Experimental Arterial Disease

I. The Reaction of the Pulmonary Artery to Minute Emboli of Blood Clot

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When emboli of rabbit blood measuring 0.5 mm. in average diameter were injected into the ear vein of healthy adult male rabbits, they either became adherent to the walls of the pulmonary arteries or impacted in them. In either case an acute arteritis accompanied by endothelial proliferation regularly occurred. This process was reversible in some instances but in others was followed by organization of emboli which resulted in eccentric fibroelastc thickening of the intima or formation of fibrous intravascular bridges. Fatty degeneration, calcification, hemorrhage, and atheroma were absent. The lesions were self-limited and healed. They were not progressive.

This paper describes experiments which were made to study the effects produced in the pulmonary arteries by the injection of small emboli of blood clot. Most previous studies of pulmonary embolism have been so exclusively concerned with large emboli that the effects produced by minute emboli have been largely overlooked. Nevertheless, recent work suggests the possibility that organization and retraction of small emboli or thrombi may result in fibrous thickening of the arterial intima. Rokitansky's suggested such a theory to explain arteriosclerosis and Duguid has recently restated it and described the morphologic evidence in favor of it.

In experiments designed to test the validity of this theory, Harrison repeatedly injected saline suspensions of small fragments of human fibrin clots, having an average diameter of 0.5 mm., into the ear veins of rabbits. He described impaction of such emboli in the small muscular and nonmuscular branches of the pulmonary arteries followed by acute arteritis, organization and shrinking of the emboli so that eventually plaque-like scars of fibroelastic tissue formed in the intima. These experiments indicate that small emboli composed of human fibrin clots can, indeed, produce changes in the rabbit's pulmonary artery which bear some similarity to arteriosclerosis, although morphologically they are by no means identical with the lesions of that disease.

It seemed important to determine whether or not small clots of rabbit whole blood would also produce this effect, since such blood clots would not be foreign to the rabbit and would more closely resemble emboli which might possibly occur spontaneously. Accordingly, the experiments herein described were performed.

Experimental Procedure

Healthy, young, adult male rabbits were used as experimental animals. They were obtained from general laboratory stock, were housed in individual cages in air conditioned animal rooms kept at a temperature of 70 F. and fed as much Purina rabbit pellets and water as they desired.

Whole blood was obtained from the left ventricle of other rabbits and allowed to clot, after which the clot was placed in a Waring Blender for one to two minutes. The average fragment resulting from this procedure measured 0.5 mm. and the largest 1.0 mm. in diameter. They were suspended in sterile normal saline so that the final volume of the suspension was about twice that of the blood from which the clots were obtained.

The blood clot suspension was injected into the ear veins of the experimental rabbits according to the schedule given in table 1 and afterwards they were killed at intervals with intravenous injections of Nembutal. Complete autopsies were done. The lungs were gently distended by injecting Zenker's formol solution into the bronchi and allowed to fix for about 16 hours. Blocks were cut from the periphery to the hilus of each lobe midway between the base and the apex. The blocks were embedded.
in paraffin, and sections of them were stained with hemotoxylin and eosin, periodic acid, and a combina-

The control group consisted of 15 healthy, male rabbits which were from the same stock, of compa-

