

# Extracorporeal Circulation: A Device for the Quantitative Study of Thrombus Formation in Flowing Blood

By H. G. Downie, D.V.M., Ph.D., E. A. Murphy, M.B., M.D.,  
H. C. Rowsell, D.V.M., Ph.D., and J. F. Mustard, M.D., Ph.D.

■ The formation of a thrombus *in vivo* is apparently governed by three factors: surface, blood flow and the nature of the constituents of blood and its coagulant activity. It is important that these three factors be kept in mind in developing techniques for the quantitation of thrombus formation. *In vitro* tests of blood coagulation are unsatisfactory because the effects of blood flow are lost and temperature and surface contact are difficult to regulate. Chandler's<sup>1</sup> method for studying thrombus formation in tubing rotated on a turntable has proven valuable in studying the histochemistry of thrombogenesis. However, this system does not readily lend itself to quantitative study and the blood is not recirculated through the body. Wessler's technique<sup>2</sup> gives useful information on the effect of stasis and hypercoagulability on thrombus formation, but it involves local arrest of blood flow. Vessel injury techniques such as those used by Ashwin et al.<sup>3</sup> and Robertson et al.<sup>4</sup> are difficult to standardize and to quantitate. In short, none of the above procedures provide a comprehensive approach to all the variables mentioned above.

The first experiments using extracorporeal circulations appear to have been those of Rowntree and Shionoya.<sup>5,6</sup> These workers, and Best, Cowan and MacLean,<sup>7</sup> who developed a similar method, studied the order of events in thrombus formation in a high-flow

pulsatile system and observed that large doses of heparin could inhibit thrombus formation. We have described elsewhere<sup>8</sup> extracorporeal circulations for the qualitative study of the effects of vessel configurations on thrombus formation. This system has been adapted for quantitative study. It was hoped that such a system would provide a much more physiological method of studying the effect of changing the characteristics of circulating blood on thrombus formation in the body.

## Methods

### THE EXTRACORPOREAL SHUNTS

The structure of the flow chamber has been discussed elsewhere in some detail.<sup>8</sup> To ensure that extraneous sources of variation and possible bias are reduced to a minimum, the exact dimensions of the connecting tubing were standardized; the dimensions are given in a scale diagram (fig. 1). Two identical precision-built flow chambers (60° bifurcation) made of high quality plastic were employed throughout, and used randomly. New connecting tubing was used for each experiment; the flow chamber was carefully washed, dried and resiliconed in a standard manner for each study.

Each experiment lasted twenty minutes. Blood flow readings (see below) were done at the beginning of the experiment, at ten minutes and at nineteen minutes. At the close of the experiment, the proximal sampler was clamped off and 50 ml of 0.85% NaCl injected just distally to the clamp. The distal sampler was then clamped and the tubing removed from the animal. The apparatus was then taken at once to a sink where the distal clamp was removed and an additional 50 ml of saline injected distal to the clamp on the proximal sampler. The tubing was then cut through as close to the flow chamber as possible. The flow chamber was then separated carefully into its two parts (fig. 2) and any material between the apposed surfaces of the halves removed. The actual channels were then gently washed with a small quantity of distilled water. The deposits

---

From the Department of Physiological Sciences, Ontario Veterinary College, Guelph, Department of Medicine, University of Toronto, Sunnybrook Hospital, Department of Veterans Affairs, Toronto, Canada.

Supported in part by Grant H-4964 from the U. S. Public Health Service.

Received for publication November 15, 1962.

SCALE DIAGRAM OF THE SYSTEM USED IN THE QUANTITATIVE STUDIES

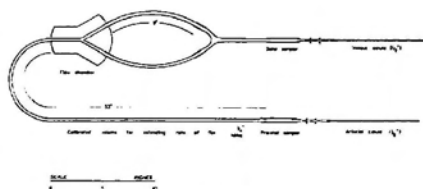


FIGURE 1

Scale diagram of extracorporeal circulation comprising flow chamber and connecting tubing.

