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The Effect of Heparin on the Bleeding Time

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Normal hemostasis depends on platelets, coagulation, and blood vessels, but the interrelations among the three parts of the mechanism are still unclear. Observations by in vivo microscopy have shown that lesions of small blood vessels are rapidly sealed by platelet plugs [Apitz (1942), M. Zucker (1947), Hugues (1953)]. The platelets first aggregate, and then undergo a peculiar change which is described as "viscous metamorphosis".

It is not known whether coagulation is necessary for the proper formation of such plugs. Generally, fibrin is not regarded as an essential constituent of the platelet plugs [Apitz (1942), M. Zucker (1947), H. Zucker (1949)], but this does not exclude a possible effect of the coagulation process on plug formation, since thrombin or intermediate products formed during coagulation may still play an important role.

In vitro studies strongly support such a concept, since platelets clump and undergo viscous metamorphosis during coagulation of platelet-rich plasma. At least three different principles formed during coagulation are said to provoke platelet clumping with or without viscous metamorphosis: product 1 [Bergsagel (1956)], activation product [Waaler (1959)], and thrombin [Bounameaux (1959)].

In vivo, the bleeding time is considered to reflect the efficiency of the platelet plugs, and the bleeding time is generally normal in congenital disorders of coagulation, suggesting that coagulation is not involved in the formation of platelet plugs. If this is so, anticoagulants should not prolong the bleeding time. The literature contains numerous reports on the effect of heparin on the bleeding time. We have summarized the results of these reports in Table 1. Essential details are often lacking, such as the exact dose of heparin, the method of administration, the interval between administration and testing, the technique

for measuring the bleeding time, and the number of observations. Taken together, however, the reports suggest that heparin in ordinary doses has no effect on the bleeding time, and, in large doses, only a slight and inconsistent effect. Repeated injections gave similar results [Copley and Robb (1942), Ganes (1942), Hjort and Stormorken (1957)].

Table 1: The effect of heparin on the bleeding time: review of the literature. The heparin dose has been calculated in mg, per kg body weight, assuming 1 mg of heparin = 100 I.U., and the average weight of man = 70 kg

Author	Species	No. of observations	Dose of heparin mg/kg	Bleeding time	
				method	result
Sköld (1936)	Man	—	ca. 0.35	—	No prolongation
Hedenius (1937)	Man	—	1.0	—	No prolongation
Heim (1938)	Man	50	ca. 0.15	—	No prolongation
Sappington (1939)	Man	20	1.0	Duke	No prolongation
		3	2—3.5		Slight prolongation
Lindgren and Wilander (1941)	Man	5	1—1.8	Ivy	No prolongation
Ganes (1942)	Man	51	1.4	Ivy	No prolongation
Roskam (1942)	Man	7	2.1	Mod. Duke	Slight but significant prolongation
Quick et al. (1948)	Man	2	0.8	—	No prolongation
Hjort and Stormorken (1957)	Man	5	2.1	Mod. Ivy	Slight prolongation
Borchgrevink and Waaler (1958)	Man	2	2.1	Mod. Ivy	Slight prolongation
Jores and Detzel (1940)	Rabbit	—	1.5	—	No prolongation
Macfarlane (1941)	Rabbit	1	—	Skin prick	No prolongation
Roskam (1942)	Rabbit	4	2.5×2	Running water	Slight prolongation
		4	2.5×2	Duke	Definite prolongation
Halse (1952)	Rabbit	—	3 "High"	Ear vein cut Duke	Slight *shortening* Prolongation
Jores and Detzel (1940)	Cat	—	1.25	—	Slight prolongation
Fulton et al. (1953)	Hamster	—	15	Observation on cheek pouch	Prolongation
Apitz (1942)	Rats	—	—	Several methods	Prolongation
M. Zucker (1947)	Rats	10	5	Observations	Prolongation in some
		4	22.5	on mesenterium	
Correll et al. (1952)	Rats	9	0.3	Tail severed	Prolongation
Copley and Lalich (1942a)	Mice	98	0.1—400	Tail vein prick	Prolongation in 4
		9	2.3—9.1	Tail vein prick	No prolongation
		6	91	Tail vein prick	Prolongation in 4
		6	230	Tail vein prick	Prolongation in 2
		7	460	Tail vein prick	Prolongation in 5
Copley and Robb (1942)	Mice	3	100	Tail vein prick	No prolongation
		7	250	Tail vein prick	Prolongation in 3
		6	500	Tail vein prick	Prolongation in 4
Apitz (1942)	Mice	—	—	Several methods	Prolongation
Lalich and Copley (1943)	Mice	7	23—46	Tail cuts	No prolongation
		36	91—182	Tail cuts	Prolongation in 6

