Upsala J Med Sci 79: 28–38, 1974

Haemostatic Plug Formation in the Rabbit Mesentery

A Methodological Study

D. BERGQVIST

Department of Experimental Medicine, Pharmacia AB, and
Department of Surgery, University Hospital, Uppsala, Sweden

ABSTRACT

A methodological study has been carried out using the rabbit mesentery for the investigation of the initial haemostatic mechanism in the microvasculature. Heaemostatic plug formation in proximal arteriolar ends differ from that in venules in many respects. The arteriolar haemostatic plug formation time is shorter than the venular and it is vessel size dependent in arterioles but not in venules. The frequency of rebleeding is higher and the rebleeding time is longer in venules than in arterioles. It is stressed that the same vessel type and size should be used in comparative studies if comparable results are to be obtained. It is also shown that most of the rebleedings appear within 10 minutes of the cessation of the primary bleeding and that the haemostatic plug formation time shows a skew distribution, which should be taken into account in statistical calculations. The importance of using an adequate experimental technique including a sharp and swift transection of the vessel is also stressed.

INTRODUCTION

The mesenteric preparation of different animal species has been used for various microcirculatory investigations, particularly since the report of the method by Chambers & Zweifach (6). Several authors have used this method when studying the initial haemostatic process (1, 7, 12, 16, 20, 21, 22, 23, 27, 29, 30, 36). Among these authors Hugues (22) made the most careful methodological study, although all have used the model to study different aspects of the platelet reaction in the early haemostatic mechanism. With a few exceptions (8, 9, 28) most investigators agree on the fundamental role played by the platelets in sealing a bleeding microvessel. Apitz (1) called the aggregate of platelets »die blutstillende Thrombose» and Zucker (36) called it »the platelet plug». Their histological findings were verified by the ultrastructural studies on the »haemostatic platelet plug» made by Kjaerheim & Hovig (27), who for the first time showed that the plug was composed of densely packed platelets. Thus many investigations, dealing with different aspects of the haemostatic mechanism have been carried out in transected and punctured mesenteric microvessels. However, there are many discrepancies in the results, especially in the evaluation of arteriolar versus venular haemostasis and the occurrence and nature of rebleedings (1, 7, 12, 16, 22, 30, 36). Because of these contradictory findings it is not possible to use the mesenteric model for haemostatic studies without further methodological investigations.

The aim of this investigation therefore was to:

1. standardise the mesenteric model for studies of the initial haemostatic mechanism
2. study the influence of vessel type and size on the haemostatic plug formation time
3. study the rebleeding pattern
4. establish the importance of these factors to form a basis for further physiological, pathophysiological and pharmacological studies.

MATERIALS AND METHODS

112 New Zealand white rabbits of both sexes (2.7± 0.5 kg), fed on a standard diet (Ewos pellets, Astra-Ewos AB, Södertälje or Teknosan pellets, Ferrosan AB, Malmö, Sweden), were used.

The animals were starved at least 12 hours before the experiment. Prior to anaesthesia bleeding time, whole blood coagulation time and haematocrit were performed in all rabbits as a screening method to exclude abnormal animals.

The bleeding time was measured in the following way: the rabbit ear was warmed for 2 minutes with a heating lamp and veins of 0.2–0.5 mm diameter

were transected. The mean of four bleeding times was recorded in every rabbit.

Whole blood coagulation time was determined by breaking off a short length of blood-filled capillary tube every 30 second until a fibrin strand had formed. Two measurements were performed in every rabbit.

Haematocrit was measured in triplicate with a micro-haematocrit centrifuge (10 000 *g* for 5 min; International Equipment Co., Boston, USA).

The animals were anaesthetised with 20% urethane in 0.9% saline (Kebo, Stockholm, Sweden) by intravenous injection (4).

Through a small midline incision the distal part of ileum was exteriorised over a siliconised glass plate in a movable, electrically heated microscope table. During the experiment the preparation was continuously superfused with thermostated Tyrode's solution of 37.5–39.0°C and pH 7.4. The bowels were covered with moistened swabs covered with plastic foil to minimise heat loss by evaporation. The excess fluid was removed by suction. With the electrically heated table and a heating lamp the rectal temperature of the animals was kept at 37.5–39.0° C during the experiment. The temperature was continuously monitored with a thermistor probe (Laboratorio Richerche Elettroniche, Milan, Italy).

For observations on the mesenteric microcirculation a Leitz BIOMED intravital microscope with a Leitz Ultrapak 6.5× objective at a total magnification of 81× was used. The diameter of the vessels was measured with a calibrated ocular graticule. The diameter was measured before transection and the vessel type was identified, i.e. arteriole or venule.

In the first part of the study (35 animals) a small opthalmic knife was used for transection of the microvessels and in the second part (77 animals) this was performed with a disposable Gillette scalpel blade, shape E/11. Vessels used were in the transparent part of the mesentery giving the upper limit of their sizes as about 110 μm. The time from transection until the bleeding stopped was recorded as well as the frequency and time for rebleedings. Every transected vessel was observed for about 20–30 minutes.

Statistical methods

The mean value, the standard deviation and the regression coefficient was calculated according to standard methods (34). The haemostatic plug formation time had a skew distribution (*vide infra*). For statistical purposes the original values are rendered more normally distributed by logarithmic transformation. In testing differences in haemostatic plug formation times between the different vessel ends the sign test was used (10), and significance was considered when $p<0.05$. In testing differences in frequencies of rebleeding the rank-sum test was used (10). Both the sign test and the rank-sum test are distribution free tests, i.e. avoid assumptions about the nature of the distribution function of the data.

Definitions

Haemostatic plug: the platelet mass formed at the ends of a transected bleeding vessel.

