deposited since the detonation in 1958 would help us to understand the rate of transfer of material from the upper atmosphere to the surface of the earth, but only a small number of measurements are being made to determine the rate of deposition. However, worldwide deposition of Rh^{102} may be roughly estimated from the Westwood results. Previous fallout data indicate ratios for the Westwood to the total worldwide deposit close to 2 for W^{185} and about 5 for Sr^{90} (*15*). Both nuclides show greater deposits in the Northern than in the Southern Hemisphere while the Rh^{102} may be expected to be more evenly distributed between the hemispheres than these two radionuclides (*1*). If a value of unity for the above ratio is assumed in the case of Rh^{102}, a total global deposit by 1 July 1962 of 0.5 Mc Rh^{102} may be calculated.

Uncertainties in this type of calculation are apparent; yet the result is in reasonable agreement with stratospheric inventories of 0.37 and 0.52 Mc of Rh^{102} calculated to be present in the altitude range 40,000 and 70,000 feet (*16, 5*) in 1960; this material may have been deposited by July 1962.

It may be speculated from our data and the deposition of Rh^{102} in precipitation at Westwood, that the nuclide will remain measurable for a number of years (*17*).

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Nitrogen Mustard: Diminution of Toxicity in Axenic Mice

Abstract. *Axenic, or germ-free, mice are more resistant to the delayed lethal effect of nitrogen mustard than normal mice. The resistance is most striking when mustard is administered at pH 2.0. The finding supports the hypothesis that intestinal bacteria play an important role in the systemic toxicity of nitrogen mustard.*

To evaluate the role of intestinal bacteria in the delayed lethal toxicity of nitrogen mustard [methyl-bis (β-choroethyl) amine-HCl] we have used a strain of axenic mice which has recently become available. We have found these animals to be a useful experimental tool.

Delayed lethal toxicity refers to that form of toxicity resulting in death of animals 4 to 10 days after treatment. It has been found that the *p*H of a solution of nitrogen mustard at the time of injection plays a critical role in this delayed lethal toxicity (*1*). Solutions at *p*H 2.0 have an LD_{50} (lethal dose for 50 percent of the inoculated group) for mice of approximately 6.5 mg/kg whereas solutions freshly made up and injected at *p*H 8.0 have an LD_{50} of 3.25 mg/kg. Nitrogen mustard at *p*H 2.0 retains full anti-tumor activity and, therefore, the therapeutic index of the compound at *p*H 2.0 is superior to that found at *p*H 8.0 (*2*). Why nitrogen mustard at two *p*H values should have two different toxicities has not been explained.

The suppression of the normal intestinal flora of rats and mice with oral or parenteral antibiotics has been accompanied by an increased tolerance for whole-body x-irradiation (*3, 4*). There is a reduction in the lethal effect of x-rays in Swiss mice when these animals are raised under pathogen-free conditions, with fecal flora containing chiefly lactobacilli (*5*). Several groups have reported that axenic or gnotobiotic conditions have been accompanied by a reduction in lethal toxicity in mice (*6*), rats (*7*), and chicks (*8*) exposed to whole-body x-irradiation. These reports stimulated an interest in determining whether axenic mice were automatically protected by their bacteria-free condition against the delayed lethal toxicity of nitrogen mustard and whether normal mice could be similarly protected by the administration of antibiotics.

In preliminary experiments Charles River CD mice were placed on ordinary diets and antibiotics were added to the drinking water. Oxytetracycline was mixed and replaced every 12 hours. Neomycin and polymyxin were replaced once daily. A single injection of a solution of nitrogen mustard was given on the seventh day after antibiotics had been started. The volume varied between 0.6 and 1.0 ml. All mice were observed until death or for a minimum of 30 days after injection. With oxytetracycline, high doses of neomycin, and polymyxin, bacterial content of feces decreased somewhat, and the lethal toxicity of nitrogen mustard diminished (Table 1). However, this protection was rarely statistically significant.

The experiment was repeated several times with axenic mice of the same strain. These mice were raised in plastic isolators; air was drawn through bacterial filters, and the mice were fed sterile water and food. Their growth curves are similar to those of normal animals. They are very sensitive to bacterial infection, and this sensitivity served as a check on their "germ-free" status. Mice were weighed and given single intraperitoneal injections of sterile nitrogen mustard solution (0.02 ml/g of body weight). Several dose

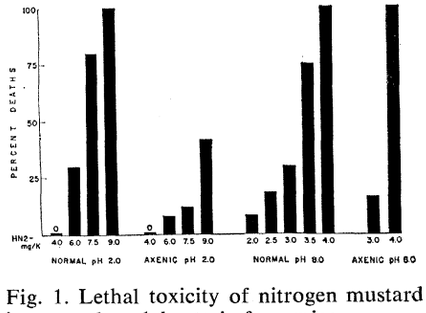

Fig. 1. Lethal toxicity of nitrogen mustard in normal and bacteria-free mice.

Table 1. Effect of oral antibiotics upon lethal toxicity of nitrogen mustard. T, total; L, lived.

