THE LOCALIZATION OF CIRCULATING IMMUNE COMPLEXES IN EXPERIMENTAL SERUM SICKNESS

The Role of Vasoactive Amines and Hydrodynamic Forces*

By WILLIAM T. KNIKER,‡ M.D., and CHARLES G. COCHRANE,§ M.D.

(From the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

Plates 19 and 20

(Received for publication 21 August 1967)

Serum sickness is a generalized acute inflammatory disease resulting from the deposition of circulating antigen-antibody (Ag-Ab) complexes in vascular structures (1, 2). The lesions observed include a proliferative and necrotic arteritis, endocarditis, myocarditis, acute glomerulonephritis, pulmonary vasculitis, cutaneous rash, synovitis and splenic granulomas. The pathologic alterations are produced by the interaction of the localized Ag-Ab complexes with many host humoral and cellular factors (3). Since the pathologic changes occur only after circulating Ag-Ab complexes accumulate in vascular structures, it was deemed important to examine the mechanisms responsible for the initial deposition of the complexes.

In previous experiments of these mechanisms performed in this and other laboratories, the deposition of circulating colloidal carbon (4) or immune complexes (5, 6) in vessel walls was brought about by increasing the permeability of small blood vessels. The large complex molecules were then deposited beneath the endothelium, along the basement membrane, suggesting that the complexes were entrapped along a filtering surface (5). In several species histamine (4, 6) and serotonin (5-HT) (4) were shown to cause the increase in permeability. In addition, overloading of the reticuloendothelial system with carbon has led to the deposition of the carbon in the intima of certain vascular structures (4).

Another factor that might influence deposition of Ag-Ab complexes is the hydrodynamic force of blood, suggested by the observation in serum sickness that coronary lesions are found frequently at aortic valve outflow areas and at major branches, particularly at bifurcations (1). It has been shown that hypertension increases the inci-

---

* This is Publication 187 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, presented in part before the American Society of Experimental Pathology, Atlantic City, April, 1966. The work was supported by United States Public Health Service Grant AI-07007 and National Multiple Sclerosis Society Grant 459.

‡ Postdoctoral Research Fellow, United States Public Health Service Training Grant 5TIG683. Present address: Department of Pediatrics, University of Arkansas, School of Medicine. Little Rock.

§ Established Investigator of The Helen Hay Whitney Foundation.

dence and severity of typical serum sickness lesions (7), and that turbulence (8) and slowed blood flow (9) predispose to endothelial injury.

The present studies were undertaken to determine the possible participation of increased permeability and of arterial hydrodynamic factors in the localization of circulating immune complexes in blood vessels in serum sickness.

Materials and Methods

Induction of Serum Sickness.—New Zealand rabbits weighing 1.9–2.8 kg were employed. Serum sickness was produced by a single intravenous injection of bovine serum albumin (BSA) labeled with ^{131}I (I* BSA), in a dose of 250 mg/kg body weight. Enhancement of immune response, measurement of the elimination of I* BSA from the plasma, and measurement of circulating I*BSA-antibody complexes were carried out as described previously (3).

Sacrifice and Morphologic Study of Tissue.—Only those animals eliminating 98.5–99.5% of their I* BSA within 8–11 days after injection were studied. Such animals terminally had circulating immune complexes in large amounts and would be expected to manifest typical lesions. The rabbits were sacrificed when less than 1.5% of the injected I*BSA remained in the plasma, and tissues were taken and examined as described before (3). 10 min before sacrifice, they received intravenously 100 mg carbon/kg body weight of Pelikan C11/143a ink (Gunther-Wagner, Hanover, Germany) that had been dialyzed against saline and contained 2% purified gelatin. Histologic changes in the kidney were scored by the system of Germuth (1). The aortic arch, high abdominal aorta (with celiac, superior mesenteric, and renal branches), and a segment of the lower abdominal aorta were taken from animals in the groups indicated. Prior to embedding in paraffin, each aortic specimen was serially sectioned at a thickness of 2–3 mm, and the sections embedded to permit study of contiguous lesions along the aorta segment's course. On these animals, one cross-section of the ascending aorta was studied by immunofluorescence.

Immunofluorescent Studies.—Studies were performed using the same methods and reagents as described before (3).

Production of Sheep Antisera to Rabbit Platelets.—Pooled rabbit platelets were obtained, washed, and suspended in a final concentration of 100,000/mm^3 (3). After incorporation into an equal volume of incomplete Freund's adjuvant, 6 ml were injected subcutaneously at monthly intervals into sheep for several months before serum was procured. The sheep antisera were stored frozen until used. The antisera had high agglutinating levels of antibodies to rabbit red cells, polymorphonuclear heterophiles (PMN's), lymph node cells, and platelets; only antibodies to platelets remained after multiple absorptions with the cross-reacting cells and rabbit plasma. Verification of complete absorption was obtained by slide agglutination in the case of cell agglutinins and by agar precipitation in the case of antibodies to plasma proteins. Subsequently the absorbed antisera were processed to obtain antibody globulin, free of macromolecular aggregates as noted previously (3).

Experimental Groups.—Unoperated rabbits were divided into five groups. The *first group*, consisting of untreated controls, received only the serum sickness–inducing regimen. The *second group*, in addition, received antiplatelet globulin in a dose sufficient to depress circulating platelets below 20,000/mm^3 until sacrifice. The first dose, 6 ml given intraperitoneally, was followed in 1 hr by 4-ml intravenously. These were given on the 5th day after BSA injection. All subsequent doses were intravenously administered, and by the time of sacrifice a total of 11–16 ml of antiplatelet globulin had been given.

