groups.

Conclusion. 1. These studies indicate that acrolein combines directly with some portion of the protein molecule to produce a conjugate that can be employed in the specific detection of acrolein by qualitative immunochemical methods.

2. The basic molecular pattern of human and rabbit serum protein is not changed when combined with acrolein at 37°C for 4 days. The addition of molecules of acrolein, however, does change the configuration of the protein molecule without loss of species characteristics. This is shown by the failure to form antibodies when rabbits are injected with acroleinized rabbit serum, and also by the response of the rabbits injected with acroleinized human serum by producing antibodies that reacted not only against acroleinized human serum but normal human serum as well. These changes in the molecular configuration were specific for acrolein regardless of the basic molecular pattern used, as evidenced by cross reactions of antiacroleinized human serum with acroleinized serum of other species.

3. Foreign protein conjugates of acrolein are necessary to produce circulating antibodies that can be detected in a test tube.

Studies are now in progress on the detection of acrolein in the blood and tissues of individuals who have suffered burns.

I wish to acknowledge the helpful advice and cooperation of Dr. Alexander S. Wiener and his laboratory personnel.

Received June 7, 1949. P.S.E.B.M., 1949, **72.**

Inhibitory Effect of Nitrogen Mustard (Bis Beta-Chloroethyl Amine) on Lesions of Experimental Serum Hypersensitiveness.* (17319)

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Experimental serum hypersensitiveness has been studied intensively because it may serve as a useful model in promoting understanding of certain diffuse vascular diseases appearing in man. The earlier investigations of experimental hypersensitiveness were concerned primarily with morphologic characteristics of the lesions and their similarity to those of the human diseases. More recent investigations, concerned with pathogenesis, have dealt with the relationship of immunologic and vascular changes.[1-4] The information obtained has led to the general conclusion that the development of humoral antibody and cutaneous hypersensitiveness is apparently related to the development of the vascular lesions. The role of immunological factors has been further investigated by determining the incidence of vascular lesions in serum-injected animals treated with drugs which may 1) prevent antigen-antibody combination or the vascular response to such combination or 2) inhibit antibody formation. Salicylates and dicumarol inhibit antigen-antibody combination *in vitro*,[5,6] but as yet there is no

* This investigation was supported by a grant from the U. S. Public Health Service.

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evidence that this effect occurs *in vivo*. That salicylates suppress the vascular lesions of serum hypersensitiveness has been reported;[4,6,7] the mechanism of this action has not been clarified. Since some of the cellular responses to antigen-antibody combination may be related to histamine release, antihistaminic drugs have been used in attempts to suppress the lesions of experimental serum hypersensitiveness. While such an effect has been reported,[8,9] our own efforts to prevent the vascular lesions with benadryl and neohetramine have been unsuccessful.[10]

Experiments designed to suppress antibody formation were undertaken in an attempt to establish the pathogenetic importance of antibody. Recent evidence of the importance of the lymphatic system in production of antibody suggested the use of lymphocytotoxic agents for this purpose, particularly since Hektoen and Corper[11] had inhibited antibody formation with sulfur mustards and recent experimental studies[12,13] have demonstrated similar effects by the nitrogen mustards. Furthermore, nitrogen mustard has been found capable of reducing immunity in chicken malaria.[14]

In the present study we have found that the nitrogen mustard, bis betachloroethyl amine (HN_2) suppresses antibody formation, the development of cutaneous hypersensitiveness, and the incidence and severity of vascular lesions in rabbits receiving one intravenous injection of 10 ml of horse serum per kg. The results obtained suggest that the appearance of vascular lesions bears a direct quantitative relationship to the antibody response of the experimental animal.

Experimental. Twenty rabbits were studied in this investigation. Ten rabbits were treated with HN_2 and received intravenous serum, while an additional 10 rabbits were given serum alone.

