Hemostatic- and Fibrinolytic-Affecting Agents in Experimental Vascular Sensitization

By Sheldon G. Cohen, M.D., and Theresa M. Sapp, M.T.

That multiple alterations in hemostasis may occur during subacute hypersensitivity reactions in the rabbit was suggested by demonstrations of physiological and histological changes during experimental procedures. An initial delay in blood coagulation was followed by the subsequent development of intraluminal aggregates of formed elements of the blood and of thrombi in association with inflammatory involvement of vascular and perivascular tissue. Since pathophysiological alterations in the histopathogenesis of cardiovascular-renal sensitization can only be partially explained by histamine and serotonin release, consideration was therefore given to possible participating roles for components of hemostatic and fibrinolytic systems. Evidence for their association with other hypersensitivity mechanisms has been offered and reviewed.

Our experimental approach through an evaluation of pharmacological influences on (1) coagulation abnormalities and (2) related histopathological changes associated with the emergence of hypersensitivity vasculitis is reported here. Agents whose properties include effects on blood coagulation, fibrinolysis and thrombolysis, and blood "sludging" were employed during responsible phases of antigen-antibody union in the rabbit.

Methods

“Serum sickness” in the rabbit, manifested by lesions of pulmonary arteritis, glomerulitis, and myocarditis, induced by the technique of passive reversed subanaphylactic sensitization, served as the basic experimental model. The procedure for this consisted of the administration of 350 mg. of a purified bovine plasma protein through the marginal ear vein. Twenty-four hours later, serum was obtained from blood withdrawn through the central artery of the ear to ascertain the presence of circulating antigen by interfacial serum precipitin tests with corresponding antiserum. The animal was then immediately subjected to challenge with 2 to 3 mg. of corresponding antibody X contained in antiserum prepared from blood taken by cardiac puncture from rabbits that had been immunized with the bovine antigen in Freund's incomplete adjuvant. This amount of antiserum was diluted in physiological saline to a total volume of 50 ml., administered by slow intravenous drip through a marginal ear vein at a rate of approximately 20 drops per minute, and adjusted in each animal to avoid precipitating overt manifestations of anaphylaxis. Twenty-four hours later, each animal was sacrificed by air embolism and multiple tissue sections were taken from the lungs, heart, and kidney and prepared for histological study.

Eighty-eight albino, male rabbits, ranging in weight from 2 to 2.5 Kg., were employed in passive reversed subanaphylactic sensitization procedures. These were divided into 11 experimental groups, each consisting of eight animals. Within each group, four members were sensitized and challenged with a bovine serum albumin (BSA) antigen-antibody system and four with a bovine gamma globulin (BGG) system. One group of eight served as controls, while each remaining group received one particular pharmacological agent in addition to the antigen and antiserum. A list of these, together with respective dosages, established and accepted as pharmacologically effective for this series, and the times of their administration are given in table 1.

Coagulation times were measured by the capillary tube method utilizing blood samples obtained by nicking an ear vein with a hypodermic needle. Prothrombin times in those experiments requiring control of Coumadin sodium dosages were measured by the method of Quick utilizing Simplastin (Warner-Chilcott).

Results

The average coagulation time of antigen-treated control rabbits was 1 minute, 22 seconds. Immediately following antiserum challenge, a rise was noted in all animals.
Table 1

List of Pharmacological Agents with Dosages, Times, and Routes of Administration, Each Given to One Group of Eight Rabbits During Reversed Passive Sensitization Procedures with Bovine Serum Proteins