The remaining groups received antagonists of vasoactive amines, beginning on the 4th or 5th day after BSA injection. The animals in the *third group* were given the antihistamine, chlorprophenpyridamine maleate (Chlor-Trimeton, kindly supplied by the Schering Corp.,

Bloomfield, N. J.), in a dose of 10 mg/kg intramuscularly every 4–5 hr, five times a day. This was the lowest dose of the antihistamine found to inhibit skin reactions 5 hr later to 10 μg histamine given intradermally to a rabbit previously injected with Evan's blue intravenously (1.0 ml/kg of a 2.5% solution). The *fourth group* received the 5-HT antagonist methysergide maleate (Sansert, kindly supplied by Mr. Harry Althouse of Sandoz Pharmaceuticals, Hanover, N. J.) in a dose of 0.5 mg/kg intramuscularly, beginning at the same time and given at the same intervals as was the antihistamine. There was no reliable way to estimate the effective dose in rabbits; however, this dose blocked skin reactions 4 hr later to 10 μg of 5-HT in rats previously given Evan's blue intradermally. Animals in the *fifth group* received combined treatment of antihistamine and serotonin antagonists in the same doses as the third and fourth groups.

Another series of rabbits was surgically manipulated to create a coarctation of the abdominal aorta.[1] Upon entering the peritoneal cavity of rabbits anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.), a segment of the abdominal aorta was dissected free of surrounding tissue. The circumference of the aorta was measured and its lumen reduced to two-thirds of its original cross-sectional area, by permanent placement of a constriction consisting of threaded Silastic (Becton-Dickinson & Co., East Rutherford, N. J.) tubing (10). The coarctation was placed approximately 1–2 cm below the renal arteries. In a "Sham-operated" group, the lower abdominal aorta was briefly constricted by threaded tubing which was then removed. After closure of the abdominal wall, all rabbits were permitted to convalesce 7–20 days (average, 14 days) before institution of the serum sickness regimen. (About 10% of the coarctation group expired in the 1st postoperative day; the survivors recovered rapidly from the surgical procedure.) Some of the animals with coarctations were treated with antiplatelet globulin and others were treated with the antagonists of both histamine and 5-HT, as outlined above.

Blood Pressure Measurement.—In most operated animals, intraarterial blood pressure measurements were obtained on the day of sacrifice. Under light Nembutal anesthesia, the exposed femoral and carotid arteries were entered with a 21 gauge needle, attached to a transducer (P23D, Statham Instruments, Inc., Los Angeles, Calif.) via plastic tubing containing heparinized saline (1.5 IU/ml). Pressure tracings were recorded on a Sanborn multichannel recorder, as were calibrating mercury manometer readings.

Hematologic Parameters of Experimental Animals.—During the treatment period, frequent total blood leukocyte, differential, and platelet counts were obtained. Hematocrit values were measured at sacrifice. Reversed passive Arthus tests using 100 μg anti-BSA nitrogen in 0.1 ml were performed on the day of sacrifice, macroscopic and histologic scoring performed as before (3).

Hemolytic Complement (C′) Activity.—Samples of blood on the day of sacrifice from animals representative of certain experimental groups were assayed for hemolytic C′ activity by a sensitive method previously described (3).

Measurement of Proteinuria.—During the final 3 days of immune elimination of I* BSA, 24-hr urine samples were tested for protein using the quantitative sulfosalicylic acid precipitation method (11). Significant proteinuria was defined as a loss in excess of 15 mg in a 24 hr period.

Bioassays of Histamine and 5-HT.—In selected animals, assay for the platelet content of the two vasoactive amines were performed by a modification of the technique described by Humphrey and Jaques (12). Before sacrifice, 9 ml of blood was obtained from the heart by siliconized plastic syringe and a 19 gauge needle. This was mixed with 1 ml of 2% disodium

---

[1] The authors are grateful to Dr. Sun H. Lee for designing and carrying out these delicate procedures.

EDTA in a siliconized tube, chilled in an ice bath. After centrifugation at 1000 rpm for 10 min, the platelet suspension was removed, and the platelets gently washed twice with a solution consisting of 9 parts saline and 1 part 2% sodium EDTA. The platelets were resuspended in saline, their concentration counted, and then the suspension was frozen at −20°C. Bioassays were performed within 3 wk on the thawed specimens; it was assumed from previous evidence (12) that the platelet amines had been released into the supernatant. The Schultz-Dale bath had a working volume of 10 ml; volumes of added test solutions varied between 0.1 and 0.3 ml. For assay of histamine content, atropinized (250 ng/ml) guinea pig intestine was used, and a standard curve of calibration obtained from graded doses of histamie in 0.1 ml volumes. Specificity of the contractions was established by complete blocking of responses with Chlor-Trimeton, 50 μg/ml. To assay platelet supernatants for 5-HT, atropinized rat uterus was used, after prior preparation of the donor with stilbestrol, 200 μg intramuscularly 18 hr before. In both assays, viability and reactivity of the test muscle was substantiated by a brisk contraction upon the addition of 1.0 μg bradykinin in 0.1 ml volumes. The platelet content of each amine was expressed in μg of free base per 1×10^9 platelets.

Skin Reactivity to Permeability Factors.—On the day of sacrifice each animal was given 1 ml of 2.5% Evan's blue dye intravenously. Within a few minutes, histamine in doses of 1 and 10 μg, kallidin in a dose of 10 μg, and normal saline were injected intradermally in 0.1 ml volumes at separated sites. 30 min later the areas of blueing were measured in two dimensions and recorded. Skin tests with 5-HT were not performed, since doses up to 100 μg rarely produced blueing in the skin of normal rabbits.

Nephrotoxic Antibody.—Antibody to rabbit glomerular basement membrane was prepared as described previously (11).

