A COMPARISON OF THE EFFECTS ON THE SHWARTZMAN PHENOMENON OF LEUKOPENIA PRODUCED BY NITROGEN MUSTARD AND BY WHOLE BODY IRRADIATION*

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In 1928 Shwartzman (1) described "a phenomenon of local tissue reactivity" in which the intradermal injection of certain bacterial products in rabbits, followed after a suitable interval of time by the intravenous injection of similar material, resulted in an intense hemorrhagic-necrotic lesion at the site of the intradermal injection. This reaction was also found to be produced by an intradermal injection of some proteins, polysaccharides, starches, and other substances, followed by an intravenous injection of bacterial products (2). Local infection with some bacteria or vaccinia virus also prepares the skin for hemorrhagic necrosis, which can be elicited by the subsequent intravenous injection of bacterial culture filtrates. The Shwartzman phenomenon thus provides a useful laboratory tool for the study of immune reactions as well as of certain host responses to infectious agents.

In an effort to elucidate the pathological dynamics which lead to hemorrhage at the site of the intradermal injection of toxin, several factors influencing this reaction have been studied. In 1948 Becker (3) reported that nitrogen mustard given intravenously to rabbits several days in advance of the intravenous injection would prevent the local skin hemorrhage of the Shwartzman reaction. This was presumed to be due to the leukopenic effect of the nitrogen mustard. The observation was confirmed and further investigated by Schlang (4) and Stetson and Good (5). Thomas and Good (6) extended these observations to include the effects of nitrogen mustard on the so called generalized Shwartzman reaction, in which two successive intravenous injections of toxin are followed by renal cortical necrosis.

The present study was designed to compare the effect of leukopenia induced by nitrogen mustard and that induced by whole body irradiation on the localized Shwartzman reaction.

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Materials and Methods

Animals.—Male and female New Zealand albino rabbits, weighing 1800 to 2500 gm. were used throughout the experiments.

Bacterial Endotoxins.—The material used to elicit the Shwartzman reaction was a filtrate of agar washings of a strain of *Serratia marcescens* (obtained from Dr. Murray Shear of the National Institute of Health). The method used for growing the cultures of *Serratia marcescens* and preparing the toxin was a modification of the method of Thomas (6), suggested by Dr. Ivan Bennett (7). Dilutions of the toxin were made in physiological saline. Since the potency of successive batches of this toxin may vary, each batch used in these experiments was first assayed biologically on four rabbits to determine the optimal dosage for intradermal and intravenous injection which would result in a maximum percentage of positive reactions and a minimum of lethality from the test itself.

Irradiation Procedure.—Each rabbit was irradiated individually in a screened wooden box. A Picker industrial x-ray unit was used. Each animal received a total whole body irradiation dose of 600 r. This amount of irradiation constitutes a dose capable of resulting in the death of 35 per cent of the exposed animals in 30 days.

The irradiation factors were as follows: 250 kv., 15 ma., aluminum parabolic filter plus 0.50 mm. copper, target skin distance 25 inches, irradiation rate 20.2 r per minute, H.V.L.[1] 2.20 mm. copper. Following irradiation daily total and differential leukocyte counts on each animal were made for 13 days. Beginning 4 days after irradiation each rabbit was given a local skin preparatory injection of 0.50 ml. *Serratia marcescens* toxin followed the next day by an intravenous injection of 1.0 ml. of the same material. Attempts to produce the localized Shwartzman reaction repeatedly were made by giving preparatory intradermal injections on days 4, 6, 8, 10, 12, and intravenous injections on days 5, 7, 9, 11, and 13 respectively after irradiation. Fifty-three rabbits were so studied. Five animals were sacrificed the day after their first intravenous injection and a section of skin through the area of hemorrhagic necrosis was removed for microscopic section. Skin sections were fixed in 10 per cent formalin and then stained with hematoxylin and eosin.

Nitrogen Mustard-Induced Leukopenia.—1.75 mg. nitrogen mustard per kilogram body weight was given intravenously to forty-two rabbits. After preliminary experiments showed that this dose of nitrogen mustard would induce a maximum leukopenia between the 3rd and 5th days after injection, attempts were made to induce successive local Shwartzman reactions beginning with the first intradermal injection on the third postinjection day. Five animals that developed a positive Shwartzman reaction after nitrogen mustard injections were sacrificed and skin sections through the area of hemorrhage were obtained for microscopic section.

