behave in somewhat similar fashion but are not regarded as of murine origin. So far there is no evidence that K-virus belongs to this group, as it does not produce any sign of neuro-muscular changes nor do Coxsackie viruses lead to pulmonary consolidation. Furthermore, Coxsackie antisera from 14 different types in Group A and Group B have failed to neutralize K-virus. A special effort has been made to determine whether K-virus might be related to the Psittacosis-Lymphogranuloma group. There is no relationship on pathologic grounds, as LCL bodies do not appear in K-virus infected tissue. Centrifugation experiments indicate that K-virus is probably smaller than any member of the psittacosis group. Furthermore, complement fixing antibodies for the psittacosis-LGV group have not been found in K-virus immune serum. Antisera to other viruses infecting mice such as PVM, LCM, and NDV fail to neutralize K-virus, which resembles none of them in the type of disease produced. On results of serologic tests, disease pattern, and on the type of endothelial proliferation seen on histologic section of infected lungs(2), it would appear that K-virus is a previously undescribed agent. Our interest has centered largely in its ability to produce acute illness. A question of importance, however, is whether latent infection with K-virus can, in later life of its murine host, lead to mammary-adenocarcinoma.

Longer term experiments essential to answering this question have been kindly set up by Dr. Howard K. Andervont. At the present time, in absence of proof from definitive experiments, the authors do not claim that K-virus is identical with the Bittner Milk Factor. It is hoped that further investigations will throw more light on the pathogenesis, mode of spread, immunology and relationships of K-virus infections in mice.

Summary. 1. Five isolations of a virus which causes a fatal pneumonitis of suckling mice by various routes of inoculation have been made from C_3H mice known to carry the Bittner Milk Agent. 2. Older suckling mice are increasingly resistant to this virus and no apparent illness has been produced by inoculation of adult mice. 3. Specific neutralizing antisera have been produced in rabbits. 4. Due to its unique behavior and failure to show serologic or other relationships to known mouse viruses, K-virus has been regarded as an hitherto undescribed agent. 5. Long term experiments have been set up to determine, if possible, whether K-virus is identical with or related to the Bittner Milk Agent.

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Received December 26, 1952. P.S.E.B.M., 1953, v82.

Occurrence of Generalized Infection of Enteric Origin in Mice Poisoned with Nitrogen Mustard.* (20045)

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The toxic effects of the nitrogen mustards on experimental animals include a number which resemble those produced by ionizing radiation(1-8). No observations seem to have been made, however, on the occurrence in animals poisoned with nitrogen mustard of spontaneous systemic infections such as have been found after exposure to moderate doses of total body x-radiation. For example: Miller, Hammond and Tompkins(9), described a high incidence of bacteremia in mice irradiated with 450 or 600 r during the second

* This investigation was carried out under a contract between the U. S. Atomic Energy Commission and the University of Chicago.

week post-irradiation, the period of greatest mortality.

Materials and methods. Methyl bis β Chloroethyl) amine hydrochloride† (HN-2) was dissolved in sterile distilled water and immediately injected intraperitoneally into Rockland Farms female RAP mice, 9 to 10 weeks old. Twenty minutes after the solution was made, any which remained was discarded and fresh solution was prepared. Preliminary experiments with doses ranging from .06 to .25 mg showed the LD_{90} (20 days) to be 0.2 mg. This dose, (0.2 mg) in 0.5 ml was used throughout the following experiments which included 610 mice, 258 of which were killed for culture. The mice had been kept under observation for one week after their arrival in the laboratory. Following injection they were housed either in groups of 20 in cages measuring 8 x 12 x 9 inches, or in groups of 30 in cages measuring 18 x 12 x 7 inches. The temperature of the mouse room was maintained at 73° to 78°F. The humidity was not controlled. They were fed a diet of mouse pellets made by Derwood Mills, according to a formula prescribed by the Argonne National Laboratory. Their water bottles were changed daily. After treatment with HN-2, 5 to 10 mice were sacrificed each day for culture of heart's blood and spleen. The mice were etherized and autopsied under aseptic conditions. As much blood as possible (0.5 to 1.0 ml) was drawn from the heart into a capillary pipette. One drop was cultured on the surfaces of a blood agar plate and an eosin-methylene blue plate, and the remainder in brain-heart infusion broth. No anaerobic cultures were made because experience had shown that mice of this strain carry very few anaerobes in their intestinal tracts, and because anaerobes were so rarely recovered from the heart's blood of mice subjected to total body irradiation(9). The spleen was removed, cut in several places and dropped into 5 ml of brain-heart infusion broth. The cultures were incubated at 37°C and examined each day. At the first appearance of bacterial growth, the cultures were streaked onto blood agar and EMB plates and the micro-organisms identified by methods previously described (9). All negative cultures were incubated for 72 hours, at the end of which time they were subcultured onto blood agar and EMB plates before they were discarded. *Mortality controls.* To obtain data on the death rate of mice receiving this dose of the drug, 2 groups of mice, totaling 144, were injected with 0.2 mg of HN-2 and observed for twenty days. The deaths occurring each day (plotted as per cent of the number of mice alive at the beginning of that day) are shown in Fig. 1.