Intravenous injections of 0.5 mg of HN_2/kg were made at 3-4 day intervals for 3 doses prior to intravenous injection of horse serum. Four additional injections at the same intervals during the subsequent 2 week period preceded sacrifice. Leucocytes were enumrated before administration of mustards, after the 5th injection (1 week after serum) and upon completion of the experiment. Qualitative serologic tests were performed by a tube precipitation procedure while quantitative antibody nitrogen (N) determinations were made colorimetrically by the technic of Heidelberger and MacPherson.[15] Skin tests, using 5 dilutions of horse serum, were graded according to the presence of hemorrhage and the area of edema and erythema observed 24 and 48 hours after intradermal injection of 0.2 ml.

Ten animals were observed in each of 2 experiments. In the first experiment antigen persisted in the circulation for the duration of observation in all but one of the animals. The antibody response is graphically represented in Fig. 1, in which the upper 5 curves represent antibody titers of serum controls while the lower 5 are for the HN_2 treated group. It is quite evident that antibody was delayed in appearance and much reduced in amount in the HN_2 treated group. This finding is even more evident in the quantitative studies of the terminal sera of all animals as indicated in Fig. 2.

The figures on each curve represent the rabbit number and, in parentheses, the calculated total antibody N content per ml of undiluted serum. Curves for the HN_2 treated group are flat and the total antibody N content does not exceed .036 mg per ml, while the content of the controls varies from a minimum of .060 to .152 mg per ml.

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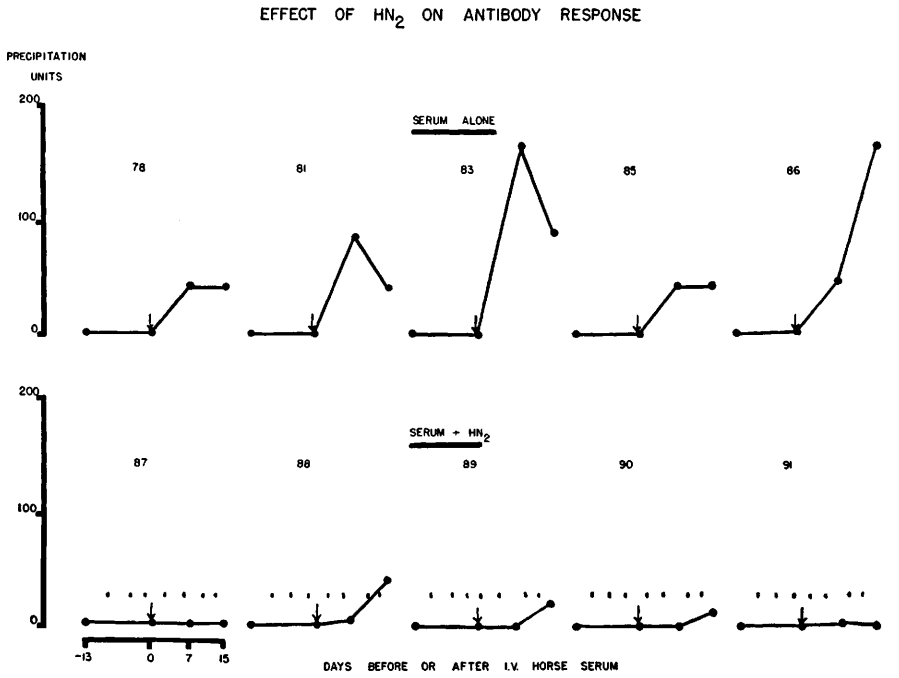

FIG. 1.
Experiment I (see text).

