Augmentation of Nitrogen Mustard Induced Leukopenia by Cortisone.* (20185)

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The administration of L-cysteine prior to the injection of nitrogen mustard (HN_2) results in a decrease in the degree of leukopenia characteristically induced by HN_2(1,2). This protective effect is dependent upon the presence within the molecule of a free sulfhydryl, amino and carboxyl group in close apposition(3). Thus, compounds with structures closely related to L-cysteine are ineffective in preventing leukopenia if this specific configuration is altered. This protective effect of L-cysteine is apparently not due to chemical inactivation of HN_2, and may be due to the protection of some substance or substances essential for leukopoiesis from destruction by HN_2. However, the possibility exists that L-cysteine may also protect tumor tissue as well as hematopoietic tissue against the effects of HN_2. Consequently, other compounds which could protect hematopoietic tissue without the possibility of interfering with the beneficial effects of HN_2 would be desirable.

ACTH and cortisone are known to produce granulopoiesis(4,5), and it is therefore possible that these compounds might be beneficial against HN_2-induced leukopenia. Furthermore, ACTH and cortisone are frequently therapeutically effective in the same diseases as HN_2, and it has been suggested that combined therapy with these 2 agents might be beneficial. Accordingly, the effect of cortisone on HN_2-induced leukopenia was investigated in rabbits. In addition, the effect of cortisone in modifying the L-cysteine protection of HN_2-induced leukopenia was also studied.

Materials and methods. The effect of cortisone on the leukopenia induced by HN_2 was studied by comparing the maximum fall in total leukocyte count and polymorphonuclear leukocyte count in rabbits receiving HN_2 alone and in rabbits receiving cortisone either before or after HN_2 administration. HN_2 [methyl bis (beta-chlorethyl) amine hydrochloride]‡ was administered intravenously in a single dose of 2.5 mg per kg body weight in all experiments. This dose invariably induces a maximum leukopenia on the third to sixth day after injection. Cortisone§ was administered intramuscularly in doses of 5 mg per kg body weight either twice daily for 2 days before HN_2 administration or once daily for 3 days following HN_2 administration (Table I). Control studies were obtained by giving intramuscular injections of either desoxycorticosterone acetate (DOCA)‖ or normal saline in place of cortisone. DOCA was administered in doses of 1.0 mg per kg body weight daily for 2 days before or for 3 days following HN_2 administration. Normal saline was given in 1.0 cc doses daily for 3 days following HN_2 (Table I). The effect of cortisone on the protective effect of L-cysteine against HN_2-induced leukopenia was investigated by giving cortisone to rabbits either before or after combined L-cysteine-HN_2 therapy. L-cysteine hydrochloride was prepared as a 20% solution(1) and administered intravenously in doses of 500 mg per kg body weight immediately before HN_2 injection. Cortisone was given in doses of 5.0 mg per kg body weight intramuscularly twice daily for 2 days before, or once daily for 3 days after combined L-cysteine-HN_2 therapy (Table II). Control studies using DOCA instead of cortisone were done. DOCA was given in doses of

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§ Saline suspension of cortisone acetate (Merck), 25 mg per cc.

‖ Desoxycorticosterone acetate in sesame oil, 5 mg per cc.

TABLE I. Effect of Cortisone on Nitrogen Mustard (HN_2)-Induced Leukopenia.

Treatment	No. of rabbits	Max fall in WBC's* (% of control count)	Max fall in PMNS* (% of control count)	Lymphopenia, (during max leukopenia) (% of control count)
Nitrogen mustard (NH_2) 2.5 mg/kg I.V.	9	82	93	77
Cortisone 5 mg/kg I.M. b.i.d. × 2 before HN_2	10	91†	96†	87†
Cortisone 5 mg/kg I.M. q.d. × 3 after HN_2	10			
DOCA 1.0 mg/kg I.M. q.d. × 2 before HN_2	9	88‡	97‡	83‡
DOCA 1.0 mg/kg I.M. q.d. × 3 after HN_2	10			
Saline 1.0 cc I.M. q.d. × 3 after HN_2	10	81	94	74
Sham operation immediately before HN_2	7	95	94	94

* Max fall $= \frac{\text{Initial count} - \text{lowest count}}{\text{Initial count}} \times 100.$

† Results essentially the same whether cortisone is given before or after HN_2 administration.
‡ Administration of DOCA either before or after HN_2 produces essentially same results.

