Effect of Nitrogen Mustard on Experimental Tuberculosis.* (23198)

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(Introduced by A. W. Wright)
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Since Hektoen and Corper's report(1) it has been known that mustard gas inhibits the formation of antibodies. With the introduction as therapeutic agents of the closely related nitrogen mustards, usually methyl bis (beta chlorethyl) amine hydrochloride (HN_2), by Gilman and Philips(2), the possibility of lowering resistance to infection or producing dissemination of an infectious process already present was postulated. HN_2 has found its greatest usefulness in the treatment of malignant lymphomas and at least one of these conditions, Hodgkin's disease, is associated with active tuberculosis frequently enough for treatment with this agent to pose a serious problem. Gellhorn and Collins(3) have reported one case of Hodgkin's disease in which they suggest that tuberculosis may have been aggravated by HN_2 treatment. One of us is familiar with 2 similar cases in which treatment with HN_2 was followed by rapid dissemination of tuberculosis. In none of these cases was there adequate study of the pre-treatment status of the pulmonary tuberculosis, and the possibility of rapid spread of tuberculosis in Hodgkin's disease without treatment is a possibility. Because of the consideration that HN_2 may be a factor in decreasing resistance to tuberculous infection, the following experiments were devised:

Methods. Guinea pigs protected by BCG were inoculated with virulent tubercle bacilli and later treated with HN_2 to determine the effect of the agent on the quiescent foci. At first it was decided to use a weight dose of HN_2 comparable to that used in humans but it was found that 0.1 mg/kilo given intraperitoneally for 6 days had little or no effect on the peripheral leukocyte count of guinea pigs. Accordingly, the dosage was gradually increased to 0.3 mg/kilo for 6 days, which was adequate to produce leukopenia but did not cause death of the animals. Forty-two guinea pigs weighing approximately 300 g each were employed. All animals were initially tested with 0.1 ml of a 1:10 dilution of "Old Tuberculin" (O.T.) and found to be negative. The animals were then divided into 6 groups of 7 animals each and treated as follows: Group I:BCG and *Mycobacterium tuberculosis* strain $H37R_v$; and HN_2; II:BCG and $H37R_v$; III: $H37R_v$ and HN_2; IV:BCG and HN_2; V:$H37R_v$ alone; VI:HN_2 alone. The various agents and treatments were administered as follows: (a) Animals receiving BCG were injected in the thigh with 0.1 ml of standard suspension.† After 30 days these animals were tested with O.T. and all were found to be tuberculin positive. (b) 60 days after BCG injection animals receiving $H37R_v$ were inoculated intramuscularly in the thigh with 0.1 ml of suspension containing 3 mg wet weight of bacteria. (c) Nitrogen mustard was given intraperitoneally, 0.3 mg/kilo on 6 consecutive days. This was started 15 days after injection of $H37R_v$. (d) Groups III through VI served as controls. (e) Total leukocyte counts, hemoglobin determinations and differential counts were performed on animals in Group VI immediately before the first injection of nitrogen mustard, 2 days after the last injection, and every 7 days thereafter for 3 weeks, 5 determinations in all being made of each animal. Blood was obtained by cardiac puncture. Two animals died during the course of bleeding. (f) Thirty days after inoculation with $H37R_v$, all animals, except those of Group VI, were sacrificed and autopsies performed. The animals were inspected for evidence of tuberculosis, and any lesions which were equivocal were removed for microscopic examination. A modification of the Steenken and Baldwin(4) and Feldman(5) schemes for recording experimental

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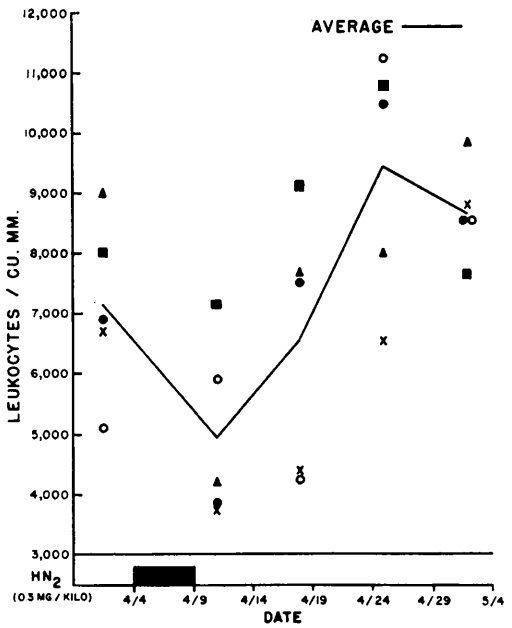

FIG. 1. Group VI. Effect upon leucocytes of 0.3 mg/kilo of nitrogen mustard given intraperitoneally on 6 successive days to guinea pigs. Each symbol represents a single animal. A higher dosage resulted in significant mortality. Lower doses were without hematological effect. Two animals died during bleeding and are not included.

tuberculosis in guinea pigs was used. Extensive involvement of an organ or regionl lymph nodes was graded as 4 with successively less involvement graded down to 0 for no evidence of lesions. The parts inspected and graded were the injection site, inguinal nodes, retroperitoneal nodes, mediastinal nodes, axillary nodes, spleen, liver, and lungs. (g) Total involvement within a group was computed and tests for statistical significance of means were performed.