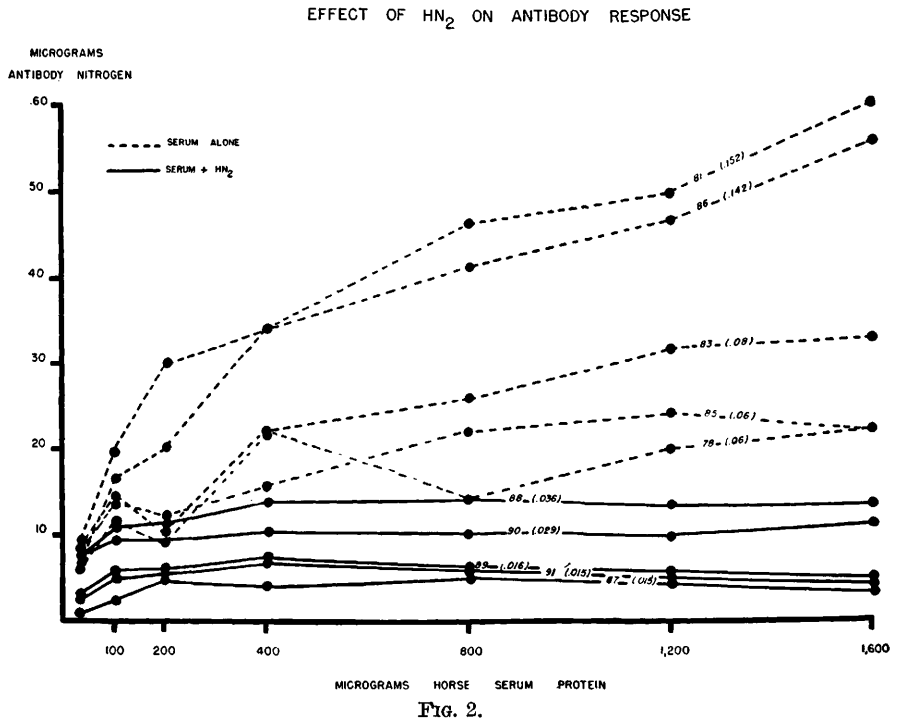

FIG. 2.
Experiment I (see text).

TABLE I.
Exp. I. Effect of HN_2 on Response to I.V. Horse Serum.

Group	Rabbit No.	Antibody† N, mg/ml	W.B.C. Before*	W.B.C. After*	Arthus‡ 1:1§	1:5	1:10	1:20	1:40	Vascular lesions
HN_2	87	.015	12,700	2,700	++	+	+	+	0	0
treated	88	.036	9,050	3,200	++	+	+	+	0	0
	89	.016	10,250	1,850	++	++	+	+	0	0
	90	.029	10,200	1,400	++++	+++	+++	++	++	0
	91	.015	9,350	2,350	+++	++	+	+	0	0
Control	78	.058			++++	++++	+++	+++	++	0
	81	.15+			++++	++++	+++	+++	+++	+
	83	.08			++++	++++	+++	+++	+	+
	85	.06			++++	++++	++	++	0	+
	86	.14+			++++	++++	++	++	++	+

* Completion of HN_2 Rx.
† Terminal serum.
‡ 13-14 days after serum.
§ Dilution of horse serum injected intradermally.

Table I summarizes the significant findings of Experiment I. The quantities of antibody N again illustrate the suppressive effect of HN_2 therapy. A significant leucopenia (of both granulocytes and lymphocytes) developed in all HN_2 treated animals. In general, Arthus reactions in the HN_2 treated group were less intense than those in the control group and therefore paralleled the antibody levels. Skin reactivity did not, however, correlate perfectly with antibody level as indicated by rabbit No. 90, which with 0.029 mg antibody N/ml yielded reactions equal in intensity to No. 86 with .14 mg/ml. None of the HN_2 treated rabbits developed vascular lesions while arterial and/or endocardial lesions of characteristic type were encountered in 4 of the 5 serum control animals. The type of arterial lesion observed is illustrated in Fig. 3.