Primary bleeding time or primary haemostatic plug formation time: the time from transection until the bleeding first stops (PHT).

Rebleeding: bleedings occurring at intervals through the initially efficient haemostatic plug.

Total rebleeding time: the total of all the rebleeding times recorded in one vessel.

Total bleeding time or total haemostatic plug formation time: the sum of the primary bleeding time and the total rebleeding time (THT).

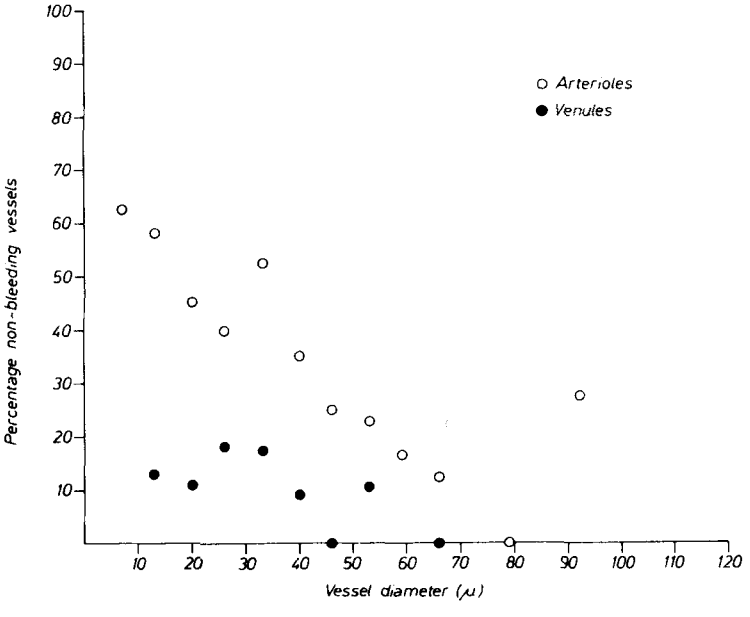

Fig. 1. The frequency (in per cent) of non-bleeding vessels in relation to the vessel diameter when an ophthalmic knife is used for the transection.

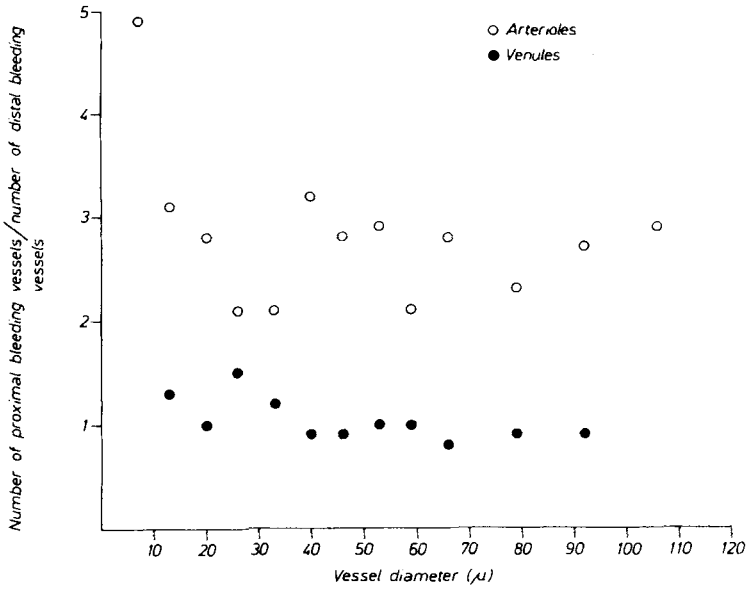

Fig. 2. The ratio of proximal to distal bleeding vessel ends after transection with a scalpel in relation to the vessel diameter.

RESULTS

The bleeding time from transected ear veins is 86±31 seconds, the coagulation time 136±78 seconds and the haematocrit 38.7±3.6. Non of the animals was rejected from the study because of an extreme value.

After transecting a microvessel in the mesentery cleanly and swiftly with a sharp knife, bleeding will immediately start from the transected vessel. Within a few seconds a haemostatic plug starts to grow because of platelets adhering to the injured vessel and aggregating to each other. The plug continues to grow until the bleeding stops after a variable length of time.

When a haemostatic plug, sufficient to stop bleeding, was formed at a proximal arteriolar end, blood will continue fo flow into the nearest vessel branch. In both venular ends and in the distal arteriolar end the blood flow will either be reversed, or stopped if anastomoses are absent. This primary haemostatic plug is not always stable and one or more rebleeding may occur. In such a case new channels are opened up through the plug and no fragmentation of the plug occurs. With a blunt blade a certain number of vessels fail to bleed at all. Fig. 1 shows the percentage of non-bleeding vessels in the different vessel size groups based on calculations from the experiments where the ophthalmic knife was used (35 animals). At least for arterioles there is a greater number of non-bleeding vessels when the diameter is small ($r=-0.85$ for arterioles; $p<0.01$. $r=0.67$ for venules; $p>0.05$).

The rest of the results are based on calculations from the animals in which sharp scalpel blades were used for transection. After transection of a venule bleeding usually occurs from both ends of the transected vessel, but it seldom occurs from the distal end of a transected arteriole as can be seen from Fig. 2. No correlation could be demonstrated between the ratio (number of proximal bleeding vessel ends per number of distal bleeding vessel ends) and the vessel size (r for arterioles is -0.37 and r for venules is 0.64; $p>0.05$).