1.0 mg per kg body weight intramuscularly daily for 2 days before or daily for 3 days after combined L-cysteine-HN_2 therapy (Table II). Total leukocyte and differential counts were done daily from the beginning of the experiment until recovery from the maximum leukopenia was evident.

Results. Administration of HN_2 alone resulted in an average maximum fall in total leukocyte count of 82% of control counts and an average maximum fall of neutrophils of 93% of control counts. The absolute lymphocyte count fell 77% of the control counts. Administration of cortisone either before or after HN_2 injection increased the average maximum fall in total leukocyte count to 91% (*i.e.* 9% more than with HN_2 alone) and the total lymphocyte count to 87% (10% more than with HN_2 alone), without any significant increase in the degree of neutropenia. In the rabbits receiving DOCA, in place of cortisone, the average maximum fall in total leukocyte, neutrophil and lymphocyte counts was 88, 97 and 83%, respectively. Administration of normal saline intramuscularly following HN injection caused no significant change in total leukocyte counts or differential counts compared to animals receiving HN_2 alone (Table I).

Rabbits given L-cysteine hydrochloride immediately prior to HN_2 administration showed an average maximum fall in total leukocyte count of only 37% of initial counts, while the average maximum fall in absolute neutrophil and lymphocyte count was only 32 and 45%, respectively (Table II). When cortisone was administered either before or after combined L-cysteine-HN_2 injections, the average maximum fall in total leukocyte count increased to 64% (27% more than with L-cysteine and HN_2 alone), and the fall in absolute neutrophil and lymphocyte counts increased to 59 and 73%, respectively (27 and 28% more than with L-cysteine and HN_2 alone). Control animals receiving DOCA instead of cortisone showed average maximum falls in total leukocyte, neutrophil and lymphocyte counts of 46, 51 and 50%, respectively (Table II).

Discussion. Although cortisone usually causes a leukocytosis in animals and in humans, it is apparent that under the conditions of this experiment, cortisone offered no protection against the leukopenia induced by HN_2 in rabbits. The administration of cortisone caused an even greater drop in total leukocyte count than in animals receiving HN_2 alone. The greater total leukopenia resulting from cortisone administration is due to an increased lymphopenia.

Endogenously produced ACTH and corti-

TABLE II. Effect of Cortisone in Modifying Protective Effect of L-Cysteine on Nitrogen Mustard (HN_2)-Induced Leukopenia.

Treatment	No. of rabbits	Max fall in WBC's* (% of control count)	Max fall in PMNS* (% of control count)	Lymphopenia, (during max leukopenia) (% of control count)
L-Cysteine 500 mg/kg I.V. immediately before HN_2 2.5 mg/kg I.V.	16	37	32	45
Cortisone 5.0 mg/kg I.M. b.i.d. × 2 before L-Cysteine and HN_2	10	64†	59†	73†
Cortisone 5.0 mg/kg I.M. q.d. × 3 after L-Cysteine and HN_2	9			
DOCA 1.0 mg/kg I.M. q.d. × 2 before L-Cysteine and HN_2	9	46‡	51‡	50‡
DOCA 1.0 mg/kg I.M. q.d. × 3 after L-Cysteine and HN_2	9			

* Max fall $= \frac{\text{Initial count} - \text{lowest count}}{\text{Initial count}} \times 100.$

† Results essentially the same whether cortisone is given before or after HN_2 administration.

‡ Administration of DOCA either before or after HN_2 produces essentially same results.

sone apparently have the same effect on HN_2-induced leukopenia as cortisone administered parenterally. Thus, when rabbits were subjected to either a splenectomy or to a sham operation, consisting solely of an abdominal incision, immediately prior to HN_2 administration, a marked increase in the degree of leukopenia was noted compared to animals receiving the same amount of HN_2 per kg body weight without an operation. In these animals, the increased leukopenia was also due to a greater lymphopenia (Table I).

