who have also encountered resistance of mammalian mucoproteins to proteolytic enzymes.

Whether the polysaccharides in these inactive complexes would, or would not, exhibit endotoxic behavior when success is achieved in liberating them from combination is not known. This is being investigated.

*Summary.* In the attempted isolation from mammalian tissues of polysaccharides with endotoxic properties, only materials devoid of such activity were obtained under conditions in which exclusion of bacteria was demonstrated.

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## Effect of Nitrogen Mustard in Rabbits Following Exposure to X-Irradiation. (26135)

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Bone marrow depression represents one of the problems encountered in the use of chemotherapeutic agents in treatment of malignant disease. At this Institution we have treated several patients with large doses of nitrogen mustard (0.95-1.8 mg/kg) for Hodgkin's disease(1). Results of such therapy suggest that patients who have received prior x-irradiation have a more severe and prolonged bone marrow depression than those not previously exposed. Because of the small number of patients and variation in the clinical setting, definite conclusions concerning this point have been difficult to reach. This report deals with the results of a comparable study done in experimental animals in an attempt to determine whether or not there is a difference in response between irradiated and non-irradiated animals following administration of nitrogen mustard.

*Methods.* Ten adult, female rabbits used in the study were divided into 2 groups of 5 each. One group was given x-irradiation while the other served as control. Two courses of x-ray were given, 250 r whole body irradiation over a 10-minute period. Following each course of exposure, a suitable period of 5-6 weeks was allowed for recovery of bone marrow as manifested by return of normal peripheral blood counts. Five weeks following the last dose of x-ray, both groups of animals were given 1.5 mg/kg nitrogen mustard intravenously. Peripheral blood counts, including red blood cell count, hemoglobin, hematocrit, white blood cell count and platelet counts (direct), were performed prior to start of the study, at weekly intervals during experiment and one week following administration of nitrogen mustard. All blood specimens were obtained by cardiac puncture without difficulty. Bone marrow aspiration was performed prior to x-ray exposure and one week following nitrogen mustard administration in the surviving animals.

*Results.* In the control group one animal sustained a cutaneous injury which became

TABLE I. Hematologic Response of Rabbits Following Exposure to X-Irradiation and Administration of Nitrogen Mustard.

| | | Control group | | | | Irradiated group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 |
| Red cell count × $10^3$ | a | 4.2 | 5.2 | 4.4 | 4.9 | 5.4 | 4.0 | 4.9 | 3.5 | 4.6 |
| | b | 3.4 | 4.2 | 4.2 | 4.6 | 4.4 | 4.8 | 5.2 | 3.4 | 4.4 |
| | c | 3.9 | 4.7 | 4.4 | 4.6 | 4.5 | 4.6 | 4.7 | 4.0 | 4.6 |
| | d | 3.7 | 4.4 | 4.3 | 4.9 | 4.3 | 3.3 | — | 3.6 | — |
| Hematocrit | a | 26.5 | 32.5 | 28.0 | 30.5 | 33.0 | 28.0 | 32.0 | 26.0 | 33.5 |
| | b | 34.5 | 37.5 | 35.0 | 40.0 | 40.0 | 34.5 | 37.0 | 27.5 | 32.0 |
| | c | 34.0 | 39.0 | 35.0 | 37.0 | 36.0 | 34.5 | 36.5 | 28.5 | 35.0 |
| | d | 33.0 | 34.0 | 33.0 | 29.0 | 34.5 | 30.5 | — | 28.5 | — |
| Hemoglobin, g % | a | 9.1 | 10.6 | 9.4 | 11.5 | 11.9 | 10.3 | 10.5 | 9.6 | 11.9 |
| | b | 10.5 | 11.4 | 11.0 | 12.5 | 12.1 | 10.1 | 11.1 | 8.8 | 9.6 |
| | c | 10.1 | 12.1 | 10.5 | 11.1 | 11.3 | 10.0 | 11.0 | 8.3 | 10.0 |
| | d | 10.3 | 11.2 | 10.1 | 9.7 | 11.4 | 10.1 | — | 8.3 | — |
| White cell count × $10^3$ | a | 10.1 | 9.4 | 9.5 | 9.0 | 8.6 | 9.8 | 5.0 | 11.1 | 6.7 |
| | b | 8.2 | 10.0 | 7.9 | 5.3 | 3.3 | 2.2 | 3.5 | 3.7 | 2.4 |
| | c | 9.7 | 12.8 | 1.7 | 15.0 | 5.7 | 8.9 | 10.0 | 13.0 | 13.0 |
| | d | 11.4 | 8.6 | 9.5 | 6.7 | 4.3 | 4.7 | — | 1.4 | — |
| Platelet count × $10^3$ | a | 200 | 415 | 280 | 377 | 483 | 530 | 390 | 315 | 330 |
| | b | 445 | 340 | 360 | 450 | 210 | 100 | 205 | 40 | 130 |
| | c | 315 | 345 | 300 | 415 | 295 | 385 | 275 | 315 | 440 |
| | d | 547 | 537 | 255 | 415 | 147 | 450 | — | few on smear | — |

a = control peripheral blood counts.
b = maximum peripheral count depression following second x-irradiation exposure.
c = peripheral blood counts prior to administration of nitrogen mustard.
d = " " " one wk following nitrogen mustard.

