The Shwartzman Phenomenon. I. Inhibitory Action of Nitrogen Mustard (HN_2)* (18036)

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The widespread use of nitrogen mustards in the treatment of neoplastic disease and the recent report on the therapeutic trial of one of these agents in glomerulonephritis(1), prompted an attempt to learn something of the mechanism of their inhibitory action on the Shwartzman phenomenon as reported by Becker(2). Shwartzman(3) considers the phenomenon of local tissue reactivity to result from a state of increased vulnerability induced at the site of the preparatory injection by the bacterial filtrate. Becker suggested that the nitrogen mustard, HN_2, as well as benzol and x-ray, acted upon vascular endothelium and rendered the endothelial cells anergic, thus preventing them from reacting to the active principle of the bacterial filtrates.

It appeared feasible to examine this suggested action of HN_2 on the endothelium by taking advantage of the rapid fixation of nitrogen mustard in the organism as shown by Karnofsky *et al.*(4). This group reported that occlusion of the circulation to the intestine for 2-5 minutes during and after the injection of nitrogen mustard protected the intestine against the usual mucosal injury. They showed, further, that occlusion of the abdominal aorta protected the femoral marrow against the action of this agent. By means of a special clamp described below, the circulation to an area of skin on the rabbit's abdomen was completely interrupted during and after the intravenous injection of HN_2 for a time considered adequate for its fixation elsewhere in the tissues. Since it had been shown(4) that occlusion of the circulation to the lower limbs during and after the injection of nitrogen mustard protected the femoral marrow and prevented the severe granulocytopenia, it appeared desirable to study the effect of this protection on the inhibitory action of HN_2.

Methods and materials. 1. Rabbits of both sexes and of various strains, weighing 1800 to 3000 g were used. All preparatory injections were made in the R.U.Q. No rabbit in these experiments received more than one injection of nitrogen mustard. 2. *Nitrogen mustard.* Methyl bis (β-chloroethyl) amine HCl† was injected intravenously 4 days before the intravenous injection of bacterial filtrate in all experiments. The agent was freshly prepared in the concentration of 1 mg/ml of saline or distilled water before each injection; the dose was uniformly 2 mg/kg. 3. *Bacterial filtrates.* The filtrates used were meningococcus filtrate 44B‡ and one prepared from the saline washings of a 24-hour growth of *E. coli* on plain agar, after the general method of Shwartzman. The intradermal (preparatory) doses were 0.25 cc of the 44B and 0.5 cc of the *E. coli*. The intravenous doses were 1 cc/kg of a 1:10 dilution of the 44B and 1-2 cc/kg of the undiluted *E. coli*. While neither of the filtrates was titrated here, the 44B(5) was known to be potent in the dilution used and the results on the controls indicate that the coli filtrate was potent as well.

4. *Circulatory occlusion.* To prevent ac-

* The opinions and assertions contained in this article are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department.

1. Chasis, H., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 565.

2. Becker, R. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 247.

3. Shwartzman, G., *Phenomenon of Local Tissue Reactivity*, Hoeber, N.Y., 1937.

4. Karnofsky, D., *et al.*, *Am. J. Path.*, 1948, v24, 275.

† The nitrogen mustard used in the earlier experiments was kindly supplied by Merck & Co. The larger part of the agent was furnished through the courtesy of Dr. H. E. Himwich of the Army Chemical Center.

‡ Kindly furnished by Dr. G. Shwartzman.

5. Shwartzman, G., personal communication.

TABLE I.
Inhibitory Effect of Nitrogen Mustard on the Shwartzman Phenomenon.
(A) Inhibitory effect of the mustard without other treatment.
(B) Effect of clamping the preparatory site.
(C) Effect of aortic occlusion.

Exp.	No. rabbits	HN_2 mg/kg	Duration stasis, min.	Intrav. filtrate	Negative	Positive*	Mild positive†	Dead before 4 hr
(A)	6	2	—	44B	5			1
	6	—	—	"		4	2	
(B)	6	2	10-14‡	*E. coli*	5			1
	4	—	—	"		4		
(C)	6	2	5-6‡	"		4	1	1

* Positive: lesion > 1 cm in diameter at 4-5 hr after intrav. inj.
† Mild positive: lesion definite, but < 1 cm in diameter at 4-5 hrs.
‡ Time indicated is measured from the completion of the HN_2 inj.

cess of HN_2 to a selected zone of skin on the abdomen, a special clamp§ was devised. The clamp consists of a patented locking wrench,‖ to the jaws of which a pair of 3/16 inch round steel rods, bent to a half circle of one-inch radius, was welded. The rods were shod with rubber tubing and applied to the tented relaxed skin and cutaneous maximus muscle of the ether-anesthetized rabbit. After the clamp had been locked into place, one cc of 5% fluorescein was injected intravenously and the abdomen examined under ultraviolet light. The complete absence of fluorescence in the occluded area was considered adequate evidence of circulatory occlusion. HN_2 was then injected and the clamp retained in place 10-14 minutes.

