Thromboplastic and Fibrinolytic Activities in Vessels of Animals

By Tage Astrup, Ph.D., and Karol Buluk, M.D.

Deposition of fibrin on the interior wall of arteries is assumed to constitute an etiological factor in the pathogenesis of arteriosclerosis.\(^1\)\(^5\) This concept was supported when normal human aortic intima was found highly thromboplastic with no, or only traces of, fibrinolytic activity.\(^6\)\(^,\)\(^7\) This observation suggested an easy deposition of fibrin after intimal injury followed by delay of removal. Fundamentally, the process may be considered a normal mechanism of tissue repair regulated by a dynamic hemostatic balance between fibrin deposition and fibrin resolution,\(^5\)\(^,\)\(^8\) operating under local conditions which favor a deposition of fibrin followed by pathological reactions.\(^9\) An advantage of this concept is that the pathogenesis of arteriosclerosis can now be studied from a different angle.

An immediate question is whether the composition of the arterial wall is the same in animals as in man. The finding that the pattern in the aorta of the monkey differs from that of man\(^7\) prompted a study of the aorta in other animal species. It was found that the hemostatic pattern in the aortic wall differs from one species to another and that none of the patterns were exactly like that found in the human aorta.\(^10\) Hence a more detailed investigation, including veins and other arteries, was undertaken.

Methods

Thromboplastic Activity. Fresh vascular tissue (200 mg) was homogenized (Potter) in 0.9% NaCl (1.8 ml) followed by freezing at \(-20^\circ\)C to complete cellular destruction. After thawing and rehomogenizing coarse particles were removed by filtration through a cotton plug. Serial dilutions were prepared in 0.9% NaCl. Clotting times were estimated at 37°C in a system consisting of 0.2 ml fresh, citrated rabbit plasma (silicone technique), 0.5 ml 0.9% NaCl and 0.1 ml tissue dilution by recalcification with 0.2 ml 0.035 M CaCl\(_2\). A preparation of human brain (Owren's P. and P-method) was used for comparison. Activities were recorded in concentrations of this human brain preparation by interpolation on the reference curve in a double logarithmic diagram and were expressed in percentages of the brain stock solution. All results are based on duplicate estimations and serial dilution curves to increase the accuracy of the interpolation. An example is shown in figure 1. The shape of the curve permits conclusions on the presence or absence of inhibitors of coagulation.

Fibrinolytic Activity. Fresh tissue (100 mg) was homogenized in 2 M KSCN (3.0 ml) followed by mechanical shaking for two hours. After centrifugation the supernatant was diluted with 7 vol of H\(_2\)O and \(\times\) HCl added to pH 1. The precipitate was redissolved in 1 ml 2 M KSCN by neutralization with solid NaHCO\(_3\). Serial dilutions in 2 M KSCN were prepared and assayed in triplicate on normal fibrin plates containing 0.12% bovine fibrin (clotted with bovine thrombin, Leo Pharmaceuticals, Copenhagen). From the dilution curves in a double logarithmic diagram the data were interpolated on a standard curve and expressed in units of a standard preparation of tissue activator (from pig hearts) per g fresh tissue, figure 2. For further details of methods see Astrup et al.\(^7\)

Material was obtained from freshly killed animals at the slaughterhouse (ox, horse, pig), from the State Serum Institute, Copenhagen (rat, pig, rabbit, monkey), or from the Anatomical Department of the School of Veterinarians (dog, cat). The three layers of the vessels were separated under water; in some cases complete separation was not possible. Only apparently normal samples were investigated.
Results

Figure 1 illustrates an assay of thromboplastic activity in rabbit aorta. Thromboplasticin concentration in the intimal layer was about 16% of the concentration of the reference solution of human brain thromboplastin. The media had about 3.5% of this concentration. All other values presented in this paper were obtained in similar manner. Adventitial tissue showed in this assay a phenomenon typical for certain tissues. The solution delayed clotting of the substrate plasma. The occurrence of clot-inhibiting material in vessel wall tissue has been observed repeatedly by previous investigators.