Nitrogen mustard		Number of mice									
		Control (water)		Oxytetracycline, 8 g/lit.		Neomycin, 1.0 g/lit.		Neomycin, 0.1 g/lit.		Polymyxin, 0.1 g/lit.	
Dose (mg/kg)	*p*H	T	L	T	L	T	L	T	L	T	L
7.5	1.9	12	4			12	7	10	3	10	5
6.0	1.9	9	2	10	5						
5.0	1.9	9	5	10*	10						
4.0	7.8	15	0	10	0						
3.5	7.8	14	0			9†	5	10	0	10‡	3
3.0	7.8	14	3	10	1						

* χ-square test, $p < 0.05$. † χ-square test, $p < 0.01$. ‡ χ-square test, $p < 0.05$.

levels were used, and nitrogen mustard was injected either at *p*H 2.0 (0.01*N* HCl) or *p*H 8.0 (0.05*M* tris buffer). Groups of 11 to 13 animals were used at each dose level. In one experiment with nitrogen mustard at *p*H 2.0, in a single dose of 7.5 mg/kg some mice were killed daily for histological examination, and others on the 5th day after injection, for studies of the peripheral blood. All axenic mice were observed daily until death or for at least 16 days after injection. This time was shorter than our usual 30-day period because of the isolation problem. At the end of the experiments cultures were taken from all cages, stool cultures were taken on survivors, and intestinal contents were taken from dead mice and from some survivors. We attempted to culture these on blood agar, EMB agar, and thioglycollate broth, but no bacterial contamination was found after incubation for 10 days at 4°, 23°, or 37°C.

The results of these experiments, compared with results of similar studies in normal mice, are given in Fig. 1. At *p*H 2.0 nitrogen mustard was markedly less toxic in axenic than in normal mice. No deaths were observed in either axenic or normal mice given 4.0 mg/kg of nitrogen mustard at *p*H 2.0. At doses of 6.0, 7.5, and 9.0 mg/kg, more normal than axenic mice died. With the χ-square test these differences were statistically significant, with p values less than 0.05 at 6.0 mg/kg, and less than 0.001 at 7.5 and 9.0 mg/kg. Where nitrogen mustard was given at *p*H 8.0 the results were somewhat different. There were fewer deaths in the axenic group than in the normal at a dose of 3.0 mg/kg, but the difference was not statistically significant. At 4.0 mg/kg, *p*H 8.0, all injected mice died in both axenic and normal groups.

Leukocyte counts on axenic mice given 7.5 mg/kg of nitrogen mustard at *p*H 2.0 and sacrificed on the fifth day after treatment showed values of 300 and 400 per cubic millimeter with no lymphocytes noted. These values were similar to the average (150 to 600 per cubic millimeter) found in normal animals given the same dose. (Mice for this part of the study were kept separate from those used in determining lethal toxicity.) Histologic examination of tissues of axenic mice confirmed extensive marrow damage lasting from 2 to 10 days after treatment; rapid recovery followed. Damage to small intestine was also seen, and marked changes in lymph nodes, thymus, and spleen were found. The results of these leukocyte counts and histologic examinations were similar to those in normal mice given the same dose of nitrogen mustard, although examinations in normal mice receiving 7.5 mg/kg were limited after 5 to 6 days because few animals survived.

Resistance of axenic mice to the delayed lethal toxicity of nitrogen mustard appears to be related to their bacteria-free status. Significant protection was seen only when the nitrogen mustard was given at *p*H 2.0. Consistently fewer deaths occurred in the normal mice receiving antibiotics than in controls, but this difference was significant statistically at only one point. The significant results support the hypothesis that intestinal bacteria play a role in the delayed lethal toxicity of nitrogen mustard. One of its effects is damage to the intestinal mucosa, and this was seen in both normal and axenic mice. Other areas of damage by mustard are marrow and formed elements of blood. With such damage the normal host may be invaded by intestinal bacteria which contribute to mortality in mice injected with nitrogen mustard. Such a mechanism has been proposed to explain death of normal animals after whole-body x-irradiation (*4*). In axenic mice the absence of bacteria would, of course, prevent such a complication. Alternatively, in mice injected with nitrogen mustard, toxic bacterial products may contribute to mortality in normal animals, and the absence of such toxic materials in axenic mice would be expected to diminish mortality. Neither of these hypotheses explains the difference in results between animals receiving mustard at *p*H 2.0 and those receiving smaller doses at *p*H 8.0. Furthermore, despite markedly diminished lethal toxicity of nitrogen mustard at *p*H 2.0 in the axenic mice, death does occur at 9.0 mg/kg and above this level, and hematopoietic depression and other tissue damage is seen. Many of the parameters of nitrogen mustard toxicity are seen in axenic mice, and the mechanisms of these forms of toxicity are unexplained.

We did not find bacteria in our axenic mice, but no effort was made to rule out the presence of viruses. It is, therefore, imprecise to consider these mice completely "germ-free" or "gnotobiotic," or to ignore possible participation of a virus, or viruses, in the results.

Accompanying the axenic status of mice, and probably related to it, are diminished levels of certain plasma and serum components. Specifically, γ-globulin is nearly absent in axenic mice, and properdin titers markedly reduced (*9, 10*). Other differences surely exist (*11*).

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