Cortisone likewise failed to augment the protective effect of L-cysteine on HN_2-induced leukopenia. On the contrary, administration of cortisone to rabbits receiving L-cysteine immediately prior to HN_2 injection resulted in a greater degree of leukopenia, neutropenia, and lymphopenia than in animals receiving L-cysteine and HN_2 alone. Cortisone, therefore, appears either to diminish the protective effect of L-cysteine or to augment the effect of HN_2 in producing leukopenia.

The administration of DOCA with HN_2 or with combined L-cysteine-HN injections also augments the resulting leukopenia. However, the effects of DOCA are not as marked as with the administration of cortisone.

Graham *et al.*(6) have found that cortisone administered immediately following irradiation in mice had no effect on the survival rate of these animals. Thiersch *et al.*(7) administered cortisone to rats daily for 2 weeks beginning on the third day following whole body radiation. They found that cortisone administered after irradiation damage was established, caused a greater depression of bone marrow and a slower rate of recovery than in animals receiving x-ray alone. On the other hand, Mirand *et al.*(8) demonstrated that both cortisone and DOCA given to mice either before or immediately after irradiation afforded protection from the lethal effects of ionizing radiation. The failure of cortisone to prevent HN_2-induced leukopenia in our studies is similar to the results of Graham and Thiersch in which cortisone failed to favorably influence the results of irradiation.

These findings indicate that no benefit can be expected from the combined use of cortisone and HN_2 as far as protection of leukocytes is concerned. Although treatment of certain diseases with both cortisone and HN_2 may be therapeutically synergistic, combined therapy must be considered dangerous from the standpoint of the severe depression of hematopoietic tissue which may result.

Summary. 1. Cortisone is ineffective in pre-

venting HN_2-induced leukopenia in rabbits. On the contrary, cortisone induces a greater leukopenia as a result of increased lymphopenia. 2. Administration of cortisone to rabbits receiving L-cysteine prior to HN_2 administration results in a more severe leukopenia and neutropenia than administration of L-cysteine and HN_2 alone. 3. The combined use of cortisone and HN_2 therapeutically may induce a dangerous depression of hematopoietic tissue.

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Growth-Promotion by Lyxoflavin. II. Relationship to Riboflavin in Bacteria and Chicks.* (20186)

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Addition of L-lyxoflavin to appropriate experimental rations increases growth of rats (1-3), chicks(3,4), and pigs(5). To explain this action it has been suggested(2,4) that lyxoflavin may have vitamin action of its own, since: (a) it has no growth-promoting action for higher animals in the absence of riboflavin, (b) its addition to diets similar to those used for assay of unidentified factors stimulates growth, and (c) it has been reported to occur naturally(6), implicating a functional role for it in metabolism. The merit of these arguments is open to question. Growth stimulation by a given substance does not prove vitamin activity, as is abundantly evident from the growth-promoting action of a variety of antibiotics. Furthermore, although lyxoflavin stimulates growth of chicks on rations used for assay of unidentified stimulants, addition of crude supplements known to contain adequate amounts of these unidentified factors did not mask the response to lyxoflavin(4) as would be expected if their growth-promoting activity was due to this substance. Finally, the evidence for the natural occurrence of lyxoflavin is inconclusive(7); unless the compound actually occurs naturally, vitamin activity can scarcely be ascribed to it.

These findings with experimental animals are superficially similar to those in bacteria. We reported previously, without confirming data, that although lyxoflavin did not permit growth of *Lactobacillus casei* in the absence of riboflavin, low concentrations of lyxoflavin did increase the growth response of this organism to limiting amounts of riboflavin(4). Lyxoflavin resembled in this respect D- and L-araboflavins(8). At higher concentrations, lyxoflavin inhibits growth by acting as a competitive antagonist of riboflavin(4). These findings, which have been confirmed by others (3,9) are extended below. The report of Shorb(9) that *Lactobacillus lactis* grows in the absence of riboflavin when lyxoflavin is supplied has been confirmed and extended.

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