† Merck & Co., Rahway, N. J.

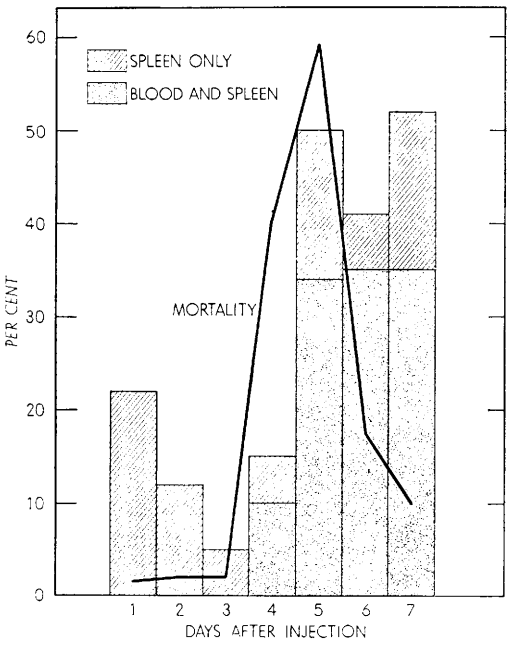

FIG. 1.
Incidence of positive cultures and mortality.

Results of blood and spleen cultures. Table I shows the number of mice from which positive cultures of blood and/or spleen were obtained each day in each series. The figures in parentheses indicate the number with positive spleen and negative blood cultures. Every mouse with a positive blood culture had a positive spleen culture as well. All but nine (13%) of the cultures were pure cultures. It will be noted that in the first three series, 18 of the 37 positive cultures contained *Salmonella enteriditis.* This disproportionately high incidence can be explained by the fact that the mice used at that time were found to contain an unusually large number of Salmonella carriers.

A diagrammatic summary of the results is

TABLE I. Daily Record of Positive Cultures of Blood and/or Spleen.

Date of inj.	No. of mice	Days after IP injection of .2 mg HN-2							
		1	2	3	4	5	6	7	8
4–10–51	130	(2)†/10*	4(3)/10	0/10	0/5		3(1)/10	6(3)/10	1/5
20	100	(2)†/5	0/5	0/10	(1)/10	7(4)/11			
26	50	(1)†/5			0/5	2(1)/8	5/8		
5–29	15					3/6			
1– 9–52	10		0/5						
16	50	0/5	0/5	(1)/5		4(1)/8			
29	70	(2)/5	(1)/5	0/5	0/5		2(1)/5		
31	50				2/5	3/5	3/5		
3– 4	80	(1)/5	0/5	0/5	(1)/5		1/6	3/7	
24	55	(1)/5	0/5	(1)/5	2/5				2(1)/4
Total mice	610	40	40	40	40	38	34	17	9
% positive		22	12	5	15	50	41	52	33

* Denominator = No. of mice killed and cultured. Numerator = No. of mice yielding positive blood and/or spleen cultures. Parentheses = No. of these mice yielding positive spleen cultures only.

† The positive cultures in the first 3 series included 18 containing *Salmonella enteriditis.*

TABLE II. Micro-Organisms Recovered from Heart's Blood and/or Spleen.

Salmonella enteriditis	39%*
E. coli	25
Proteus	20
Paracolobactrum	16
Pseudomonas aeruginosa	4
C. pseudotuberculosis murium	4
α Streptococci	1

* % of all strains recovered. Positive cultures of both heart's blood and spleen from a single mouse were counted as one strain.

presented in Fig. 1 which shows that a high incidence of bacteremia (33-35%) occurred during the 5th, 6th and 7th days. During this period, a number of additional mice (6-17%) were found to have bacteria in their spleens. A fairly high percentage of the mice killed in the first 2 days were also found to have infected spleens.

