should be remembered that thrombocythemias are often observed in conditions, such as the myeloproliferative disorders (leukemias [31, 32], erythremias, etc.) (13), which are often accompanied *per se* by a thrombophilic modification of the thrombelastographic pattern (12).

Our study was carried out by treating animals by means of various procedures, which are able to considerably elevate the platelet count. Thrombelastographic determinations were carried out under such conditions and the results correlated with the platelet counts. The results obtained have indicated that no thrombophilic tendency occurred in the presence of the thrombocythemic state, nor the thrombelastographic signs of a platelet impairment could be detected.

Material and methods

1. Production of experimental thrombocythemias. The following procedures were used to produce the experimental thrombocythemias: a) pantetheine, a combination of pantothenic acid and cysteamine (beta-mercaptoethylamine); the commercially available compound pantetine (Maggioni), resulting from the disulfuric binding of two molecules of pantetheine, was used;

b) serotonin (5-hydroxy-triptamine);

c) testosterone propionate;

d) progesterone;

e) somatotropic hormone (STH);

f) orchidectomy.

Serotonin, testosterone and progesterone were kindly supplied by Vister (Casatenovo Brianza), STH by Choay (Milano), pantetine by Maggioni (Milano).

The various substances were administered according to the scheme of Table 1, in which are also indicated the animals used in each experiment and the frequency of blood examinations. The orchidectomy was carried out by means of aseptic operation under the usual conditions.

2. Blood samples. For coagulation studies, oxalated blood (one part of sodium oxalate 0.1 M and nine parts of blood) was obtained by means of heart puncture in rabbits and by means of aortic puncture in guinea pigs, in the living, slightly anesthetized animal, after laparotomy. Guinea pigs were then sacrifed. A group of 5 animals was taken for each determination, when guinea pigs were used. Not less than eight to ten milliliters of oxalated blood were taken for each sample, in order to insure reliable results in thrombelastographic determinations. Capillary blood was used for platelet counts.

3. Methods. Thrombelastographic determinations, by means of the Hartert thrombelastograph (17, 18), and platelet counts were carried out according to the methods described elsewhere (9, 10).

4. Evaluation of the results. The mean values of the platelets counts and of the maximal amplitude of the thrombelastograms are reported in Tables 2 and 3. The values referring to each single determination are indicated in Fig. 1—6 (platelet counts plotted against maximal amplitude).

Table 1:

Procedure	Animal (N.)	Mean weight	Daily dose	Duration of the treatment	Blood samples taken after:
Pantetine	Rabbits (7)	2500 g	40 mg/kg	8—10 days	0, 3, 5, 7, 9, 11 days
Serotonin	Rabbits (11)	2500 g	44 mg/kg	(single dosis)	0, 4, 8, 12, 24 hours
Testosterone	Guinea pigs (30)	300 g	15 mg/kg	25 days	0, 5, 10, 15, 20, 25 days
Progesterone	Guinea pigs (30)	300 g	15 mg/kg	25 days	0, 5, 10, 15, 20, 25 days
STH	Guinea pigs (30)	300 g	10 U/kg*)	25 days	0, 5, 10, 15, 20, 25 days
Orchidectomy	Rabbits (18)	2500 g	—	—	0, 20, 40, 60 days

*) Evans units.

Results

The following results were obtained:

1. *Platelet counts.* In all experimental conditions, a more or less marked thrombocythemia was obtained. In Table 2, the mean values referring to the platelet counts at the various intervals, are reported. After the administration of pantetine, the maximal rise of the platelet counts was obtained after 10 days, i.e. after 1000 mg of drug. The highest individual value was 2 000 000. The rise of platelets after serotonin was not as marked, but reached individual values as high as 1 280 000. The increase of platelet counts after the sexual hormones was a little less pronounced, insofar as the highest peaks were 1 070 000 for testosterone and 740 000 for progesterone. The administration of STH produced a considerable increase of platelets in some experiments, with maximal peaks around 1 400 000. Orchidectomy also caused a considerable degree of thrombocythemia 60 days after the operation, while less pronounced modifications were observed previously.