The results of the quantitative antibody determinations on the terminal sera of Experiment II are summarized in Table II and Fig. 4. Three of the HN_2 treated group failed to develop any antibody despite the fact that antibody N production by the controls was considerably greater than in the first experiment. One of the 2 remaining HN_2 treated rabbits (5) in this experiment, however, produced amounts of antibody greater than 4 of the 5 controls and the other (M-4) more than that produced by HN_2 treated rabbits of the first experiment. Among the HN_2 treated rabbits, 3 and M-1, which had developed evident leucopenia under treatment, developed no antibody, yielded completely negative Arthus reactions and failed to develop vascular lesions. Rabbit M-5 which died 8 days after serum injection developed evident vascular lesions, for which we have no satisfactory explanation. Rabbits 5 and M-4 showed lesser degrees of leucopenia and developed considerably more antibody than was common to the HN_2 treated groups; they also exhibited evident positive Arthus reac-

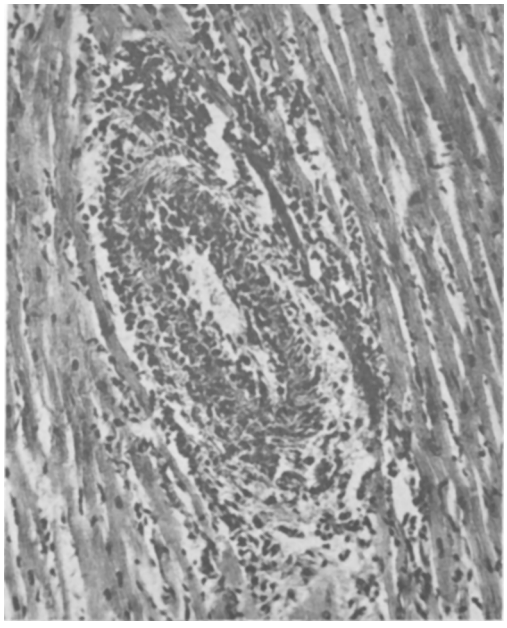

FIG. 3.
A myocardial artery (from a serum control animal) exhibiting marked acute panarteritis, and well-defined medial necrosis.

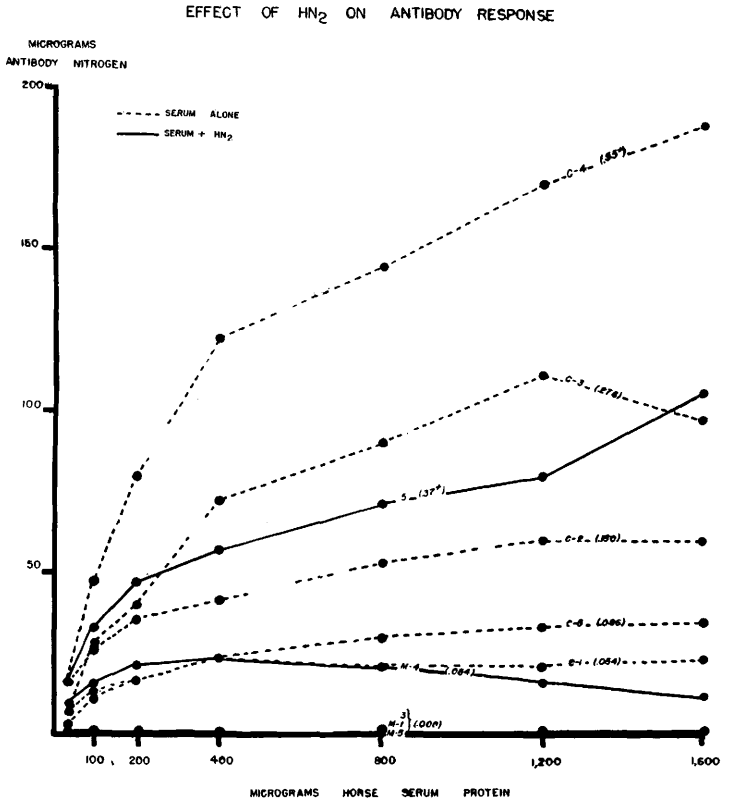

FIG. 4.
Experiment II (see text).

tions and slight vascular lesions. It was our impression that these rabbits, reacting almost exactly as untreated rabbits, had been relatively unresponsive to HN_2.