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Times of administration</th>
<th>Prechallenge</th>
<th>Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin sodium (Panheparin, Abbott; Liquaemin, Organon)</td>
<td>30 mg./Kg.</td>
<td>1 hour</td>
<td>1, 3, and 5 hours</td>
<td></td>
</tr>
<tr>
<td>Coumadin sodium (Warfarin, Endo)</td>
<td>20 mg./Kg.</td>
<td>48 hours</td>
<td>immediate</td>
<td>immediate</td>
</tr>
<tr>
<td>Ethylenediamine tetraacetic acid sodium—EDTA (Endrate disodium, Abbott)</td>
<td>5 mg./Kg.</td>
<td>immediate</td>
<td>immediate</td>
<td></td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>800 mg. in 50 ml aqueous solution</td>
<td>Continuously from 20 minutes pre- through 20 minutes postchallenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protamine sulfate (Lilly)</td>
<td>10 mg./Kg.</td>
<td>immediate</td>
<td>immediate and 30 minutes</td>
<td></td>
</tr>
<tr>
<td>Heradimetrine bromide (Polybrene, Abbott)</td>
<td>5 mg./Kg.</td>
<td>immediate</td>
<td>immediate and 30 minutes</td>
<td></td>
</tr>
<tr>
<td>Fibrinolysin (Actase, Ortho)</td>
<td>5,000 units/Kg.</td>
<td>immediate</td>
<td>immediate and 2 hours</td>
<td></td>
</tr>
<tr>
<td>Streptokinase—SK (Varidase, Lederle)</td>
<td>50,000 units</td>
<td>immediate</td>
<td>immediate and 2 hours</td>
<td></td>
</tr>
<tr>
<td>Trypsin* (Armour)</td>
<td>5 to 10 mg./Kg.</td>
<td>immediate</td>
<td>immediate and 2 hours</td>
<td></td>
</tr>
<tr>
<td>Hydroxychloroquine sulfate (Plaquenil, Winthrop)</td>
<td>8 mg./Kg.</td>
<td>immediate</td>
<td>4 hours</td>
<td></td>
</tr>
</tbody>
</table>

*Trypsin was given intraperitoneally: 5 mg./Kg. in four animals, and 10 mg./Kg. in four. All other drugs were administered intravenously.

Averaging 5 minutes, 54 seconds. A gradual lowering then followed, averaging 2 minutes, 50 seconds within 15 minutes; 2 minutes, 30 seconds within 45 minutes; and returning to normal limits within 105 minutes postchallenge. The efficacy of heparin-neutralization action of protamine and hexadimetrina was indicated by evidence of their own anticoagulant effects causing elevated coagulation times when given in excess dosage. Exogenous heparin administration resulted in complete inability of the blood to clot throughout the two-hour periods between successive dosages. Coumadin sodium resulted in elevation of prothrombin times persisting throughout the entire experimental period. Fibrinolytic-affecting agents failed to influence coagulation times in comparison with the control group. Histopathological evidence for the efficacy of anticoagulant agents was demonstrated by the small focal areas of alveolar and intraseptal hemorrhage found within pulmonary parenchyma. Lesions demonstrated in the lungs, kidneys, and heart were typical of those noted and described in previously reported studies.1-3 Briefly, these included the following:

**Arteritis**

The small pulmonary arteries and arterioles were chiefly involved in segmental fashion. These exhibited varying degrees of perivascular and adventitial infiltrations, chiefly with eosinophilic granular cells and with lesser numbers of polymorphonuclear leukocytes. Often these cells were observed in nodular-granulomatous-like collections about por-
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tions of the affected vessel. Perivascular cellular infiltrates often extended into adjacent pulmonary parenchyma. Eosinophils were also seen infiltrating the intima and through the media. Edema, disruption in the morphological continuity and early proliferation of endothelial cells occurred within the intima. Intraluminal thrombus and cellular aggregate formation were frequently noted. Adventitial and medial edema were evident but rarely were mural necrotic changes demonstrated.

Glomerulitis

Renal lesions consisted of glomerular alterations characterized by leukocytic infiltrations, edema of the basement membrane and endothelial cells, and increased glomerular cellularity. Myocarditis

Myocardial lesions consisted of scattered collections of eosinophilic granular cells within the interstitial tissue. Occasionally, cellular infiltrations within focal areas of the right ventricular wall were associated with other inflammatory changes consisting of interstitial edema and necrosis of myocardial fibers.

Within each of the 11 experimental groups, there were no significant differences noted in hemostasis or histopathological alterations among those rabbits subjected to BSA and those to BGG antigen-antibody systems.

A summary of microscopic pathological findings for the eight experimental groups of sensitized-challenged and treated animals is given in table 2.

Table 2

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Arteritis</th>
<th>Glomerulitis</th>
<th>Myocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Heparin</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Coumadin</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>EDTA sodium</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Protamine</td>
<td>8</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hexadimetrine</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fibrinolysin</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Streptokinase 1 and 5a*</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>8</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

*a = attenuated lesions. This classification applied where only one to two vessels in the multiple lung section were affected and to a very mild and often equivocal degree, with a light, narrow cellular perivascular ringing, and the absence of medial and/or intimal involvement.

vors disruption in the integrity of the vascular endothelial barrier.9 Thus, initial attempts were directed to neutralization of the endogenous mechanism of prolonged clotting following subanaphylactic sensitization. However, heparin antagonists were ineffective in preventing endothelial damage and subsequent penetration and infiltration of inflammatory cells through the walls of the involved vasculature.