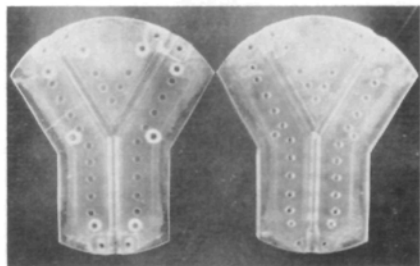


FIGURE 2

The flow chamber. The two surfaces are apposed during the experiments and held together by through and through screws. After each experiment, the deposit is collected from the channels only.

which were examined histologically were not exposed to distilled water. The deposit within the lumen of the flow chamber was then carefully detached, transferred into a clean dried beaker of known weight, and after it had been dried the whole was weighed on a balance graduated to read in 1/100th of a mg. Although not done in the present study, the deposits can be analyzed chemically.

#### BLOOD FLOW

This was measured in the following fashion: before the experiment, a part of the proximal connecting tubing (fig. 1) which contained exactly 20 ml was determined by direct calibration and its ends defined by suitable marks. The length of time required for a 2 ml bubble of air to traverse the measured length of tubing gives an estimate of the rate of flow of blood. In each experiment, the first estimate of flow was based on the length of time, measured with a

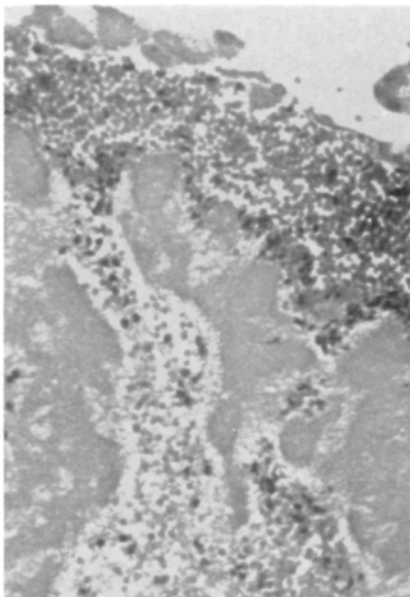


FIGURE 3

A microscopic section of a deposit stained with H & E. The pale amorphous areas are fused platelets. In between are red and white cells and a mesh of fibrin. H & E  $\times 605$ .

stopwatch, required for the head of the column of blood to pass through the calibrated portion. The results of this reading and the two bubble readings at the 10th and 19th minutes proved in general to be very consistent, (table 1). The actual rate of flow is computed from the formula  $V = \frac{1.2}{T}$  where V = volume of flow in liters per minute and T the number of seconds required by the flow to traverse the measured distance.

#### EXPERIMENTAL ANIMALS

Since these experiments require a large flow of blood through the extracorporeal circulation, it is necessary that a fairly large animal be used to avoid circulatory embarrassment. Further it is desirable for dietary studies that omnivores be used. Since these requirements are met by the pig, this animal has been used throughout.

The pigs were lightly anaesthetized with iv pentobarbital (150 mg/kg body weight). Except in experiments designed to evaluate the effects

TABLE 1  
Flow Rates and Weight of Deposit Formed in Control Animals

At start	Estimated flow (liters/min)			Dry weight of deposit	
	At 10 minutes	At 19 minutes	Mean	Weight $\mu$ g	Log <sub>10</sub> weight $\mu$ g
0.67	0.60	0.60	0.62	1	0.000
0.50	0.41	0.50	0.47	10	1.000
0.48	0.50	0.46	0.48	200	2.301
0.67	0.50	0.55	0.57	200	2.301
0.60	0.60	0.60	0.60	300	2.477
0.24	0.24	0.27	0.25	300	2.477
0.60	0.24	0.23	0.36	300	2.477
0.48	0.29	0.32	0.36	400	2.602
0.67	0.52	0.43	0.54	500	2.699
0.48	0.32	0.31	0.37	700	2.845
0.80	0.67	0.60	0.69	700	2.845
0.57	0.52	0.55	0.55	800	2.903
0.60	0.34	0.32	0.42	900	2.954
0.55	0.43	0.41	0.46	1500	3.176
0.67	0.55	0.60	0.61	1800	3.255
0.50	0.35	0.38	0.41	1800	3.255
0.67	0.50	0.46	0.54	1800	3.255
0.43	0.33	0.32	0.36	3500	3.544
Mean	0.57	0.44	0.44	0.48	2.576

Correlation coefficient between weight of deposit and rate of flow is  $-0.2134$ ,  $P < 0.4$ .

of various anticoagulants, these drugs were not used. A description of the surgical preparation of the animals is given in detail in a previous communication.<sup>8</sup>

#### STA ACTIVITY EXPERIMENTS

Serum rich in serum thrombotic accelerator (STA) activity was prepared from pigs after the method of Wessler.<sup>9</sup> Non-activated plasma was prepared from blood taken into silicone-coated glass containers through siliconed stainless steel needles. In this case, trisodium citrate was used as the anticoagulant instead of potassium oxalate. The blood was centrifuged at 3,200 rev/min (Relative Centrifugal Force 2304) at 4°C for 15 minutes and the supernatant plasma removed and transferred to silicone-coated containers pending use.

