LOCALIZATION OF COLLOIDAL SUBSTANCES IN VASCULAR ENDOTHELIUM. A MECHANISM OF TISSUE DAMAGE

I. Factors Causing the Pathologic Deposition of Colloidal Carbon *

Baruj Benacerraf, M.D.; Robert T. McCluskey, M.D., and Dorothy Patras, M.D.
From the Department of Pathology, New York University College of Medicine, New York, N.Y.

The classical description by Aschoff[1] defined a group of cells, designated as the reticuloendothelial system (R.E.S.), which are characterized by their highly phagocytic behavior toward colloidal particles and dyes. He included in this system the Kupffer cells, the cells lining the sinuses of the spleen, lymph nodes and bone marrow, and the endothelial cells in the adrenal and anterior pituitary. These cells are normally concerned with the removal of particulate matter from the blood. The phagocytic capacity of endothelium in other locations has been a subject of controversy.[2] Foot[3] has reported phagocytosis of India ink in pulmonary vessels. Domagk and Neuhaus,[4] as well as Klostermeyer,[5] claimed that the endothelial cells of pulmonary capillaries and to a lesser extent of glomeruli were phagocytic for bacteria. This was denied by Pratt[6] who considered the bacteria in the lungs to be within macrophages rather than in capillary endothelium.

The arrest of colloids in sites other than the R.E.S. can be explained on the basis of two distinct mechanisms. First, it may be caused by the coagulation or flocculation of the suspension investigated in the circulation and its consequent arrest in capillaries, and secondly, it may be the result of alteration of the endothelium itself whereby it becomes phagocytic or sticky for the circulating colloid.

The work of Halpern, Benacerraf and Biozzi[7] emphasized the importance of using tracer colloidal suspensions which remain well dispersed in the blood in a wide range of concentration and which are devoid of toxicity. They demonstrated that the abnormal localization of India ink observed in lung capillaries was caused by a process of intravascular coagulation of fibrin brought about by the shellac in the preparation.

However, even if a preparation is used that is stable in the circulation and does not cause intravascular clotting, the endothelium of blood vessels can still be shown to accumulate carbon under certain circum-

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stances.[8] The endothelial cells of capillaries and venules in the skin have been found to take up carbon or dyes at the site of injection of inflammatory agents such as histamine.[8,9] Jancso[8] suggested that histamine "activates" endothelium to become phagocytic. Whatever the exact mechanism, the occurrence of stickiness of endothelium for circulating colloids as well as platelets and leukocytes is a well known feature of the inflammatory reaction.

The purpose of this study is to investigate the deposition of colloidal particles in other sites than the R.E.S. under the influence of: dosage of colloid employed; blockade of the R.E.S.; the systemic effects of vasoactive amines and soluble antigen-antibody complexes in antigen excess.

It is generally accepted that the phagocytosis of particulate matter by the macrophage system is beneficial to the organism. However, the significance of the arrest of potentially harmful or toxic colloidal materials or bacteria in other sites by the mechanisms investigated in the present study has not been sufficiently emphasized as a mechanism of tissue damage.

Material and Methods

Use was made of a nontoxic stable suspension of carbon C11-1431a from the firm Günther, Wagner, of Hanover, Germany. This preparation, made of particles about 250 Å in size, contains approximately 100 mg. of carbon per ml. in a solution of partially hydrolyzed gelatin and phenol. For most of the experiments this preparation was diluted with a solution of gelatin, as previously described,[10] to further guarantee the stability of the suspension. When large doses were to be administered, the preparation was dialyzed against water to remove the phenol. This carbon suspension has been used to explore the phagocytic function of the R.E.S.[11] The clearance of these efficiently phagocytized carbon particles follows an exponential function of time, $C = C_0 10^{-KT}$ where C = concentration of carbon at time T. The constant K expresses the rate of phagocytosis and varies inversely with injected dose. $K \times D$ = constant, which illustrates the saturating effect of phagocytized carbon on the R.E.S. All experiments were performed on adult white male Swiss Webster mice. The animals were sacrificed by decapitation, and sections were prepared of heart, lung, liver, spleen, kidney, stomach, skin, and, in some cases, aorta and brain.

Antigen-antibody complexes were prepared from rabbit antiserums against 3 times recrystallized hen ovalbumin or bovine serum albumin (BSA). The serums were analyzed for antibody by the quantitative precipitin technique.[12]

The antiovalbumin serum contained 1.44 mg. per ml. of antibody

protein. Ten ml. of this serum were precipitated at the equivalence point by antigen. After several washings in saline and centrifugation, the precipitate was redissolved in 150 mg. ovalbumin in 4 ml. of saline. Five ml. of anti-BSA containing 2.3 mg. of antibody protein per ml. were precipitated with BSA at its equivalence point and subsequently redissolved with 25 mg. of BSA in 1 ml. of saline. The soluble complexes were injected as described below.