The next step was concerned with those agents that might influence the subsequent development of thrombi or micro-emboli. However, the promotion of good anticoagulant effects at the levels of action of exogenous heparin, Coumadin, citrate, and EDTA and the reported "desludging" action of hydroxychloroquine10 were without histopathogenetic influence. It is possible that the strong thrombus-promoting effect of an intravascular cellular aggregate11 and/or embolic antigen-antibody precipitate12 serving as a nidus may partially account for irreversibility.

Discussion

Although evidence for heparin release6 and fibrinolytic activity7-8 in anaphylactic reactions has been documented, their exact roles are ill-defined. It is not understood whether these associations represent cause or effect phenomena. However, there is reason to suspect that both these mechanisms might influence the vascular tissue response to immune processes as represented by the experimental model studied here.

It is known that the action of heparin fa-
The failure of citrate and EDTA to suppress these tissue manifestations of the immune process through possible interference with calcium participation in the in vivo complement system\textsuperscript{13} is also noteworthy.

A role for rabbit platelets in antigen-antibody reactions has been demonstrated.\textsuperscript{14, 15} A study of experimental vascular sensitizing procedures in platelet-deficient animals could therefore be a significant contribution. However, all attempts to induce selective thromboctopenic states in rabbits, including the use of ristocetin described by Gangarosa and co-workers,\textsuperscript{16} were unsuccessful.

Attempted enhancement of fibrinolysis and thrombolysis through the use of the human "plasminogen-activated" serum fraction\textsuperscript{17} was without influence on the ultimate histopathological picture. Only with streptokinase could a definite attenuating effect on the development of lesions of periarteritis and panarteritis be demonstrated. Although SK is also capable of plasminogen-activation in the rabbit,\textsuperscript{18} modification of vascular tissue damage may be attributed to its anti-inflammatory properties.\textsuperscript{19} Similarity of action of trypsin,\textsuperscript{20} however, was not evident in this instance.

**Summary**

The effects of hemostatic- and fibrinolytic-affecting pharmacological agents upon the experimental model of hypersensitivity vasculitis of the rabbit were studied. The technique of passive reversed subanaphylactic sensitization with bovine serum albumin and gamma globulin antigen-antibody systems was utilized in the production of lesions of pulmonary periarteritis and panarteritis, and myocarditis. Experimental procedures included attempts at inducing simultaneous: (a) anticoagulant effects at the levels of action of heparin, coumadin sodium, ethylene-diamine tetra-acetic acid, and sodium citrate; (b) neutralization of endogenous heparin release with protamine and hexadimetrine bromide; (3) enhancement of fibrinolytic systems with plasmin, streptokinase, and trypsin; (d) blood "desludging" with hydroxychloroquine sulfate. Only streptokinase with anti-inflammatory properties exerted a suppressive and attenuating effect on the development of lesions of periarteritis and panarteritis.

**Acknowledgment**

Technical assistance in the conduct of laboratory procedures in this study was rendered by Arlene R. Gallia, student laboratory assistant.

**References**


_Circulation Research, Volume IX, July, 1961_


BOOK REVIEWS


This monograph represents mostly a summary of the author's experience with the fetal electrocardiogram. The technique, the limits of accuracy, the normal fetal electrocardiogram in various fetal presentations and in multiple pregnancy, are discussed in detail. The value of the fetal electrocardiogram in the diagnosis of fetal life, congenital heart disease, and the assessment of the state of fetal heart in labor and near delivery is stressed and illustrated with original records. The introduction contains some remarks on the history of electricity and on bioelectric phenomena in tissue, which do not pertain to the discussed subject. Furthermore, the formulation in the chapter on "Bioelectric Phenomena in Tissue" are not explained well, making the text too technical and not sufficiently clear for M.D.'s. Nevertheless, the book achieves its goal, namely, stressing the value of fetal electrocardiography as a new and valuable diagnostic aid.


This monograph aims to provide a practical guide to the investigation and care of cerebrovascular accidents and is based on clinical experience with 320 patients. The account is in four sections: clinical examination of the patient; a scheme of differential diagnosis to elucidate the cause of a cerebrovascular accident; a clear account of the physiological and pathological problems involved, with particular reference to the role of collateral circulation; and a guide to the evaluation of the patients' chances of rehabilitation.

The authors are to be congratulated on the clarity of their guidance. They show that clinical examination is the basis of investigation of cerebrovascular disease, and though complementary examinations may be sometimes essential, they can never supplant clinical examination. The book is illustrated with clear diagrams, and the reproductions of numerous cerebral arteriograms are of a very high standard.

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