Glomerulonephritis in Pressor Drug-Enhanced Serum Sickness of Rabbits.* (30677)

SIGMUND L. WILENS AND DOLORES HENDERSON
Laboratory Service and Research Division, New York Veterans Administration Hospital, New York

In a recent investigation(1) it was found that repeated intravenous injections of blood pressure elevating drugs following a single large intravenous injection of horse serum in rabbits regularly caused marked enhancement of the lesions of serum sickness and the appearance of manifestations, such as verrucous endocarditis and acute synovitis, not ordinarily seen in this experimental condition. Glomerulonephritis when present was unusually severe but its incidence was not significantly increased.

In the present study pressor agents were given at 2 time intervals only, either shortly after intravenous injection of foreign serum when the concentration of circulating antigen in the blood stream is high or shortly before the development of serum sickness when antibodies appear in increasing concentration. Unexpectedly, it was found that the incidence of glomerulonephritis was greatly influenced by the time of administration of blood pressure elevating drugs. The present report records this finding.

Materials and methods. Thirty-four young adult rabbits weighing from 1800 to 2500 g were injected intravenously with 15 cc/kg body weight of whole pooled horse serum (Difco). Eleven of these were injected intravenously with 0.1 cc levophed (0.2% levartereonal bitartrate, Winthrop) at 2 hours, 0.1 cc aramine (10 mg/ml metaraminal bitartrate, Merck Sharp and Dohme) at 4 hours and 0.1 cc adrenalin (1 mg/ml epinephrine hydrochloride, Parke Davis Co.) at 6 hours after horse serum injection. They were given 3 injections in the same order 2 hours apart on the second day. Eleven other rabbits were given the same sequence of 3 injections at 2-hour intervals on the sixth, seventh and eighth days after horse serum injection. The remaining 12 rabbits were given a total of 12 injections of pressor agents, 3 on the first day and 3 on each of the 6th, 7th and 8th days after horse serum administration. The dosage and time intervals between injections were kept constant.

The days of injection of pressor agents were staggered in this way to determine at what period they might have an effect on the development of glomerular damage. Immediately after injection of horse serum, antigen levels in the circulation are high but insignificant amounts of antibody have formed. During the 6th to 8th days, however, antigen levels drop rapidly and antibody levels rise (2,3).

Three different pressor agents were used because each is believed to cause elevation of blood pressure in a somewhat different manner. It is well known that rabbits rapidly become tolerant to repeated adrenalin injections and will not respond unless the dose is continuously increased. It was hoped that the use of 3 agents would elicit more consistent responses to each one. Blood pressure readings by the ear capsule method were attempted but did not yield reliable results because the central ear arteries usually contracted promptly after injection of the pressor agents.

The doses employed were close to the maximum that could be given safely. In preliminary trial experiments, with pressor agents only larger doses or ones given at shorter intervals caused many immediate deaths. The kidneys of rabbits that died during these preliminary experiments or were sacrificed 11 days after repeated injections were examined histologically and none showed inflammatory changes in the glomeruli. The sequence with which the drugs were given was decided arbitrarily.

Seven additional rabbits were given 15 cc/kg body weight of horse serum and received no pressor agents. All rabbits were sacrificed 11 days after horse serum injection and the

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TABLE I. Incidence of Glomerulonephritis in Rabbits Injected Intravenously with Horse Serum and Pressor Agents.

Pressor agents (days of injection)	No. of animals	Diffuse glomerulonephritis			Focal glomerulo-nephritis	No glomerulo-nephritis
		Marked	Moderate	Mild		
1 & 2	11	7	3	1	0	0
6 - 8	11	0	0	0	6	5
1 & 6-8	12	2	3	0	6	1
none	7	0	0	2	1	4

kidneys were examined by routine histological methods.

Results. All 11 rabbits that were given pressor agents only on the first and second days after horse serum had diffuse glomerulonephritis. In 7 these lesions were very severe and in 3 moderately severe. In all 10 enlargement and increased cellularity of the tufts was much more pronounced than is usually seen when diffuse glomerulonephritis develops in rabbits that are given horse serum but no pressor drugs. The eleventh rabbit also had diffuse inflammatory changes in the glomeruli but these were much milder and only slightly more pronounced than in the 2 control rabbits receiving only horse serum that also developed diffuse glomerulonephritis.

None of the 11 rabbits given pressor agents only on the 6th, 7th and 8th days developed diffuse glomerulonephritis although many of these had severe inflammatory lesions in the heart, lungs and various arteries. In 6, however, limited focal glomerular lesions were noted in glomeruli situated close to the medullary portion of the kidney.

