(cross-reacting) antigens or other factors.

Summary. In the donor/recipient mouse strain combination C57B1→A, 5-15 × 10^6 neonatal thymus cells cause a G.V.H. reaction in 86% of newborn recipients. This does not differ significantly from the incidence obtained with adult thymus cells in the same dose range.

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Effect of Administration of Alkylating Agents or 6-Mercaptopurine on Serum Level of Deoxyribonuclease I in Leukemia Patients.* (28641)

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The factors influencing human deoxyribonuclease (DNase) I activity have been studied(1). A method was described for assay of this enzyme by following the rate of release of acid soluble deoxyribose-purine-containing fragments from an incubation mixture consisting of serum deoxyribonucleic acid (DNA), Mg++ and pH 7.3 buffer. Under these conditions, *in vitro* addition of nitrogen mustard (methyl-*bis*(beta-chlorethyl) amine hydrochloride) noncompetitively inhibited human serum DNase I. However, extracts prepared from human leucocytes inhibited the enzyme activity in a competitive manner(1).

Since nitrogen mustard, Myleran and 6-mercaptopurine (6-MP) are commonly used in the treatment of leukemia patients, it was of interest to determine the *in vivo* effects of these compounds on serum DNase I levels. In the present study, determinations of serum DNase I were made on normal human beings, on patients with carcinoma and on patients with leukemia. In the group of leukemia patients, DNase I levels and leucocyte counts were measured before and after therapy with nitrogen mustard, Myleran or 6-MP.

Methods. DNase I assay on serum: DNase I determinations were made on the serum samples essentially according to the procedure reported earlier(1). Two ml of serum was incubated with 2 mg deoxyribonucleic acid (DNA, sodium salt, calf thymus, Hammersten, highly polymerized; Mann Research Laboratories Inc. New York) 3 mg $MgCl_2 \cdot 6H_2O$, 0.5 ml of 0.1 M tris (hydroxymethyl) aminomethane buffer pH 7.3, and 0.1 ml toluene in a total volume of 3 ml. After 20 hours of incubation at 45°C, determinations of DNase I were made by measuring deoxyribose-purine-containing fragments in the trichloroacetic acid-soluble fraction. Eight-tenths ml of 55% trichloroacetic acid (TCA) was added to the incubation mixture and the

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TABLE I. Effect of Malignancy on Human Serum DNase I Levels.

Subjects	No. of cases	Deoxyribose-purine-containing fragments released* Mean (μg)	Range (μg)
Normal	15	189 ± 45	135-303
Carcinoma	23	168 ± 30	111-222
Chronic leukemia†	11	183 ± 44	123-243

* See text for details of DNase I assay procedure.

† Of 11 patients, 10 were chronic myelocytic leukemia cases and 1 chronic lymphatic leukemia.

precipitate was centrifuged and discarded. TCA-soluble supernatant was extracted 3 times with ether to remove excess of TCA. The aqueous extract was assayed for deoxyribose according to the colorimetric procedure of Allfrey and Mirsky(2). Deoxyribose was used as the standard. The observed DNase I activities as measured by the above procedure are reported to be proportional to the amount of serum added to the system(1).

Hematologic: The routine method for measuring leukocyte count is described by Cartwright(3). Diagnosis of leukemia was based on clinical features, leukocyte counts and examination of Wright-stained smears of the peripheral blood and bone marrow aspirate. Carcinoma patients were diagnosed by histological and radiological methods.

Results. In Table I are listed the results of serum DNase I levels in normal humans, or in patients with carcinoma or chronic leukemia. The means, standard deviations and ranges for DNase I values for normal subjects and leukemia patients are not significantly different. However, serum DNase I values were slightly lower in the carcinoma group than in normal subjects. In Table II are given the results of serum DNase I levels and total leukocytes of 4 leukemia patients before and after therapy with nitrogen mustard or Myleran. Case 1 was a chronic lymphatic leukemia patient who received a single injection of 0.4 mg nitrogen mustard/kg body weight. DNase I determinations were made on this patient before nitrogen mustard injection and 24 hours after the injection. Case 2 received 8 mg Myleran therapy per day for 6 months while cases 3 and 4 received 8 mg Myleran per day for 3 months. Following therapy with nitrogen mustard or Myleran serum DNase I levels and total leukocytes were lowered in all 4 cases.

