TABLE II. Heat Balance Calculations.

Item 1	Heat loss, kg cal/hr		Heat input, kg cal/hr					
	Thorax	94	Metabolism	221				
	Legs	128	Skin temp.	42				
	Remainder	41	Rectal	0				
		263		263				
Item 2	Heat loss (shivering), kg cal/hr		Heat input (shivering), kg cal/hr		Heat loss (exercise), kg cal/hr		Heat input (exercise), kg cal/hr	
	Thorax	100	Metabolism	87	Thorax	97	Metabolism	255
	Legs	82	Skin temp.	79	Legs	105	Skin temp.	0
	Remainder	0	Rectal	16	Remainder	49	Rectal	−4
		182		182		251		251

and joints(9,10). In these experiments, the steady state was not reached during the first 2 or 3 hours of cooling. Metabolism was eventually doubled at 10°C. In these experiments skin temperature dropped at a rate comparable to that reported by Horvath *et al.* (11), whose subjects were well-clothed individuals exposed to −40°C with a heat input of 74 kg cal/hr/m^2 at 2 hours' and 85 kg cal/hr/m^2 at 3 hours' exposure. The increase in heat input with exercise is of the same order of magnitude as that reported by Horvath for clothed men.

Summary. Exercise after cooling is accomplished at a greater energy cost than exercise during cooling. Over-all direct heat loss is less during exercise after cooling.

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Phosphatase Activity of Hematopoietic Tissues of Nitrogen Mustard-Treated Animals.* (22226)

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Previous studies in this laboratory(1) have shown that whole body x-irradiation markedly inhibits citric acid synthesis *in vivo* by the spleens and thymus glands of rats and mice. Treatment of animals with nitrogen mustards caused a similar inhibitory effect on citric acid formation by hematopoietic tissues (2). More recently we found(3) that sublethal and lethal doses of x-irradiation produce marked increases in the adenosine triphosphatase and 5-nucleotidase activity of the spleens and thymus glands. In view of the known similarities between x-irradiation and the nitrogen mustards in some of their actions on mammalian tissues we were interested in ascertaining whether these chemical agents produced changes in phosphatase activity similar to those which

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result from x-irradiation. To test this possibility various doses of the hydrochlorides of methyl *bis*(2-chlorethyl)amine (HN2) and ethyl *bis*(2-chlorethyl)amine (HN1) were administered intraperitoneally to rats and the adenosine triphosphatase and 5-nucleotidase activity of the spleens and thymus glands was measured at various intervals. Some measurements of the adenosine triphosphatase activity of the tissues of mice treated with HN2 were also made. The results of these experiments demonstrated that the nitrogen mustards resemble x-irradiation(3) in their effects on the phosphatase activity of hematopoietic tissues.

Materials and methods. Young male Sprague-Dawley rats (175-225 g) and male Carworth Farms mice (20-30 g) were used for this study. The animals were maintained in an air-conditioned laboratory at 65-75°F and were fed Rockland Rat Diet. Freshly prepared aqueous solutions of HN1 and HN2 were administerd intraperitoneally. For the enzyme assays the animals were sacrificed by decapitation at intervals after treatment with the nitrogen mustards and cold, aqueous homogenates of spleen and thymus glands were prepared. The adenosine triphosphatase assays were performed by the method of DuBois and Potter(4) using 1% homogenates of mouse spleen, mouse thymus glands and rat thymus glands and 0.5% homogenates of rat spleen. Duplicate assays using 2 levels of tissue were made. For the measurements on rat spleen 0.5 mg and 1 mg of tissue were used and for the assays on the thymus glands of both species and mouse spleen 1 mg and 2 mg of tissue were employed. The adenosine triphosphatase activity was expressed as units, *i.e.*, the micrograms of inorganic phosphorus liberated from adenosine triphosphate/mg of tissue during a 15-minute incubation period. The 5-nucleotidase assays were performed using a method developed in this laboratory(5). For measurement of the 5-nucleotidase activity of rat spleen and thymus glands 2% homogenates were prepared and quantities of homogenate containing 2 mg and 4 mg of tissue were used. Inorganic phosphorus measurements were made by the method of Fiske and SubbaRow(6).