The total and primary haemostatic plug formation times for different vessel sizes are given in Table I and this is demonstrated graphically in Figs. 3 and 4. It can be seen, that the haemostatic plug formation time, both the total and the primary, increases with increasing arteriolar size. There is a good correlation between arteriolar diameter and the haemostatic plug formation time (Table II). On the other hand there is no correlation between venular size and haemostatic plug formation time (Table II). From Figs. 3 and 4 and Table I it can also be seen that the total venular rebleeding time is much greater than the total rebleeding time for the proximal arteriolar end, and that there is a great variation as far as the distal arteriolar end is concerned.

The haemostatic plug formation time for distal

Table I. *Total (THT) and primary (PHT) haemostatic plug formation times for arterioles and venules*
The mean value and S.D. are given

Vessel size (μm)	Arterioles				Venules			
	Proximal end		Distal end		Proximal end		Distal end	
	THT	PHT	THT	PHT	THT	PHT	THT	PHT
7	37±49	27±29	62±73	56±67				
13	35±36	31±35	102±127	86±88	200±256	166±238	277±281	232±266
20	41±46	32±31	138±216	117±209	270±300	201±273	278±268	224±246
26	45±43	37±35	113±203	88±197	290±304	221±279	232±255	195±245
33	66±54	49±42	178±259	177±260	262±218	176±187	293±287	245±278
40	75±73	62±65	257±318	252±317	189±181	145±128	353±322	283±306
46	85±75	67±61	385±396	214±324	250±239	126±134	391±302	295±308
53	99±86	77±62	262±332	158±276	221±204	149±134	386±314	310±307
59	99±71	88±68	206±281	188±280	254±273	195±269	318±287	261±277
66	123±103	101±98	363±364	314±354	252±287	229±296	347±312	260±279
79	139±91	121±89	234±270	234±270	383±310	314±287	353±293	283±275
92	164±99	147±92	366±365	365±377	264±271	229±261	302±266	230±243
106	199±109	184±106	436±330	409±350				

arteriolar ends is significantly longer than that for the proximal ends ($p<0.001$), but the corresponding venular haemostatic plug formation times do not differ significantly ($p>0.05$). The haemostatic plug formation time for proximal arteriolar ends is statistically shorter than that for venules ($p<0.001$). The difference between venular and distal arteriolar haemostatic plug formation time is insignificant for the primary time ($p>0.05$) and of borderline significance for the total time ($0.01<p<0.05$). The difference in haemostatic plug formation times between arterioles and venules diminishes with increasing vessel size (Figs. 3 and 4).

If the frequency of rebleeding is plotted against the total rebleeding time the origo represents an absolutely stable haemostatic plug. In proximal arteriolar and venular ends there is a correlation between the frequency of rebleeding and the total rebleeding time (r 0.77 and 0.86; $p<0.01$, see Fig. 5). There is no correlation between the frequency of

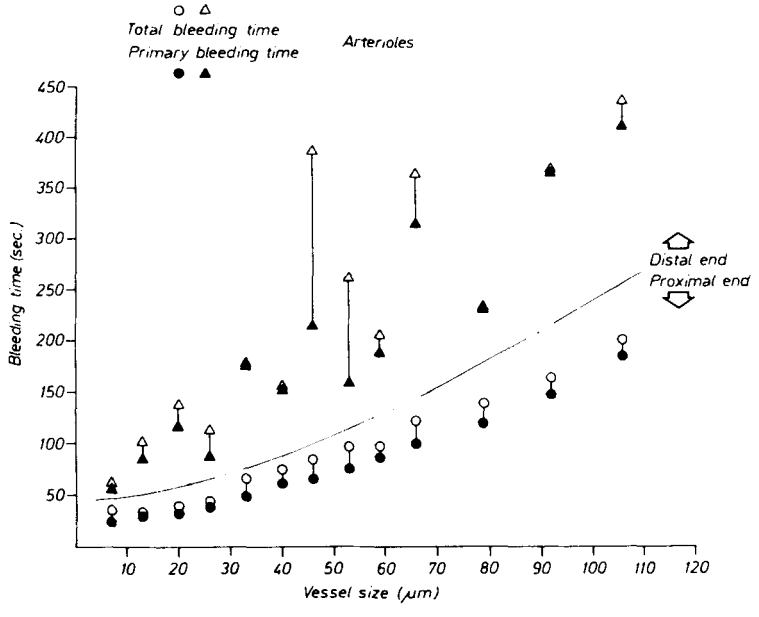

Fig. 3. The arteriolar haemostatic plug formation time in relation to the vessel diameter.

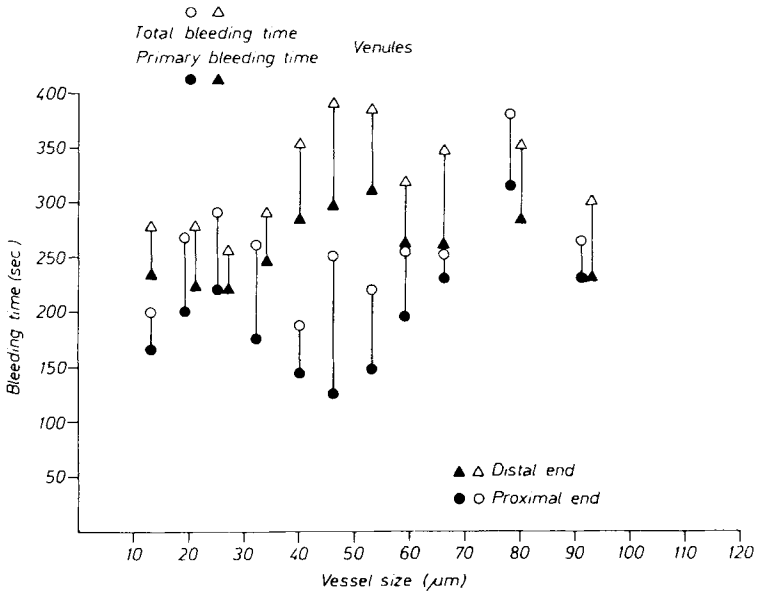

Fig. 4. The venular haemostatic plug formation time in relation to the vessel diameter.