RESULTS

The Role of Increased Permeability in the Localization of Circulating Immune Complexes.—

The localization of circulating carbon in vessel walls during serum sickness: In order to find if a state of increased permeability existed in arterial walls during the onset of lesions in serum sickness, 10 rabbits undergoing this disease were injected with purified carbon (100 mg/kg, intravenously) 10 min before sacrifice. Histologic examination of the coronary arteries revealed deposits of carbon only in areas where lesions were partially or fully developed. In early lesions, i.e. in lesions showing only endothelial proliferation taken early in the course of disease, the carbon was frequently seen lying freely between intimal cells and against the luminal side of the lamina elastica (Fig. 1). The carbon failed to pass through the lamina elastica and was not seen in the media in those lesions in which structural damage to the lamina elastica had not yet occurred. However, if PMN's were present and the lamina elastica was ruptured (see reference 3), the carbon passed through into the media and even into the adventitia. Thus, the intact intimal lamina elastica appeared to act as a barrier to the passage of carbon particles (and by inference, other macromolecules) entering the wall of an artery that was developing inflammation. Carbon was also noted in glomeruli, but glomeruli from normal rabbits receiving carbon also contained carbon, and accurate definition of its position in the glomerulus could not be made.

The effect of histamine and serotonin antagonists and of platelet depletion on the

localization of circulating complexes and on the development of serum sickness lesions: Experiments were next designed to test the effect of antagonists of vasoactive amines and of depletion of platelets, the main reservoir of these amines, on the deposition of immune complexes from the circulation into the tissues. Rabbits that were prepared for serum sickness were divided into four treatment groups and one control group on the 4th day prior to the development of lesions. The treatment groups included animals depleted of platelets and others that received antagonists of histamine and of 5-HT. The effects of these measures on the experimental animals are shown in Table I.

16 rabbits were treated with sheep antibodies to rabbit platelets. These animals showed a marked inhibition of arterial and cardiac lesions with the exception of the myocarditis. Localization of antigen-antibody complexes in glomeruli was greatly reduced, and the glomerular lesions and proteinuria were also inhibited. In rabbits treated with both histamine and serotonin antagonists an even more pronounced diminution in both cardiovascular lesions and glomerular lesions was noted. Out of 11 animals, only one inflamed artery and one site of myocarditis could be found. Of particular interest, proteinuria failed to develop in most of these rabbits and only a small amount was present in those animals affected. Localization of immune complexes in glomeruli, as determined by fluorescent techniques, was greatly diminished by the treatment with both the amine antagonists. Rabbits treated with either amine antagonist alone showed partial suppression of cardiovascular and renal lesions, the inhibition being less than that noted with combined antagonists or platelet depletion.

Experiments were performed to find complexes deposited in walls of large vessels in control and treated rabbits. The ascending aorta was studied by the fluorescent antibody technique in 21 rabbits. As was anticipated from the low incidence of such lesions, only six areas of endothelial proliferation were found; each contained BSA and rabbit γ-globulin (Fig. 2). Four of the six lesions occurred in control animals and two in rabbits treated with both antagonists. None of the aortas that were free from lesions contained fluorescence.

The effect of treatment on formed elements of the blood and on complement levels: Table II shows the effect of platelet-depleting measures and combined histamine and serotonin-inhibiting treatment on blood cells. Terminal measurements on the day of sacrifice are recorded. Hemolytic complement activity in animals from all groups was markedly lowered, ranging from 0 to 28.6 units, in an assay system where 50–100 $C'H_{50}$ units/ml is normal. Control rabbits with serum sickness averaged 17.0 units, while platelet-depleted animals and combined-drug–treated animals averaged 8.5 and 9.4 units, respectively. However, as noted below, abundant C'3 was found in Arthus reactions induced in animals of all groups.

Tests were also carried out on the total histamine and serotonin content of the platelets in treated and control rabbits. Platelets were obtained at the time

TABLE I

Incidence and Severity of Serum Sickness Lesions in Treated and Control Rabbits

	Control-no treatment (10 rabbits)		Platelet-depleted (16 rabbits)		Treatment with antagonists of*					
					Histamine and serotonin (11 rabbits)		Histamine (8 rabbits)		Serotonin (5 rabbits)	
	Rabbits affected		Rabbits affected		Rabbits affected		Rabbits affected		Rabbits affected	
	(%)	Severity‡	(%)	Severity‡	(%)	Severity‡	(%)	Severity‡	(%)	Severity‡
Cardiovascular lesions										
Coronary arteries and heart										
Arterial endothelial proliferation	90		44		9		37		60	
Arterial subendothelial deposits	80		12		9		37		40	
Arterial medial necrosis	80		19		9		25		40	
Arterial fibrinoid deposits	70		12		9		25		20	
Endocardial proliferation	90	Severe	50	Moderate	54	Mild	50	Mild	80	Moderate
Myocarditis	90	Moderate	88	Moderate	9	Mild	50	Mild	40	Mild
Lung										
Arterial endothelial proliferation	80	Severe	56	Moderate	27	Mild	87	Moderate	60	Moderate
Medial PMN infiltration (arteries)	20		12		9		25		20	

	Renal lesions									
Glomerular fluorescence deposits§	+–++ Coarse‖		0–+ Fine		0–+ Very fine		0–+ Fine		Not studied	
Glomerular endothelial proliferation (average score)	2.2+¶		1.7+		1.0+		1.2+		2.2+	
Petechiae	70	Marked	44	Moderate	27	Mild	62	Mild	60	Mild
Terminal 24 hr proteinuria	513 mg (37–1305)		86 mg (0–300)		4.8 mg (0–22.5)		218 mg (0–1350)		431 mg (37–1500)	

* Antihistamine, chlorphenylamine; antiserotonin, methysergide. For dosage, see Materials and Methods.

‡ Gradations of severity: mild, one to two lesions in studied sections; moderate, three to four lesions; and severe, five or more lesions.

§ Grading of amount of fluorescence along the basement membrane in glomeruli: 0, no fluorescence; +, visible but not strong fluorescence; ++, intermediate fluorescence; and +++, maximal fluorescence seen in serum sickness.