Figure 2 shows an assay of fibrinolytic activity in dog aorta. The adventitial coat yielded a solution with 9% of the concentration of the standard solution, equal to 3.4 units per ml. Since 100 mg tissue had been used per ml final solution, the concentration of plasminogen activator was 34 units per g fresh tissue. Similarly the solution of the media yielded about 2% of the concentration of the standard solution corresponding to 0.8 units per ml and 8 units per g tissue. The intima gave 5.4% and 21 units per g tissue.

The thromboplastic and fibrinolytic activities of individual samples of aorta are recorded in table 1. The variation between individuals of the same species is great, confirming earlier observations. Nevertheless, there are characteristic differences in the distribution of thromboplastic and fibrinolytic activity between the species. Thus rat aorta often contained a thromboplastin inhibitor in

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FIGURE 2

In view of the frequent occurrence of fibrinous material in the vessels in the form of arterial and venous thrombi or as mural deposits, it is surprising that interest has been aroused only recently in the estimation of those factors in the vessel wall, which could influence fibrin formation and fibrin dissolution. In the vessel wall, as in any other tissue in the body, injury leading to cell death and tissue necrosis is followed by reparative processes, which tend to restore normal conditions. Fibrin formation and production of fibrinous deposits, followed by connective tissue formation take place in every tissue following local injury. However, a lesion on the interior wall of a vessel differs from injuries at most other places in the body. The vascular system contains a large amount of fibrinogen and clot-producing components, which can be delivered rapidly to the site of injury by the circulating blood. There is, therefore, a greater potential for production of fibrin, when injury occurs in a tissue exposed to the blood stream. Hence, in order to elucidate the processes of tissue repair it is important to know how cellular material, released by the injury, influences clot formation and clot dissolution.

Bernheim was, to our knowledge, the first to become aware of this problem. In 1910 he presented observations on the relation of the blood vessel wall to coagulation of the blood. He prepared saline extracts of vessels (especially aorta) from dog, chicken, and pig and demonstrated enhanced clot formation after addition of this material to freshly drawn...
individual estimations on different animals recorded for each species. Thromboplastin concentration expressed in percentages of the human brain solution used for comparison. Fibrinolytic activity expressed in units of plasminogen activator per gram of fresh tissue.

Inh.: presence of thromboplastin inhibitor. A: adventitial layer. M: tunica media. I: tunica intima. Where separation of the layers could not be accomplished the combined layers were assayed as indicated by brackets. In some smaller animals samples were pooled for each assay. Number of animals in parentheses.

Samples of homologous blood. The vessel extracts from pigs were tested also on dog and rabbit blood with positive results. The following remarks are quoted from his long-forgotten paper: "In the blood-vessel wall itself there is a substance which, once the vessel is injured, probably aids in the formation of a clot. And if this is true, those engaged in the field of vascular surgery have still another problem to consider in connection with the already very difficult and trying technique. The final solution of this problem, however, rests with the pharmacologists and physiologists, as in the case with many other problems of modern medicine."

Campbell was probably the next to deal with the thromboplastic activity of blood vessels. He separated the three coats of bovine thoracic aorta and prepared extracts by grinding with saline, followed by filtration through gauze and centrifugation. The activity of the clear supernatant fluid was estimated on bovine oxalated plasma. Adventitia was found to be most active, with media less active, and intima slightly less active still. The discrepancy, between his results and ours, in which media was found to be the most active, could be caused by the different extraction procedures. Complete extraction of thromboplastic activity is difficult and requires intermediary freezing.7

Development of the concept of a dynamic hemostatic balance regulating fibrin deposition and fibrin dissolution, and its immediate