Table 2:

Procedure	Blood samples*) 1	2	3	4	5	6
Pantetine	348	550	722	815	957	1136
Serotonin	437	808	717	1021	362	
Testosterone	341	301	496	528	932	909
Progesterone	341	510	544	656	622	627
STH	341	480	540	580	610	1128
Orchidectomy	440	450	360	640		

*) Blood samples taken at the intervals, which are indicated in Table 1.

2. *Maximal amplitude.* The modifications of the maximal amplitude can be visualized in the mean values, which are reported in Table 3. As a general rule, the administration of hormones did not significantly modify the maximal amplitude, with respect to both the mean values and the individual values. There was a greater degree of variability in the other experimental conditions, i.e. administration of pantetine and serotonin, and orchidectomy, but not much beyond the control values. As a whole, the modifications of the maximal amplitude exhibited a slight tendency to the increase of the values, but within very modest limits.

Table 3:

Procedure	Blood samples*) 1	2	3	4	5	6
Pantetine	67	68	66	66	66	63
Serotonin	53	57	57	52	54	
Testosterone	68	70	73	70	70	72
Progesterone	68	68	70	69	69	69
STH	68	67	66	68	74	71
Orchidectomy	64	62	66	66		

*) Blood samples taken at the intervals, which are indicated in Table 1.

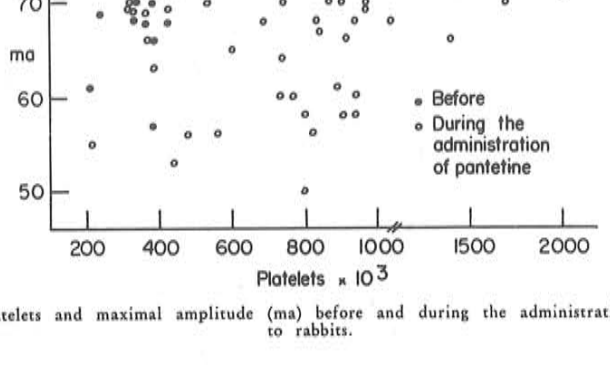

Fig. 1: Platelets and maximal amplitude (ma) before and during the administration of pantetine to rabbits.

3. *Correlations between platelet count and maximal amplitude.* By plotting the values of the platelet counts against those of the maximal amplitude, no significant correlations could be detected, as evidenced in Fig. 1—6. There were

very high platelet levels, which were accompanied by low values of the maximal amplitude and vice versa. It was not possible to demonstrate that the highest values of the platelet counts were consistent with a significant increase of the maximal amplitude on the basis of either the individual values or the mean values.

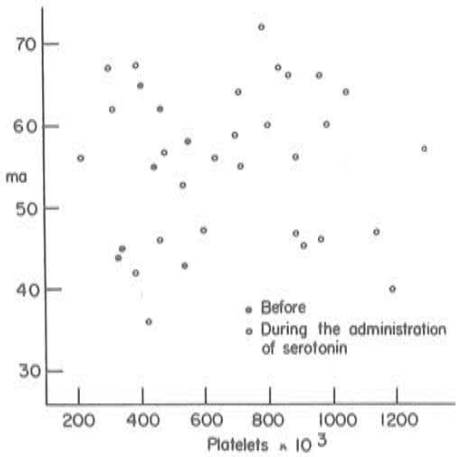

Fig. 2: Platelets and maximal amplitude (ma) before and during the administration of serotonin to rabbits.

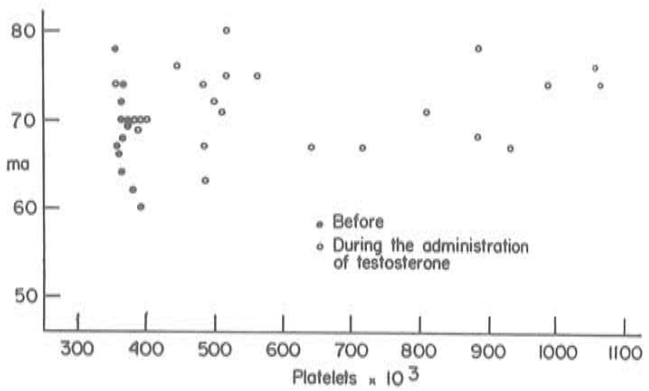

Fig. 3: Platelets and maximal amplitude (ma) before and during the administration of testosterone to guinea pigs.