Thus, the *in vitro* experiments suggest an effect of coagulation on clumping and viscous metamorphosis of platelets, while the *in vivo* studies apparently have not confirmed such an effect. We have therefore reexamined the effect of heparin on the bleeding time in three species. Under standardized conditions we found that heparin in large doses markedly and consistently prolonged the bleeding time.

Materials and Methods

Heparin. Heparin 5 per cent "A-L", Oslo, Norway, was used. The preparation contains 100 I.U. per mg of heparin, or 5000 I.U. per ml solution. If necessary, heparin was diluted in physiological saline before injection.

Experiments in man. Before and 15—30 minutes after intravenous injections of heparin into healthy male volunteers, the following tests were done: platelet count, bleeding and clotting times. At least five days passed between the injections, and the doses were given in random order without informing the observer about the dose. The platelets were counted in venous blood by the method of Brecher and Cronkite (1950), using two pipettes and two chambers, counting at least 200 platelets in each chamber. The bleeding time was recorded as the mean of three cuts using a modified Ivy technique [Hjort and Stormorken (1957)]. The average of 35 tests in 30 normal individuals was 6.2 minutes with a range of 2—12.5 minutes. When heparin had been given, there was often renewed bleeding from the cuts, and two bleeding times were then recorded: the time from the cut until complete cessation for 60 seconds (bleeding time), and the total number of minutes during which there was bleeding in the half hour following the cut (total bleeding time). The whole blood clotting time was measured by the method of Hjort and Stormorken (1957). The average of 60 tests on 36 normal individuals was 4.5 minutes with a range of 2.7—7.0 minutes.

Experiments in rats. Male rats weighing 180—300 gm were used. They were anesthetized by an intraperitoneal injection of Nembutal (Abbott Lab., North Chicago, Ill., USA), 4 mg per 100 gm body weight. Heparin was injected into a tail vein, 0.1 ml per 100 gm body weight. *Nine minutes* later, the platelets were counted in tail vein blood by the method of Brecher and Cronkite (1950), using one pipette and one chamber and counting at least 400 platelets in the chamber. *Ten minutes* after the injection, the posterior edge of one ear was quickly cut off with a new Gillette surgical blade. The removed segment measured about 6 by 1 mm. The ear was immediately placed in a tube containing saline at 38° ± 0.5° C. After a short lag period, the blood flowed in 10—20 thin streams from the wound. The bleeding increased rapidly, and then decreased more slowly. The bleeding time was measured from the time of cutting until all streams had stopped for at least 60 seconds. In untreated rats, there was often renewed bleeding 1—5 minutes after the endpoint, but these bleedings lasted only for 15—25 seconds, and were ignored in determining the endpoint. The amount of blood lost was initially measured, but this was discontinued since there was no close correlation between blood loss and bleeding time. If the bleeding stopped within 10 minutes, the bleeding time was examined for the other ear also. In 50 determinations on 25 untreated rats we found a mean bleeding time of 2'25" with a range of 1¼—4¾ minutes. One determination was excluded, since the ear bled for more than 15 minutes; the other ear of this rat gave a normal bleeding time. *Thirty minutes* after the injection, the abdomen was opened, and about 4 ml blood was aspirated from vena cava into a dry, siliconized syringe. One ml was then measured into each of two tubes, and the whole blood clotting time measured at 37° C by the method of Hjort and Stormorken (1957).