Occlusion of the aorta was done by a method based on a suggestion by Karnofsky, *et al.*(4). The aorta was obstructed by pressing a length of thick-walled rubber tubing on the anterior abdominal wall of the ether-anesthetized rabbit and seating it in the groove formed by the transverse processes of the lumbar vertebrae. The tubing was held in place, on the aorta, by wrapping a clinical blood pressure cuff, folded longitudinally, about the lower abdomen of the rabbit and thereafter inflating the cuff to about 260 mm Hg. After inflation, the HN_2 was injected and the occlusion maintained for varying periods.

§ Designed and constructed by Mr. J. F. Bronson, Master Mechanic, NMRI.

‖ Vise grip manufactured by Petersen Co., Dewitt, Neb.

Results. Results of following experiments are summarized in the table.

1. *Inhibitory effect of HN_2 without other treatment.* In a group of 11 rabbits, 5 of which received HN_2 4 days before the intravenous injection of filtrate, all controls were positive at 4-5 hours and all HN_2 animals were negative.

2. *Effect of excluding HN_2 from the site of the preparatory injection.* Clamping the local area with exclusion of HN_2 from the endothelium of the preparatory site did not prevent the inhibition in any of the 5 rabbits tested with potent filtrates.

3. *Effect of the occlusion of circulation to the lower limbs.* Of the 6 animals injected intravenously with potent filtrates 4 days after the aortic occlusion, one died 3 hours after the intravenous injection, and at this time showed petechial hemorrhages at the site of the preparatory injection. The remaining 5 animals showed mild to moderately strong positive reactions at 4-5 hours.

Discussion. The experiments reported here confirm the inhibitory action of HN_2 on Shwartzman phenomenon, but fail to support the hypothesis that the inhibition is mediated by action on the vascular endothelium, rendering it unable to respond to bacterial filtrates. Clamping the skin in such a manner as to deny HN_2 access to the area which was to be the site of the preparatory injection, failed to prevent the inhibitory effect. It has been further shown that although the HN_2 was permitted to reach the site of the preparatory injection in even greater concen-

tration than normal, the inhibitory effect was markedly reduced when the lower limbs were excluded from the action of the agent. On the basis of these findings, it is postulated that the inhibitory effect of HN_2 on the Shwartzman phenomenon is the result of the systemic rather than a local action of the agent.

Studies in progress on the peripheral blood and on the histologic changes in the bone marrow after the administration of HN_2, suggest that protection of the marrow may be a factor in the decreased inhibition seen after the aortic occlusion.

Summary and conclusions. 1. Inhibition of the Shwartzman phenomenon by HN_2 is confirmed. 2. Protection of the preparatory site from HN_2 fails to influence the inhibition. 3. Protection of the lower limbs from the action of HN_2 by aortic occlusion decreases or prevents the inhibition.

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Effects of X-Rays on Size of Yeast Cells.* (18037)

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Enlargement of cells after exposure to ionizing radiation has been observed in many types of biological material, although it is only infrequently mentioned in the literature (1-4). There are two general explanations for this enlargement. First, it may be due to swelling, which can be defined as enlargement resulting chiefly from selective increase of one of the cell constituents, namely water. Second, it may be due to nonselective and continued formation of protoplasm and other cell constituents with a simultaneous slowing of the division rate.

The purpose of this work has been to determine whether the increased size of x-irradiated yeast cells is selective or nonselective. To obtain evidence bearing on this question, irradiated and nonirradiated cells have been compared with respect to two properties. The first is their specific gravity. This quantity is a fair indication of the amount of nonaqueous materials in the cells, since most of these are heavier than water. If the irradiated cells have a significantly lower specific gravity than the nonirradiated ones, this indicates that enlargement is at least partly due to simple swelling. On the other hand, if there is no significant difference between the specific gravities, this indicates that enlargement is due to nonselective increase in amounts of aqueous and nonaqueous material. The second basis of comparison is total nitrogen content, which may be taken as a rough index of the amount of protoplasm in the cells. If the nitrogen content of the irradiated cells is significantly lower than that of the controls, this indicates selective enlargement whereas substantially identical nitrogen contents are evidence for nonselective enlargement.

The yeast was a single-cell strain of *Saccharomyces cerevisiae* (Schenley No. 52). The cells were grown at 28°C in an aqueous solution containing 2% glucose and 0.3%

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