‖ Coarse or fine refers to the apparent size of the deposited immune complexes in affect glomeruli. Fluorescent reagents included fluorescent anti-RGG, anti-BSA, and anti-rabbit C′ (β_{1C}-globulin).

¶ For grading system, see reference 1.

of sacrifice when lesions in control animals were near the maximum. The results are shown in Table III.

Skin testing procedures to evaluate responsiveness: To determine the ability of the experimental animals to respond to unrelated inflammatory agents and gauge the completeness of amine antagonism in the treated groups, intradermal tests of histamine and, as control, kallidin, were performed on the day of sacrifice. In Table IV average diameters of blueing 20–30 min after injections of 0.1 ml of vasoactive agent are recorded. Serotonin, in a dose as high as 100 μg, failed to induce significant blueing in rabbit skin.

TABLE II

Hematologic Parameters in Rabbits with Serum Sickness at Sacrifice

	Controls (no treatment)	Platelet-depleted	Antihistamine and antiserotonin
Hematocrit	40 (38–41)	25 (15–36)	39 (27–47)
Platelet count per mm^3	149,000 ($65–250 \times 10^3$)	14,000 ($0–45 \times 10^3$)	159,000 ($35–385 \times 10^3$)
Total Leukocytes per mm^3 (average)	10,430	10,200	14,950
PMN'S, % (average)	58	53	56
Lymphocytes, % (average)	36	44	37
Eosinophiles, % (average)	0	1	0
Basophiles, % (average)	3	1	2
Monocytes, % (average)	3	1	5

Inflammatory reaction to nephrotoxic antibody and immune precipitates (Arthus reaction) in normal and treated rabbits: Considering the lowered incidence and severity of reactions in the treated rabbits, it was important to determine if the treated rabbits were capable of mobilizing an inflammatory reaction to an immunologic stimulus once fixed in the tissues. Two reactions were chosen in which antigen and antibody could be made to react in the tissues despite the treatment: nephrotoxic nephritis and Arthus reactions. In the first of these, sheep antibody to rabbit glomerular basement membrane (nephrotoxic serum) was injected intravenously into normal rabbits and rabbits treated as above. Five normal untreated rabbits yielded an average of 400 mg protein in the urine in the first 24 hr following injection of nephrotoxic serum. Four rabbits treated with both antihistamine and antiserotonin in doses identical to those

used in the serum sickness experiments yielded an average of 410 mg proteinuria in the first 24 hr after nephrotoxic serum. Three platelet-depleted rabbits had an average of 1155 mg proteinuria in the same period; this elevated value resulted from a massive value of 2300 mg in one of the rabbits, the other two

TABLE III

*Serotonin and Histamine Contents of Platelets in Treated and Control Rabbits during Serum Sickness**

Group	No. of rabbits	Serotonin μg per 1 × 10^9 platelets		Histamine μg per 1 × 10^9 platelets	
		Average	Range	Average	Range
Control	5	59	32–93	52	12–93
Platelet-depleted	5	0‡		0‡	
Combined antihistamine and antiserotonin	3	40	9–46	112	100–133
Antihistamine	4	35	17–53	112	71–214
Antiserotonin	2	24	single rabbit	78	57–100

* Platelets obtained at time of sacrifice. All rabbits, including controls, given serum sickness regimen.

‡ Plasma with few remaining platelets collected and treated as if platelets were present. Platelet counts in the actual samples tested for the presence of vasoactive amines were not performed.

TABLE IV

*Reactions to Intradermally Applied Inflammatory Agents in Control and Treated Rabbits with Serum Sickness**

Test material	Untreated controls	Platelet-depleted	Antagonist of:		
			Histamine and serotonin	Histamine	Serotonin
Histamine, 10 μg	9.8	9.4	2.8	1.3	9.2
Histamine, 1 μg	7.5	7.0	0.8	0	7.4
Kallidin, 10 μg	10.4	7.5	8.4	8.6	11.6
Normal saline	0.5	0	0	0	

* Average diameters of blueing skin reaction in mm. Each figure represents the average of reactions of 5–11 rabbits.

rabbits in this group losing 550–600 mg protein per 24 hr in the urine. Hematuria was not present. With fluorescent antibodies, abundant sheep gamma globulin was found along the glomerular basement membranes in a linear deposit in all rabbits. In the second reaction attempted, RPA test sites were placed on rabbits of each group shown in Table I on the day prior to or the day of sacrifice. 100 μg

N anti-BSA was employed. In the reactions that ensued, a mild to moderate diminution in the edema of the macroscopic reaction was observed in each treated group. Histologically, however, the difference was undetectable. PMN infiltration, hemorrhage, and vascular necrosis were apparent in all groups. By fluorescent antibody techniques, BSA antigen and, of importance, β_{1C}-globulin (rabbit C'3) were localized in affected venules in all groups. In the group treated with both antagonists, a slight reduction in the amount of BSA deposition was apparent, which may account for the slight difference in macroscopic reaction in this group.

TABLE V

Incidence of Serum Sickness Arteritis in Regions of the Aorta

Location	Sham-operated	Coarctation of the abdominal aorta		
	Untreated control (7 rabbits)	Untreated control (7 rabbits)	Anti-Histamine and 5-HT (7rabbits)	Platelet-depleted (8 rabbits)
	%	%	%	%
Coronary ostia	71	71	0	25
Aortic arch	43	43	29	25
Carotid and inomminate arteries	29	0	0	12
Upper abdominal aorta	43	43	14	0
Superior mesenteric and renal arteries	29	57	0	0
Coarctation site	—	43	43	37
Below (0.5 cm) coarctation site	—	71	14	12

The Importance of Hydrodynamic Factors in the Localization of Circulating Complexes in Arteries.—

The possible influence of intraarterial hydrostatic forces on the deposition of circulating immune complexes in serum sickness was studied. This was carried out by (*a*) comparing the incidence of lesions in zones of the aorta or its branches where turbulence of blood occurs with a zone of relatively even blood flow; and (*b*) by surgically creating a coarctation of the abdominal aorta to artificially induce an area of irregular luminal hydrodynamic forces.