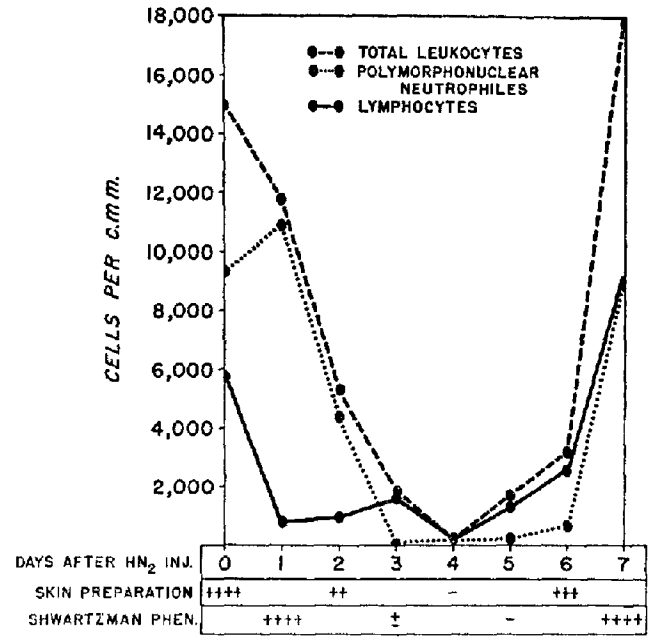
Blood Counts.—White blood counts were done, using 5 per cent acetic acid colored with gentian violet as a diluent. Differential counts were made on smeared slides stained with Wright's method.

EXPERIMENTAL RESULTS

Control Results.—A typically positive localized Shwartzman reaction occurred in 60 to 71 control rabbits (85 per cent). Of those rabbits which failed to give a positive reaction, total WBC and differential counts were done for only two animals. The total WBC counts were 9,000 and 12,000 respectively. The totals of the polymorphonuclear leukocytes and the lymphocytes each exceeded 2000 cells/c.mm. after both the skin preparatory intradermal and provocative intravenous toxin injections.

[1] H.V.L., half value layer.

Correlation of Leukopenia Induced by Nitrogen Mustard and Inhibition of the Shwartzman Reaction.—Preliminary experiments indicated that administration of 1.75 mg. of nitrogen mustard per kilogram of body weight resulted in a leukopenia paralleling that shown in the data of Stetson *et al.* (5) and of



TEXT-FIG. 1. Hematologic changes and susceptibility to Shwartzman phenomenon in a rabbit receiving an intravenous dose of HN_2 amounting to 1.75 mg. per kilogram body weight. The circulating lymphocytes reach a low level during the first 24 hours, while the polymorphonuclear leukocytes are not significantly depressed until the 3rd day.

The degree of skin preparation taking place was estimated on the basis of the amount of erythema, edema, and induration prior to the intravenous challenge, and was roughly graded from − to ++++. The intensity of the Shwartzman phenomenon occurring after the intravenous challenging injection was similarly recorded, − indicating no hemorrhage, ± indicating scattered petechiae, +++ indicating confluent petechiae and purpura, and ++++ indicating typical extensive hemorrhagic necrosis.

Graef *et al.* (8). The earliest hematological change in the peripheral blood is a lymphopenia, which is most marked at about 18 to 20 hours. This is followed by a sharp drop in the absolute number of circulating polymorphonuclear leukocytes, which reaches a minimum level at about 90 hours. The characteristic leukopenia developing in a rabbit given 1.75 mg. of HN_2 per kilo is illustrated in Text-fig. 1.