Experiments

The Effect of Dosage on the Distribution of Carbon Particles

It has been established that when 16 mg. of carbon per 100 gm. of body weight are injected intravenously into mice, the carbon particles are cleared within an hour by the R.E.S. About 90 per cent of the injected carbon is found in the liver and spleen. No carbon is found in the endothelium of kidney, lung, heart, or skin.[11,13] The same results were observed in the present study (Fig. 1).

It was found that the repeated daily injection of 16 mg. of carbon per 100 gm. for 5 days in a group of 10 mice did not result in deposition of carbon in sites outside the R.E.S. In order to investigate the effect of overloading the R.E.S. on the distribution of injected carbon, 4 doses of 70 mg. of carbon per 100 gm. were injected into 12 mice in 48 hours. Another group of 12 mice received the same dose once a day for 6 days. This severe overloading of the R.E.S. resulted in death of 7 of the 24 mice in spite of the nontoxic properties of the carbon suspension, presumably because of increased susceptibility to infection. Despite a marked increase in capacity of the R.E.S. as evidenced by the enlargement of the liver and spleen to twice their normal combined weights, the distribution of the carbon particles under these conditions was quite different from what was seen with the smaller dose. The endothelial cells of arteries, veins and capillaries in many locations were heavily laden with carbon particles. In the liver itself, carbon was seen not only in markedly enlarged Kupffer cells, but also within the endothelium of sinuses, hepatic and portal veins, and to a lesser extent in the hepatic cells themselves, where it had a finely granular appearance (Fig. 2). In the spleen there was marked hyperplasia and increased phagocytosis in the red pulp. In the lung, carbon was present in macrophages within alveoli and in the endothelial cells of pulmonary arteries and capillaries (Fig. 3). The kidney showed a striking accumulation of particles within glomeruli (Fig. 4) and also within endothelium of blood vessels of all sizes. In the heart, granules of carbon were seen within the endothelium lining the chambers and covering the heart valves (Fig. 5) and within the endothelium of coronary arteries. The

larger arteries such as the aorta showed phagocytosis by endothelial cells (Fig. 6).

Thus it can be seen that with prolonged and excessive administration of colloidal particles, phagocytosis by endothelium can be demonstrated in regions where it is not normally observed. The phagocytic properties of the cells of the R.E.S. can therefore be considered as an especially active function of endothelium in general. The accumulation of carbon in areas other than the R.E.S. varied in intensity and was most marked in glomeruli, small vessels of the heart, pulmonary arteries, and most small veins. As will be shown, these are in general the areas in which the endothelium can be most easily activated.

The Effect of Previous R.E.S. Blockade on the Distribution of Carbon Particles

In the experiments described above, the overloading of the R.E.S. caused by the repeated injections of carbon may have been an important determining factor in the altered distribution observed. To investigate this further, a group of 5 mice received injections of 3 doses of thorotrast, 1 ml. per 100 gm., every 48 hours. Such a course of treatment with thorotrast has been shown to cause effective saturation of the phagocytic activity of the R.E.S.[14] Twenty-four hours after the last dose, the mice received 16 mg. of carbon per 100 gm.

In these mice, the amount of carbon in the R.E.S. was less than that usually seen (Fig. 7), and carbon particles were found in endothelium in other locations, particularly in renal glomeruli (Fig. 8). All the cells of the R.E.S. were markedly swollen and contained pale pink or yellow granules, representing phagocytized thorium dioxide. In the spleen there were numerous nodular accumulations of swollen macrophages in the form of granulomatous lesions (Fig. 9). The glomerular cells were markedly swollen and contained pale pink or yellow material, representing thorotrast, as well as carbon (Fig. 8).

The Effects of Vasoactive Amines on the Distribution of Carbon Particles

The work of Jancso[8] and Biozzi, Mene and Ovary[9] has emphasized the role of histamine in producing activation of the endothelium of blood vessels of the skin to phagocytize colloidal carbon. These effects have been studied locally and are associated with increased capillary permeability, which is also a feature of the inflammatory reaction. Experiments were performed to ascertain whether the systemic administration of histamine, serotonin or adrenalin would cause deposition

of carbon in the vascular endothelium of various organs, when 16 mg. of carbon per 100 gm. were injected, a dose which is normally removed exclusively by the R.E.S. Serotonin was injected intravenously in doses of 0.25, 0.5, and 1.5 mg. Histamine dihydrochloride was given in doses of 5 mg. intravenously or intraperitoneally; the mouse is notably resistant to the lethal effect of histamine. Adrenalin was administered intraperitoneally in doses of 0.05 and 0.1 mg. Combinations of these substances were also used in some instances, employing the same doses. Within a few minutes after the injection of these agents, the carbon suspension was injected slowly and the animals sacrificed one to two hours later.