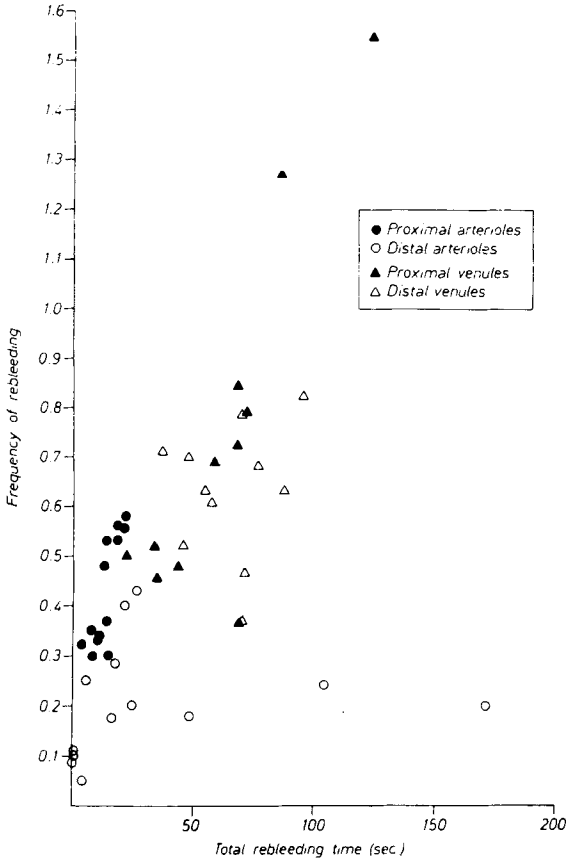

Fig. 5. The stability of the haemostatic plug is shown by plotting the frequency of rebleeding against the total rebleeding time. Origo represents an absolutely stable plug.

rebleeding and the vessel size, nor is there any correlation between the total rebleeding time and vessel size (Table II). The frequency of rebleeding in venules is significantly higher than in arterioles ($p<0.01$).

Figs. 6 and 7 show the number of rebleeding plugs expressed as a percentage of the total in relation to the time between the cessation of primary bleeding and the start of the first rebleeding. Within 10 minutes after the formation of the primary haemostatic plug almost all (>97%) rebleedings have occurered, and within 5 minutes more than 70% of the rebleedings have occurred. Every line represents one vessel size and the time for rebleeding to occur is independent of the vessel size.

The distribution of the haemostatic plug formation times is skew, that is, even in normal rabbits some vessels bleed for a very long time. The longest haemostatic plug formation times are seen in distal arteriolar ends and in venules. In Figs. 8 and 9 the distribution curves are given for vessels of the sizes 26 and 53 μm. The skew distribution is rendered more normal after logarithmic transformation.

DISCUSSION

Since urethane is known to induce haemolysis (5) with the release of adenosine diphosphate, ADP, the use of urethane anaesthesia in haemostatic studies may be theoretically unacceptable. ADP is,

Table II. *Correlation coefficients between the vessel diameter in μm and different parameters for the four vessel segments studied*

NS: not significant.
$p<0.001$

Correlation (r value) between vessel size and	Proximal arteriolar end	Distal arteriolar end	Proximal venular end	Distal venular end
Primary haemostatic plug formation time	0.99***	0.92***	0.51 NS	0.23 NS
Total haemostatic plug formation time	0.99***	0.84***	0.42 NS	0.31 NS
Frequency of rebleeding	0.29 NS	0.06 NS	−0.34 NS	−0.48 NS
Total rebleeding time	0.60 NS	0.01 NS	−0.19 NS	0.57 NS