Table I gives the results of attempts at inducing a localized Shwartzman reaction in 42 rabbits after administration of nitrogen mustard. The leukocyte

counts were taken a the time of the skin preparatory injection of *Serratia marcescens* toxin. It will be seen that when the polymorphonuclear leukocyte count was less than 1200 cells/c.mm. only 18 per cent of the animals had a positive Shwartzman reaction. The percentage was nearly identical when the lymphocyte count was less than 1200 cells/c.mm. Similarly when the total WBC count was less than 2000, only 10 per cent of the animals gave positive

TABLE I

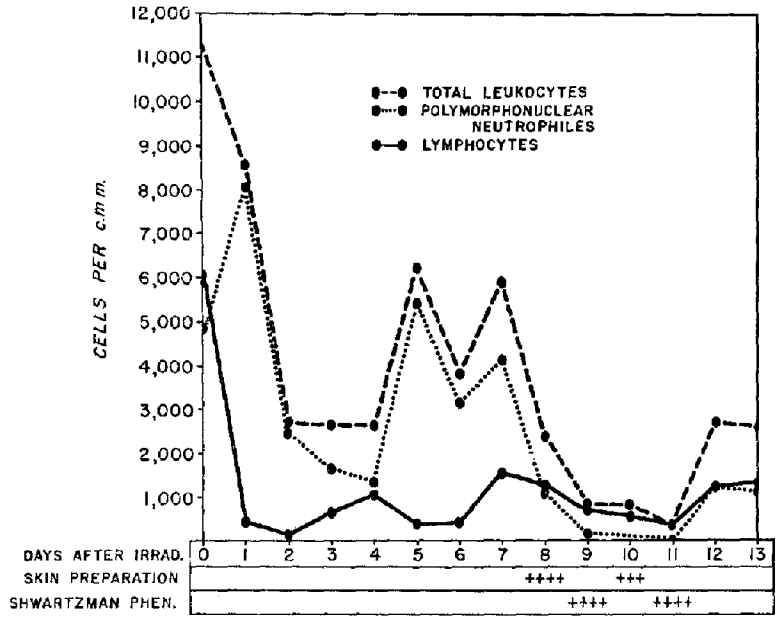
Relationship between the Numbers of Circulating Leukocytes and Susceptibility to the Shwartzman Phenomenon in Rabbits Treated with Nitrogen Mustard

Type of cell counted	Leukocyte count at time of skin preparatory injection cells/c.mm.	Shwartzman reaction	
		No. of animals in group	No. giving positive reaction
Polymorphonuclear leukocytes	Over 1200	8	8 (100 per cent)
	Less than 1200	34	6 (18 " ")
	(400–1200)	5	1 (20 " ")
	(100–400)	5	1 (20 " ")
	(Less than 100)	24	4 (17 " ")
Lymphocytes	Over 1200	26	11 (42 " ")
	Less than 1200	16	3 (19 " ")
	(400–1200)	12	2 (17 " ")
	(100–400)	3	1 (33 " ")
	(Less than 100)	1	0 (0 " ")
Total WBC	Over 2000	22	13 (59 " ")
	Less than 2000	20	2 (10 " ")
	(1000–2000)	7	0 (0 " ")
	(Under 1000)	13	2 (15 " ")

reactions. These findings confirm those of Becker (3) and Stetson and Good (5). However, Stetson concluded, "that inhibition of the Shwartzman phenomenon could be correlated with a reduction in the number of circulating granulocytes, rather than with the number of lymphocytes." Our data suggest no such distinction between the two types of cells.

Relationship between the Number of Circulating Leukocytes and Susceptibility to the Shwartzman Phenomenon in Rabbits Treated with Whole Body Irradiation. —Determination of WBC and differential counts daily after a 600 r whole body irradiation showed that between the 3rd and 7th day both the lympho-

cyte and polymorphonuclear leukocyte counts were usually at their lowest. Consequently, in subsequent observations, counts and injections of toxin were made primarily during this post-irradiation period. Text-fig. 2 illustrates the characteristic leukopenia developed after a 600 r whole body irradiation. Table II gives the results of concurrent blood counts in attempts to induce the localized Shwartzman reaction in 53 rabbits following whole body irradiation with 600 r. It will be seen that there are comparable numbers of animals in each of the groups of animals in this table as in Table I. In this group, how-



TEXT-FIG. 2. Hematologic changes and suseceptibility to Shwartzman reaction in a rabbit following whole body irradiation (600 r). The degrees of skin preparation and localized skin Shwartzman reaction are graded the same as in Text-fig. 1.

ever, the incidence of positive Shwartzman reactions in rabbits with total polymorphonuclear leukocyte counts below 1200 is almost five times that in rabbits whose luekopenia was induced by nitrogen mustard. The same differences between the two groups are found in animals with total lymphocyte counts below 1200 cells/c.mm. In the two groups, it can be noted for animals with total polymorphonuclear leukocyte counts of less than 100 cells/c.mm. there were still 75 per cent positive reactions in the irradiated animals.