The experiments were performed several times on groups of at least 4 mice for each substance or combination of substances tested. Since in some mice given adrenalin or serotonin, thrombi were observed, similar investigations were also carried out using animals given 100 to 200 U.S.P. units of heparin (Organon) intravenously, 15 minutes before the experiments.

In all of the experiments most of the carbon was phagocytized by the R.E.S. in the usual fashion, but the agents studied caused deposition in various other sites as will be described below.

Histamine. Abnormal deposition of carbon in histamine-treated animals was seen as follows: Fine granules of carbon were found in endothelial cells of glomeruli but not elsewhere in the kidneys. The capillaries and venules of the heart and the serosa of the stomach also contained carbon in endothelial cells. No more than traces of carbon were found in the vessels of the skin or lung. Pretreatment with heparin did not alter these effects of histamine.

Adrenalin. The changes observed with adrenalin were striking. There was marked accumulation of carbon in the venules and capillaries in the renal medulla (Fig. 10) and a small amount in glomeruli. The venules and capillaries in the gastric mucosa seemed to have their lining coated with carbon (Fig. 11). In the lung and to a lesser extent in the kidney, carbon was present in the form of large intravascular clumps, suggesting thrombus formation. Pretreatment with heparin reduced considerably the accumulation of carbon in the form of clumps, but did not reduce the amount of carbon in renal glomeruli or in the gastric mucosa.

Serotonin. Serotonin caused relatively slight abnormal deposition of carbon. As in the case of adrenalin, carbon was found in the vessels of the renal medulla and in the form of clumps in the lung. In the latter location, this was largely prevented by pretreatment with heparin.

The combination of serotonin and adrenalin increased the severity of adrenalin effects, and in this situation also the formation of thrombi in the lung and kidney was largely prevented by heparin.

The Effect of Soluble Antigen-Antibody Complexes on the Distribution of Carbon

Histamine and serotonin are released from mast cells as a result of antigen-antibody interaction. Germuth[15] has shown that soluble antigen-antibody complexes, when injected intravenously into guinea pigs, caused anaphylactic reactions due to liberation of agents such as histamine. In order to investigate the effects of release of endogenous histamine and related substances on the distribution of carbon, 6 mice received injections of soluble antigen-antibody complexes. Three mice were given 3.5 mg. of anti-BSA in the form of soluble complexes with BSA, and 3 other mice received 2.5 mg. of antiovalbumin in the form of soluble complexes with ovalbumin. Five minutes later 16 mg. of carbon per 100 gm. were slowly injected intravenously, and the animals were sacrificed about two hours later.

The changes observed in carbon distribution were more striking than with any of the pharmacologic agents investigated. In the kidney there were many fine granules in glomerular endothelial cells (Fig. 12). In the heart, carbon was seen in the endocardium, including the heart valves (Fig. 13), in coronary arteries and in the small blood vessels, especially in the epicardial fat (Fig. 14). There was considerable accumulation of carbon in the venules and capillaries throughout the wall of the stomach (Fig. 15). In the skin and subcutaneous tissues there was marked accumulation of carbon in the endothelium of small blood vessels (Fig. 16). In the lung there was virtually no carbon, indicating that there had been no intravascular clotting.

Discussion

Repeated injections of colloidal materials leading to prolonged elevation of blood levels resulted in phagocytosis by endothelial cells in sites other than the R.E.S. The histologic findings clearly demonstrated the presence of colloidal carbon within vascular endothelial cells. Although this occurred in virtually all endothelial cells, storage of carbon was more prominent in certain regions than in others; venules and renal glomeruli showed the most striking accumulation.

The data provide evidence for the phagocytic properties of endothelium outside of the R.E.S., a problem which has been discussed frequently.[2] Under normal circumstances the great avidity of the R.E.S. for colloidal particles and its great capacity do not allow the

demonstration of these properties. These findings are in agreement with the recent observation of Buck who found accumulation of thorium dioxide within endothelial cells of large arteries in rabbits following the administration of large doses of thorotrast.[16]

Aside from circumstances where there is overloading of the R.E.S., phagocytosis by vascular endothelium is known to occur at sites of inflammation and after local injections of histamine.[8,9] The present study was not concerned with the mechanism by which this was brought about. The activation of endothelium may be the result of altered function of the cells, or a consequence of a change in the endothelial surface which renders it sticky for a variety of materials and which may represent the first step in phagocytosis. Whatever the mechanism, this phenomenon may be of importance in the pathogenesis of disease states which depend upon the localization of injurious colloidal or particulate material, in such sites as heart valves, renal glomeruli or arteries.[17]

The systemic administration of serotonin, adrenalin, or histamine causes localization of carbon in the vessels of the heart, kidney, and stomach. The effects of serotonin or adrenalin may be determined in part by the occurrence of intravascular clotting or by changes in hemodynamics. Infusion of adrenalin has been shown to accelerate blood clotting *in vivo*.[18] The systemic administration of histamine appears to result in abnormal localization attributable to changes in the endothelium itself, especially in renal glomeruli and small vessels of the heart.