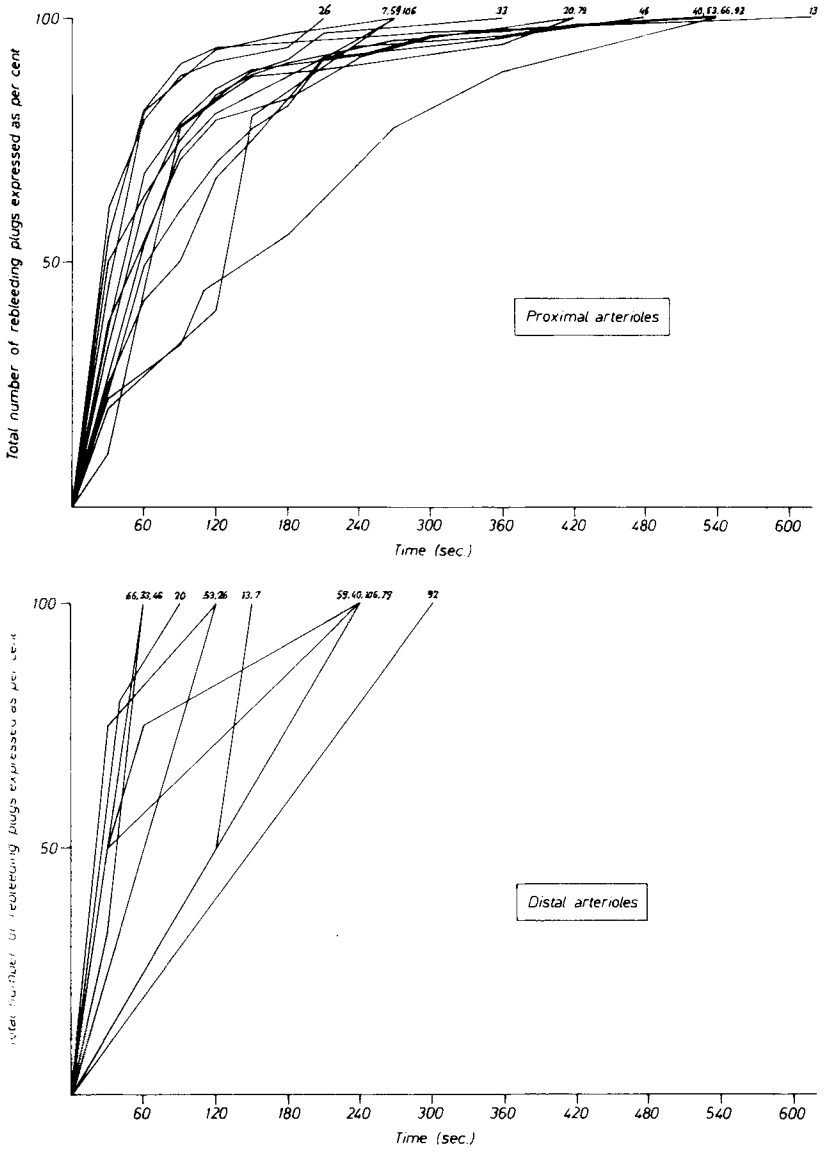

Fig. 6. The number of rebleeding arteriolar haemostatic plugs expressed as per cent of total number of rebleeding plugs plotted against the time between the first arrest of bleeding and the start of the first rebleeding. Every line represents one vessel size (diameter given above the curve).

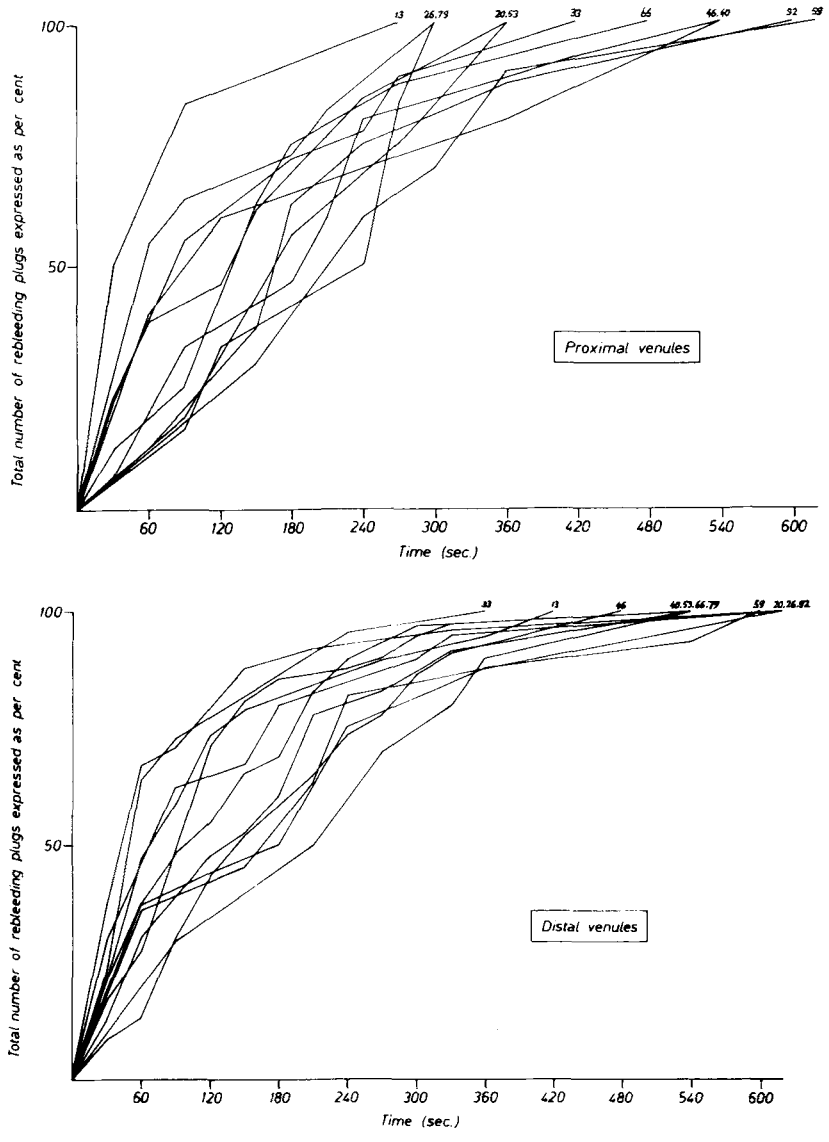

Fig. 7. The number of rebleeding venular haemostatic plugs expressed as per cent of total number of rebleeding plugs plotted against the time between the first arrest of bleeding and the start of the first rebleeding. Every line represents one vessel size (diameter given above the curve).

however, rapidly metabolized and removed from plasma (17, 18, 24). We have evaluated the effect of different anaesthetic agents on the formation and stability of the haemostatic plug using the mesenteric model and found no significant differences between urethane, chloralose and mebumal sodium anaesthesia or neurolept analgesia (4). As rabbits anaesthetised with urethane were stable in acid–base balance and blood pressure, and as urethane was the agent most easy to handle in our hands, we decided to use it in our further studies.