1. The abdominal aorta, between the segmental branches, contained no lesions in seven sham-operated untreated control rabbits. By contrast, in areas of the aorta where turbulence would be expected, inflammatory lesions were readily demonstrated (Table V, column 1). In addition, the endocardium of the semilunar valves and the intima of the base of the aorta were affected in all animals. Whereas lesions of the coronary ostia often included medial inflammation, lesions elsewhere in the aorta and its branches generally were limited to the intima, PMN's infrequently reaching the media. The characteristic feature of early aortic or branch lesions was an elevation of endothelial

cells from the underlying internal elastic lamina. This was followed by the infiltration of PMN's into the subendothelial spaces. It was common for lesions of the aorta near outflows of arterial branches to extend contiguously through the outflow orifice and down the branches (Fig. 3). Circulating carbon did not deposit in such lesions to the same degree as seen in coronary arteries, although the percolation of carbon particles between cells and the accumulation of carbon along the underlying membrane was apparent. Fluorescence observations of five intimal lesions from the aortas of five rabbits indicated that immune complexes were present only in the lesions. The amounts of antigen and antibody were never great.

2. Coarctations were surgically created in the abdominal aortas of 11 rabbits at a point 1–2 cm below the renal arteries. In the zone of constriction thus created, intimal lesions, morphologically similar to those described in part 1 above were noted in 73% of the animals. In 12 sham-operated rabbits with serum sickness, only one site of inflammation was found at the manipulated segments. Intraarterial blood pressure measurements were performed in all rabbits. In those with coarctations, average systolic pressure readings of 120 mm Hg (range 98–160) were observed in the carotid arteries, and 86 mm (52–108) in the femoral arteries. In the sham-operated animals, the corresponding values were 114 (88–160) and 105 (48–160). The average gradient between carotid and femoral pressures was 34 mm Hg in rabbits with coarctations, and only 9 mm Hg in the sham-operated controls. Other than the development of lesions at the site of aortic constriction, animals with coarctations developed cardiovascular and renal lesions with an incidence and severity expected of unoperated rabbits. Microscopic examination of the site of coarctation for reaction to the surgical procedure revealed only a small amount of chronic inflammation, limited to areas around sutures in the adventitia.

Treatment by platelet depletion or antihistamine and antiserotonin in rabbits with surgical coarctation undergoing serum sickness: In order to find whether the hydrodynamic factors that appeared to enhance localization of circulating complexes depended upon the release of vasoactive amines, rabbits with coarctations were treated with antiplatelet globulin or antihistamine and antiserotonin as previously described. The incidence of lesions in areas of blood turbulence in such treated and control rabbits is shown in Table V. It may be noted that both treatments, especially the combined use of amine antagonists, effectively prevented expected intimal lesions in most areas. However, in two areas of disturbed channel flow, the incidence of lesions was not greatly affected by treatment. One such area was the aortic arch, a point of marked contour change. The other was the site of coarctation, not withstanding the fact that lesions immediately downstream were largely inhibited by treatment (Table V).

DISCUSSION

The Role of Increased Vascular Permeability and Vasoactive Amines in the Deposition of Circulating Immune Complexes in Arteries and Glomeruli.—

Previous studies in mice (4) and guinea pigs (5, 6) have shown that circulating colloidal carbon and antigen-antibody complexes deposit in vessels during a state of increased permeability. The increased permeability was brought about by the release of vasoactive amines from their reservoirs in the tissues and by injections of the amines. The complexes and carbon were found to aggregate in the subendothelial space of small vessels where their passage through the vessel wall was impeded by the basement membrane. Evidence indicating that the large size of antigen-antibody complexes accounts for their retention by the basement membrane is presented in the accompanying article (13). The net charge of the complexes and other macromolecules was found not to influence their ability to localize along vascular basement membranes, and no specific affinity was observable between circulating complexes and the membranes.

The localization of antigen-antibody complexes in vessel walls is known to be responsible for the lesions of serum sickness. In the present studies, the deposition of complexes appears to be induced by mechanisms similar to those mentioned above. This was suggested by the following evidence:

1. When colloidal carbon was injected during the formation of the arterial lesions, it became localized, along with the complexes, in the developing intimal lesions. When intimal proliferation alone was noted, the carbon filtered between endothelial cells and lined up along the lamina elastica interna. This membrane appeared to act as a barrier to the further passage of macromolecules suggesting, as before (5), that a process of filtration was occurring. It was not until PMN's entered the site and caused disruption of the lamina elastica (as noted previously, reference 3), that the carbon was able to pass freely through this membrane into the media.

2. When substances known to increase vascular permeability (histamine and 5-HT) were antagonized, the deposition of circulating immune complexes in glomeruli and arteries and the development of lesions was markedly or completely inhibited (Table I). Only a single focus of coronary arterial inflammation was found in 11 treated animals. The development of endocardial, pulmonary arterial, and myocardial lesions was markedly inhibited. Of particular note, glomerulitis and proteinuria were at a minimum or were absent while urine output was unchanged. While the explanation of this inhibition may lie in a combined effect of the 5-HT and histamine antagonists, other effects of the treatment must be considered. For example, the 5-HT antagonist employed, methysergide maleate, has among its side effects the inhibition of histamine release, and although the rabbits were reactive to kallidin, it is also not possible to rule out inhibition of some other mediator. A direct protective action

on the vessel by the amine antagonists would appear unlikely, however, in view of the similar inhibitory effect of platelet depletion on deposition of circulating complexes (below).