The most interesting results were seen after the administration of soluble antigen-antibody complexes which are known to cause liberation of endogenous histamine, serotonin, and possibly other substances from mast cells.[15] There was striking accumulation of carbon in glomerular endothelium and the small blood vessels of the heart, similar to what occurred after the administration of histamine, but in addition there was marked deposition of carbon in small blood vessels in the skin and in and around the stomach. It is reasonable to relate the findings with the large number of mast cells in these areas. The phenomena described may have some bearing on the pathogenesis of lesions of experimental serum sickness, since antigen-antibody complexes, which are handled as unstable colloids by the R.E.S.[19] circulate during the period when lesions appear and are localized at the sites of tissue damage.[17,20]

Summary

Phagocytosis of colloidal carbon by vascular endothelium of blood vessels, glomeruli and heart valves was demonstrated after injection of amounts of carbon which overloaded the reticuloendothelial system.

Blockade of the R.E.S. by thorotrast resulted in deposition of carbon in the glomeruli, and in endothelium in other locations, following injection of carbon in amounts normally cleared by the R.E.S.

Systemic administration of histamine caused abnormal localization of carbon particles in vascular endothelium, especially in glomeruli and small blood vessels of the heart. Treatment with serotonin or adrenalin also brought about abnormal localization of carbon, which was partly prevented by prior administration of heparin.

Soluble antigen-antibody complexes caused marked abnormal deposition of injected carbon in glomeruli, heart valves, and small blood vessels in the skin and stomach.

The significance of these observations with respect to the production of tissue damage is discussed.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Liver from a mouse given 16 mg. of carbon per 100 gm. and sacrificed one hour later. The Kupffer cells are loaded with carbon. × 175.

FIG. 2. Liver from mouse given 4 injections of 70 mg. of carbon per 100 gm. in 48 hours. The Kupffer cells are markedly swollen and loaded with carbon. Carbon is also present within endothelium in portal vein and hepatic cells. × 200.

FIG. 3. Pulmonary artery from mouse shown in Figure 2. Carbon particles are found within endothelial cells. × 300.

FIG. 4. Kidney from mouse given 70 mg. of carbon per 100 gm. daily for 6 days. The glomeruli show marked accumulation of carbon. × 300.