It must be borne in mind, however, that our haemostatic studies were made while the mesenteric preparation was continuously superfused with Tyrode's solution. The superfusion milieu is standardised as far as possible, for ions, pH, temperature and flow. Because of the superfusate it must be stressed, that the haemostatic plug formation time obtained with the present method may not be comparable to bleeding times obtained with other techniques, for instance described by Duke (11) or Ivy (25). Comparisons between these two types of techniques should thus be very careful. Using a continuous superfusate, blood and various tissue factors are washed away. There are also differences between the tissues themselves,

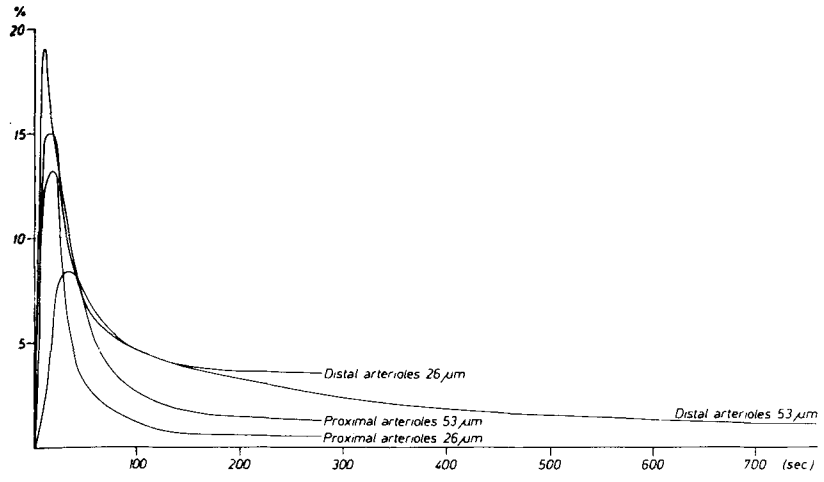

Fig. 8. The distribution of the arteriolar haemostatic plug formation time shown for two vessel sizes.

i.e. anatomical localisation, thickness, forces of pressure and pulling and numbers of transected vessels.

Another factor of importance is the transection technique. The phenomenon of nonbleeding vessels has been described before but not the difference between vessel types (1, 22, 33, 36). Endothelial adhesion was previously considered to be the main haemostatic mechanism in capillaries (15, 33), but Jørgensen & Borchgrevink (26) showed histologically that platelet plugs were formed in capillaries too. From our study it is evident (Fig. 1), that the frequency of non-bleeding vessels, when using a blunt knife, is somewhat higher in arterioles than in venules, and that the frequency is correlated to the diameter of the arteriole. The theory of endothelial adhesion and the frequent occurrence of non-bleeding vessels are probably due to the fact that the importance of sharp knives and swift transections in previous haemostatic experiments was not considered. In conclusion, these results made us use disposable knife blades in the main study. The frequency of non-bleeding vessels could then be neglected and could well be compared with Hugues' (22) results.

Some authors studying the initial haemostatic mechanism have used puncture of microvessels instead of transection (7, 14, 19, 36). In those studies the size of the puncture hole, the thickness and elasticity of the vessel wall and the adjacent tissue will probably influence the bleeding time. When a vessel is punctured there is a combination of a haemostatic plug outside the vessel and a microthrombus inside it.

After transection of a venule bleeding occurred from both ends of the transected vessel, while bleeding was less common from the distal than from the proximal arteriolar end, which almost

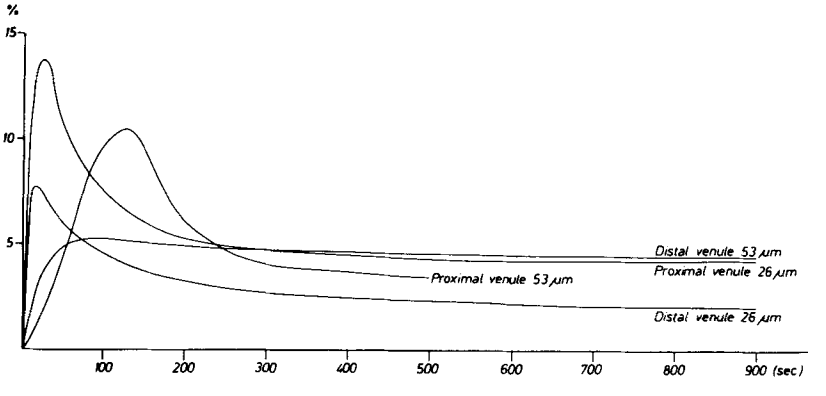

Fig. 9. The distribution of the venular haemostatic plug formation time shown for two vessel sizes.

always bled (Fig. 2). Although this phenomenon has been briefly mentioned by various authors (1, 13, 22, 33, 36), no systematic study of this problem has been carried out. Fig. 2 shows that the number of observations from distal arteriolar ends is less than from the other vessel ends, a fact that must be remembered in the further discussion. The pressure differences, that exist between proximal and distal arteriolar ends, could in part explain this observation. A pressure difference should also exist between the both venular ends, but this will not be so pronounced as in arterioles and probably is of no importance since the venular ratio is near 1.

There are contradictory results in the literature as far as the relationship between the haemostatic plug formation time and vessel type and diameter is concerened (1, 22, 33, 36). Hugues (22) observed the importance of the vessel type, but apart from his study and as far as the vessel size is concerned, no systematic studies on these problems have been carried out. Hugues (22) found the longest bleeding times in proximal arteriolar ends, whereas bleeding times from the other vessel segments were of the same magnitude and about half that seen in the proximal arteriolar ends. In his calculations Hugues did not use the 11% of the transected vessels, where the bleeding time exceeded 10 minutes, which was seen in venules and distal arteriolar ends–a finding that fits well with our experience (*vide infra*). This is probably the explanation why Hugues found the primary haemostatic plug formation time referred to above, where the proximal arteriolar end showed the longest bleeding time. He pointed out that the most reproducible results were obtained from the proximal arteriolar ends, but we suggest that valuable information also can be obtained when the venules are taken into consideration. Thus we found aspirin to have no effect on arteriolar haemostatic plug formation time, whereas venular haemostatic plug formation time was significantly shortened (2).