Bacteria obtained from blood and spleen cultures. A classification of the bacteria recovered from cultures of heart's blood and spleen is presented in Table II. All except *Corynebacterium pseudotuberculosis murium* have been found to be normal inhabitants of the intestinal tract of this strain of mice. As already mentioned, most of the cultures of Salmonella were obtained from the mice in the first 3 series which contained a high incidence of carriers of this micro-organism.

Discussion. These experiments demonstrate the development of generalized systemic infection in a considerable number of mice which had been poisoned with nitrogen mustard and killed for culture of heart's blood and spleen. It should be pointed out that mice which died spontaneously were discarded without culture. The maximum incidence of bacteremia occurred on the 5th, 6th and 7th days, much earlier than in mice subjected to total body irradiation(9). This period did not coincide exactly with the period of greatest mortality as it did in the irradiated mice. However, the data for the nitrogen mustard mortality curves were not obtained from mice which were cultured, as in the case of irradiated mice, but were obtained from two groups which were given nitrogen mustard solely for the purpose of determining the daily death rate. A much larger proportion of mice poisoned with nitrogen mustard was found to have positive spleen (and negative blood) cultures than was the case in the irradiated mice, particularly during the first two days.

As in the case of the irradiated mice, the bacteria recovered from heart's blood and spleen were all members of the normal intestinal flora with the possible exception of *Corynebacterium pseudotuberculosis murium* which was not encountered in any cultures of the intestinal content of these mice.

It must be emphasized that the data herein reported are based on mice poisoned with an LD_{90} of nitrogen mustard. No therapeutic experiments were made to determine whether it would be possible to control the development of bacteremia by administering suitable anti-

biotics, and if so, whether such treatment would significantly reduce the mortality.

Korol(10) obtained equivocal results in one experiment in which mice poisoned with nitrogen mustard were treated with streptomycin, but the treatment was not continued beyond the 4th day. In one unpublished experiment Korol(11) found a slight reduction in mortality in mice treated for 12 days with 7000 μg of streptomycin a day.

While the development of bacteremia may hasten the death of animals poisoned with nitrogen mustard, it seems doubtful that its importance as the cause of death is as great as it appears to be after moderate doses of total body x-radiation.

Summary. 1. Nine to 10 weeks old female mice, averaging 27 g in weight were injected intraperitoneally with 0.2 mg (approximately 7 mg per kilo = LD_{90}) of nitrogen mustard (Methyl bis (β-chloroethyl) hydrochloride). Cultures of heart's blood and spleen of mice sacrificed for that purpose were made at daily intervals. 2. Bacteremia was found to be present in over $\frac{1}{3}$ of the mice on the 5th, 6th and 7th days. A number of additional mice on these days (6-17%) showed positive cultures of spleen only. The species of bacteria recovered from blood and/or spleen were all members of the normal intestinal flora, with the exception of *Corynebacterium pseudotuberculosis murium*. 3. Generalized infection of enteric origin seems to play a less important role as a cause of death in mice poisoned with nitrogen mustard than in mice subjected to moderate doses of total body x-radiation.

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Received January 3, 1953. P.S.E.B.M., 1953, v82.

Growth Stimulation of *Lactobacillus gayoni* by N-D-Glucosylglycine.* (20046)

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(Introduced by R. Wulzen.)
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Early studies on the nutrition of *Lactobacillus gayoni*(1) provided evidence for a "gayoni factor", inasmuch as this organism could not be cultured on existing synthetic media unless supplemented by liver or yeast fractions. Subsequent experiments(2,3) showed that yeast nucleotides and unusually high levels of pteroylglutamic acid were required in addition to the active principle of liver or yeast. Recently, it has been found possible to replace in part the nutrients in liver or yeast by glutamine or asparagine, although maximum growth could not be obtained(4).

The growth of *L. gayoni* also has been found to be dependent upon heating the complete

* This work was supported by a grant from Swift and Co., published with the approval of the Monographs Publications Committee, Paper No. 214, School of Science, Department of Chemistry. A preliminary report was presented before the American Chemical Society, Atlantic City, Sept. 1952.