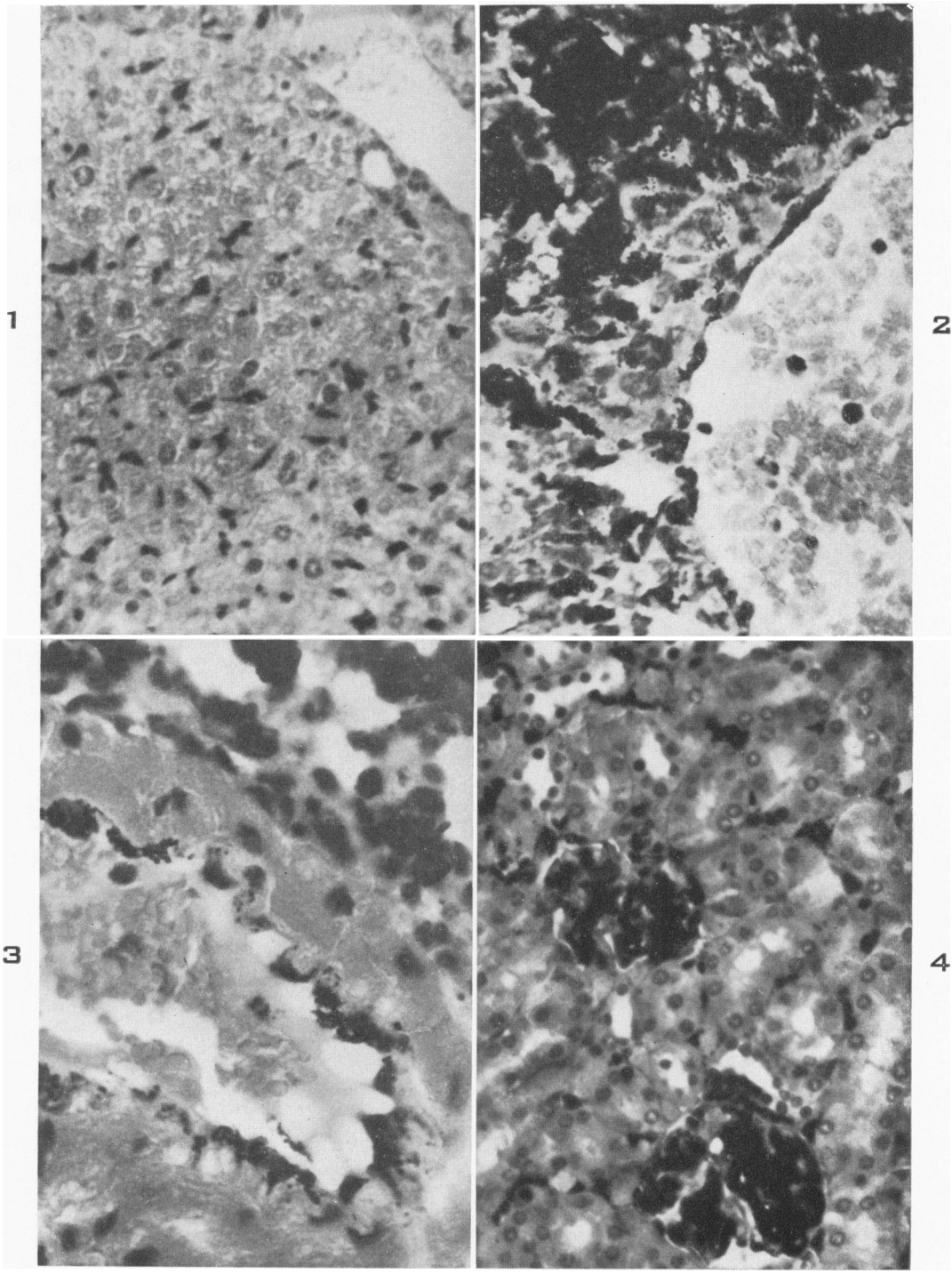
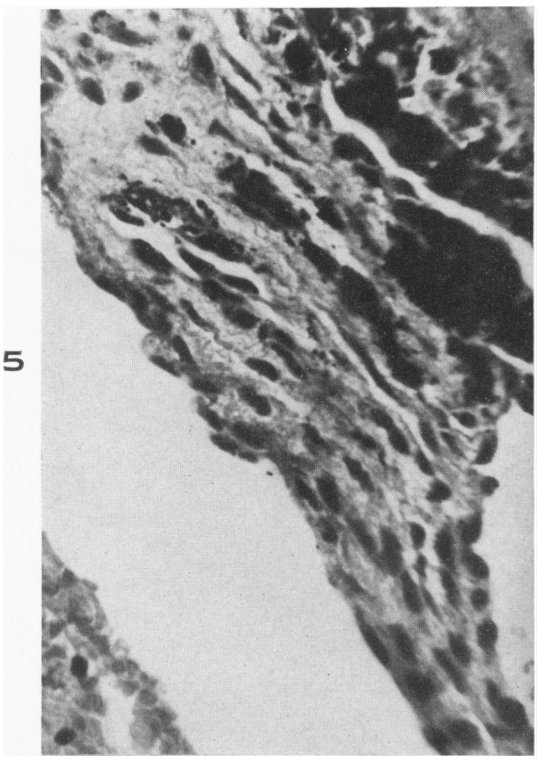


FIG. 5. Mitral valve from mouse given 70 mg. of carbon per 100 gm. for 6 days. There is marked irregular deposition of carbon within the valve leaflet. × 385.

FIG. 6. Aorta from mouse given 4 injections of 70 mg. of carbon per 100 gm. in 48 hours. Fine particles of carbon are present in endothelial cells. × 650.

FIG. 7. Liver from mouse given 3 injections of thorotrast every 48 hours, followed by 16 gm. of carbon per 100 gm. The mouse was sacrificed 4 hours after the carbon injection. The Kupffer cells are swollen and contain yellow or pink granules (thorotrast) and only a small amount of carbon. × 175.

FIG. 8. Kidney from mouse shown in Figure 7. The glomerular cells are swollen and contain yellowish pink granules (thorotrast) and some carbon particles. × 650.



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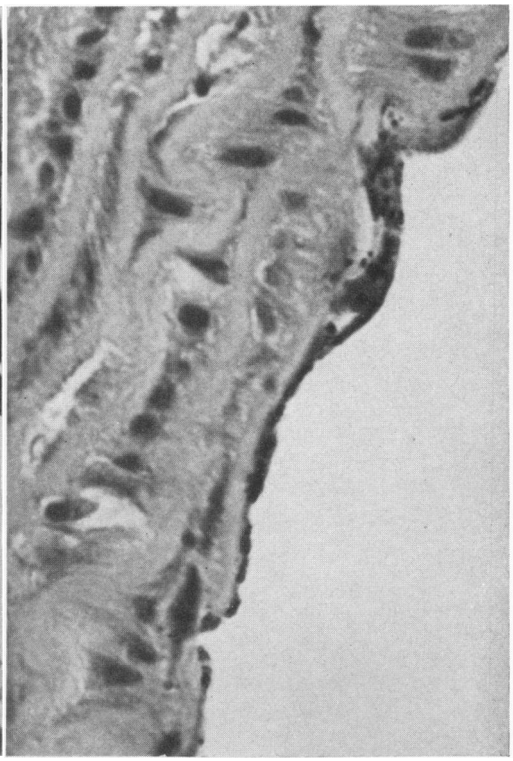

