

Production of Generalized Shwartzman Reaction in Rabbits with Nitrogen Mustard-Induced Granulocytopenia.* (32126)

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(Introduced by E. P. Cronkite)

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Thomas and Good(1,2) in 1952 reported on complete inhibition of the generalized Shwartzman reaction in rabbits made leukopenic by pretreatment with nitrogen mustard. Preparation and triggering of the reaction were made with endotoxin either from *Serratia marcescens* or from meningococci. Hjort, MacGehee and Rapaport(3) confirmed these findings in 1964 with rabbits prepared for the reaction with thorotrast, and with endotoxin as a trigger. When thrombin infusion was used as a trigger, no inhibition was observed. They concluded that leukocytes may take an active part in the conditioning of the intravascular clotting, which is an essential part of the generalized Shwartzman reaction. The following experiments were planned to reproduce this effect and to find out if an inhibition could be abolished by replacement of granulocytes.

Materials and methods. Thirty-eight young, adult rabbits averaging 2.5 kg in weight were used during this study between February-April, 1965. They were fed with a pellet diet and water. One group of 20 rabbits was injected with 5 mg nitrogen mustard by cavacatheter(4) 2-5 days prior to the preparative dose of endotoxin. The circulating granulocyte count (CGC) at the day of the preparative endotoxin injection ranged from 3,950/mm³ to less than 10/mm³ depending upon the time after the injection of the nitrogen mustard. The other group of 18 rabbits were controls. Their CGC at the time of the preparative dose of endotoxin ranged from 1,600/mm³ to 12,800/mm³.

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The generalized Shwartzman reaction was induced by 2 intravenous injections of purified lipopolysaccharide from *E. coli* 0111 cell walls[§](5), the preparative dose being 100 µg, and the triggering dose 1,000 µg 24 hours later. Twenty-four hours after the triggering dose all animals were sacrificed. Kidneys, liver, spleen, heart and lung were studied for intravascular fibrin deposition and hemorrhagic necrosis(6).

For 3 days prior to injection of nitrogen mustard or of lipopolysaccharide, each of the rabbits received one daily injection of 27.5 mg of pyrrolidone-methyl-tetracyclin suspended in oil subcutaneously in order to reduce the mortality from invading enteric organisms during the leukopenic period.

Results. Eleven of the 18 rabbits in the control group (61%) developed the generalized Shwartzman reaction, whereas 8 out of the 20 rabbits in the nitrogen mustard treated group were positive (40%). This difference is statistically not significant (Chi² 0.95). There is some reduction in frequency of positive reactions in those rabbits having granulocyte counts below 1,000/mm³ (Chi² 1.78). P value of 0.05 requires Chi² of 3.84. A far greater number of rabbits would be needed to determine if this difference is significant. From Fig. 1 it is obvious that there is no threshold granulocyte count below which the generalized Shwartzman phenomenon cannot be demonstrated, even at granulocyte counts of 20/mm³ and less the reaction was reproducible.

The analysis of the incidence of positive reactions as related to the time elapsing between nitrogen mustard and the preparative injection of lipopolysaccharide is given in Table I. If there is an interval of 3 days between nitrogen mustard and lipopolysac-

[§] Kindly supplied by Dr. O. Luederitz, Max Planck Institute for Immuno-Biology, Freiburg/Breisgau.

TABLE I. Incidence of Generalized Shwartzman Reaction at Various Time Intervals Between Nitrogen Mustard and the Preparative Injection of Lipopolysaccharide.

Rabbit No.	Time interval days	Granulocyte level per mm ³			Shwartzman reaction
		before nitrogen mustard	before preparative dose of LPS	before provocative	
441	2	2,050	1,150	100	—
443	2	4,800	2,250	2,100	+
444	2	2,750	3,950	700	+
386	3	3,350	50	50	—
388	3	2,650	<10	10	—
389	3	3,000	<10	<10	—
391	3	2,350	200	10	—
416	3	3,050	150	250	+
418	3	3,650	50	100	—
427	3	4,500	50	250	—
402	4	2,200	50	50	—
403	4	2,800	10	<10	+
404	4	2,600	100	1,150	+
405	4	2,900	10	10	—
430	4	6,150	10	50	+
431	4	4,500	<10	10	—
440	4	3,950	50	10	—
439	4	2,150	150	50	—
428	5	3,150	<10	<10	+
429	5	4,250	50	100	+

charide, only 1 out of 7 rabbits gave a positive response as compared to 3 out of 8 at 4 days' interval. One of the 7 rabbits tested on day 3 showed heavy deposition of fibrin in renal glomerular capillaries characteristic of the generalized Shwartzman reaction. Chi² with 10.4 shows a significant inhomogeneity with P near 0.001 between these two groups.

Discussion and summary. The present results resemble those which Johnstone and Howland(7) obtained with nitrogen mustard and with total body irradiation applied before the local Shwartzman reaction. Their conclusion that there was no threshold granulocyte concentration below which the local Shwartzman reaction cannot be produced was opposed to the earlier findings of Stetson and Good(8) and others(3,9). The situation regarding the generalized Shwartzman reaction has not yet been re-examined.