Histological Sections through Skin Showing Positive Shwartzman Reaction in a Rabbit Which Previously Received Whole Body Irradiation and a Rabbit Which Had Been Treated with Nitrogen Mustard.—Fig. 1 is a section through the

dermis of a nitrogen mustard–treated rabbit showing part of a positive Shwartzman reaction. Fig. 2 is a higher power magnification of the same section, showing an area of focal inflammation. There are small groups of inflammatory cells, largely polymorphonuclear leukocytes and some degeneration of connective tissue. Figs. 3 and 4 are low power and high power sections through the dermis at a "Shwartzman site" from a rabbit receiving whole body ir-

TABLE II

Relationship between the Numbers of Circulating Leukocytes and Susceptibility to the Shwartzman Phenomenon in Rabbits Treated with Whole Body X-Irradiation

Type of cell counted	Leukocyte count at time of skin preparatory injection cells/c.mm.	Shwartzman reaction	
		No. of animals in group	No. giving positive reaction
Polymorphonuclear leukocytes	Over 1200	5	4 (80 per cent)
	Less than 1200	48	42 (88 " ")
	(400–1200)	18	17 (94 " ")
	(100–400)	14	13 (93 " ")
	(Less than 100)	16	12 (75 " ")
Lymphocytes	Over 1200	12	10 (83 per cent)
	Less than 1200	41	36 (88 " ")
	(400–1200)	30	27 (90 " ")
	(100–400)	9	7 (78 " ")
	(Less than 100)	2	0 (0 " ")
Total WBC	Over 2000	12	10 (83 per cent)
	Less than 2000	41	36 (88 " ")
	(1000–2000)	21	20 (95 " ")
	(Less than 1000)	20	16 (89 " ")

radiation. There is diffuse acute inflammation, characterized by the presence of large numbers of polymorphonuclear leukocytes, hemorrhage and degeneration of connective tissue. Fig. 5 is a section of the subepidermal region of the same section used to make Fig. 1. There is marked lack of subepidermal inflammation and hemorrhage. Fig. 6 is a section through the subepidermal region of the same section used to make Figs. 3 and 4. Marked hemorrhage and inflammation are seen.

DISCUSSION

Stetson and Good (5) cited the following observations in support of their thesis that depression of the total number of circulating polymorphonuclear

leukocytes is the decisive factor in suppression of the localized Shwartzman reaction in rabbits given nitrogen mustard.

In rabbits given 1.75 mg. nitrogen mustard per kilogram of body weight, the hematologic changes day by day were as follows: On the 1st day the total WBC remained over 7000, polymorphonuclear leukocytes were 6000, but the lymphocytes dropped to less than 500. At this time the rabbits were capable of reacting positively either to a skin preparatory or to an intravenous provocative injection of toxin ordinarily capable of producing the typical local hemorrhagic necrosis of the localized Shwartzman reaction. On the 2nd day the lymphocyte count was only slightly higher and the total WBC and polymorphonuclear leukocyte counts were but slightly lower. On this day a somewhat less intense, but nevertheless still significant, Shwartzman reaction could be produced in the rabbits. By the end of the 3rd day there was a drop in the total polymorphonuclear leukocyte count to less than 500 cells/c.mm. The lymphocyte count had increased to nearly 1900 and the total WBC was 2400 cells/c.mm. On this day and for the next 3 days the rabbits which had received injections of nitrogen mustard showed no positive Shwartzman reaction. They had proved resistant both after the preparatory injection into the skin and after the intravenous provocative injection.

The findings described in the present report corroborate that there is suppression of the localized Shwartzman reaction on the 3rd, 4th, 5th, and 6th day after the intravenous injection of nitrogen mustard in a dosage of 1.75 mg. per kilogram of body weight. But one cannot say whether the rabbits' ability to have a typical localized Shwartzman reaction under these circumstances is related to the total leukocyte count or to the total polymorphonuclear leukocyte count. Indeed, the data in Table I indicate a closer correlation between the total WBC count and the percentage of animals showing positive reactions than between the polymorphonuclear leukocytes as such, the lymphocytes, and the positive reactions.