The results of administration of 6-MP to the chronic myelocytic leukemia patients on levels of human serum DNase I are given in Table III. DNase I determinations were made on the patients prior to and at the end of therapy. All the patients received 150 mg 6-MP per day. Case 1 received therapy for 2 weeks, case 2 for 3 months, case 3 for 2 months, case 4 for 7 days and case 5 for 2 months. At the end of 6-MP therapy, serum DNase I levels and total leukocyte counts were lowered in all 5 cases as shown in Table III.

Discussion. There is a slight lowering in the level of human serum DNase I of patients with carcinoma in comparison with normal humans or patients with leukemia. These results confirm the earlier observations of Gavosto *et al*(4) or of Wroblewski and Bodansky(5) who reported a somewhat lower serum DNase I activity in cancer patients in comparison to normal humans.

Following administration of nitrogen mustard, Myleran or 6-MP therapy to leukemia

TABLE II. Effect of Administration of Nitrogen Mustard and Myleran on Levels of Human Serum DNase I.

No.	Therapy*	Deoxyribose-purine-containing fragments released* Before therapy (μg)	After therapy (μg)	Lowering (%)	Pre-therapy total leucocytes cells/mm³	Post-therapy total leucocytes cells/mm³	Leucocytes lowering (%)
1	Nitrogen mustard	129	66	50	281,800	11,900	96
2	Myleran	129	84	35	207,000	6,700	97
3	"	189	144	24	37,000	11,750	68
4	"	243	174	28	84,000	16,500	80

* See text for details of therapy and DNase I assay procedure.

TABLE III. Effect of Administration of 6-Mercaptopurine (6-MP) on Levels of Human Serum DNase I.

No.	Therapy*	Deoxyribose-purine-containing fragments released* Before therapy (μg)	After therapy (μg)	Lowering (%)	Pre-therapy total leucocytes cells/mm³	Post-therapy total leucocytes cells/mm³	Leucocytes lowering (%)
1	6-MP	162	144	11	85,200	9,500	88
2	6-MP	210	129	39	245,000	4,100	98
3	6-MP	123	48	61	81,400	3,500	96
4	6-MP	201	162	19	133,500	3,700	97
5	6-MP	222	153	31	240,000	15,600	94

* See text for details of therapy and DNase I assay procedure.

patients, there was a lowering of total leukocytes and also a lowering of human serum DNase I. *In vitro* addition of nitrogen mustard or of extracts prepared from human leukocytes is reported to inhibit human serum DNase I activity(1,6). However, *in vitro* addition of Myleran or of 6-MP to the human serum does not inhibit human serum DNase I activity(7). Further, the *in vitro* concentrations of nitrogen mustard required to inhibit serum DNase I levels are much higher in comparison to the levels that would be present in the circulating blood following therapy with nitrogen mustard in treatment of leukemia. These results would rule out a direct *in vivo* inhibitory effect of the therapeutic agents on serum DNase I levels.

Administration of nitrogen mustard, Myleran or 6-MP, however, may cause a lowering of serum DNase I by an indirect effect on the DNase I inhibitor or inhibitors. The leukocytes which are broken down following therapy may result in an elevation of the concentrations of serum DNase I inhibitors (1,6) which are present in the leukocytes and which would be liberated into the serum. However, this hypothesis may not be entirely correct since the extent of the drop in leukocyte count following therapy does not correlate with the lowering of DNase I levels. Further studies are planned to correlate DNase I level with the leukocyte count by determining DNase I levels during the course of therapy rather than at the end of treatment.

Summary. Serum deoxyribonuclease I (DNase I) is slightly lowered in untreated carcinoma patients in comparison with normal subjects. However, there are no significant differences between the serum DNase I of untreated leukemia patients and normal subjects. A reduction in number of leukocytes following therapy with 6-mercaptopurine, nitrogen mustard or Myleran in leukemia patients is accompanied by a lowering in serum DNase I activity. A possible mechanism for the lowering of DNase I activity is discussed.

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