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**TABLE 1**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Thromboplastic Activity</th>
<th>Fibrinolytic activity</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>M</td>
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<tr>
<td>Mac. rhesus (3)</td>
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<td>18</td>
</tr>
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<td>Java monkey (3)</td>
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<td>10</td>
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<td>Horse (4)</td>
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<td>10</td>
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<tr>
<td>(aorta desc.)</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Pig (4)</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Dog (5)</td>
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<td>0</td>
</tr>
<tr>
<td>Guinea pig (20)</td>
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<td>3</td>
</tr>
<tr>
<td>(each 5 animals)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Rat (15)</td>
<td>1 Inh. Inh.</td>
<td>1 Inh. Inh.</td>
</tr>
<tr>
<td>(each 5 animals)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ox (3)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Calf (2)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Foetus (1)</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Rabbit (6)</td>
<td>0 Inh. Inh.</td>
<td>0 Inh. Inh.</td>
</tr>
<tr>
<td>Cat (1)</td>
<td>6</td>
<td>0</td>
</tr>
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</table>

Circulation Research, Volume XIII, September 1965
TABLE 2

<table>
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<tr>
<th>Conc in %</th>
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<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.1</th>
<th>1.5</th>
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<tr>
<td>A</td>
<td>&gt;24 h</td>
<td>56</td>
<td>23</td>
<td>13</td>
<td>10^6</td>
<td>8^6</td>
<td>7^6</td>
<td>5^6</td>
</tr>
<tr>
<td>M + I</td>
<td>&gt;24 h</td>
<td>84</td>
<td>36</td>
<td>36</td>
<td>11^4</td>
<td>11^5</td>
<td>6.5</td>
<td>5^6</td>
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</tbody>
</table>

Clotting times at 37°C recorded in minutes and seconds. Suspensions of aortic tissues prepared as described before. Dilutions of suspension prepared with saline and expressed in percentages of the stock solution. 0.1 ml tissue suspension was added to a mixture of 0.2 ml citrated rabbit plasma diluted with 0.5 ml 0.9% NaCl and recalcified with 0.2 ml 0.030 M CaCl₂ solution. A: adventitial layer. M + I: combined media and intima.

significance for the thrombogenic theory of the pathogenesis of arteriosclerosis, it is important to obtain data elucidating the distribution of thromboplastic and fibrinolytic activities in the vessel wall. Our first assays were performed on normal human aorta, and on apparently normal samples from atheromatous aortas of varying grades of severity in order to see whether there were any differences in the activities of the parts of the aorta not directly involved in the pathological process. The most striking observation was the high thromboplastic activity of the intima with no, or rarely a trace of, fibrinolytic activity. These findings made it clear that there is in man, after tissue injury, an enhanced tendency to deposit fibrin on the aortic surface, with no simultaneous enhancement of fibrin dissolution. The high thromboplastic activity of the human arterial intima was confirmed by other investigators. The absence of fibrinolytic activity in the central layers of human arteries and its presence in adventitia was also confirmed. While human arteries are rather similar in their patterns of distribution, veins form a separate group. Veins have lower thromboplastic activity and fibrinolytic activity extends into their intima.

The patterns differ still more in animal species as is shown in table 1. The presence of fibrinolytic activity in the aortic intima (and media) of the rhesus and Java monkeys confirms our earlier observation that distribution in these animals differs from that in human aorta. This is emphasized by the low thromboplastic activities found in the layers of the monkey aorta. The pattern resembles that of dog, though here the thromboplastic activity is nearly zero. The latter results are significant in the evaluation of data obtained in experiments on the vascular system of dogs. They raise questions concerning the extent to which results obtained on dogs in experimental vascular surgery, and in studies on thrombosis, can possibly reflect the situation in man.

The pattern in the horse shows high fibrinolytic activity in adventitia and some in the two other coats. It is interesting that thromboplastic activity is also high. In the latter respect the horse resembles the pig, where likewise adventitia is high in fibrinolytic activity, but media and intima are completely devoid of fibrinolytic activity. Bovine material is fairly high in thromboplastic activity, but fibrinolytic activity is nearly absent.

