permit the destruction of viral infectivity while at the same time preserving the antigenicity. No reaction conditions below a 24-48-hour period of treatment have been found which would achieve this double objective. However, treatment of the soluble antigens of influenza and mumps with 5% by volume of liquid ethylene oxide at 4°C for 48 hours followed by a 30-minute period at room temperature to allow for ampoule filling and then immediate lyophilization has been found to yield antigens which when reconstituted to their original volume with distilled water are non-infective and have undergone no significant decrease in complement-fixation titer in the process.

At the time of writing, antigen samples treated as described above have been stored in the cold room at 4°C for 3 months without significant loss of titer.

Summary. An investigation has been conducted of the use of liquid ethylene oxide for the preparation of stable non-infective soluble antigens of influenza and mumps. It has been found that treatment of these antigens with 5% by volume of liquid ethylene oxide at 4°C for 48 hours, then for 30 minutes at room temperature, followed by immediate lyophilization, is a satisfactory method to accomplish this purpose.

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Influence of Nitrogen Mustard upon Reactions to Bacterial Endotoxins: Shwartzman Phenomenon and Fever.* (19859)

IVAN L. BENNETT, Jr.† AND LEIGHTON E. CLUFF.‡

From the Department of Medicine, Emory University School of Medicine.

Becker(1) found that the Shwartzman phenomenon could not be elicited in rabbits a few days after injection of nitrogen mustard (hereafter referred to as HN_2) or benzol or after total body x-ray irradiation. This effect has been confirmed by Schlang(2) and by Stetson and Good(3). Although Becker postulated that the effect of HN_2, benzol and x-ray was due to the production of an "anergic" state in cells of the vascular endothelium. Stetson pointed out that all 3 of these forms of treatment are followed in a few days by a profound neutropenia. His experiments showed that the period of Shwartzman non-reactivity after HN_2 or benzol coincides with the time of maximum depression of circulating granulocytes. Furthermore, prevention of leukopenia by aortic compression in HN_2-treated animals or by administration of sulfapyridine in benzol-treated rabbits eliminated the period of Shwartzman non-reactivity. Stetson concluded that the presence of an adequate number of circulating granulocytes is necessary for the elicitation of the Shwartzman reaction and that the inhibitory effect of HN_2 and benzol is due to suppression of these cells. Weis-

* Supported in part by a grant from Baxter Laboratories.

† Present Address: Yale University School of Medicine, New Haven.

‡ Present address: Rockefeller Institute for Medical Research, New York.

berger(4) reported that rabbits given l-cysteine showed less depression of circulating granulocytes after HN_2 than untreated animals.

The present report describes the effect of pretreatment with l-cysteine upon HN_2 inhibition of the Shwartzman phenomenon. Injection of colloidal materials such as thorotrast abolishes natural resistance in rabbits to the Shwartzman reaction as well as resistance acquired in the course of repeated elicitation of the reaction(5,6). The mechanism of this effect is not entirely clear although an effect on the reticulo-endothelial system may play a part. Its influence on the non-reactivity produced by HN_2 was investigated. Bacterial toxins capable of eliciting the Shwartzman phenomenon produce transient leukopenia and fever when injected intravenously in rabbits (7). It was therefore thought that observation of the effect of HN_2 induced leukopenia upon the febrile response to bacterial pyrogens might yield some information about the importance of circulating leukocytes in the febrile reaction.