In his study Hugues (22) also studied the vessel contraction in connection with haemostatic plug formation. He found no correlation between the degree of contraction and the bleeding time.

We found that the haemostatic plug formation time is longer and the rebleeding tendency greater in venules than in arterioles, and that the time for haemostatic plug formation is significantly longer in the distal than in the proximal arteriolar end. These investigations clearly show, that in studies of haemostatic plug formation one must be careful to differentiate between arterioles and venules as well as taking into account the vessel diameter at least in arterioles. It can be concluded, that the same vessel size and type should be used in comparative studies, if comparable results are to be obtained. This fact has not always been taken into consideration (12, 16, 30).

The literature concerning the nature and occurrence of rebleedings is contradictory (1, 7, 12, 16, 20, 22, 30, 36), and no systematic study on this phenomenon has been made.

The rebleeding always started with the opening up of new channels through the formed plug as pointed out by Zucker (36). However, some authors have remarked on the frequency of fragmentation both in rebleedings (35) and during the growth of primary plugs (31, 32).

We found the lowest frequency of rebleeding in the distal arteriolar ends, but the length of the total rebleeding time varied. This time and the frequency of rebleeding was fairly constant for the proximal arteriolar end, while both parameters were longer for the two venular segments. Both proximal vessel ends showed some correlation between the frequency of rebleeding and the total rebleeding time. On the other hand the rebleeding pattern was independent of the vessel size in the range investigated. Our results, showing that rebleedings are more frequent in venules, speak against the hypothesis claimed by Aptiz (1), that pressure is of prime importance for the rebleedings to occur. Thus it is also of considerable importance to define the vessel type when studying the rebleeding pattern, whereas the vessel size seems to be of less importance within the range under investigation. We have found, that the rebleeding pattern is a useful parameter, for instance in pharmacological studies. Thus, the rebleeding frequency was significantly decreased in rabbits treated with aspirin (2), ether (4) and sulfinpyrazone or phenylbutazone (unpublished results), but the haemostatic plug formation time was normal in all groups except the aspirin group in which the venular haemostatic plug formation time was shortened.

As can be seen from Figs. 6 and 7 the majority of rebleedings (>97%) occurred within 10 minutes of the formation of the primary haemostatic plug. Using this experimental model system it would thus

be sufficient in normal, untreated rabbits to observe for a period of 10 minutes after primary haemostatis.

The distribution of haemostatic plug formation time has not been studied in detail before, although Hugues (22) found that in 11% of transected venules and distal arteriolar ends the bleeding continues for more than 10 minutes. In these cases he found fragmentation from already formed plugs or no plugs at all. In our studies it was extremely rare to find a total absence of plugs. On the other hand the growth rate of the plugs in venules and distal arteriolar ends was very slow, when bleeding was prolonged (3). As can be seen from Figs. 8 and 9 the haemostatic plug formation time shows a skew distribution in our material, the skewness being more pronounced for venules and distal arteriolar ends. Since prolonged bleeding times are a part of the normal bleeding time distribution it is our opinion, that they must be included in calculations on haemostatic plug formation time. In our material logarithmic transformation of the haemostatic plug formation time render it more normally distributed and in our opinion this method should be used, when statistical comparisons are made.

CONCLUSIONS

1. A swift and clean transection with a sharp knife should be used in experiments on haemostatic plug formation.

2. The ratio of bleeding proximal ends to bleeding distal ends is between 2 and 3 for arterioles and about 1 for venules. This ratio is not correlated to the vessel size.

3. Total and primary haemostatic plug formation time in proximal arteriolar ends are statistically shorter than those in venules and distal arteriolar ends.

4. Total and primary haemostatic plug formation time in arterioles but not in venules are correlated to the vessel diameter.

5. The difference in haemostatic plug formation time between arterioles and venules decreases with increasing vessel size.

6. The total rebleeding time is longer in venules than in proximal arteriolar ends. Distal arteriolar ends show a variable pattern.

7. The frequency of rebleeding in proximal arteriolar and venular ends is correlated with total rebleeding time.

8. There is no correlation between vessel diameter and total rebleeding time or frequency of rebleeding.

9. The frequency of rebleeding is lowest for distal arteriolar ends, followed by the proximal arteriolar ends and both venular ends.

10. Most rebleedings (>97%) have occurred within 10 minutes after the formation of the primary haemostatic plug.

11. The haemostatic plug formation time shows a skew distribution which can be rendered more normal by logarithmic transformation.