**Table 1.—Experimental Data**

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Weight</th>
<th>Injection schedule cc. per week</th>
<th>Survival time in days after</th>
<th>Gross findings</th>
<th>Microscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5</td>
<td>Last dose</td>
<td>First dose</td>
<td>Distribution</td>
</tr>
<tr>
<td>WB1</td>
<td>2200</td>
<td>2.0 2.0</td>
<td>0 7</td>
<td>Pulmonary embolism</td>
<td>3+</td>
</tr>
<tr>
<td>WB4</td>
<td>2400</td>
<td>1.8 1.8 1.8 2.0</td>
<td>0 21</td>
<td>Pulmonary embolism</td>
<td>4+</td>
</tr>
<tr>
<td>WB2</td>
<td>1935</td>
<td>2.0 1.5 1.5 1.5 1.5</td>
<td>38 70</td>
<td>2+</td>
<td>+</td>
</tr>
<tr>
<td>WB3</td>
<td>2385</td>
<td>2.0 1.5 2.0 1.8 1.5</td>
<td>38 70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WB11</td>
<td>2780</td>
<td>2.0*</td>
<td>3 min. 3 min.</td>
<td>Pulmonary embolism</td>
<td>3+</td>
</tr>
<tr>
<td>WB13</td>
<td>2660</td>
<td>2.0*</td>
<td>3 min. 1</td>
<td>Pulmonary embolism</td>
<td>4+</td>
</tr>
<tr>
<td>WB8</td>
<td>2110</td>
<td>2.0*</td>
<td>3 min. 1</td>
<td>Pulmonary embolism</td>
<td>4+</td>
</tr>
<tr>
<td>WB5</td>
<td>2310</td>
<td>1.5*</td>
<td>1 2</td>
<td>Massive pulmonary infarcts</td>
<td>3+</td>
</tr>
<tr>
<td>WB10</td>
<td>2420</td>
<td>2.0*</td>
<td>1 1.5</td>
<td>3+</td>
<td>+</td>
</tr>
<tr>
<td>WB6</td>
<td>2150</td>
<td>1.5*</td>
<td>3 4</td>
<td>3+</td>
<td>+</td>
</tr>
<tr>
<td>WB7</td>
<td>1925</td>
<td>2.0*</td>
<td>7 8</td>
<td>2+</td>
<td>+</td>
</tr>
<tr>
<td>WB9</td>
<td>2250</td>
<td>2.0*</td>
<td>16 17</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>WB12</td>
<td>2460</td>
<td>2.0*</td>
<td>27 28</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>WB14</td>
<td>2220</td>
<td>2.0*</td>
<td>60 60</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Injections given in 12 hr. periods.

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of Weigert's and Van Giesen's stains. Selected sections were stained for iron and hemoglobin and sections cut frozen were stained for neutral fat with Sudan III.
injections of 2 cc. of normal saline at intervals of three hours in order to observe any changes which might be produced in the arterial wall by the injection of the suspension fluid used in the experiments.

**Results**

**Control Series**

Examination disclosed small perivascular granulomas in the periphery of the lungs of about 15 per cent of the untreated control animals (fig. 1). The affected vessels were small arteries or arterioles, frequently showing marked endothelial proliferation, and the exudate was composed of lymphocytes, small mononuclear cells and a few eosinophils. No evidence was found of spontaneous disease of the large and medium sized arteries. The pulmonary arteries of animals receiving repeated intravenous injections of sterile saline were entirely normal.

**Experimental Series**

Fourteen rabbits were used in the experiments (table 1). Ten received three or four injections of 2 cc. of the saline suspension of blood clots at intervals of two hours and 4 received injections twice weekly for as long as five weeks. The lungs were examined at intervals of three minutes to 70 days. After the injections, 9 animals were sacrificed during the first two weeks and 5 subsequently.

**Gross Findings.** At autopsy recent infarcts were discovered in the upper and middle lobes of the right lung of one animal dying about 36 hours after the injections. Five animals, which succumbed within a few minutes after the last injection, had large emboli in the right ventricle and main pulmonary artery. The right ventricle was markedly dilated and the lungs were moderately congested. Such large emboli were thought to have arisen from conglutination and propagation of injected emboli.

**Microscopic Findings.** Arterial lesions were widespread in the lungs of all animals examined during the first two weeks. They appeared within a few hours and persisted for about two weeks, after which they healed with variable amounts of scarring. For convenience of discussion, several variants of the acute lesion will be described separately, although in reality they occurred simultaneously. No vascular lesions were discovered in organs other than the lungs.

1. Impaction of Emboli without Demonstrable Alteration of the Artery. The earliest lesions were discovered in rabbit WB11, which died suddenly three minutes following a single injection of 2.0 cc. of blood clot suspension. The emboli had become impacted in both the muscular and nonmuscular arteries of all lobes. The muscular arteries appeared as though contracted in spasm about the emboli, for the internal elastic lamina was wrinkled, the endothelial cells were standing on end and the muscle fibers were short and robust. Numerous vacuoles were present both between and within the muscle fibers, the contents of which were negative for neutral fat, glycogen and mucin. One other animal, WB6, which was killed three days after the last injection, also showed impaction of emboli without other significant changes in the arteries.