Materials and methods. White male rabbits of mixed breed weighing 2-3 kg were used. Hair was removed from the abdomen with electric clippers and successive intradermal injections were made in alternate sides of the abdomen beginning in the right upper quadrant. Material used to elicit the Shwartzman reaction was a filtrate of agar washings from a culture of *Serratia marcescens*(8). The intradermal preparatory dose was 0.5 ml of 1/10 dilution and the intravenous dose, given 24 hours later, was 1.0 ml of 1/25 dilution. With this dosage, approximately 90% of normal animals tested showed typical Shwartzman lesions. In some of the experiments on fever, a vaccine containing 1.0 billion typhoid bacilli per ml was used and in others P-25 polysaccharide from *S. marcescens* (obtained from Dr. M. J. Shear) was employed. HN_2 (mechlorethamine hydrochloride, Merck) was freshly prepared in a concentration of 1.0 mg/ml in distilled water. This solution was injected rapidly into a marginal ear vein in a dose of 5.0 mg. l-Cysteine hydrochloride was prepared in 20% solution, neutralized to pH 7.0 by addition of sodium hydroxide. This was injected in a dose of 650 mg/kg intraperitoneally in rabbits, 20 minutes before administration of HN_2. Leukocyte counts were performed a few minutes before injection of HN_2 or cysteine, and daily thereafter. The maximum leukopenic effect was usually apparent from the third to fifth day, following the pattern which has been described frequently (3,4,9). On days when P-25, *S. marcescens* filtrate, or typhoid vaccine was to be given, counts were done before injection. Skin lesions were classified as positive only if there appeared an area of confluent hemorrhage at the prepared site. In experiments on fever, animals were placed in wooden stalls, secured with loose-fitting collars and rectal temperatures were taken every 30 minutes for 6 hours by means of ordinary clinical thermometers. In order to facilitate comparison of temperature responses before and after HN_2, fever curves were charted on 3/16 inch graph paper and, using the temperature at the time of injection as a base line, the area beneath each curve was measured with a Keuffel and Esser compensating planimeter (No. F4236). The vernier reading of the planimeter was taken as the "fever index", an expression of the height and duration of fever. Thorotrast, a 25% suspension of thorium dioxide (Heyden Co.), was injected slowly intravenously in a dose of 9.0 ml 8 hours after the intradermal preparatory injection for the Shwartzman reaction and 16 hours before the intravenous provocative injection.

Certain difficulties should be mentioned. Of 78 rabbits given HN_2 alone, 49 died before experiments could be completed. Of these, 20 died after HN_2 injection only and 29 after one or more injections of one of the bacterial materials employed in the study. Of 67 rabbits given HN_2 after pretreatment with cysteine, 43 died before completion of experiments, 25 after cysteine and HN_2 only and 18 after one or more injections of bacterial toxin. Despite the neutralization of cysteine solutions to pH 7.0, injection of this material into the peritoneal cavity of rabbits was attended by evidence of considerable irritation, at times accompanied by mild circulatory collapse within a few minutes after injection. It was found in later experiments that these

symptoms could be ameliorated by making the intraperitoneal injection while the animals were under light nembutal anesthesia.

Experimental. Effect of l-cysteine upon inhibition of the Shwartzman phenomenon by HN_2. Forty rabbits which had demonstrated positive Shwartzman reactions when tested with *S. marcescens* filtrate were divided into 2 equal groups designated A and B. Animals in group A were given only HN_2 intravenously and those in group B, after pretreatment with l-cysteine, were also given HN_2. Beginning on the first day after injection of mustard, the 2 animals showing the lowest leukocyte counts in each group were tested for capacity to exhibit the Shwartzman phenomenon. After the skin reaction was noted, animals were then allowed to rest until the 9th day after HN_2 injection when intradermal filtrate was again given, followed by intravenous provocative dose on the 10th day. This procedure was continued, 2 animals of those remaining in each group being tested daily and all animals being retested on the 9th and 10th days. Because of deaths before completion of the 10-day experimental period, it was necessary to carry out this procedure in 2 additional groups of animals. Animals given HN_2 alone showed marked leukopenia from 2 to 6 days later and during this period the Shwartzman reaction was negative. All animals showed typical Shwartzman reactions when retested on the 9th and 10th days. In contrast, the leukopenic effect of HN_2 was much less in the cysteine-treated animals of group B and with one exception, an animal whose count fell to 400 on the second day, all animals given cysteine responded to injection of *S. marcescens* filtrate with hemorrhagic necrosis of the Shwartzman type. Table I shows the results obtained in group B.

TABLE I. Effectiveness of Premedication with l-Cysteine in Preventing Inhibition of Shwartzman Reactivity by HN_2. Note that the only negative reaction was in an animal with leukopenia.