Control rabbits in this experiment exhibited a fairly wide variation in total antibody N and no evidence of leucopenia at the completion of the experiment. The severity of the vascular lesions developed by 4 of the controls was directly correlated with antibody level, the mildest lesions occurring in rabbit C-5 and the most severe in C-4. The correlation between the intensity of the Arthus reactions and the level of antibody was again imperfect, there being considerable variability in skin responsiveness.

In general, the 2 experiments reveal that the intensity of the vascular lesions correlated fairly directly with the levels of antibody N in the terminal serum samples, whether the animals had been treated with HN_2 or not.

The dividing line between the appearance of vascular lesions and their absence is at approximately .06 mg antibody N/ml. All rabbits with greater amounts of antibody developed vascular lesions, including one HN_2 treated rabbit (5) of experiment II. All animals with less than .05 mg antibody N/ml failed to develop vascular lesions, with the exception of the one to which reference has already been made (M-5).

Discussion. The vascular lesions of experimental serum hypersensitiveness occur in animals treated in a manner known to induce the anaphylactic and Arthus type of hypersensitiveness. The importance of antibody in the latter 2 hypersensitive states has been repeatedly demonstrated[16–19] and it would, therefore, seem logical to conclude *a priori*

16 Opie, E. L., *J. Immunol.*, 1924, **9**, 231.
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18 Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1941, **40**, 127.
19 Kabat, E. A., *Am. J. Med.*, 1947, **3**, 535.

TABLE II.
Exp. II. Effect of HN_2 on Response to I.V. Horse Serum.

Group	Rabbit No.	Antibody N, mg/ml	W.B.C. Before	W.B.C. After	Arthus 1:5	Arthus 1:10	Arthus 1:20	Arthus 1:40	Arthus 1:80	Vascular lesions
HN_2	M-1	0	8,600	1,200	0	0	0	0	0	0
treated	M-4	.054	11,950	3,600	+++	+++	++	+	∓	±
	3	0	11,500	2,400	0	0	0	0	0	0
	5	.37+	9,000	4,500	++	++	+±	+±	+	±
	M-5*	0	9,300	2,900			Not done			+
Control	C-1	.05	—	6,650	++	++	±	±	0	0
	C-2	.15	—	6,700	++	++	+±	0	0	±
	C-3	.276	—	5,750	++++	++++	+++	+++	+	+
	C-4	.55+	—	10,000	+++	++	++	+±	+	+
	C-5	.086	—	6,400	++	+	+	∓	0	±

* Died 8 days after serum I.V.

that the development of vascular lesions by the serum-injected rabbit would also be related to the development of antibody. Nevertheless, several previous studies are not in agreement with this conclusion.[3,7]

In the present experiments, the nitrogen mustard, bis beta-chloroethyl amine induced leucopenia and a parallel suppression of both antibody formation and vascular lesions. While suggestive, these studies, however, are not interpreted as establishing the role of anti-protein antibody in the pathogenesis of the experimental vascular lesions. The nitrogen mustards are powerful inhibitors of a number of enzyme systems and of a variety of tissue metabolic functions; some of the systems affected may possibly play a role in the pathogenesis of the vascular lesions, independent of the antibody present. It would be desirable, in defining the role of antibody, to reproduce the lesions by passive transfer methods, to study the effects of x-rays, and to extend the present observations to include larger groups of animals before attempting to formulate recommendations regarding management of related human diseases.

Summary. Periodic intravenous injections of the nitrogen mustard, bis beta-chloroethyl amine, suppressed both antibody formation and the development of vascular lesions in rabbits injected intravenously with a massive dose of horse serum. The incidence of vascular lesions was directly correlated with the amount of antibody produced, since all rabbits with terminal amounts of antibody in excess of 0.06 mg nitrogen per ml exhibited vascular lesions. Cutaneous hypersensitiveness failed to develop in rabbits which did not produce detectable amounts of antibody.

Received June 8, 1949. P.S.E.B.M., 1949, **72**.