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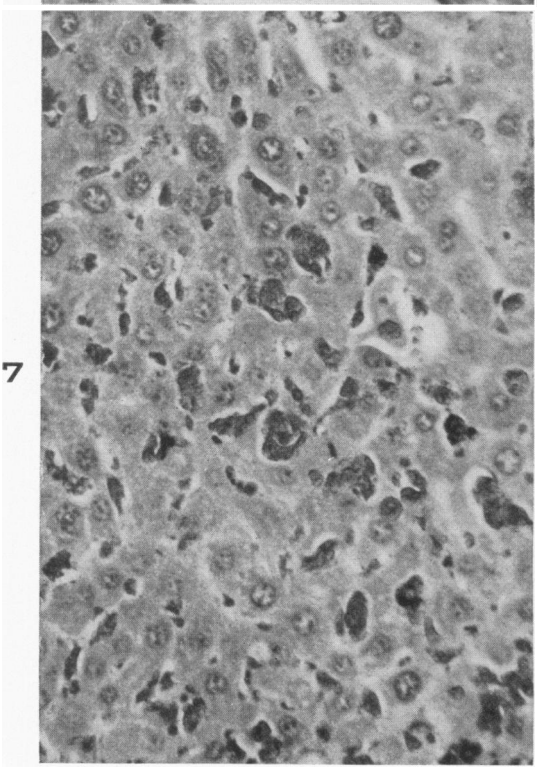

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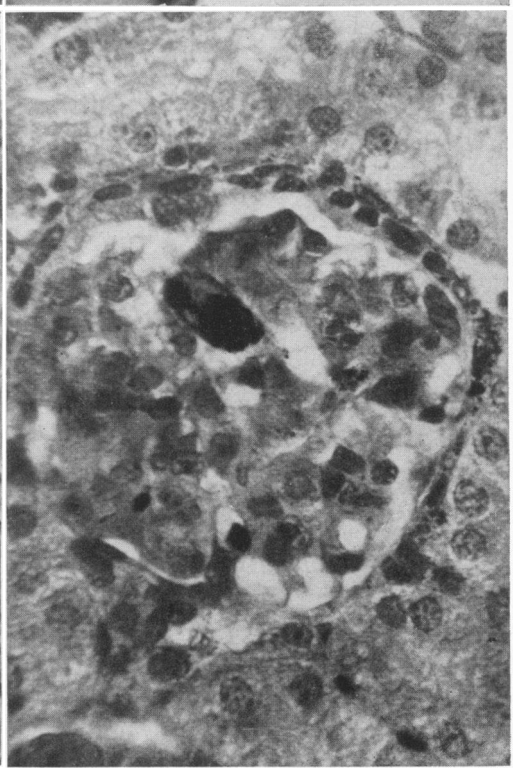

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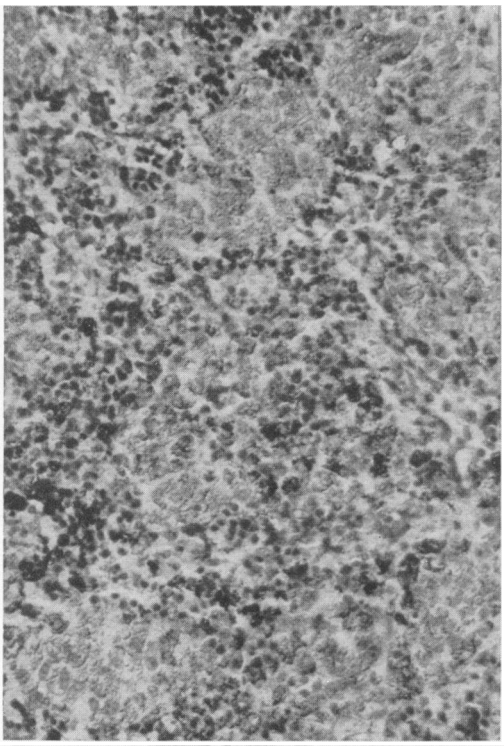
FIG. 9. Spleen from mouse given 3 injections of thorotrast every 48 hours followed by 16 mg. of carbon per 100 gm. There are nodular aggregates of mononuclear cells containing thorotrast. Only a small amount of carbon is present. × 175.

FIG. 10. Renal medulla from mouse given 0.1 mg. of adrenalin followed by carbon, 16 mg. per 100 gm. The mouse was sacrificed one hour later. The lining of the blood vessel in the center is covered with carbon. × 450.

FIG. 11. Gastric mucosa from mouse shown in Figure 10. There is carbon lining the endothelial surface of the small blood vessels. × 175.

FIG. 12. Kidney from mouse given soluble antigen-antibody complexes (BSA-anti-BSA) followed by carbon, 16 mg. per 100 gm. The mouse was sacrificed two hours later. The glomerular cells are swollen and contain fine particles of carbon. × 450.