Examination of the data in Table II, on the other hand, reveals that the rabbits in which the total WBC and polymorphonuclear leukocyte counts had been lowered by whole body irradiation gave positive Shwartzman reactions in at least as high percentages as in normal rabbits.

From these data one may conclude (*a*) that the presence of normal numbers of circulating polymorphonuclear leukocytes is not an obligatory prerequisite condition to the occurrence of the localized hemorrhagic necrosis of the Shwartzman phenomenon, and (*b*) that the mechanisms of action in this relation of nitrogen mustard and of whole body irradiation differ. Efforts to elucidate these differences are being made.

In reviewing the data of Becker (3) from which he concluded that the Shwartzman reaction was "completely suppressed in some rabbits and partially suppressed in others by pretreatment with total body x-ray irradiation," the following facts stand out. When he gave 300 r whole body irradiation, he did not observe suppression of the Shwartzman reaction. When he gave 800 r,

he observed "two plus positive reactions" in seven out of thirteen animals. This was a sixfold increase in positive reactions over the rate in animals in his study which received nitrogen mustard.

Study of microscopic sections (Figs. 2 to 6) from nitrogen mustard-treated animals and irradiated animals suggests that some factor other than the presence or absence of polymorphonuclear leukocytes is decisive in determining the susceptibility to the localized Shwartzman reaction.

The possibility must be considered that important differences exist in the vascular effects or reactions of the irradiated and nitrogen mustard–treated animals. A careful search of sections of skin, spleen, and kidney of animals in the irradiated and mustard-treated group showing a positive localized Shwartzman reaction, has revealed some intravascular and subendothelial deposits of fibrinoid material, however, no consistent differences could be perceived. However, examination of the Shwartzman site of the irradiated animal does show a tremendous infiltration of polymorphonuclear leukocytes which is not present in the nitrogen mustard–treated animal. This is all the more remarkable in view of the low numbers of circulating leukocytes in both groups.

SUMMARY AND CONCLUSIONS

The effect of leukopenia on the susceptibility of rabbits to the localized Shwartzman reaction was studied in those receiving whole body irradiation and nitrogen mustard respectively. Of animals receiving nitrogen mustard in which the total polymorphonuclear leukocyte count was less than 100 cells/c.mm. only 17 per cent developed positive local Shwartzman reactions whereas 75 per cent of irradiated animals with total polymorphonuclear leukocyte counts less than 100 cells/c.mm. had positive reactions. A local Shwartzman reaction occurred in 60 of 71 control rabbits (85 per cent). Animals of both the treated groups showed positive Shwartzman reactions at a time when their total polymorphonuclear leukocyte counts were less than 500 cells/c.mm.

From the findings, it is concluded (*a*) that the presence of normal numbers of circulating polymorphonuclear leukocytes is not an obligatory prerequisite condition to the localized hemorrhagic necrosis of the Shwartzman phenomenon, and (*b*) the mechanisms of action of nitrogen mustard and whole body irradiation on body tissues differ in relation to the Shwartzman reaction.

Dr. George Casarett reviewed the microscopic sections and Mr. Leon Schwartz the prepared photomicrographs. Mrs. Florence VanSlyke and Miss Patricia Ewart supervised the irradiation of the animals. Dr. Solomon Michaelson and Miss Ruth Brooks gave valuable advice and technical assistance.

