The Response of Normal and Malignant Lymphoid Tissue to Methyl-Bis-(β-Chloroethyl)amine and Ethyl Carbamate (Urethane) in Adrenalectomized and Non-Adrenalectomized Mice

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In a recent review Gilman and Philips (3) reported that nitrogen mustards in general have a depressant cellular effect which is most striking in tissues containing rapidly proliferating cells. Ethyl carbamate (urethane) is another drug that acts on dividing cells. Guyer and Claus (4) have shown that there is a marked reduction in the number of mitotic figures in the corneas of urethane-treated rats and mice. Colchicine effects an inhibition of cellular growth primarily as a result of mitotic arrest in the metaphase. These 3 agents have found clinical and laboratory usefulness in leukemia, presumably due to the cellular effects mentioned above.

The studies of Selye (8) clearly indicate that stimulation of the adrenal gland, as seen in the "alarm" reaction, plays a role in lymphoid regression. It has, therefore, been natural to assume that possibly the lymphoid regression produced by the above chemicals is at least in part a result of direct or indirect adrenal stimulation resulting from drug administration. It has been shown by Bass and Freeman (1) that a nonspecific stimulus induced by intraperitoneal administration of ethyl alcohol results in regression of malignant lymphoid tissue. On the other hand, Karnofsky, Graef, and Smith (5) have shown that in the adrenalectomized rat, nitrogen mustards produce reduction in the size of the spleen, thymus, and lymph nodes. The adrenal hypertrophy observed by Ludewig and Chanutin (6) studying nitrogen mustards and by Murphy and Sturm (7) studying urethane in rats, therefore, may be interpreted as either unrelated to the lymphoid regression produced by these agents or as of only minor significance.

The present investigation was undertaken to study further: (1) the effect of these agents on normal and malignant lymphoid tissue and (2) the role of the adrenal glands in producing lymphoid regression in urethane and nitrogen mustard-treated mice.

EXPERIMENTAL

Comparative effects of colchicine, urethane and methyl-bis (β-chloroethyl) amine hydrochloride.1 C3H mice bearing transplanted 6C3HED lymphomas were used for our study. This tumor has proved very satisfactory as we have not observed spontaneous regression in our control animals. Therefore, an evaluation of therapy on small groups of mice has been possible, as any regression or maintained inhibition could be considered a result of medication. The dose selected for each drug was one which was lethal to approximately 10 per cent of the animals treated. This dosage assured a maximum therapeutic effect.

Fig. 1 gives the results of therapy with urethane, colchicine, and methyl-bis (β-chloroethyl)-amine (HN2), both alone and in combination, on tumor growth. Change in tumor size is indicated as the difference between the size of the tumor on any given day and that at the time therapy was begun. Tumor size was arbitrarily considered to be one-half the length plus the width expressed in millimeters. The order of increasing activity for the individual drugs tested is: HN2, urethane, and colchicine. The regression obtained with HN2 plus colchicine is no greater than with colchicine alone. This is to be expected since the regression resulting from colchicine therapy alone is so striking. Although the difference between the results with urethane alone and with urethane plus HN2 is not great, there seemed to be a slight potentiation of action.

When C3H mice became unavailable, we continued our studies on Rockland white mice. The response of the normal lymphocyte of the spleen and thymus under various conditions of therapy

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1 Source of HN2—Methyl bis (β chloroethyl) amine hydrochloride was obtained from Merck and Co., Rahway, N. J., through the courtesy of Dr. C. P. Rhoads, Chairman, Committee on Growth, National Research Council.

2 These mice were raised in our laboratory from breeders obtained from Carworth Farms.
was investigated. Adult male mice of approximately the same age were selected. All animals were sacrificed by severing the cervical cord after 4 days of therapy. Wet weights of thymus and spleen were recorded. Adrenal weights, also obtained, will be discussed later. Treatment schedules were the same as those employed for the C3H mice, but the dosage was increased to correspond with the higher LD50 of the respective agents for white mice.

Fig. 2 shows the results obtained. The spleen and thymus of animals treated with urethane and HN2 were significantly smaller than those of the controls. Organ weights after combined therapy averaged slightly less than after the administration of a single agent.

To determine whether combined therapy would be as effective when nonlethal doses were used, a group of littermates of the Akm strain2 were similarly studied.

Fig. 3 shows that each mouse receiving combined therapy has a smaller spleen and thymus than its littermates receiving either drug alone. Although there were no deaths at the dosages employed, organ size with the combined therapy was generally equal to, if not greater than, that obtained with lethal doses of either urethane or HN2 alone. This observation suggests the advantages of using these drugs in combination. By such a technic regression of normal lymphoid tissue can be obtained without danger of lethality.

Effects of urethane and HN2 in adrenalectomized animals—It has been reported by Karnofsky and his co-workers (5) that HN2 is effective in reducing the size of the lymph nodes, thymus and spleen in adrenalectomized rats. Since no data was presented on the malignant lymphocyte changes of HN2-treated animals following adrenalectomy, we have undertaken to compare the effect of both urethane and HN2 on malignant and normal lymphoid tissue in adrenalectomized mice.