Discussion and Conclusions

The results obtained can be discussed first of all by taking into consideration the characteristics of the experimental thrombocythemias which were produced

by using the various procedures. The rise of platelet count could be easily predicted after serotonin and after pantetine on the basis of previous reports. As far as serotonin is concerned (3, 6, 20), it was suggested that a stimulation of the

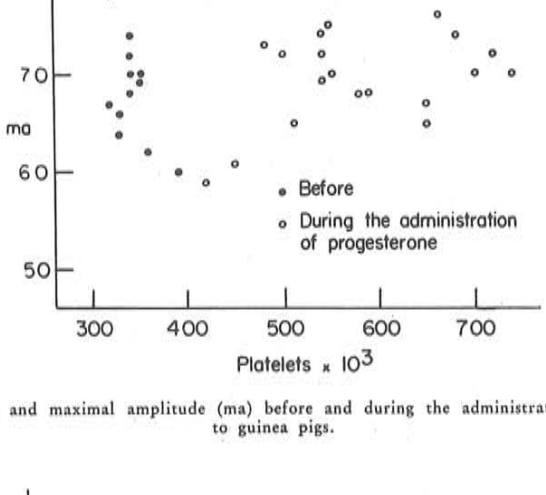

Fig. 4: Platelets and maximal amplitude (ma) before and during the administration of progesterone to guinea pigs.

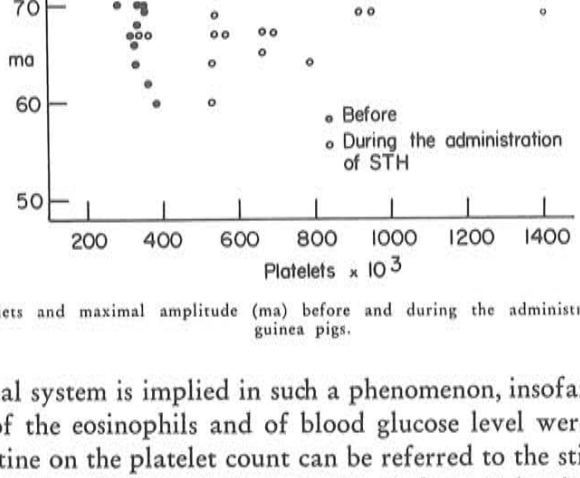

Fig. 5: Platelets and maximal amplitude (ma) before and during the administration of STH to guinea pigs.

pituitary-adrenal system is implied in such a phenomenon, insofar as concomitant modifications of the eosinophils and of blood glucose level were observed. The action of pantetine on the platelet count can be referred to the stimulating action of pantothenic acid, a component of pantetine. Such an action is, however, much more pronounced in pantetine (1, 15). The findings of a marked thrombocythemia after the administration of progesterone and of testosterone was unex-

pected, although these hormones had been already used in therapeutics for the treatment of thrombocytopenias, and some experimental data had indicated a moderate effect in increasing the platelet level.

The thrombocythemia after orchidectomy was observed also unexpectedly in the course of researches on the hormonal regulation of blood coagulation (11). Hypophysectomy is also followed by a rise of platelet count, which is, however, less pronounced than after orchidectomy. Such findings were confirmed by some recent, similar experiments (27).

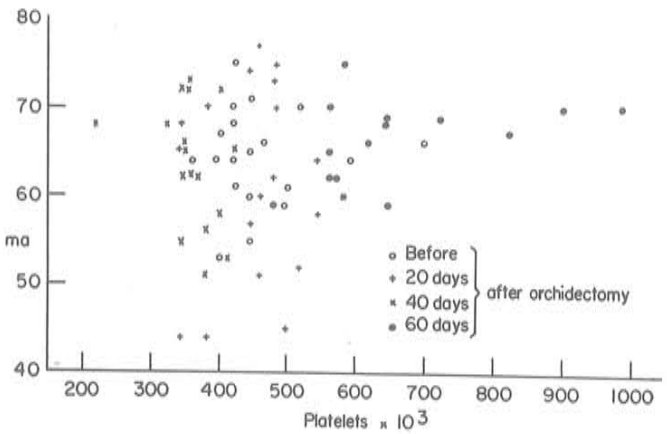

Fig. 6: Platelets and maximal amplitude (ma) before and after orchidectomy in rabbits.