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EXPLANATION OF PLATES

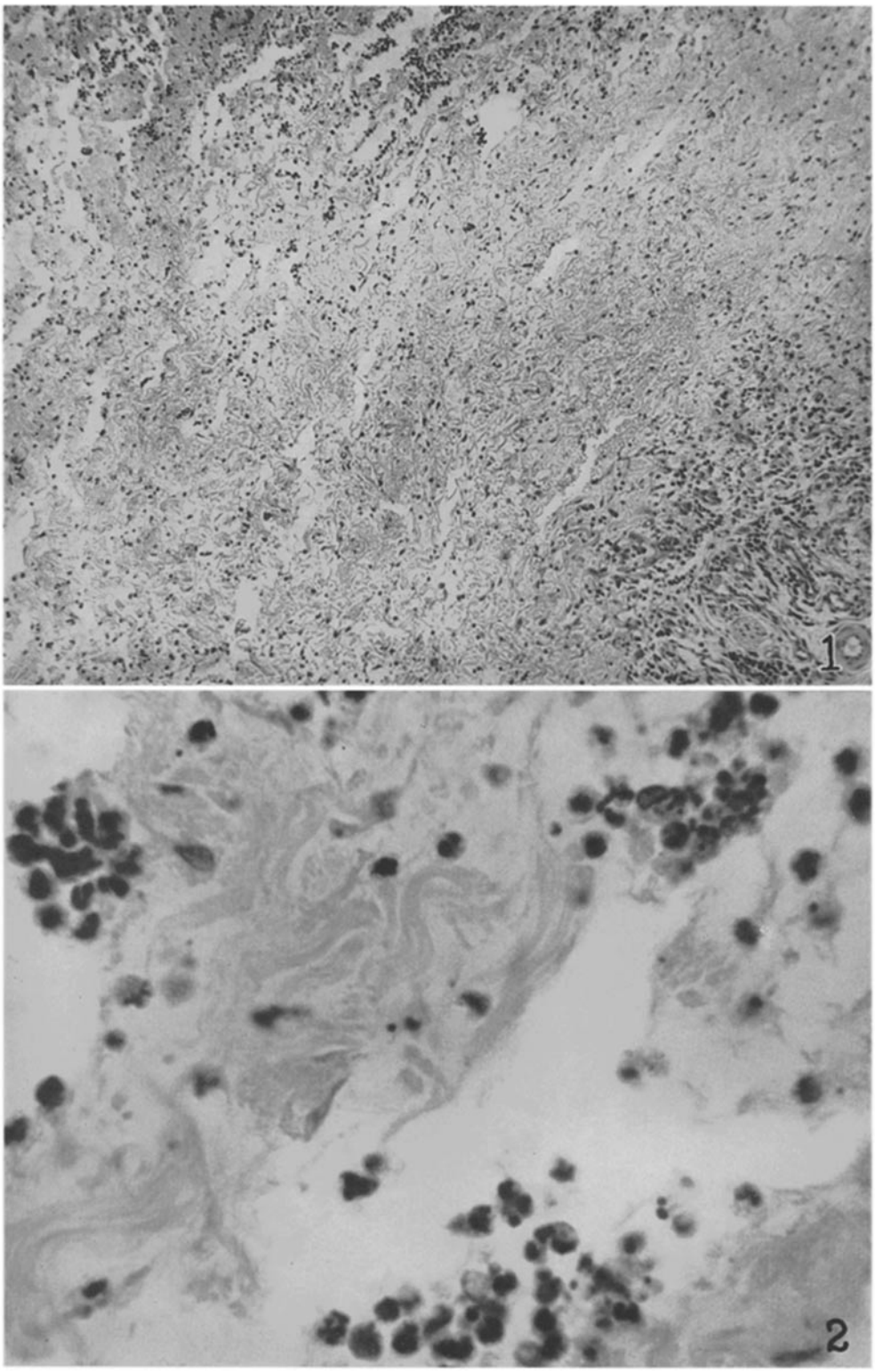
The photomicrographs were made after the skin had been fixed in 10 per cent formalin for several days.

PLATE 33

FIG. 1. Section through dermis at site of positive Shwartzman reaction from a rabbit which had received nitrogen mustard, to show the focal inflammation and edema. Section made on day after intravenous injection of *Serratia marcescens* toxin, when total polymorphonuclear leukocyte count was less than 400 cells/c.mm. Hematoxylin and eosin. × 80.

FIG. 2. Higher power photomicrograph of the same section as Fig. 1, showing region at edge of focal inflammation with small groups of polymorphonuclear leukocytes and some edema of the connective tissue. Hematoxylin and eosin. × 410.

FIG. 3. Section through dermis at site of positive local Shwartzman reaction from a rabbit receiving whole body irradiation. Total polymorphonuclear leukocytes at time were less than 400 cells/c.mm. Moderately cellular inflammation and edema of the connective tissue can be seen. Hematoxylin and eosin. × 80.

