Shwartzman Phenomenon. III. Modifications of Nitrogen-Mustard Suppression.* (19846)

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The suppressive action of nitrogen mustard on the Shwartzman phenomenon is well established(1-3) and observations on protection of the femoral marrow(2,3) as well as studies of peripheral blood smears and histologic sections(3), indicate that the mechanism of suppression hinges on the granulocytopenia which regularly follows adequate mustard treatment in the rabbit. Despite the high correlation between the presence of the heterophil granulocyte and the occurrence of the phenomenon, the definitive demonstration of the role of the granulocyte is yet to be presented. In its absence it seems of interest to report on some experiments which further strengthen the correlation noted above. The first of these is based on the observation of Weisberger and Heinle that parenteral cysteine confers marked protection against the leucopenia induced by nitrogen mustard(4). These studies were repeated and extended to examine the effect of cysteine protection against the suppressive action of this substance on the Shwartzman phenomenon.

A second approach to the correlation was found in the accidental observation that the hematological response of the rabbit seemed to be altered by relatively mild inflammation. The latter was induced by sterile bacterial filtrates injected intradermally about the time of the mustard injection. This apparent alteration in response and the attendant effect on the Shwartzman phenomenon were studied by means of daily intradermal injections of colitoxin.

Methods and materials. Male, albino rabbits weighing between 1800 and 3000 g were housed in separate cages and given routine laboratory feed and water *ad lib.* (In a few cysteine experiments, sex was not recorded.) *Nitrogen mustard.* Methyl bis β-chloroethyl amine hydrochloride (HN_2) was injected intravenously. Solutions were freshly prepared in the strength of 1 mg/ml in isotonic saline. Two mg/kg of the agent were injected 4 days before the intravenous provocative injection of colitoxin. *Cysteine protection.* Two g of cysteine hydrochloride were dissolved in about 20 ml of distilled water and neutralized to nitrazine paper with 30% NaOH. The solution was injected intravenously 3 to 13 minutes before the injection of HN_2. In some experiments cysteine hydrochloride was given in doses of 750 mg/kg. *Regular skin preparation used in cysteine protection.* The abdomen of the rabbit was lathered and shaved just prior to the intradermal preparatory injection of 0.5 ml of colitoxin (the sterile supernate of a centrifuged 7-day-old brain-heart infusion broth culture of *E. coli*, containing about 0.4% phenol). *Daily intradermal injections.* The abdominal skin of the rabbit was lathered and shaved shortly after the HN_2 injection. Thereafter the animal received an

TABLE I. Modification of Suppressive Action of HN_2 on the Shwartzman Phenomenon.

Group	Modifying treatment	HN_2	Pos*	Neg	Dead‡
1	Cysteine intrav. before HN_2	2 mg/kg	6	4	2
2	Control sol. intrav. before HN_2	"	0	9†	0
3	Daily intradermal inj. of colitoxin	"	3	7	0
4	" " " " saline	"	0	6	3
5	Controls for groups 1-4	—	10	2	3

* Any unequivocal reaction even petechial.

† Controls sol. consisted of equivalent vol of isotonic saline (5 rabbits); equivalent vol of hypertonic 3.35% saline (2); approx. 1 g/kg of Na ascorbate in 5% sol. (2).

‡ Dead before elapse of 4 hr after provocative inj.

* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

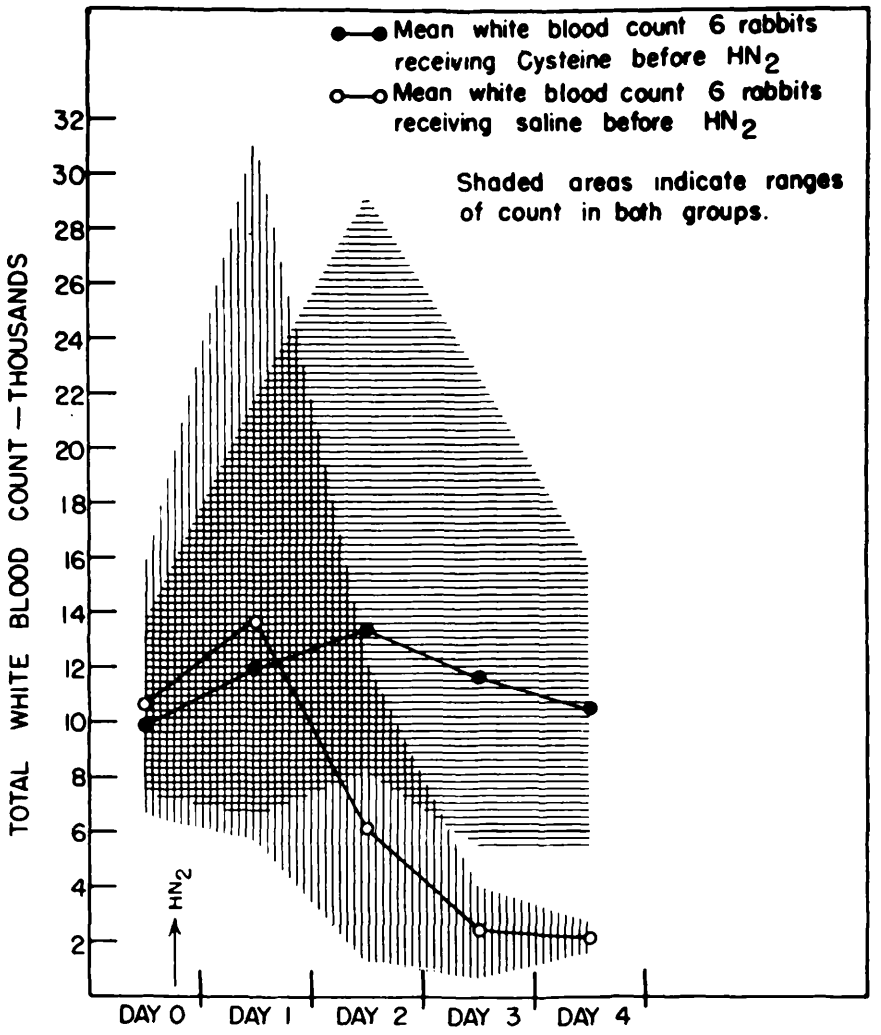

FIG. 1. Protective action of cysteine against HN_2-induced leucopenia.

intradermal injection of 0.5 ml of colitoxin. A similar injection was made in a different area of the abdomen each day, sparing the right upper quadrant for the fourth intradermal injection which served as a routine preparatory injection for the Shwartzman phenomenon, provoked by an intravenous injection of colitoxin on the next day, the fourth after HN_2. Control animals received 0.5 ml sterile isotonic saline in place of colitoxin except for the last, which consisted of a preparatory injection of 0.5 ml colitoxin. *Provocative injections.* One ml/kg of undiluted colitoxin was injected intravenously 4 days after the HN_2 and 24 hours after the preparatory skin injection. In the earlier cysteine experiments agar washings of 24-hour growths of *E. coli* were used in place of colitoxin. *Blood studies.* White blood counts and differential smears were made daily on most HN_2-treated rabbits. Wright's stain was used for the preparation of the differential slides. One hundred cells were counted on each slide.

Results and discussion. The experimental results relating to the Shwartzman phenomenon are summarized in Table I. Fig. 1 indicates that 750-945 mg/kg of cysteine given 3-13 minutes before 2 mg/kg of HN_2, strongly protects against the leucopenic effect of that