Results. On a dose regimen of 0.3 mg HN_2/kilo body weight given intraperitoneally on 6 consecutive days it was possible to produce significant lowering of the total leukocyte count in groups of guinea pigs similar to those employed in the experiment (Fig. 1). The normal range of leukocyte counts in guinea pigs is between 5,000 and 10,000 cells/cu mm. While average counts are only slightly below the normal minimum, it can be seen that in 4 of the 5 individual animals there was a significant drop. Because the bleeding schedule for blood counts was set at 7-day intervals, it was conjectured that blood loss might affect the counts. Another group of animals not subjected to HN_2 treatment and bled at 7-day intervals did not reveal any decrease in total leukocytes.

The results given in Table I disclose no demonstrable difference in amount or spread of tuberculosis in groups of treated and untreated animals. The findings in various control groups substantiate the effectiveness of the various agents used.

Discussion. Various explanations for resistance of previously infected subjects to dissemination of tubercle bacilli or reinfection or reactivation of disease under certain conditions have been proposed. Evidence supporting or disproving these various explanations has been conflicting and the problem has not yet been solved. Therefore, speculation as to the reasons for the observed exacerbation of quiescent tuberculosis following treatment with HN_2 has not been attempted. What we have attempted was to ascertain if the course of experimental infections in animals would be affected by treatment with HN_2. It was postulated that when dissemination of disease in patients followed administration of HN_2, resistance had previously been good and that subsequent spread was due to a breakdown of that resistance. In Groups I and II in our study an attempt was made to set up similar conditions. When Group I animals were subjected to challenge with HN_2 no measurable difference in dissemination was observed between these animals and those of Group II. The other groups included in the study served as controls for the variables in the experiment.

In a previous experiment with larger num-

TABLE I. Mean Amount of Tuberculosis Observed in Groups I through V.

Groups	I	II	III	IV	V
Injection site	4.0	4.0	4.0	0	4.0
Inguinal nodes	.3	1.0	1.1	0	1.3
Retroperitoneal nodes	.8	.7	2.1	0	2.0
Mediastinal nodes	.7	0	1.7	0	2.0
Axillary nodes	0	0	0	0	.1
Spleen	0	.1	2.1	0	2.7
Liver	0	0	.4	0	1.3
Lungs	0	0	0	0	.8
Total	5.8	5.8	11.4	0	14.2

Group I—BCG, $H37R_v$, HN_2. Group II—BCG, $H37R_v$. Group III—$H37R_v$, HN_2. Group IV—BCG, HN_2. Group V—$H37R_v$.

bers of animals, the dose of nitrogen mustard was smaller, having been predicated on that used in humans. While the results obtained were identical with those in the present study, they were open to question because no significant leukopenia could be demonstrated with this dose.

In the present study the dose of HN_2 was calculated to cause a significant drop of leukocytes without causing death. The difference in dosage which will accomplish this effect was found to be very critical, since the animals remained well and healthy on one dose and were killed quickly when the dose was increased very slightly. It is believed, therefore, that HN_2 was given in an amount which would affect the animals since leukopenia occurred regularly in our test group.

In Groups I and II the animals were made resistant to subsequent challenge with virulent tubercle bacilli by prior inoculation with BCG. Reference to Table I indicates that virulent organisms were confined to the injection site and adjacent nodes, a situation analogous to that of patients with quiescent tuberculosis. Treatment with HN_2 appears to have had no effect on animals thus treated, since the amount and distribution of the infection were essentially the same.

In Groups III and V the animals were also inoculated with virulent organisms but did not have the protection of BCG. The guinea pigs receiving HN_2 appeared to have less dissemination of the disease than those not receiving it. A statistical analysis of the data‡ in these groups reveals the difference between the mean amount of tuberculosis present in treated and untreated animals to have no significance.

No effect was evident when animals of Group IV, which were given BCG alone, were subjected to treatment with nitrogen mustard.

Summary. Nitrogen mustard administered intraperitoneally to guinea pigs in a dose of 0.3 mg/kilo for 6 days was sufficient to produce significant leukopenia but had no appreciable effect on amount or spread of tuberculous infection nor did it reduce the protection produced by prior vaccination with BCG.

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Method for Simultaneous Estimation of Pregnane-3α, 17α, 20α-Triol and Pregnane-3α, 17α, 20α-Triol-11-One in Urine. (23199)

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Cases of adrenogenital syndrome due to adrenal hyperplasia excrete large amounts of C-20-methyl steroids such as pregnane-3α, 17α, 20α, triol(1), and pregnane-3α, 17α, 20α, triol-11-one(2), in the urine. Although milligram amounts of pregnane-3α, 17α, 20α-triol are also excreted in cases of adrenal virilizing tumor(3) and small quantities of this steroid are present in the urine of normal persons(4), the presence of pregnane-3α, 17α, 20α-triol-11-one has been established so far only in cases of adrenal hyperplasia(2). In all the above publications the presence of both compounds was established by isolation and identification. However, until recently

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