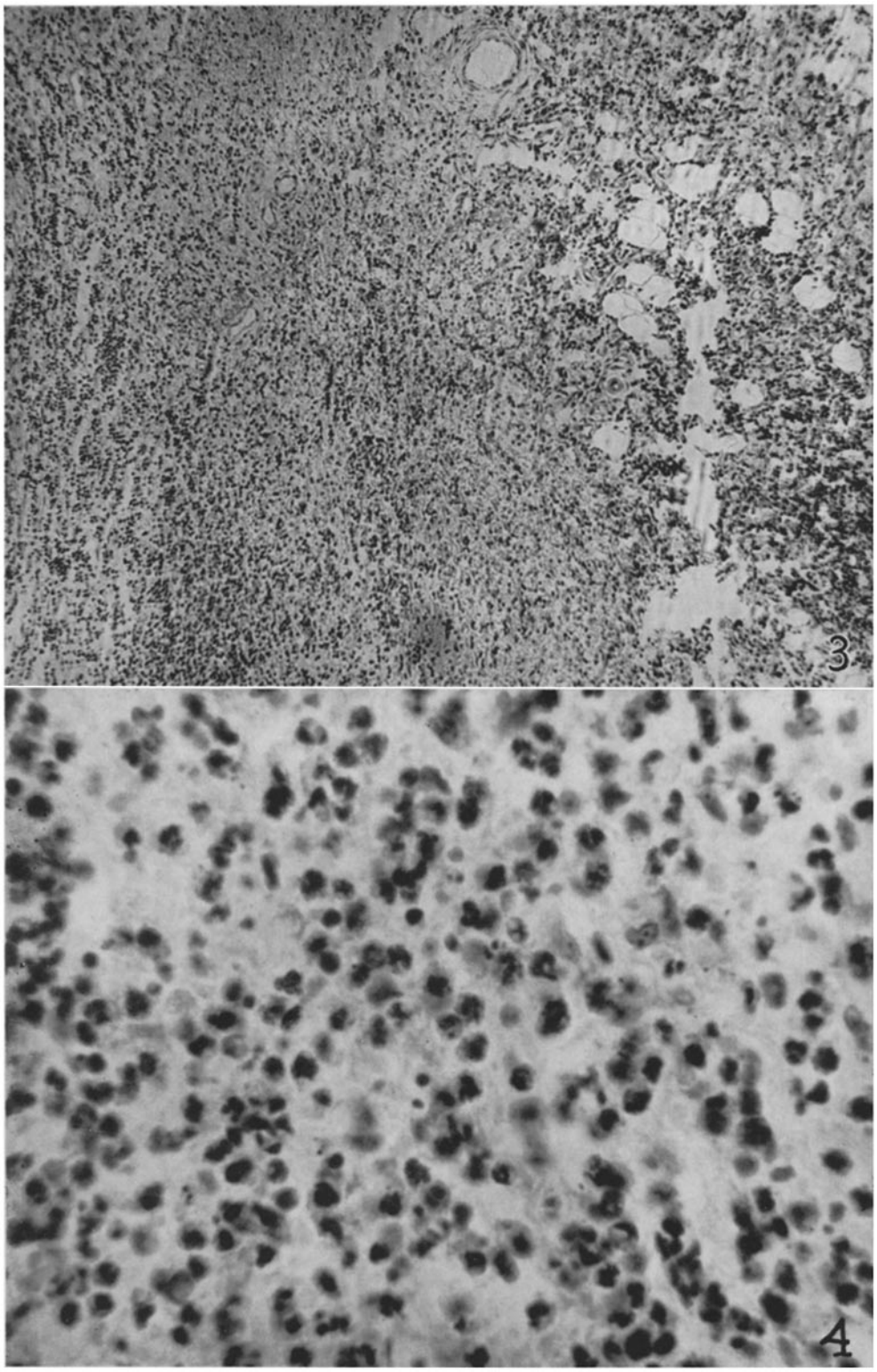


(Johnstone and Howland: Shwartzman phenomenon of leukopenia)

PLATE 34

FIG. 4. Higher magnification of Fig. 3 showing the numerous cells, largely polymorphonuclear leukocytes. Hematoxylin and eosin. × 410.