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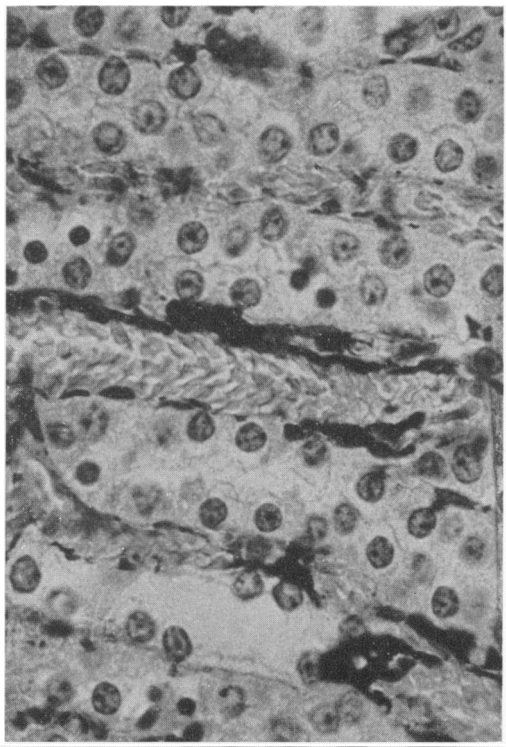

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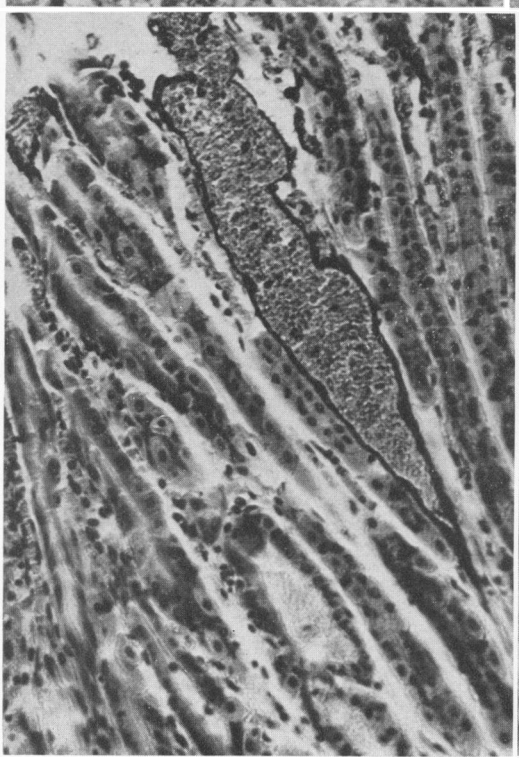

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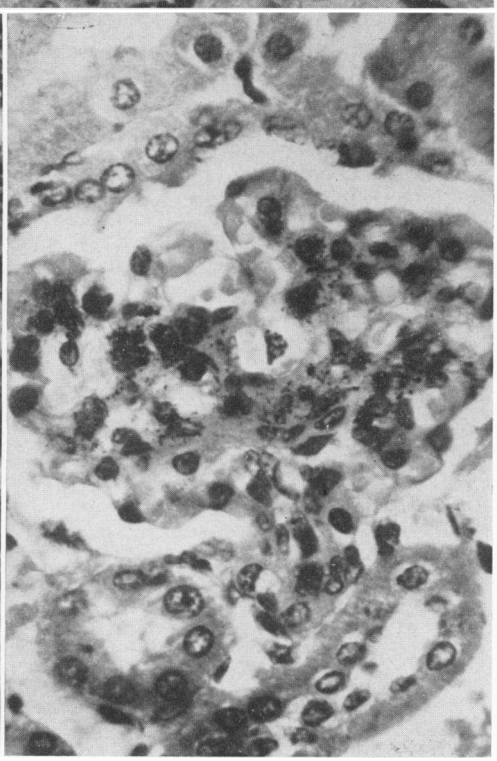

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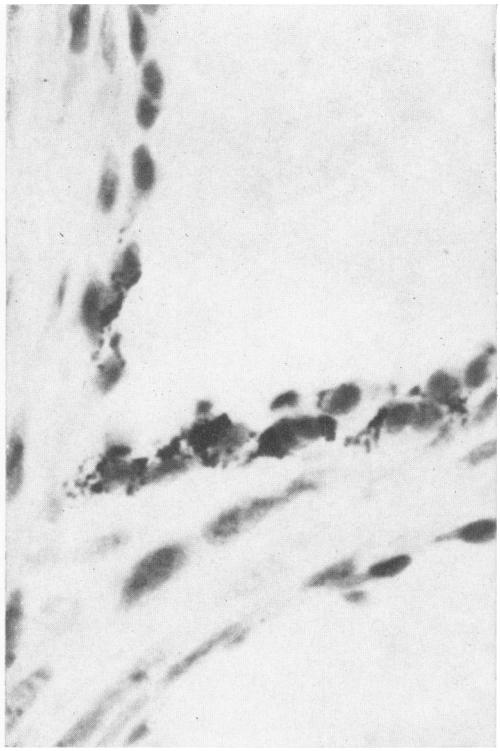
FIG. 13. Mitral valve from mouse given soluble antigen-antibody complexes (ovalbumin-antiovalbumin) followed by 16 mg. of carbon. The mouse was sacrificed two hours later. Some of the endothelial cells of the valve contain carbon particles. × 650.