2. Impaction of Emboli with Acute Arteritis. Outspoken acute arteritis of large muscular arteries was discovered in 6 of the 9 rabbits which were sacrificed within two weeks of injection. An early stage consisted of accumulation of polymorphonuclear leukocytes around the embolus in the lumen (fig. 2). In other vessels there was an acute, exudative arteritis either with or without necrosis at the site of the impaction (fig. 4). The perivascular lymphatic space was frequently distended with exudate and sometimes the neighboring lung tissue was also inflamed. Swelling and disarray of the endothelial cells were often conspicuous and occasionally were found at a distance from the embolus. Small, nonmuscular arteries were similarly affected. Frequently, mononuclear cells, which presumably came from the endothelium, migrated into the embolus. The elastica was unaltered except when medial necrosis occurred.

3. Hyperplasia of the Vascular Endothelium. Increase in size and number of the endothelial cells occurred in 8 of the 9 animals and was such a striking change that it deserves special mention. Both large and small arteries were affected. Frequently there were other evidences of acute inflammation, but this was not
PLATE 1

FIG. 1. Control rabbit A19, right lower lobe. Small perivascular granulomas occurred in the periphery of the lungs of about 15 per cent of the control animals. The perivascular exudate is made up of mononuclear cells and a few eosinophilic leukocytes and, in addition, there is endothelial hyperplasia of the intima. Van Giesen and elastica stains, × 360.

FIG. 2. Rabbit WB1, right upper lobe. This animal died one hour after the second injection of 2 cc.
invariable and in 2 rabbits, WB6 and WB7, hyperplasia was the only discoverable evidence of vascular injury. In some cases the hyperplasia was focal and in others diffuse (figs. 3 and 5); the cells might remain in the intima or they might move into the embolus. The cytoplasm of the affected cells was swollen, granular and vacuolated, but contained no demonstrable fat, glycogen or glycoproteins. The nuclei were large and hyperchromatic and, although mitoses were not encountered, fusion of cells was observed in some of the emboli. Occasionally, especially in small arteries, the elastica did not show its specific staining properties or was fragmented. Commonly the hyperplasia was accompanied by a dense accumulation of mononuclear cells around the affected vessel.

4. Adhesion of Emboli to the Intima and Endocardium. In 2 rabbits, WB8 and WB10, emboli became adherent to the intima of large muscular arteries and formed small mural thrombi which were covered with endothelium and partly organized at the site of attachment (fig. 6). A similar embolus was discovered adherent to the endocardium of the right ventricle of rabbit WB8. This clot was not covered with endothelium, but mononuclear cells had wandered into the base of it (fig. 7). One other instance of mural embolism has been seen in a rabbit injected intravenously with a saline suspension of small fragments of human fibrin clot and filter paper fibers and will be reported in detail in another paper.

5. Healing of the Lesions (figs. 8 to 11). The acute arteritis lasted for one to two weeks and then began to heal. The clots shrank as they became organized and covered by endothelium, and the exudate disappeared. Many arteries were completely restored to normal, but in some, focal fibrous intimal or medial scars remained to mark the site of injury. Thus, of 7 rabbits which were allowed to live for more than a week after the injections, arterial scars were found in 4. The number of arteries involved seemed to depend upon the time of examination, since after a month only a few scars remained; this suggests that the lesions were self-limiting and not progressive.

**DISCUSSION OF RESULTS**

The results of these experiments are in essential agreement with those of Harrison. However, since clots of blood were used in the present experiments, instead of the clots of human fibrin employed by Harrison, additional information has been obtained. Thus it has been shown that small emboli of blood clot from the same species of animal will produce lesions in the pulmonary artery which are similar to those produced by fibrin clots from a species which is foreign to the rabbit. This finding suggests the possibility that small emboli of an animal’s own blood might damage the pulmonary artery. Such a possibility must, of course, remain a theory until a source for such emboli is demonstrated, which has certainly not been done in our experiments. However, the work of Knisely, Bloch, Eliot and Warner on the sludging of blood indicates a possible source of such emboli and should be further investigated from this point of view.