Experiments in mice. White mice weighing 20—25 gm were used. Heparin was given subcutaneously, and the bleeding and clotting times were measured 15—20 minutes later. For the

bleeding time, the mouse was put in a cylinder stoppered at both ends with perforated corks, one for fresh air, the other to let the tail out. The tail was prewarmed in water of 38° C. 1½ cm from the tip a vein was cut with a sharp Gillette blade. The tail was immediately dipped into saline at 38° C and the bleeding observed. In order to measure the amount of bleeding per unit time a slightly different method was used. Several small glass tubes, each containing 3½ ml of saline were put in a waterbath at 38° C. The tail was put successively into each tube for a fixed period of time, 15 or 30 seconds, until the bleeding stopped. A trace of saponin was then added to each tube, and hemoglobin was measured as oxyhemoglobin in a Ljungberg colorimeter (AB Lars Ljungberg, Stockholm, Sweden). To measure the whole blood clotting time, the heart was opened, and a capillary pipette was filled with blood. At intervals, a small piece of the pipette was broken off. The clotting time was recorded as the time from the filling of the pipette until the first appearance of a fibrin thread.

Results

a) *Man.* Table 2 and Figure 1 show the effect of heparin on the platelet count and on the bleeding and clotting times. Heparin did not significantly lower the platelet count, but in doses over 2 mg per kg it did prolong the bleeding time. The bleeding characteristically came in waves, often stopping for a few minutes, and then starting again.

Table 2: The effect of heparin on platelet count, bleeding and clotting times in man. The figures in brackets denote the total number of minutes during which there was bleeding in the half hour following the cut

Person	Heparin mg/kg	Whole blood clotting time	Bleeding time, min.	Platelet count, in 1000's per cmm
1	0	3½'	5½	262
	1	25'	5	—
	2	54'	8½ (14)	268
	3	> 24 hours	11½ (18)	241
	7	> 24 hours	> 30 (> 30)	240
2	0	3'	8	323
	2	65'	7½	295
	7	> 24 hours	15½ (24)	286
	10	> 24 hours	20½ (26)	313
3	0	4'	4½	187
	1½	> 6 hours	5	195
	3	> 24 hours	10 (15½)	172
	7	> 24 hours	19 (27)	177
4	0	3'	5	280
	2	100'	12½ (24½)	279
	5	> 24 hours	5½ (20½)	270

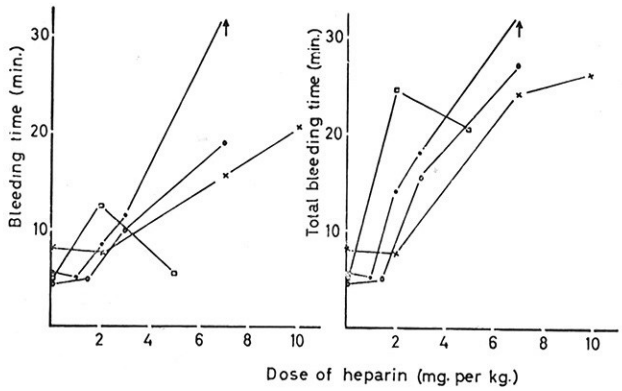

Fig. 1: The effect of heparin on the bleeding time in man

b) *Rats.* Table 3 gives the results for heparinized rats. With a heparin dose of 2 mg per kg the bleeding was continuous, uniform, and generally quite pro-

Table 3: The effect of heparin on platelet count, bleeding and clotting times in rats

Heparin dose	Rat no.	Platelets in 1000's per cmm	Whole blood clotting time in min.	Bleeding time	
				in min.	comments
2 mg/kg	1	620	> 40	> 20	Bleeding is rapid and even
	2	695	> 40	> 20	
	3	910	> 40	> 20	
	4	745	> 40	> 20	
1 mg/kg	1	1052	32	> 20	Bleeding occurs in waves, but never stops completely
	2	542	> 40	> 20	
	3	652	> 40	> 20	
	4	860	> 40	> 20	
0.5 mg/kg	1	675	37	> 20	Bleeding occurs in waves, and often stops for 15—60 secs.
	2	935	40	16½	
	3	862	37	15	
	4	772	14	18	
0.25 mg/kg	1	650	15	2, 2½	Endpoint difficult to determine because of frequent relapses. Most ears startet to bleed again later on
	2	820	14	8½, 4	
	3	920	18	6, 8	
	4	630	18	2¼, 5½	
0 mg/kg	1—15				Endpoint is not difficult to determine, but relapses are not rare
Average		744	4.3	2.4	
Range		540—1010	3—6½	1¼—4½	

fuse. After 1 mg per kg, the bleeding came in waves without stopping completely. With smaller doses, the bleeding stopped for a few seconds or even a minute or two, and then started again, lasting from 30 seconds to a few minutes. In many experiments not reported here we increased the dose in small steps from 0 to 0.5 mg of heparin per kg, hoping to demonstrate successive increases in the bleeding time. However, we did not succeed in this since the endpoint was too difficult to determine accurately after injections of small amounts of heparin. We have therefore only included one group of rats receiving 0.25 mg/kg to illustrate this point.