Although these experimental conditions imply some modifications of the physiologic balance, they were not accompanied by a pathologic situation, such as happens in thrombocythemic leukemias or polycythemias. It was, therefore, possible to study the effect of the increased platelet level on that characteristic of the thrombelastogram, which is usually referred to the variations of platelet activity, i.e. the maximal amplitude. On the basis of other observations it can be stated that the variations of the other coagulation factors were not as pronounced as those of platelets. Only a rather marked decrease of fibrinolytic activity could be observed after the administration of testosterone and progesterone. In other words, there were no other factors which could modify the maximal amplitude of the thrombelastogram. In spite of the marked increase of the platelet counts, the variations of the maximal amplitude could be not referred to these modifications. In contradistinction to the findings in vitro, in which a rather definite proportion exists between platelet count and maximal amplitude (12, 19), the increase of the platelet concentration up to 2 millions (serotonin) was not followed by a proportional increase of the maximal amplitude. On the other

side, it could be not demonstrated that such an excess of platelets caused an impairment of that properties of the clot, which are responsible for the maximal amplitude.

Furthermore, the functional activity of these platelets should be considered normal, as far as it may influence the maximal amplitude: for instance, some platelet functional defects (e.g. thrombasthenias) may be accompanied by a decrease of the maximal amplitude (10, 19).

These findings can be evaluated in connection with the physiopathologic significance of the maximal amplitude under these experimental conditions. The thrombocythemic state, within the limits of our observations, did not seem to influence the maximal amplitude, either in the sense of an increase (thrombophilic tendency) or in the sense of a decrease (platelet functional defect even in the presence of an increased platelet level, and/or inhibiting effect of an excess of platelets).

Such experimental thrombocythemias do not reproduce, therefore, the thrombelastographic patterns, which are observed in pathologic conditions, as already mentioned. Other factors should be also involved in determining, under such conditions, the thrombophilic aspect of the thrombelastogram or its altered shape (see 34). The influence of leukocytes was taken into consideration (22, 40), and may play a role in such a phenomenon. Our observations are indicating that the thrombocythemic state *per se* is not able to cause consistently such alterations, at least within the platelet level of 1.5—2.0 millions.

Summary

Experimental thrombocythemias were produced in animals by injecting pantetine, serotonin, progesterone, testosterone, STH, and by carrying out orchidectomy. Thrombelastographic determinations of the maximal amplitude were carried out under such conditions and the results correlated with the platelet counts. No significant increases of the maximal amplitude could be observed, even in the presence of very high platelet levels, nor the thrombelastographic signs of a platelet impairment could be detected.

Résumé

Des thrombocythémies expérimentales sont produites chez l'animal par injection de pantétine, sérotonine, progestérone, testostérone, STH et également par

orchidectomie. Une étude parallèle de l'amplitude maxima du thrombélastogramme et du nombre des thrombocytes a été effectuée. Aucune augmentation significative de l'amplitude maximale n'a été observée, même pour les taux de plaquettes les plus élevés. Aucun signe d'anomalie des plaquettes dans le thrombélastogramme n'a pu être détecté.

Zusammenfassung

Experimentelle Thrombozythämien wurden bei Tieren durch die Injektion von Pantetin, Serotonin, Progesteron, Testosteron, STH und durch Orchidektomie erzeugt. Es wurde die Maximal-Amplitude im Thrombelastogramm bestimmt und mit der Plättchenzahl verglichen. Es konnte keine signifikante Zunahme der Maximal-Amplitude selbst bei sehr hohen Plättchenzahlen nachgewiesen werden. Auch konnte kein Zeichen eines Plättchen-Defekts im Thrombelastogramm beobachtet werden.

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