Further evidence against nonspecific inhibitory effects of the treatment on the development of vascular inflammation was obtained. When rabbits were treated with the antagonists of vasoactive amines (or depleted of platelets) they were still capable of responding strongly to tissue-fixed immune reactants as exemplified by nephrotoxic nephritis and Arthus reactions. This was of special importance in the studies of arteries. Here, for technical reasons, it was difficult to detect deposition of complexes and reliance was placed on the absence of morphologic lesions as a mark of diminished deposition.

3. When the most important reservoir of vasoactive amines in the circulation, i.e. platelets (12, 14), was removed in rabbits subjected to serum sickness, localization of immune complexes and the development of arterial and glomerular lesions was markedly suppressed. The inhibition of lesions was not complete, but it must be noted that platelets were not completely eliminated and that other sources of permeability factors, such as circulating leukocytes (12), were not affected by the treatment.

This mechanism for localization in blood vessel walls of materials from the circulation apparently is applicable to macromolecules only. 7S antibody to rabbit glomerular basement membrane deposited in glomeruli despite inhibition of vasoactive amines and depletion of platelets. This supports the notion that circulating macromolecules require some local change in vascular function in order to be deposited. If this concept is correct, the complexes may function actively in the sense that they increase permeability through release of permeability factors, but their actual localization would be a passive phenomenon.

Circulating macromolecular immune complexes may be important in the pathogenesis of certain diseases of humans other than serum sickness. Among these are acute glomerulonephritis, disseminated lupus erythematosis, rheumatoid arthritis, hypersensitivity vasculitis, and periarteritis nodosa. It will be of interest in the future to learn if increased vascular permeability and passive entrapment of immune complexes in vessels play a role in the development of the inflammatory lesions of these diseases. In human cases of serum sickness, it has been noted that large doses of antihistamines controlled certain symptoms and inhibited the skin rash (15).

In the models previously studied in mice and guinea pigs (4–6), mast cells appeared to be the reservoir of vasoactive amines. These amines could be released either by antigen-antibody complexes or by a mast cell–disrupting agent to bring about localization of complexes (5). However, in rabbits the platelets are known to contain the great majority of vasoactive amines (12, 14, 16), there being no mast cells in arteries and glomeruli. In addition, the white cells contain less than 5% of the total blood histamine. It has been

shown that immune complexes bring about the clumping (14, 17, 18) and lysis (18–20) of platelets, and that vasoactive amines are released in their presence (14, 16, 19). A heat-labile factor in plasma apparently is involved in this release (16, 21, 22). One might postulate that in serum sickness, antigen-antibody complexes circulate, bring about the clumping of platelets and the release of permeability factors from them. Indeed, blood taken from rabbits developing serum sickness does contain platelet clumps (17). With the release of these factors, the permeability of vessels increases, leading to heightened filtration of plasma and localization of the complexes on the filtering vascular membranes. Of the permeability factors, the vasoactive amines have been shown most commonly to affect venules. The present evidence suggests that when released intravascularly these or other agents may also alter the permeability of the intima in arteries, glomerular capillaries, and endocardium. The reason for the different anatomical location of the permeability effect is not clear, although the role of platelets in the release of the permeability factors and the hydrodynamic forces placed on the platelets in the arteries may well be important. Clumping of platelets in vivo, induced by immunologic or non-immunological means, reportedly cause certain lesions similar to those of serum sickness (17, 23, 24); however, the present studies do not support the concept that clumping alone is responsible for lesions. In spite of adequate numbers of platelets and a full complement of circulating complexes, animals given the serum sickness regimen but treated with antagonists of vasoactive amines did not manifest lesions of serum sickness. It is thus difficult to implicate platelets in the formation of these lesions aside from their possible role in the release of permeability factors leading to localization of circulating complexes.

The Role of Hydrodynamic Forces in the Deposition of Circulating Immune Complexes in Arteries.—

Arterial lesions in serum sickness frequently were found in areas where turbulent hydrodynamic forces arise, such as at branching points, and at areas of abrupt contour change; e.g., the aortic arch. In addition, a surgically treated coarctation of the abdominal aorta in rabbits brought about typical intimal lesions at the point of constriction, and immediately above and below. A considerable drop in blood pressure was noted below the coarctation in these rabbits. Studies of the mechanism by which the hydrodynamic forces induced deposition of complexes suggested that the dependence upon release of permeability substances, as found in studies of other lesion sites, may exist in areas of high blood turbulence. Antagonism of vasoactive amines or depletion of platelets at a time when lesions generally would be starting markedly inhibited development of intimal inflammatory reactions in arterial branches and at aortic regions of outflow (Table V). However, the antagonists to vasoactive amines failed to prevent formation of inflammatory foci in the intima

at the coarctation site or along the arch of the aorta. It is possible that shearing forces at such points of high turbulence are sufficient to reduce endothelial integrity mechanically (see reference 8), permitting ingress of macromolecules into arterial walls in the absence of known chemical mediators. In addition, it is possible that the inhibitory measures invoked were insufficient to be effective in these highly susceptible zones.

SUMMARY

In serum sickness, mechanisms by which circulating immune complexes become localized in the walls of vessels and glomeruli have been studied. In affected arteries, morphologic observations showed that circulating marker particles of carbon would rapidly deposit along the luminal surface of the internal elastic lamina. This, as in previous studies, suggested an increase in vascular permeability during which large molecules were capable of being trapped by a filtering membrane in the vessel wall.

In attempts to prevent the increase in vascular permeability, rabbits were treated with antagonists of histamine and serotonin. Such treatment markedly inhibited the localization of immune complexes in glomeruli, the development of proteinuria, and glomerular endothelial proliferation. Cardiovascular lesions also were largely prevented from developing. Depletion of platelets, the principal reservoir of vasoactive amines, had a similar though less pronounced effect. While the deposition of immune complexes was inhibited, allergic inflammation in general was not, since normal rabbits treated as above were found capable of developing full Arthus reactions and acute nephrotoxic nephritis.