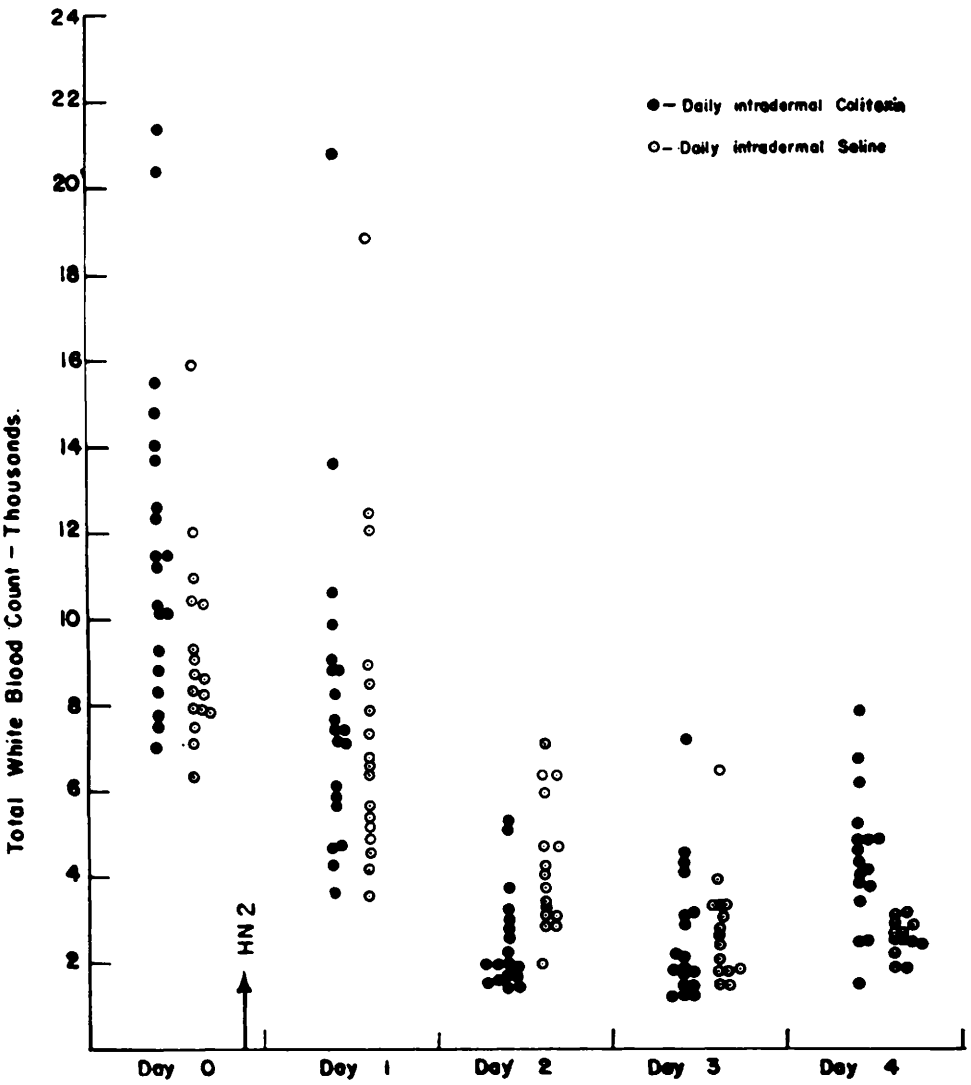

FIG. 2. Modification of mustard-induced leucopenia by inflammation.

agent. Differential smears show that the granulocyte counts remain high, ranging from 1855 to 16800/cmm on the third day, and from 1055 to 8860/cmm on the fourth day. The data in Table I indicate that with the sustained peripheral blood count is seen a concurrent disappearance of the usual suppressive action of the HN_2 on the Shwartzman phenomenon.

Fig. 2 indicates that the daily intradermal injection of 0.5 ml of colitoxin, started after the administration of HN_2, conspicuously alters the hematological response which normally follows 2 mg/kg of HN_2. The regular pattern of leucopenia, following this dose of mustard, is demonstrated in the saline control points in Fig. 2, as has been reported(3,4).

It appears then that the decreased suppressive activity of HN_2 in both the experimental conditions set out above is associated with the presence in the peripheral blood of granulocytes in some quantities at a time when they are ordinarily quite scarce. Further, the slight decrease in the suppressive action of the HN_2 in the second group is associated with a condition in which granulocyte return is also slight.

The last experiment is of interest in another

direction, in that a relatively simple process (inflammation induced by intradermal colitoxin) seems to speed appreciably the return towards a normal blood picture in the rabbit receiving HN_2. The significance of the data remains to be determined, but it appears that inflammation may change the rate of development and the rate of discharge from the marrow of the cell or cells that give rise to the normal heterophils in the peripheral blood. This problem is being further studied and details will be presented elsewhere.

Conclusions. 1. The protective action of cysteine against the leucopenia induced by HN_2 is confirmed and extended to the suppressive action of that agent on the Shwartzman phenomenon. 2. The regular pattern of leucopenia may be considerably altered by repeated small inflammatory reactions induced after the administration of HN_2. The suppressive action of the mustard on the Shwartzman phenomenon appears to decrease when such alteration is produced. 3. Additional support for the correlation between the presence of leukocytes (probably granulocytes) and the production of the Shwartzman phenomenon is supplied by these experiments.

Following completion of this manuscript, it was learned that Dr. Ivan Bennett had also completed a study on the protective action of cysteine against nitrogen mustard suppression of the Shwartzman Phenomenon.

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Failure of "Tween 80" to Alter Fecal Fat Excretion in Bile Fistula Dogs. (19847)

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The wetting agent "Tween 80" (polyoxyethylene sorbitan monooleate) has been reported to correct steatorrhea in a child with celiac disease when doses of 40 mg/g dietary fat were given(1). It has also been pointed out that the fat absorptive defect in sprue and in celiac disease resembles that in patients and in experimental animals deprived of bile, in that in these conditions fecal fat excretion is approximately linearly related to dietary fat intake and unabsorbed fat is largely hydrolyzed(2). If the steatorrheas of celiac disease and of biliary deficiency have a common cause, "Tween 80" would be expected to reduce fecal fat excretion in both conditions. Furthermore, if the solubilizing properties of bile salts are chiefly concerned in the facilitation of fat absorption by bile, "Tween 80" would be expected to reduce fecal fat excretion in bile deficient animals since sodium glyco- and taurocholate have been shown capable of so doing(3). The present study was undertaken to test these two implications.

Methods. External bile fistula dogs were fed diets consisting of either "Pard" alone or "Pard" plus 25 g of lard, containing a total of 15 and 40 g of triglyceride fat/day, respectively. Either 2 or 4 grams of "Tween 80" were added to each of these diets. The mixtures of food and "Tween 80" were given in one daily feeding for 7 days, the last 5 days of which all feces were collected, blended, and analyzed for petroleum ether soluble material extracted in 5 hours(4). Seven dogs received 2 of the test mixtures and one dog was tested once.

Results. As summarized in Table I, neither dose of "Tween 80" reduced fecal fat excretion below the expected limits for untreated bile fistula dogs.

Discussion. Although "Tween 80" has been shown to reduce fecal fat excretion in 4 partially gastrectomized patients with steatorrhea(6) and to reduce the fat concentration in the feces of 3 out of 4 additional gastrectomy patients(7), it does not appear that