In addition to the impaction of emboli followed by acute arteritis, organization of the emboli, and fibrous scarring of the intima, which Harrison described, the present experiments have disclosed adhesion of emboli to the intima of large vessels without impaction, and in one instance adhesion of an embolus to the endocardium of the right ventricle. Formation of bridge-like fibrous adhesions in the lumen of large branches of the pulmonary artery has also been observed. These are thought to be the

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**FIG. 3.** Rabbit WB5, left upper lobe, one to two days after injection. Slight swelling and disarrangement of endothelial cells with a small focus of hyperplasia and perivascular exudate. Periodic acid stain, × 300.

**FIG. 4.** Rabbit WB1, left lower lobe (see fig. 2). An embolus impacted in a large muscular artery showing focal acute inflammation, necrosis, and perivascular exudate. Hematoxylin and eosin, × 275.
Fig. 5. Rabbit WB1 (see fig. 2). Endothelial hyperplasia in a small artery with an impacted embolus. Periodic acid stain, X 300.

Fig. 6. Rabbit WB10, right middle lobe. Adhesion of an embolus to the intima of a large muscular
counterpart of the intravascular bridges which occasionally form in the human pulmonary artery as a result of thrombosis or embolism.\(^7\)

When these experiments were undertaken it was thought that the use of blood clots instead of clots of human fibrin might lead to the production of atheromas through decomposition of the blood and that in this way lesions closely resembling spontaneous arteriosclerosis might be produced. Such, however, did not occur. Atheromas did not form, neither fat nor calcium was deposited, and hemorrhage did not occur. Only an occasional scar was vascularized and in this case the capillary walls appeared normal. These findings are in keeping with those of previously published experiments in which whole blood was injected into the media of large arteries.\(^8\) In these experiments, too, scars and not atheromas resulted.

These experiments are of interest when considered in relation to the growing body of evidence indicating that organization of minute emboli or thrombi may lead to significant injury of either the arterial intima or of the endocardium. Mention has already been made of the observations of Duguid\(^1\) which indicate that some forms of arteriosclerosis may possibly be causally related to organized mural thrombi. Recently Heard\(^2\) has described a similar close association between mural thrombosis and arteriosclerosis of the renal arteries. In a study of Lambli’s excrescences, Magarey\(^5\) has presented evidence which he interprets as indicating that they form as the result of the organization of partially attached deposits of fibrin on the surface of the heart valve. He also reports that “in mitral stenosis deposits of fibrin undergoing organization were found on the surface of the valve, including the angles between the cusps,” and suggests that this process of organization might contribute to the progressive development of the stenosis. Thus it is apparent that there is morphologic evidence which suggests the possible importance of organization of minute emboli or thrombi in the pathogenesis of certain disorders of the cardiovascular system. The evidence that such a train of events can occur under certain experimental conditions has been set forth in the experiments described in this paper and in the paper by Harrison. However, that such a sequence of events actually does take place naturally has not been established.

**Summary**

The reaction of the pulmonary arteries to minute emboli of whole rabbit blood clots was studied in rabbits. It was discovered that the emboli either became impacted in the lumen or adherent to the wall of the pulmonary arteries and were there organized. During the process of organization, varying degrees of acute arteritis and endothelial hyperplasia occurred. In some arteries healing was accomplished by shrinking of the emboli and eccentric fibrous thickening of the intima. Occasionally such areas were supplied with discrete capillaries. Defects in the media were repaired by collagenous scars. Organization of emboli also resulted at times in the formation of intravascular bridges. Fatty degeneration, calcification, bleeding into the lesions or atheroma formation did not occur and there was no evidence that the lesions were progressive.

In one case an embolus became adherent to the endocardium of the right ventricle.

The results are discussed in the light of recent work indicating that organization of minute emboli or thrombi of blood or fibrin may contribute to the development of certain disorders of the cardiovascular system.
Fig. 9. Rabbit WB2, right upper lobe, 38 days after the last and 70 days after the first injection. Fibrous intimal plaque in a large artery. Van Giesen and elastica stains, $\times$ 275.

Fig. 10. Rabbit WB2, right upper lobe. Fibrous intimal plaque in a small artery. Van Giesen and elastica stains, $\times$ 300.

Fig. 11. Rabbit WB4, right lower lobe, 21 days after the first injection and immediately after the last of four injections. Impaction of an embolus in a medium sized muscular artery with destruction and fibrous repair of the media and elastica. Van Giesen and elastica stains, $\times$ 100.
REFERENCES


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