Hydrodynamic factors were noted to be important in determining the location of arterial lesions. Studies of aortas from unmodified rabbits and from those with surgically induced coarctations of the abdominal aorta revealed intimal lesions concentrated at areas of high turbulence, such as at branches, bifurcations, outflows and zones of configurational change. Lesions in these areas were also largely inhibitable by depletion of platelets or by antagonists of histamine and serotonin.

BIBLIOGRAPHY

1. Germuth, F. H., Jr. 1953. A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J. Exptl. Med.* **97**:257.
2. Dixon, F. J., J. J. Vazquez, W. O. Weigle, and C. G. Cochrane. 1958. Pathogenesis ot serum sickness. *A. M. A. Arch. Pathol.* **65**:18.
3. Kniker, W. T., and C. G. Cochrane. 1965. Pathogenetic factors in vascular lesions of experimental serum sickness. *J. Exptl. Med.* **122**:83.
4. Benacerraf, B., B. T. McCluskey, and D. Patras. 1959. Localization of colloidal

substances in vascular endothelium. A mechanism of tissue damage. I. Factors causing the pathologic deposition of colloidal carbon. *Am. J. Pathol.* **35**:75.

5. Cochrane, C. G. 1963. Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels. I. Structural studies. *J. Exptl. Med.* **118**:489.
6. Cochrane, C. G. 1963. Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels. II. Pathogenetic and pharmacodynamic studies. *J. Exptl. Med.* **118**:503.
7. Fisher, E. R., and J. Bark. 1961. Effect of hypertension on vascular and other lesions of serum sickness. *Am. J. Pathol.* **39**:665.
8. Scharfstein, H., W. H. Gutstein, and L. Lewis. 1963. Changes of boundary layer flow in model systems: Implications for initiation of endothelial injury. *Circulation Res.* **13**:580.
9. Sakai, Y., and J. J. Lewis. 1965. A study of platelet aggregation and blood velocity *in vitro*. *J. Thorac. Cardiovasc. Surg.* **49**:982.
10. Lee, S. H. 1959. The production of experimental ascites and experiences in treatment with the rectus wick operation. *Surg. Forum.* **9**:557.
11. Hammer, D. K., and F. J. Dixon. 1963. Experimental glomerulonephritis. II. Immunologic events in the pathogenesis of nephrotoxic serum nephritis in the rat. *J. Exptl. Med.* **117**:1019.
12. Humphrey, J. H., and R. Jaques. 1954. The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species. *J. Physiol. (London).* **124**:305.
13. Cochrane, C. G., and D. Hawkins. 1968. Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. *J. Exptl. Med.* **127**:137.
14. Waalkes, T. P., and H. Coburn. 1959. The role of platelets and the release of serotonin and histamine during anaphylaxis in the rabbit. *J. Allergy.* **30**:394.
15. Goodman, L. S., and A. Gilman. 1956. The Pharmacologic Basis of Therapeutics. The MacMillan Co., New York. 2nd edition. 663.
16. Humphrey, J. H., and R. Jaques. 1955. The release of histamine and 5-HT (serotonin) from platelets by antigen-antibody reactions (in vitro). *J. Physiol. (London).* **128**:9.
17. Hughes, A., and R. S. Tonks. 1959. The role of microemboli in the production of carditis in hypersensitivity experiments. *J. Pathol. Bacteriol.* **77**:207.
18. Miescher, P., N. S. Cooper, and D. Hurez. 1960. The *in vitro* action of antigen-antibody complexes on thrombocytes anderythrocytes. *In* Cellular Aspects of Immunity, Ciba Foundation Symposium. Little Brown and Co., Boston. 450.
19. Barbaro, J. F. 1961. The release of histamine from rabbit platelets by means of antigen-antibody precipitates. I. The participation of the immune complex in histamine release. *J. Immunol.* **86**:369.
20. Gocke, D. J., and A. G. Osler. 1965. *In vitro* damage of rabbit platelets by an unrelated antigen-antibody reaction. I. General characteristics of the reaction. *J. Immunol.* **94**:236.
21. Gocke, D. J. 1965. *In vitro* damage of rabbit platelets by an unrelated antigen-antibody reaction. II. Studies of the plasma requirement. *J. Immunol.* **94**:247.

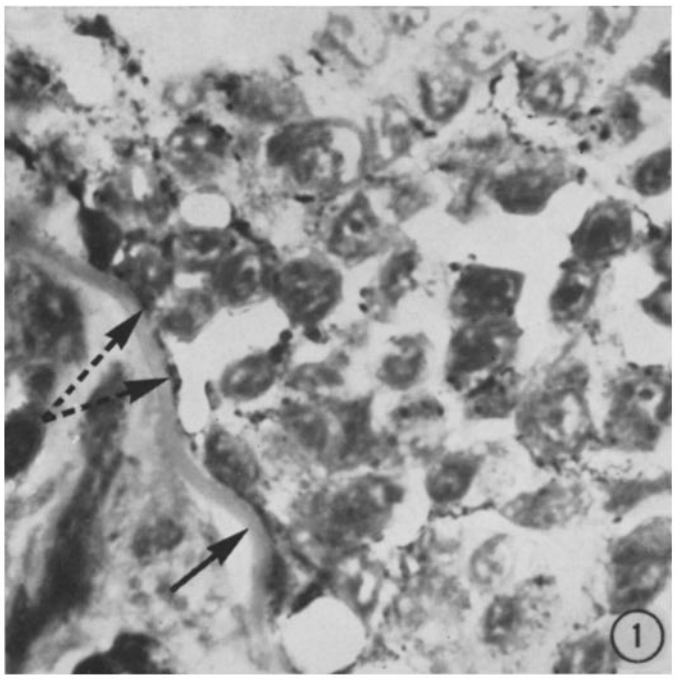
22. Barbaro, J. F. 1961. The role of plasma in the release of histamine. *J. Immunol.* **86**:377.
23. Hughes, A., and R. S. Tonks. 1962. Intravascular platelet clumping in rabbits. *J. Pathol. Bacteriol.* **84**:379.
24. Krantz, J. C., Jr., and C. J. Carr. 1965. Present status of platelet aggregation. *New England J. Med.* **272**:89.