c) *Mice.* Table 4 shows the correlation between the dose of heparin and the bleeding and clotting times. Doses up to 20 mg of heparin per kg did not prolong the bleeding time, in spite of a markedly prolonged clotting time. Larger doses of heparin, however, did prolong the bleeding time: with 400 mg per kg the bleeding did not stop at all in more than half the mice.

Table 4: The effect of heparin on the bleeding and clotting times in mice

No. of mice	Heparin mg/kg	Bleeding time		Whole blood clotting time	
		mean (secs.)	range (secs.)	mean	range
28	0	49	24—110	51 secs.	15—145 secs.
10	1	—	—	115 secs.	56—276 secs.
10	5	65	32—105	> 4 hours (< 18 hours)	
10	20	60	37— 75	> 18 hours	
25	100	84	39—239	> 18 hours	
29	100 × 2[1]	120	37—332	> 18 hours	
10	200	212	47—> 600[2]		
10	400	376	57—> 600[2]		

[1] 4 hours' interval.

[2] When calculating the mean, > 600 seconds was taken *as* 600 seconds.

In non-heparinized mice the bleeding usually increased during the first 30 seconds, and then steadily decreased until it stopped. This was the same with both deep and superficial cuts. When heparin was given in a dose which gave a measurable prolongation of the bleeding time, the bleeding was similar. However, when heparin was given in a dose which was large enough to give a very prolonged bleeding time, the bleeding came in waves (see Fig. 2).

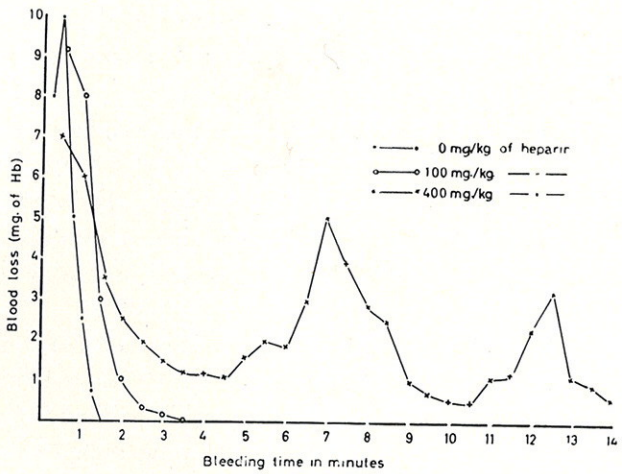

Fig. 2: Blood loss from tail cuts in mice. See text for a description of the technique

Discussion

The bleeding from a bleeding time wound in man is primarily stopped by platelet plugs [H. Zucker (1949)]. Histologically, these plugs are similar to the head of white thrombi. Studies on the formation of platelet plugs may therefore shed light on both the arrest of bleeding and the mechanism of thrombosis.

One essential problem in this field concerns the role of coagulation in the formation of adequate platelet plugs. *In vivo* microscopy has suggested that there are two steps in this formation: aggregation of platelets, and viscous metamorphosis [Apitz (1942), Hugues (1959)]. Prolongation of the bleeding time in the absence of thrombocytopenia may therefore be caused either by a failure of the platelets to aggregate or by interference with viscous metamorphosis.

We have found that heparin in sufficiently large doses always prolongs the bleeding time in men, rats and mice. To obtain this effect, we had to give more than 2 mg per kg to men, more than 0.5 mg per kg to rats, and more than 200 mg (subcutaneously) per kg to mice. These differences probably depend both on species and on the technique. Smaller doses often prolonged the bleeding time, but their most conspicuous effect was a change to a wavelike type of bleeding: the bleeding may be prolonged, but waxing and waning, or it may stop for short periods and then start again, several times. Therefore, the techni-

que is important, and an insensitive method may fail to demonstrate abnormal bleeding. We believe that these points explain the variable results reported in the literature.