One minute after the start of a flow chamber experiment, the serum or plasma was injected by a silicone-coated syringe into the distal sampler. The average dose, given over three minutes was 2.7 ml/kg body weight; the pigs ranged from 18.5 kg to 104 kg. None of the animals showed any untoward reactions during or following the injection of the material.

#### COAGULATION TESTS

The following coagulation tests were carried out by techniques that have been described:<sup>10</sup>

whole blood clotting in glass (WBCT-G) and in silicone (WBCT-S), the one-stage prothrombin time (OSPT), plasma thromboplastin time (PTT) (the activity of diluted plasma in the thromboplastin generation test) and the platelet adhesive index (PAI). Blood samples for the coagulation tests were taken from the exposed carotid artery just prior to the insertion of the cannula.

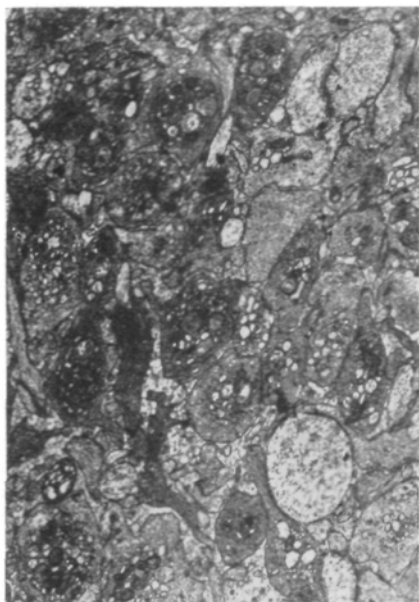
#### STATISTICAL CONSIDERATIONS

Examination of the data in the experimental and control animals showed that the weights of the thrombi were lognormally distributed. Accordingly, while the actual weights are given in the tables, in all calculations the logarithms of the weights have been used. Means of the values have been transformed back into the original scale and the resulting values in the tables are, therefore, geometric means. Clotting time proved to be lognormally distributed, plasma thromboplastin time was harmonic normally distributed and the other tests were normally distributed. The distribution of the results for the in vitro clotting tests are in keeping with our findings from studies in man.<sup>10</sup>

#### Results

##### HISTOLOGY OF THROMBI

The earliest recognizable component seen on the surface was granular material which on



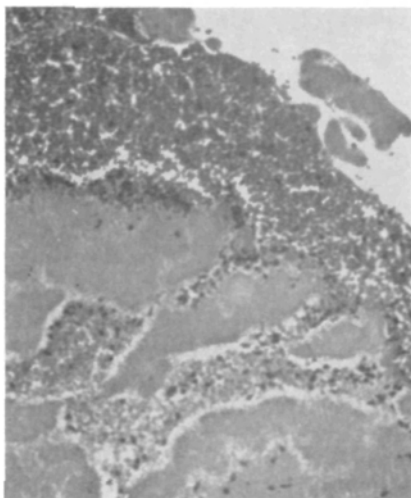
**FIGURE 4**

An electron microphotograph of a deposit. The globular masses are platelets, which have clumped but still have intact membranes. Some vacuolation or bleb formation has occurred but for the most part their internal structure seems intact. The granular material in the spaces where the platelet membranes are not adherent appears to be plasma. No fibrin was detected between the platelet membranes.  $\times 6,650$ .

microscopic examination was found to consist primarily of blood platelets (fig. 3). This was confirmed by electron microscopy (fig. 4). More advanced thrombi were composed of platelets, red cells, white cells and fibrin. Histological examination of these thrombi showed them to have a laminated appearance (fig. 5), consisting of alternating layers of clumped platelets, and fibrin containing red and white cells.