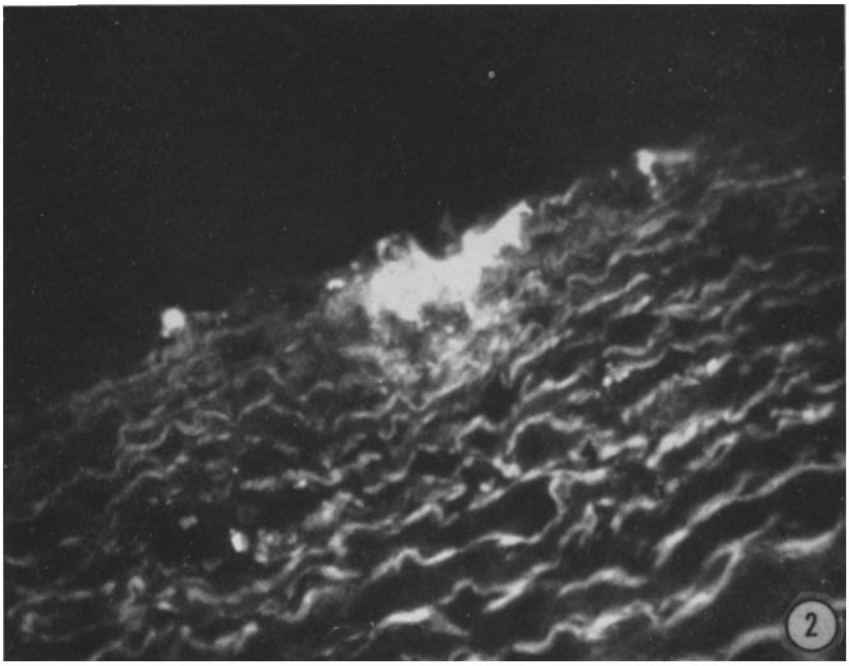
EXPLANATION OF PLATES

PLATE 19

FIG. 1. Photomicrograph of an artery of a rabbit with serum sickness. Intimal proliferation is apparent in the right hand of the figure. The solid arrow denotes the intact intimal elastic lamina that separates diseased intima from underlying media. Purified carbon was injected intravenously 20 min before sacrifice. Broken arrows point to carbon particles lying beneath intimal cells along the luminal surface of the elastic lamina. Other carbon particles are observed between intimal cells. The distribution of carbon is similar to that in venules known to be in a state of increased permeability (see text). Hematoxylin and eosin. × 1300.

FIG. 2. Fluorescent photomicrograph of the ascending aorta taken from a rabbit with serum sickness. The section was treated with fluorescent anti-RGG and reveals a deposit of immune complexes. Early intimal proliferation was observed in hematoxylin-eosin–stained sections. Wavy fluorescence is the blue autofluorescence of elastic fibers in the aortic wall. × 600.

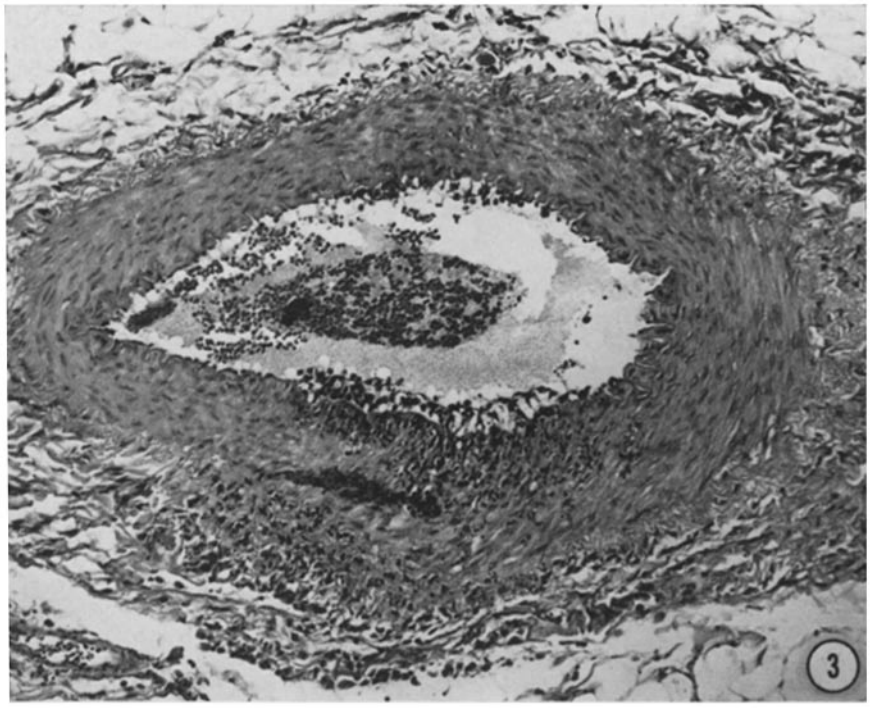




(Kniker and Cochrane: Mechanism of localization of circulating complexes)

PLATE 20

FIG. 3. Photomicrograph of the renal artery at the point of branching from the aorta. An early intimal lesions is seen at the top, with lifting of the endothelial layer, presumably by edema fluid. A more extensive lesion is seen along the bottom of the arterial wall, with lifting of the endothelial cell layer from the elastic lamina and infiltration of leukocytes through all layers of the wall. Hematoxylin and eosin. × 125.



(Kniker and Cochrane: Mechanism of localization of circulating complexes)