Both Rockland white and F1 C3H hybrid mice were used in this study. The Rockland mice were given 0.025 mgm. per mouse of desoxycorticosterone acetate in sesame oil subcutaneously on the day of operation; beginning on the second day they
Fig. 2.—Thymus and spleen weights following nitrogen mustard and urethane therapy. Rockland Farms white adult male mice were treated as outlined below and were sacrificed at end of fourth day of therapy. C = solvent controls for group U+N. U = urethane, U.S.P., dissolved in distilled water, 152 mgm. per 100 gm. of body weight daily for 2 days, followed by 100 mgm. per 100 gm. daily for 2 days. N = nitrogen mustard dissolved in isotonic sodium chloride 6 mum. per kgm. on first day of therapy and 3 mum. per kgm. on third day. U+N = urethane and nitrogen mustard in quantities stated for individual U and N groups. HN2 was administered 8 hours after injection of urethane. All injections were made intraperitoneally. (SE = standard error).

Fig. 3.—Effect of urethane and nitrogen mustard therapy on spleen and thymus weight in 6 week old Akm strain mice. Solid symbols represent female mice; open symbols male mice. Each litter is represented by a different symbol. C = solvent control for U+N group. U = urethane dissolved in distilled water, dose 50 mgm. per 100 gm. of body weight. N = nitrogen mustard dissolved in isotonic sodium chloride, 1.5 mgm. per kgm. on alternate days. U+N = urethane and mustard in quantities stated for individual U and N groups. HN2 was given 8 hours after injection of urethane. All injections were made intraperitoneally. Animals were sacrificed after 4 days of therapy.
were maintained on 0.9 per cent NaCl as the drinking water. The C3H hybrids were not given desoxycorticosterone acetate and were maintained on saline as a source of fluid from the time of operation. Adrenalectomy was performed under ether anesthesia. Although immediate operative mortality was negligible, deaths were frequently encountered during the experimental period.

Fig. 4 A shows that tumor inhibition occurs in adrenalectomized mice treated with urethane and HN₂; this is evident even with the reduced drug dosages which are necessary with such animals. Fig. 4B shows the decreases in spleen size observed in urethane and HN₂-treated Rockland white and C3H hybrid mice. Although the differentials are not marked with this lower dosage, it is clear that the same pattern of organ response as observed in nonadrenalectomized mice is obtained. From the data presented it is evident that both urethane and HN₂ are effective "lympholytic" agents in adrenalectomized animals.

Ludewig and Chanutin (6) have shown that there is an enlargement of the adrenal gland in HN₂-treated rats. This is associated with a decrease in cholesterol ester and an increase in the protein and water content of the glands. Results obtained with mice in this laboratory are shown in Table I. Mice treated with both HN₂ and urethane show an increase in wet and dry adrenal weight as compared with the controls. Although chemical analyses were not performed, the data presented suggest that the adrenal weight-response to urethane administration may be on the same basis as that following HN₂ administration.

Since atrophy of malignant and normal lymphoid tissue occurs when adrenalectomized mice are treated with urethane and HN₂, the adrenal hypertrophy resulting from administration of these...
agents cannot be taken as evidence that these chemicals have their major action through production of an "alarming stimulus."

Further evidence that the action of HN₂ and urethane is a direct one on the affected cell, is offered by Guyer and Claus (4), who have shown that nearly all mitotic forms can be eliminated in the corneas of rats and mice treated with urethane, and by Friedenwald and associates (2) who have shown that HN₂ acts on the corneal cells in the premitotic phase.

To determine whether cellular activity similar to that seen on the cells of the cornea was also evident in lymphoid tissue, the effect of injected urethane was studied. The reduction of the mitotic activity in the spleen of a C3H mouse treated with urethane is shown in Fig. 5. Iron hematoxylin, which has an affinity for nuclear material of cells in stages of mitosis, was employed to demonstrate altered nuclear activity. It seems evident that the urethane-treated animal has few cells that are stained with this dye, whereas large amounts of the dye are taken up by the lymphocytes of the untreated animal. The stained cells are largely in the prophase stage of mitotic activity. This evidence indicates that the findings of Guyer and Claus for the mouse cornea also apply at least qualitatively to the mouse spleen. That nitrogen mustard has a direct action on blood-forming organs was reported in the review of Gilman and Philips. (3).

REFERENCES


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* Urethane was dissolved in distilled water; nitrogen mustard in isotonic saline.
† Mean = total weight of pooled adrenals.
‡ Mean = number of adrenal pairs in pool.


DESCRIPTION OF FIGURE 5

Fig. 5—Photomicrographs of spleens of Rockland white male mice. A. Normal mouse injected intraperitoneally with 0.23 ml. water. B. Normal mouse treated with one dose of urethane dissolved in water, 152 mgm. per 100 gm. of body weight intraperitoneally. Both animals were sacrificed 8 hours after injections were made. Iron hematoxylin stain. Mag. × 165.
Fig. 5
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