Only 5 of the 12 rabbits given pressor agents both on the first day and on the 6th, 7th and 8th days had diffuse glomerulonephritis of a degree comparable to that observed in 10 of the 11 rabbits given pressor agents only on the first 2 days. In 3 of these the lesion was severe and in 2 moderately severe. Six others had a few focal lesions in centrally placed glomeruli similar to those observed in the group that received pressor agents only on the 6th to 8th days.

Two of the control rabbits had mild diffuse inflammatory glomerular changes. In one other, minimal focal areas of increased cellularity were noted in several glomeruli. The remaining 4 rabbits in this group had normal kidneys. The composite findings are shown in Table I.

Discussion. The development of renal glomerular lesions in rabbit serum sickness is to a large extent unpredictable(2). When present they are usually mild and transient. Hawn and Janeway(4) believed that the different antigens in foreign serum were responsible for the varied distribution of lesions. Dixon, Feldman and Vazquez(5) felt that individual variations in antibody production in different rabbits can influence the types of lesions that develop. McCluskey and Benacerraf(6) produced glomerular inflammatory changes in mice by intravenous administration of soluble antigen-antibody complexes prepared *in vitro*. The exact amounts given were measured precisely yet not all of the injected mice developed lesions. It is possible that factors other than antigen and antibody levels in the blood may be involved.

The findings of diffuse glomerulonephritis in all 11 rabbits given blood pressure elevating agents shortly after horse serum injection is too unusual to be considered accidental. Especially striking is the absence of diffuse glomerular lesions in rabbits given pressor agents on the 6th to 8th days. This difference depended clearly on time of administration and not on number of injections. The rabbits in the first group received only 6 such injections, those in the second group were given 9 injections.

The implication of these findings is that the pressor drugs were effective in enhancing renal damage if given only when antigen levels in the blood were high. When antibody levels are rising, however, they may prevent the development of glomerular damage. When given at both periods the development glomerulonephritis is unpredictable but when present is much more severe than is usually

seen in serum sickness of rabbits not injected with pressor drugs.

Pressor agents may possibly influence the reactions to foreign serum by causing increased penetration of antigenic materials into intact glomeruli, by damaging glomeruli mechanically while antigens are circulating in the blood or by exerting an adjuvant effect on the antigen-antibody reaction. However, the sera of rabbits given pressor agents following a single large intravenous injection of horse serum had remarkably low titers of precipitating anitbodies on the 11th day as tested by the capillary tube method. They were generally in range of 1:160 or 1:320 and were no different than in rabbits given horse serum and no pressor agents.

No satisfactory explanation can be given for the observation reported from the limited data available. Further investigations may disclose the mechanism involved. However, the use of pressor drugs in association with serum sickness in rabbits provides a convenient technique for producing renal glomerular lesions with considerable regularity.

Summary. Repeated intravenous injections of blood pressure elevating drugs given shortly after a single large intravenous dose of horse serum markedly increased the incidence and severity of diffuse glomerulonephritis in rabbits sacrificed on the 11th day. Only minimal glomerular lesions were observed if the pressor agents were given at a later period. The implication of these findings is that these agents augment the development of renal damage in serum sickness when administered at a time when circulating antigen levels in the blood are high and that they may suppress this reaction when given at a time when antigen levels are rapidly falling and antibody levels rising.

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A Transplantation Assay for Mouse Cells Responsive to Antigenic Stimulation by Sheep Erythrocytes.* (30678)

J. C. KENNEDY,† L. SIMINOVITCH, J. E. TILL AND E. A. MCCULLOCH
(Introduced by Raymond C. Parker)
Departments of Medical Biophysics and Medicine, University of Toronto and Ontario Cancer Institute, Toronto, Canada

An important part of the immunologic response of normal mice to injected sheep erythrocytes is the generation of large numbers of hemolysin-producing cells which may be enumerated by means of the plaque-formation technique of Jerne and collaborators(1,2). The role of cell proliferation in the production of plaque-forming cells is indicated by the great increase in number of these cells observed in the spleens of mice following immunization with sheep red cells(2) and by our observation that the ability of mice to respond to the injection of sheep erythrocytes by the production of plaque-forming cells exhibits a sensitivity to gamma rays that is of the same order as that found for mammalian cell proliferation(3). These results have been interpreted to mean that plaque-forming cells have progenitors within the spleens of mice, and that these progenitor cells are capable of responding to antigen by

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† Research Fellow of Nat. Cancer Inst. of Canada.