Large doses of dicoumarol may also prolong the bleeding time [Lalich et al. (1943), van Cauwenberge and Jaques (1958)]; and the mean bleeding time of a group of hemophiliacs was significantly longer than in normals, although still within normal limits [Borchgrevink and Waaler (1958)]. Lewalle et al. (1959) found a decreased resistance of the hemostatic plugs in patients with coagulation defects.

Heparin inhibits viscous metamorphosis [Zucker and Borrelli (1959)], and this explains the effect of *moderate* doses, since viscous metamorphosis is probably necessary for making the plug impermeable to blood. *In vivo* microscopy has shown that such doses do not prevent plug formation. In fact, the plug is often larger than normal, but it is friable, small parts are often detached, and it remains permeable to blood [Apitz (1942), M. Zucker (1947), Hugues (1953)]. Copley and Lalich (1942 b) mention that the clot resistance is often reduced in heparinized mice with a normal bleeding time. Finally, it should be mentioned that moderate doses of heparin do not prevent white thrombus formation in arterio-venous shunts [Shionoya (1927), Best et al. (1938)]. These observations are in agreement with our findings with such doses: an irregularly prolonged and often wavelike bleeding from the cuts. Taken together, all these observations suggest that coagulation is not necessary for the aggregation of platelets, but it *is* necessary for the production of a firm plug by means of viscous metamorphosis and fibrin formation. This conclusion is identical to that of Roskam (1942).

If this is the only explanation, it is difficult to understand how there can be a difference in the effect of two doses of heparin, when the smaller one prolongs the whole blood clotting time to more than 24 hours. This may be due to very favourable clotting conditions within the platelet plug, but it is more likely that *large* doses also interfere with the aggregation of platelets. Such doses gave profuse, continuous bleeding in our rats, which we may take as an indication that platelet plugs were not formed. This was, however, not seen in men and mice. *In vivo* microscopy has given direct evidence that the plug may fail to form in the presence of such doses [M. Zucker (1947), Hugues (1953), Fulton et al. (1953), Berman et al. (1955)], and experiments with arterio-venous shunts led to the same conclusion [Best et al. (1938)]. Thus, heparin may also prevent the aggregation of platelets, but excessive doses are needed for this effect.

Summary and Conclusions

1. Moderate doses of heparin often prolonged the bleeding time in men, rats and mice. The bleeding was wavelike, and a sensitive method was necessary to demonstrate the prolongation.

2. Large doses gave a prolonged bleeding time, and the bleeding was often profuse and continuous.

3. Heparin did not produce thrombocytopenia. Moderate doses probably prevent viscous metamorphosis and fibrin formation, resulting in large but inefficient platelet plugs. Large doses may also interfere with the aggregation of platelets, preventing the formation of platelet plugs.

Résumé

1. Des doses modérées d'héparine prolongent souvent le temps de saignement chez l'homme, le rat et la souris. Le saignement est irrégulier, en forme de vague et une méthode sensible est nécessaire pour démontrer la prolongation du temps de saignement.

2. Les fortes doses d'héparine prolongent le temps de saignement et provoquent des hémorragies profuses et continues.

3. L'héparine ne provoque pas de thrombocytopénie. Les doses empêchent probablement la métamorphose visqueuse et la formation de la fibrine avec, comme résultat, la formation de bouchons plaquettaires grands mais inefficaces. Les fortes doses d'héparine peuvent interférer avec l'agréggation des plaquettes et empêcher la formation des bouchons plaquettaires.

Zusammenfassung

1. Die Blutungszeit wurde durch Heparin in mittleren Dosen beim Menschen, bei der Ratte und der Maus oft verlängert. Die Blutung war wellenförmig, und man muß daher eine empfindliche Methode verwenden, um die Verlängerung nachzuweisen.

2. Die Blutungszeit wurde immer von Heparin in großen Dosen verlängert; die Blutung war meistens kräftig und andauernd.

3. Heparin verursachte keine Thrombozytopenie; unsere Befunde können wahrscheinlich folgendermaßen erklärt werden: Mittlere Dosen von Heparin reduzieren die Widerstandsfähigkeit der Plättchenpfropfen durch eine Hemmung

der viskösen Metamorphose und der Fibrinbildung. Größere Dosen verhindern wahrscheinlich die Bildung von Plättchenpfropfen durch eine Hemmung der Aggregation der Plättchen.

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