#### CONTROL ANIMALS

The rate of flow varied between 0.20 and 0.70 liters/minute (table 1). The difference in flow rate between the initial values and



**FIGURE 5**

A microphotograph showing bands of platelets alternating with bands of fibrin containing red and white cells. H & E  $\times 355$ .

those at 10 and 19 minutes may be related to differences in technique or in animals at 10 and 19 minutes. In the control animals, no significant correlation was found between rate of flow within the limits used and the amount of deposit formed (table 1). This, of course, does not mean that flow is unimportant, but that within the limits of the present study, it was not found to correlate with the amount of deposit. Deposits grow slowly at first but the process gradually accelerates. The deposit extends distally and, to some extent, medially and proximally from the initial nidus which usually forms at the classical site,<sup>8</sup> the bifurcation. The sequence of events is illustrated by a series of photographs taken at intervals during the course of an experiment (fig. 6). The taking of such photographs is made possible by clamping the proximal sampler and washing the blood out of the flow chamber system with 0.85% NaCl. The saline is not allowed to enter the jugular vein but is discharged to the exterior by clamping the venous can-

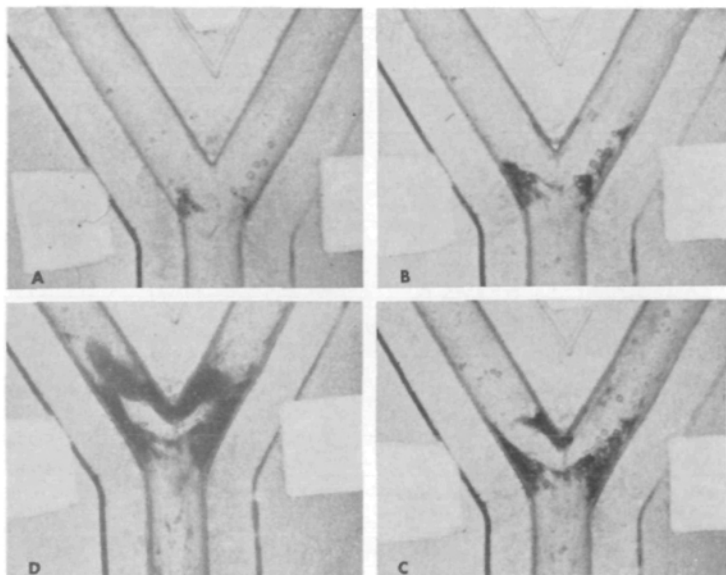


FIGURE 6

The extent and distribution of a deposit at a bifurcation. The photographs were taken at 10-minute and 15-minute intervals.

Figure 6A after 10 minutes flow, figure 6B after 25 minutes flow, figure 6C after 30 minutes flow and figure 6D after 45 minutes flow. Flow is from the bottom.

nula and disconnecting the coupler between the venous cannula and efferent limb of the flow chamber. After the photograph has been taken the circulation is reestablished by recoupling and removing both clamps.

The geometric mean dry weight of thrombus formed was 0.377 mg (the antilog of the main log 2.576) with 95% confidence limits of 0.18 and 1.00 mg. The extreme values were: 0.001 mg and 3.5 mg.

#### RELATIONSHIP BETWEEN COAGULATION TESTS AND THROMBUS FORMATION

Correlation coefficients are presented in table 2. Two tests are significantly correlated with the weight of deposit. The higher values for the platelet adhesive index (denoting greater coagulant activity) are associated with heavier deposits. Short clotting times in silicone-coated glass tubes are also associated with heavier deposits. Thus, there is rough

Table 2  
*Correlation Between the Weight of Deposit and Various In Vitro Coagulation Tests*

Test	n	Correlation coefficient	
Whole blood clotting time (glass)	17	-0.17	
Prothrombin time	15	+0.25	
Plasma thromboplastin time	15	+0.30	
Adhesive index	15	+0.53	< 0.05
Whole blood clotting time (silicone)	13	-0.56	< 0.05

**TABLE 3**  
*Effect of STA Activity on Deposit Formation*

Treatment	n	Geometric mean weight of thrombus—mg	
Plasma infusions	9	0.632	
Serum infusions	9	4.480	$P < 0.01$

correspondence between more coagulable blood as estimated by these two tests and tendency to thrombus formation in flowing blood as judged by the studies in the extracorporeal circulations.