The rabbit aorta has practically no fibrinolytic activity in any of its layers, but thromboplastic activity is extremely high in media and intima. It is surprising that there is little or no thromboplastic activity in adventitia and even a weak inhibitor may be present (fig. 1). The rat is again different. Some fibrinolytic activity is present and in most of the samples there is an inhibitor of thromboplastin (table 2). Hence the rat should not respond as easily to vascular injury as the rabbit, which seems to agree with general experience with these animals.

In table 1 human aorta is included with average values as published earlier. It is seen that the potential ability to form fibrin is high in man and the pattern similar to that of the rabbit. At the same time the capacity to produce lysis is low, even if high in adventi-
TABLE 3

Thromboplastic and Fibrinolytic Activity in Different Vessels of Some Animal Species

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>M</th>
<th>I</th>
<th>A</th>
<th>M</th>
<th>I</th>
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<tr>
<td>Ox</td>
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<td>170</td>
<td>53</td>
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<td></td>
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<tr>
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<tr>
<td>Calf foetus</td>
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<td>25</td>
<td>7</td>
<td>36</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Horse</td>
<td>16</td>
<td>50</td>
<td>100</td>
<td>190</td>
<td>133</td>
<td>61</td>
</tr>
<tr>
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<td>20</td>
<td>8</td>
<td>61</td>
<td>247</td>
<td>105</td>
</tr>
<tr>
<td>Arteria pulm.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox</td>
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<td>40</td>
<td>25</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calf</td>
<td>10</td>
<td>75</td>
<td>20</td>
<td>34</td>
<td>0</td>
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<tr>
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<td>33</td>
<td>5</td>
<td>296</td>
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<td>30</td>
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<tr>
<td>Vena pulm.</td>
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<tr>
<td>Calf</td>
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<td>120</td>
<td>11</td>
<td>3</td>
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<td></td>
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<tr>
<td>Horse</td>
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<td>40</td>
<td>304</td>
<td>190</td>
<td></td>
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</tbody>
</table>

Individual estimations recorded. For details see text and table 1.

The observed differences may explain why, in animals, the response to injury differs from that in man. Arteriosclerosis and atherosclerosis have a distribution in animals, which differs with the species. Some recent observations may be related to the findings described in the present paper. Arteriosclerosis occurs frequently in young cattle with Johne's disease. Since cattle belong to the species with the highest capacity to respond...
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to injury with fibrin deposition it can be expected that arteriosclerotic lesions will occur earlier and more frequently than in some other animals. Similarly, atherosclerosis has been observed in some herbivorous wild animals (buffalo, hippopotamus, wild cow) with a very low fat intake. These animals belong to species which, like cattle, can be assumed to be predisposed to fibrin deposition. The fact that wild monkeys (baboon) show lesions resembling early human atherosclerosis, though less pronounced, can possibly be attributed to a lower tendency to form fibrin on the intima.

Though the material is still incomplete, the data already obtained suggest a clue to many observations on the pathophysiology of thrombosis and vascular disorders. The results may assist investigators to select the right species of animal for a particular type of experiment or study, and evaluation of experimental data should be facilitated. The results also warn against drawing extensive conclusions from data obtained on only one animal species. It is seen that regulation of fibrin deposition after intimal injury involves a variety of systems, which when referring to the aorta are as follows:

1. Lack of tissue thromboplastin (typical example, dog).
4. Presence of a powerful, though latent, humoral system of fibrinolysis (man).

Summary

Thromboplastic activity and concentration of plasminogen activator were assayed in the coats of arteries and veins from a number of species. The widely differing patterns found in these species suggest great variation in the tendency to deposit fibrin on the interior surface of the vessels. Several modes of regulating fibrin deposition after intimal injury exist in the animal organism. These observations may assist the evaluation of data obtained in experiments on animals, and they demonstrate certain limitations with respect to conclusions on pathology in man.

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