Days after HN_2	WBC on day of: Skin inj.	I.V. inj.	Reaction
1, 2	4250	5550	+
	2050	5200	
2, 3	400	1100	—
	1350	8000	
	1300	2050	
3, 4	2400	2750	
	1600	5600	
4, 5	3100	10450	
	2050	24000	
5, 6	4600	6450	
	5600	7950	
6, 7	5000	14960	
	5600	3500	
7, 8	6700	17880	
	6250	5100	
	3100	4150	
	5050	9050	+
	1950	2200	
	15850	15440	
	4300	11750	
	6450	7000	
	8600	3350	
9,10	12840	11120	
	9950	7500	
	10400	1200	
	7300	11600	
	9200	9500	
	4850	9700	
	12000	7100	
	8700	13450	

Failure of thorotrast injection to alter HN_2 inhibition of the Shwartzman reaction. Eight animals that had demonstrated positive Shwartzman reactions with *S. marcescens* filtrate were given 5.0 mg HN_2 intravenously and followed with daily leukocyte counts. On the 4th day, at which time 6 animals showed peripheral counts of less than 1000, all received bacterial filtrate intradermally and, 8 hours later, each one was given thorotrast. Twenty-four hours after skin preparation, leukocyte counts were repeated and *S. marcescens* filtrate was given intravenously. None showed a positive Shwartzman reaction at the prepared skin site, indicating that thorotrast does not overcome the inhibitory action of HN_2. Four of the 8 animals were retested with *S. marcescens* filtrate on the 9th and 10th days after HN_2 injection, at which time their leukocyte counts had risen to normal figures. All reacted with typical skin hemorrhage.

Effect of HN_2 leukopenia upon pyrogen-induced fever. The febrile response of rabbits after injection of 0.05 ml of typhoid vaccine or 0.005 mg of P-25 polysaccharide was measured and, after a rest period of 1 week, animals were given 5 mg of HN_2 intravenously. Daily leukocyte counts were performed in all animals and, when the total peripheral count fell below 300/mm^3, the injection of vaccine or P-25 was repeated and temperatures recorded. Leukopenic animals responded

TABLE II. Fever Indices after Injection of .05 ml Typhoid Vaccine in Rabbits before and after HN_2. Peripheral leukocytes were reduced below 300/mm³ by HN_2.

	Before HN_2	After HN_2
	168	156
	192	176
	243	216
	186	220
	212	197
	161	191
	144	163
	212	176
	149	170
	198	162
Avg	186.5	182.7

to pyrogen injection with brisk fevers which could not be distinguished from those before HN_2 injection. Table II compares the febrile responses of 10 rabbits to typhoid vaccine before and after HN_2 treatment. Similar results were obtained in 6 animals given P-25 polysaccharide. These findings indicate that the production of fever by bacterial materials is not dependent upon the presence of a normal number of leukocytes in the peripheral circulation.

Discussion and summary. The finding of Stetson and Good that the period of inhibition of the Shwartzman phenomenon which follows injection of HN_2 in the rabbit coincides with the time of maximum depression of circulating leukocytes was confirmed. In addition, the prevention of severe leukopenia by cysteine resulted in retention of Shwartzman activity, further evidence for the role of the leukocyte in the Shwartzman reaction. Although injection of colloidal materials abolishes natural or acquired resistance to the Shwartzman phenomenon, thorotrast did not influence the Shwartzman non-reactivity produced by HN_2. The failure of HN_2-induced leukopenia to influence the febrile response of rabbits to pyrogenic bacterial materials indicates that pyrogen fever is not dependent upon the presence of normal numbers of circulating leukocytes.

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Beneficial Effects of Vitamin B_{12} and Aureomycin in Rats Given Large Doses of Cortisone.*† (19860)

JOSEPH MEITES.‡§

From the Department of Physiology and Pharmacology, Michigan State College, East Lansing, Mich.

On corn-soybean meal or corn-powdered milk diets, vit. B_{12} or antibiotics have been shown to be partially or completely effective

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