#### THE EFFECT OF CHANGES IN BLOOD COAGULATION ON THROMBUS FORMATION

This can be illustrated from the results obtained in pigs in which serum rich in serum thrombotic accelerator (STA) activity was injected at the start of the experiment. STA is an activity described by Wessler.<sup>9</sup> He found that after blood had clotted, the serum formed contains a factor which accelerates blood coagulation and enhances thrombus formation in isolated, and therefore, static, vascular segments. Plasma, if carefully prepared, contains little of this activity.

Two comparable groups of nine pigs were studied. In one group, an injection of serum was given and in the other plasma was given. The weights of deposits formed are shown in table 3. The mean weight of deposit was markedly enhanced after the serum injection; in contrast the plasma injection produced only a slight increase over the control values.

#### Discussion

These studies on thrombus formation in a flowing system have reconfirmed the classic observation of Zahn,<sup>11</sup> Bizzozero,<sup>12</sup> Eberth and Schimmelbusch<sup>13</sup> and Fulton and associates<sup>14</sup> in *in vivo* studies and of Rowntree and Shionoya<sup>5, 6</sup> and Best, Cowan and MacLean<sup>7</sup> in extracorporeal shunts. They showed that a thrombus formed in flowing blood begins with the deposition of platelets with the subsequent accumulation of other blood cells and plasma proteins. The lognormal distribution of the weight of the deposits formed suggests that growth in the weight of the thrombus is at

least within limits exponential and this is borne out by the serial observations in the course of an experiment (fig. 6). It is obviously difficult to produce more direct evidence on this point. The statistical implications of this have been discussed elsewhere.<sup>15, 16</sup>

This technique although clearly imperfect presents a convenient method for quantitating tendency to thrombus formation while preserving the characteristics of blood in the intact vascular system. We feel that the factors preserved which may be important are the pulsatile character of the flow, the continual replenishment of blood coagulation factors (both anticoagulant and procoagulant), and maintenance of temperature, of pH and of gas tension.

Wessler and associates<sup>2, 9</sup> have shown that the intravascular infusion of serum with high STA activity promotes the formation of thrombi in clamped segments of arteries or veins distant from the site of the original injection. Our studies complement their observation in that while the surface of the flow chamber is foreign, a high rate of blood flow is maintained and, therefore, the factor of stasis is eliminated.

The weight of the deposit formed after serum infusion is considerably enhanced. It seems reasonable to attribute this to increased activity of the blood coagulation mechanism. Certainly the effects of plasma infusion are trivial by comparison. Wessler and associates<sup>17</sup> have demonstrated in their static vascular segments that STA activity promotes thrombus formation even in animals made thrombocytopenic, from which they conclude that the platelet may be of minor importance in this type of thrombosis. They are careful to point out, however, that it cannot be inferred from such experiments that platelet participation is not of major importance in flowing blood. Our studies show that serum presumably rich in STA activity influences thrombus formation in flowing blood but it remains to be seen whether this also occurs in experiments on the thrombocytopenic animal.

Apart from *in vivo* studies of platelet

survival, little attempt has been made to quantitate the effects of procoagulant and anticoagulant substances on the tendency to form thrombi in flowing blood. Heavy reliance has been placed on *in vitro* tests of coagulation as an index of this, but there has been little verification. While the extracorporeal circulation like the test tube presents a foreign surface, the properties of flowing blood are preserved. By comparing the weight of thrombus formed with the results from sundry *in vitro* clotting tests, it is possible to choose tests which are informative. In this study, the clotting time in silicone and the platelet adhesive index gave the best correlations. In a study of the effect of dicumarol on thrombus formation, the platelet adhesive index and clotting time in silicone were again found to give good correlations.<sup>18</sup> This to a large extent corroborates the conclusion reached in studies in man reported in an earlier paper.<sup>10</sup>

### Summary

A standardized extracorporeal shunt system has been described. It allows examination of the sequence of events in thrombus formation and it provides a means of quantitating it in flowing blood. It further allows assessment to be made of the usefulness of classical *in vitro* clotting tests in thrombogenesis. The platelet adhesive index and clotting time in silicone showed the best correlation with the amount of thrombus formed.

Pig serum presumably rich in serum thrombotic accelerator (STA) has been shown to enhance thrombus formation from flowing blood.

Within the limits of these experiments, the rate of blood flow has been found to be of little importance.