secondarily infected, was resistant to therapy and had to be sacrificed. The remainder of the animals in this group tolerated the nitrogen mustard well and showed no significant depression of the peripheral blood count. Bone marrow specimens were diluted with peripheral blood but showed normal activity of all cell types. They remained well throughout the study, and there were no deaths following nitrogen mustard.

There were 3 deaths among the 5 animals of the irradiated group. One of these showed evidence of generalized debility with weight loss and lethargy during the recovery period following x-irradiation despite return of the peripheral blood count to normal levels. On the day following injection of nitrogen mustard, the animal was found dead in its cage. At autopsy no specific organ abnormality was noted. The second animal which died showed lethargy 4 days post-injection. A blood count at this time showed a marked neutropenia and a marked depression of platelets on the peripheral smear. Marrow aspirate showed depression of granulocytic and megakaryocytic activity. The animal died on the following day and autopsy revealed massive hemopericardium and hemorrhage into the left lung, which was felt was due to the trauma of cardiac puncture and associated thrombocytopenia. The third animal showed no immediate ill effects of nitrogen mustard therapy, but was found dead in its cage 4 days post-injection. Autopsy revealed no gross cause of death. Hematologic data are shown in Table I.

*Discussion.* Our experience with administration of large doses of nitrogen mustard to patients with Hodgkin's disease had suggested that previous treatment with x-irradiation, even in those with apparent recovery of normal marrow function, caused severe and prolonged post-mustard marrow depression. Mrazek and Wachowski(2) have also suggested that previous treatment with irradiation or chemicals was the factor most directly related to the occurrence of peripheral blood depression in patients who received doses of nitrogen mustard. The Council on Pharmacy and Chemistry of the American Med. Assn.(3) has stated that "a severe and even uncontrollable depression of the hemopoietic

system may follow the usual dose of nitrogen mustard, particularly in patients with widespread disease and in those patients who have had previous treatment with other similar agents or x-ray."

In an attempt to clarify the problem of this effect of previous exposure to x-ray and subsequent response to nitrogen mustard, a similar experimental study was devised utilizing the rabbit as the experimental animal. There was a marked difference in response of the two groups. In the control group which received no previous x-ray, but did receive 1.5 mg/kg nitrogen mustard, there were no deaths and no depression of hemopoietic activity. In the x-irradiated group, with all animals showing normal hemopoietic activity prior to receiving nitrogen mustard, 3 of 5 animals died following injection. In 2 of these, the cause of death was not demonstrated at autopsy. Blood counts were not obtained prior to death. In the third, bone marrow and peripheral blood count revealed marked depression of hemopoietic activity and death secondary to massive hemopericardium. That this complication was, in part, due to thrombocytopenia is suggested by the fact that no evidence of bleeding due to cardiac puncture in the control group was noted.

*Summary.* Rabbits which had been exposed to whole body x-irradiation showed a significant mortality rate following injection of 1.5 mg/kg body weight nitrogen mustard in contrast to the control group where no deaths occurred. This difference was noted even though bone marrows of the x-irradiated group were functionally normal when nitrogen mustard was administered.

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## Effects of Brucellaphage on Extracellular and Intracellular *Brucella abortus*.* (26136)

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During studies on the activity of a brucellaphage(1) capable of lysing smooth *Brucella abortus* it was observed that UV-inactivated phage preparations retained their ability to inhibit multiplication of *Br. abortus* organisms. Similar findings have been reported for *Escherichia coli*(2). Efforts now have been made to ascertain whether these inhibitory effects were caused by the protein moiety of the phage, and also whether such effects could be exerted intracellularly as well as extracellularly. An opportunity to examine this latter aspect was afforded by concurrent studies on intracellular multiplication of *Br. abortus* within monocytes maintained *in vitro*(3-5).

*Methods.* Smooth organisms of *Br. abortus*, strain SA-S(4), were maintained and grown using tryptose agar, buffered beef extract broth (BBE), or, as described below, within monocytes. Brucellaphage(1) was propagated by mixing a BBE suspension of 24-hour-old *Br. abortus* organisms with phage in the ratio of 1:5, allowing adsorption for 30 mintues at 37°C, and plating the mixture upon tryptose agar. Twenty-four hours later the surface of the agar was washed with saline, a few ml of chloroform was added to the washings to kill all remaining viable brucellae, and the mixture was allowed to stand overnight at 4°C. Phage plus bacterial debris were then sedimented by centrifugation,

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