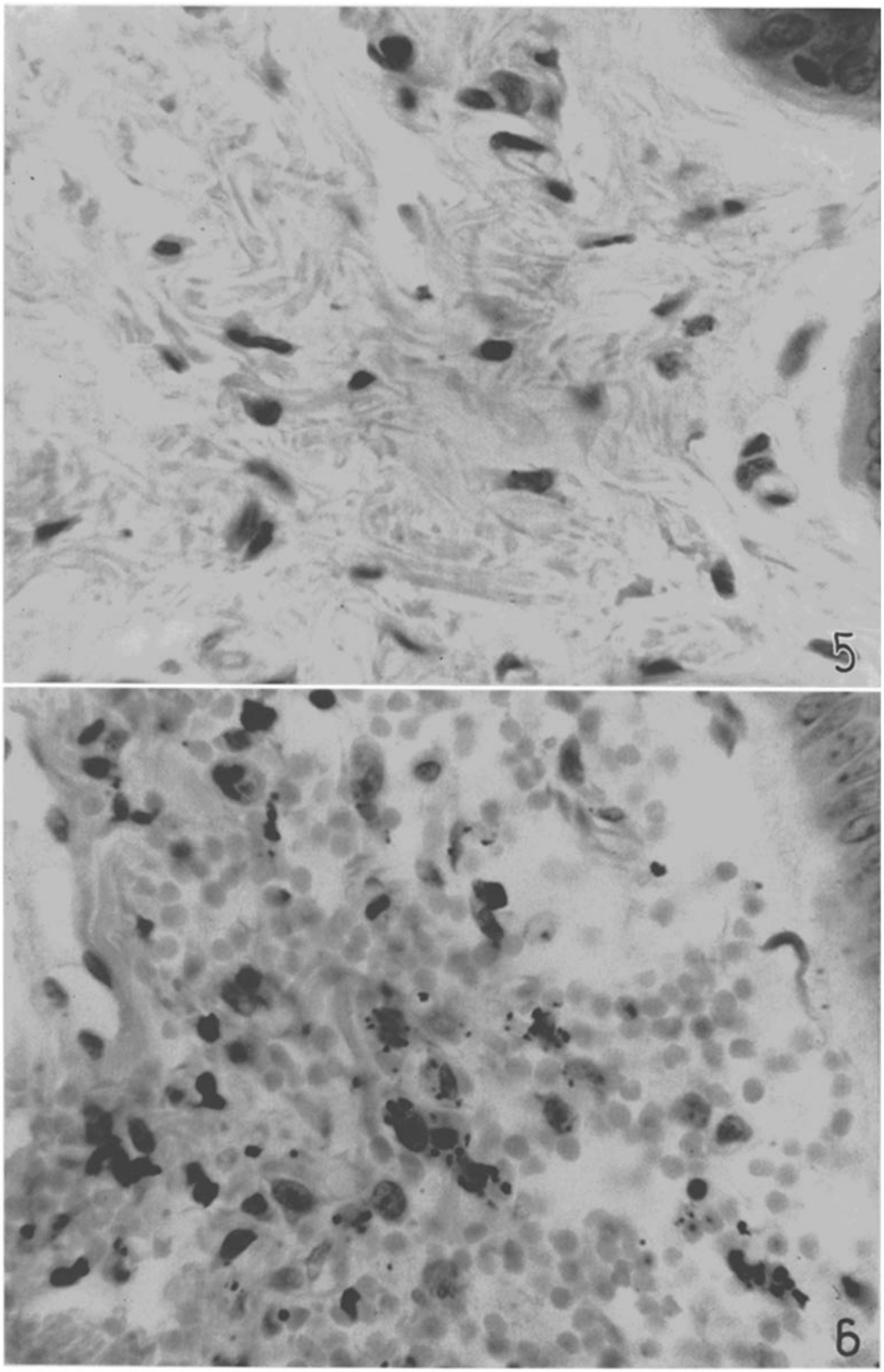
FIG. 5. Section of subepidermal region at "Shwartzman site" from rabbit receiving nitrogen mustard: to illustrate lack of subepidermal inflammation and hemorrhage. Hematoxylin and eosin. × 410.



(Johnstone and Howland: Shwartzman phenomenon of leukopenia)

PLATE 35

FIG. 6. Section of subepidermal region at site of Shwartzman raction, illustrating the hemorrhage and inflammation observed in a rabbit receiving whole body irradiation. Hematoxylin and eosin. × 410.



(Johnstone and Howland: Shwartzman phenomenon of leukopenia)