### Acknowledgment

We wish to thank the Chairman of the Ontario Hydro-Electric Power Commission and the members of the hydraulics division who provided us with the flow chamber models which they made expressly for this purpose: Mr. I. Grinyer who prepared the electromicrophotograph, and Mrs. I. Newton, Mr. L. Pinter, Mr. M. Kovaks, and Mr. J. Von Hugo for their technical assistance.

### References

1. CHANDLER, A. B.: *In vitro* thrombotic coagulation of the blood. A method for producing a thrombus. *Lab. Invest.* 7: 110, 1958.
2. WESSLER, S.: Studies in intravascular coagulation. III. The pathogenesis of serum-induced venous thrombosis. *J. Clin. Invest.* 34: 647, 1955.
3. ASHWIN, J. G.: The effect of drugs on experimental thrombogenesis. In *Anticoagulants and Fibrinolysis*, ed. MacMillan, R. L. and Mustard, J. F. Toronto, Macmillan Co. of Canada, 1961, p. 117.
4. ROBERTSON, H. R., MOORE, J. R., AND MERSEREAU, W. A.: Observations on thrombosis and endothelial repair following application of external pressure to a vein. *Canad. J. Surg.* 3: 5, 1959.
5. ROWNTREE, L. G., AND SHIONOVA, T.: Studies in experimental extracorporeal thrombosis formation. Method for direct observation of extracorporeal thrombus formation. *J. Exp. Med.* 46: 7, 1927.
6. SHIONOVA, T.: Studies in experimental extracorporeal thrombosis. III. Effects of certain anticoagulants (heparin and hirudin) on extracorporeal thrombosis and on the mechanism of thrombus formation. *J. Exp. Med.* 46: 19, 1927.
7. BEST, C. H., COWAN, C., AND MACLEAN, D. L.: Heparin and the formation of white thrombi. *J. Physiol.* 92: 20, 1938.
8. MURPHY, E. A., ROWSELL, H. C., DOWNIE, H. G., ROBINSON, G. A., AND MUSTARD, J. F.: Encrustation and atherosclerosis: The analogy between early *in vivo* lesions and deposits which occur in extracorporeal circulations. *Canad. Med. Assoc. J.* 87: 259, 1962.
9. WESSLER, S., REIMER, S. M., AND SHEPS, M. C.: Biologic assay of a thrombosis-inducing activity in human serum. *J. Appl. Physiol.* 14: 943, 1959.
10. MURPHY, E. A., AND MUSTARD, J. F.: Dicumarol therapy. Some effects on platelets and their relationship to clotting tests. *Circulation Res.* 8: 1187, 1960.
11. ZAHN, F. W.: Untersuchungen über Thrombose. *Central Bl. f. Med. Wiss.* 10: 129, 1872.
12. BIZZOZERO, J.: über einen neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. *Virch. Arch. Path. Anat.* 90: 261, 1882.
13. EBERTH, C. J., AND SCHIMMELBUSCH, G.: Thrombose nach Versuchen und Leichen Befunden. Stuttgart. Enke. 1888.
14. BERMAN, H. J., AND FULTON, G. P.: Platelets in the Peripheral Circulation, in *Blood Platelets*, ed. Johnson, S. A., Monto, R. W., Rebnick, J. W., and Horn, R. C. Boston, Little, Brown and Co., 1961, p. 7.

15. MURPHY, E. A.: Evaluation of clinical data. Improvement of efficiency by simple transformation. *J. Chron. Dis.* In press.
16. MUSTARD, J. F., MURPHY, E. A., ROWSELL, H. C., AND DOWNIE, H. G.: Factors Influencing Thrombus Formation In Vivo. *Am. J. Med.* 33: 621, 1962.
17. WESSLER, S.: Factors involved in the initiation of thrombosis, *In Anticoagulants and Fibrinolysins*, ed. MacMillan, R. L., and Mustard, J. F. Toronto, Macmillan Co. of Canada, 1961, p. 108.
18. MURPHY, E. A., DOWNIE, H. G., ROWSELL, H. C., AND MUSTARD, J. F.: The effects of dicumarol and heparin therapy on platelet economy. The relationship of these changes to thrombus formation. *In Anticoagulants and Fibrinolysins*, ed. MacMillan, R. L., and Mustard, J. F. Toronto, Macmillan Co. of Canada, 1961, p. 68.