FIG. 14. Heart from mouse shown in Figure 13. The small vessels in the pericardial adipose tissue contain carbon in their endothelial cells. × 300.

FIG. 15. Stomach wall from mouse given soluble antigen-antibody complexes (BSA-anti-BSA) followed by carbon, 16 mg. per 100 gm. The mouse was sacrificed two hours later. The small vessels in the muscular wall contain carbon in their endothelial cells. × 140.

FIG. 16. Skin from mouse shown in Figure 15. The venules show accumulation of carbon within endothelial cells. × 300.



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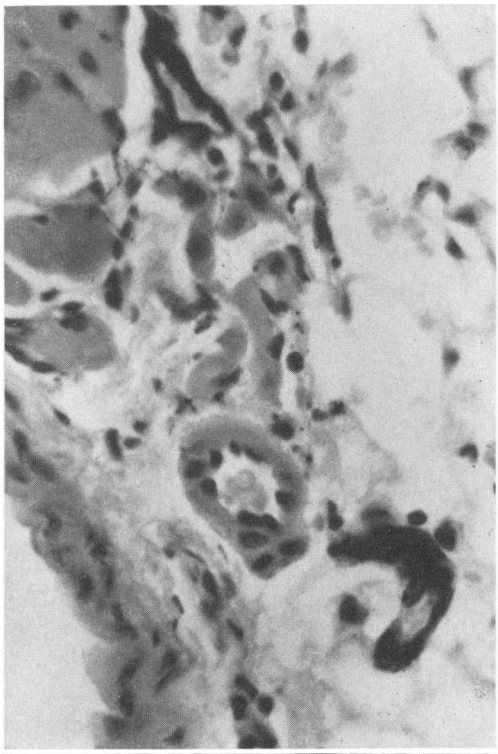

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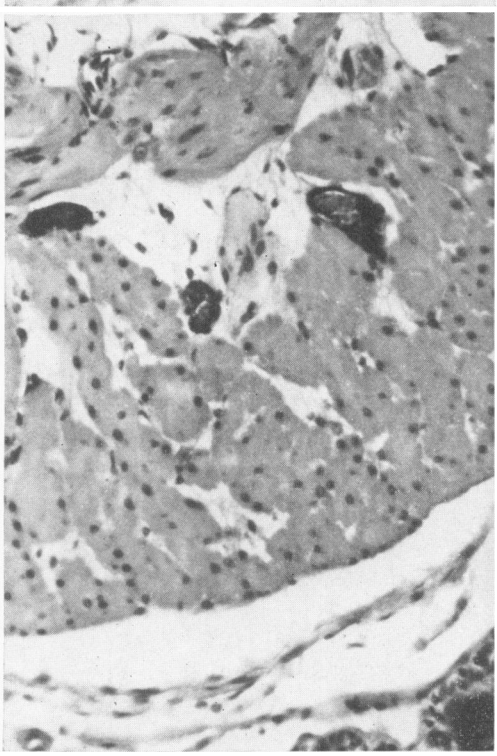

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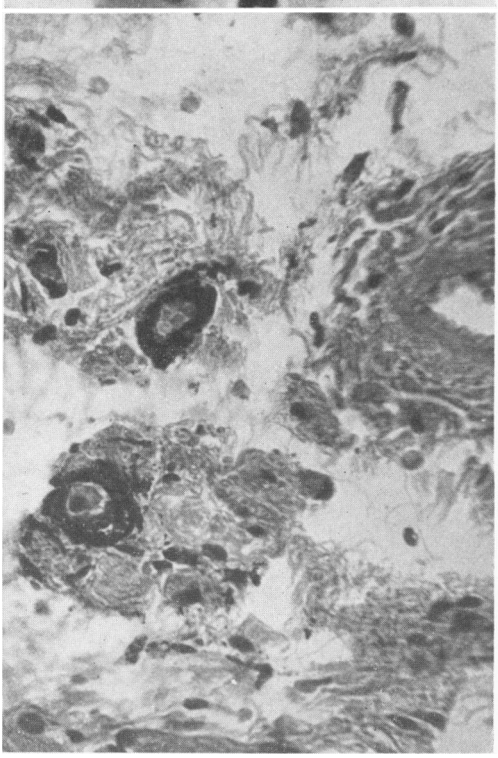

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