REFERENCES

1. Apitz, K.: Die Bedeutung der Gerinnung und Thrombose für die Blutstillung. Virchows Arch Path Anat *308:* 540, 1942.
2. Arfors, K.-E., Bergqvist, D., Bygdeman, S., McKenzie, F. N. & Svensjö, E.: The effect of inhibition of the platelet release reaction on platelet behaviour *in vitro* and *in vivo*. Scand J Haematol *9:* 322, 1972.
3. Bergqvist, D. & Arfors, K.-E.: The growth rate of haemostatic plugs in the rabbit mesentery. Proc. VII Conf. Microcirculation, Aberdeen, 1972. Karger, Basel, 1973. p. 186.
4. Bergqvist, D., McKenzie, F. N. & Arfors, K.-E.: Influence of various anaesthetic agents on the formation and stability of haemostatic plug in the rabbit mesentery. Upsala J Med Sci, in press.
5. Bree, M. & Cohen, B. Effects of urethane anaesthesia on blood and blood vessels in rabbits. Lab Animal Care *15:* 254, 1965.
6. Chambers, R. & Zweifach, B. W.: Topography and function of the mesenteric capillary circulation. Am J Anat *75:* 173, 1944.
7. Chen, T. I. & Tsai, C.: The mechanism of haemostasis in peripheral vessels. J Physiol *107:* 280, 1948.
8. Cruz, W. O.: Studies on hemostasis *in vivo*. Henry Ford Hospital Symp. Blood Platelets. Little, Brown & Co., Boston, 1961.
9. Cruz, W. O.: The significance of a smooth muscle component in hemostatis. Proc Soc Exptl Biol Med *119:* 876, 1965.
10. Dixon, W. J. & Massey, F. J., Jr: Introduction to Statistical Analysis, McGraw-Hill, New York, 1957.
11. Duke, W. W.: The relation of blood platelets to hemorrhagic disease; Description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. J Am Med Assoc *55:* 1185, 1910.
12. Evans, G., Packham, M. A., Nishisawa, E. T., Mustard, J. F. & Murphy, E. A.: The effect of acetylsalicylic acid on platelet function. J Exptl Med *128:* 877, 1968.
13. French, J. E., Macfarlane, R. G. & Sanders, A. G.: The structure of haemostatic plugs and experimental thrombi in small arteries. Brit J Exptl Path *45:* 467, 1964.

14. Herrmann, R., Frank, J. D. & Marlett, D. L.: An *in vivo* technique for assessing the formation of a hemostatic platelet plug. Proc Soc Exptl Biol Med 128: 960, 1968.
15. Herzog, F.: Die Rolle der Capillaren bei der Blutstillung. Pflügers Arch Gesamt Physiol *207:* 476, 1925.
16. Hirsh, J., Buchanan, M., Glynn, M. & Mustard, J. F.: Effect of streptokinase on hemostatis. Blood *32:* 726, 1968.
17. Holmsen, H. & Stormorken, H.: Kinetic studies on the breakdown of adenosine diphosphate in human plasma. Scand J Clin Lab Invest Suppl. 84, p. 138. 1965.
18. Holmsen, I. & Holmsen, H.: Breakdown of adenosine nucleotides in whole blood. III Congr Int Soc Thromb Haemost. Washington 1972. Abstract volume p. 206.
19. Honour, A. J. & Mitchell, J. R. A.: Platelet clumping in injured vessels. Brit J Exptl Path *45:* 75, 1964.
20. Hovig, T., Rowsell, H. C., Dodds, W. J., Jørgensen, L. & Mustard, J. F.: Experimental hemostatis in normal dogs and dogs with congenital disorders of blood coagulation. Blood *30:* 636, 1967.
21. Hovig, T., Dodds, W. J., Rowsell, H. C. & Mustard, J. F.: The transformation of hemostatic platelet plugs in normal and factor IX deficient dogs. Amer J Path *53:* 355, 1968.
22. Hugues, J.: Contribution à l'étude des facteurs vasculaires et sanguins dans l'hémostase spontanée. Arch Internat Physiol *41:* 565, 1953.
23. Hugues, J. : Métamorphose visqueuse des plaquettes et formation du clou hémostatique. Thromb Diath Haemorrh *3:* 34, 1959.
24. Ireland, D. M. & Mills, D. C. B.: Degradation of adenosine diphosphate in plasma. Biochem J *92:* 30P, 1964.
25. Ivy, A. C., Shapiro, P. F. & Melnick, P.: The bleeding tendency in jaundice. Surg Gynec Obstet *60:* 781, 1935.
26. Jørgensen, L. & Borchgrevink, C.: The platelet plug in normal persons. I. The histological appearance of the plug 15 to 20 minutes and 24 hours after the bleeding and its role in the capillary hemostasis. Acta Path Microbiol Scand *57:* 40, 1963.
27. Kjaerheim, Å. & Hovig, T.: The ultrastructure of haemostatic blood platelet plugs in rabbit mesenterium. Thromb Diath Haemorrh *7:* 1, 1962.
28. Macfarlane, R. G.: Critical review: the mechanism of haemostasis. Quart J Med N S *10:* 1, 1941.
29. Marr, J., Barboriak, J. J. & Johnson, S. A.: Relationship of appearance of adenosine diphosphate, fibrin formation and platelet aggregation in the haemostatic plug *in vivo*. Nature *205:* 259, 1965.
30. Packham, M. A., Warrior, E. S., Glynn, M. F., Senyi, A. S. & Mustard, J. F.: Alteration of the response of platelets to surface stimuli by pyrazole compounds. J Exptl Med *126:* 171, 1967.
31. Roskam, J.: Role of platelets in the formation of a hemostatic plug. Blood platelets. Little, Brown & Co., Boston, Mass., 1961.
32. Roskam, J., Hugues, J., Bounameaux, Y. & Salmon, J.: The part played by platelets in the formation of an efficient hemostatic plug. Thromb Diath Haemorrh *3:* 510, 1959.
33. Sanders, A. G.: *In vivo* observations on haemostasis in the hamster. Symp. Zool Soc Lond *27:* 109, 1970.
34. Snedecor, G. W. & Cochran, W. G.: Statistical methods. The Iowa State Univ. Press. Ames, Iowa, USA. 6th ed. 1967.
35. Stormorken, H. & Owren, P. A.: Physiopathology of hemostatis. Sem Hemat *8:* 3, 1971.
36. Zucker, M. B.: Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprothrombinemic rats. Amer J Physiol *148:* 275, 1947.

Received May 16, 1973

Address for reprints:

David Bergqvist
Department of Experimental Medicine
Pharmacia AB, Box 604
S-751 25 Uppsala 1
Sweden