Thomas and Good in all their experiments gave a preparative dose of endotoxin either 3 days after nitrogen mustard, and none of the 15 rabbits developed the reaction, or they gave nitrogen mustard 12 hours or 6 days prior to endotoxin, and with normal granulocyte counts there was a high incidence of generalized Shwartzman reaction.

The experiments, however, show a significant inhibition concomitant with granulocytopenia only 3 days after nitrogen mustard. If the animals were challenged 4 or 5 days after nitrogen mustard with the preparative dose

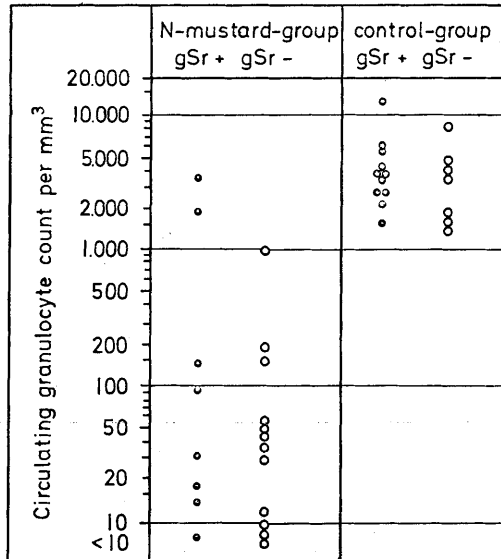


FIG. 1. Distribution of generalized Shwartzman reactions (closed circles) and negative rabbits (open circles) in the nitrogen mustard treated group as compared to the control group. Arrangement following the granulocyte level prior to preparative injection of lipopolysaccharide.

of lipopolysaccharide no inhibitory effect was seen.

It must be concluded from these observations that the inhibitory effect of nitrogen mustard upon the ability of rabbits to react to endotoxins with the peculiar syndrome of generalized Shwartzman reaction, cannot only be due to granulocytopenia. The extent of granulocytopenia 4 and 5 days after nitrogen mustard is the same as on day 3, whereas the incidence of the generalized Shwartzman reaction differs significantly. These findings in part correspond to the unimpaired ability of nitrogen mustard treated leukopenic rabbits to produce an equal fever response to typhoid vaccine as obtained more than a week earlier in the same rabbits(9).

In rabbits made granulocytopenic with nitrogen mustard no granulocyte replacement is necessary to make the animals ready to react with the generalized Shwartzman reaction to two appropriately spaced injections of enterobacterial lipopolysaccharide.

Only if the preparative dose of lipopolysaccharide was given 3 days after nitrogen mustard is there some inhibition.

1. Thomes, L., Good, R. A., J. Exp. Med., 1952, v95, 409.
2. ———, *ibid.*, 1952, v96, 605.
3. Hjort, P., MacGehee, W., Rapaport, S., Proc. 10th Congr. Internat. Soc. Haematol., Stockholm, 1964.
4. Rodriguez-Erdmann, F., Pflueger Arch. Ges. Physiol., 1959, v269, 306.
5. Westphal, O., Luederitz, O., Angew. Chem., 1954, v66, 407.
6. Borle, A., Krecke, H. J., Miller, F., Sitte, H., Immunopathology 1st Internat. Symp. Seelisberg, 1959, p339.
7. Johnstone, D. E., Howland, J. W., J. Exp. Med., 1958, v108, 431.
8. Stetson, C. A., Good, R. A., *ibid.*, 1951, v93, 49.
9. Bennett, I. L., Jr., Cluff, L. E., Proc. Soc. Exp. Biol. & Med., 1952, v81, 304.

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Effect of C₂₁-Methyl Steroids on the *Musculus complexus* and Hatching of The Chick.* (32127)

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The *Musculus complexus*, termed "hatching muscle" by Fisher(1), has been implicated in the hatching process of birds by several writers. The muscle is paired, originates on cervical vertebrae 3 to 5 and on the fascia covering underlying cervical muscles, and inserts broadly on the parietal bones at the back of the skull. In the chick it increases rather abruptly in size and weight,

beginning 3 or 4 days before hatching, then decreases again to a somewhat normal appearance in about the same number of days after hatching. The increase is attributed to an influx of fluid which is perhaps in part lymph, since the lateral portions of the muscle are enclosed by 2 large lymph glands. When it is infiltrated with fluid the *M. complexus* appears very non-muscular, being quite mucoid and yellowish in color, and it is apparently unable to contract appreciably. Smail(2) has suggested a mechanism whereby pipping (the initial breaking of the egg shell) is brought about when the enlarging muscle pushes the back of the head away from the shell of the egg, forcing the beak tip against the opposite side until the pressure there breaks the shell. No muscular movement is necessary, the work by the *M.*

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