Results. In a previous study in which HN1 and HN2 were administered to rats under conditions comparable with those used for the present experiments the LD_{50} of the hydrochloride of HN1 was 0.75 mg/kg and LD_{50} for HN2 hydrochloride was 1.5 mg/kg when the compounds were given intraperitoneally (2). To measure the influence of HN2 on the adenosine triphosphatase activity of the spleens and thymus glands of rats sublethal doses of 0.5 mg/kg and 0.75 mg/kg were given to 2 series of animals. At daily intervals for 5 days 4 animals at each dosage level were sacrificed for adenosine triphosphatase measurements on the spleens and thymus glands. The results of these measurements are shown in Fig. 1 in which each value on the curves is the average for assays on the tissues of 4 animals.

Administration of 0.75 mg/kg of HN2 hydrochloride resulted in an increase in the adenosine triphosphatase activity of the spleen to 186% of normal in 2 days. The spleens of rats which received 0.5 mg/kg of HN2 hydrochloride exhibited a maximum increase to 161% of normal in 3 days. Gradual reversal of the alteration in enzyme activity followed the initial increase and the rate of reversal was more rapid with the lower dose of HN2. The thymus glands exhibited a much smaller increase in enzyme activity during the observation period after these doses of the nitrogen mustard. However, in other experiments in which higher doses of HN2 were administered considerable increases in the adenosine triphosphatase activity of the thymus glands were noted. Thus, after 2 mg/kg of HN2 hydrochloride the adenosine triphosphatase activity of the thymus glands increased to 179% of normal in 3 days.

TABLE I. Comparison of the Increase in Adenosine Triphosphatase Activity of Spleens of Rats 72 Hours after Various Doses of HN1 and HN2.

Nitrogen mustard (mg/kg)	Adenosine triphosphatase activity (μg P/mg tissue/15 min.) HN1	HN2
.5	36.4 (34.3-38.0)	27.5 (26.4-28.9)
.75	44.6 (40.1-46.5)	30.3 (28.2-32.5)
1.0	47.4 (43.5-49.6)	35.6 (34.4-37.6)

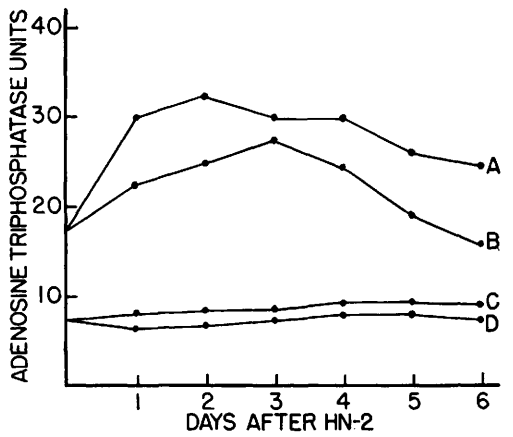

FIG. 1. Effect of methyl *bis*(2-chlorethyl)amine (HN2) hydrochloride on adenosine triphosphatase activity of spleens and thymus glands of rats. A, spleen 0.75 mg/kg; B, spleen 0.5 mg/kg; C, thymus glands 0.75 mg/kg; D, thymus glands 0.5 mg/kg.

To ascertain whether a relationship exists between the toxicity of nitrogen mustards and their ability to increase the adenosine triphosphatase activity of the spleens of rats a comparison of the effects of equivalent doses of HN1 and HN2 was made. For these measurements rats were given 0.5, 0.75 and 1 mg/kg of the hydrochlorides of HN1 and HN2 intraperitoneally. Groups of 4 animals at each dosage level were sacrificed at 72 hours for adenosine triphosphatase measurements on the spleens. The results of these measurements are shown in Table I in which the average and the range of values for each group are presented. The amount of increase in adenosine triphosphatase activity produced by each of the nitrogen mustards was dependent upon the dose and HN1 was more effective than HN2 at comparable dosage levels in agreement with the higher toxicity of HN1 to rats.

The effects of HN2 on the adenosine triphosphatase activity of mouse tissues was measured after administration of single doses of 2, 5 and 10 mg/kg of the hydrochloride intraperitoneally. The results of adenosine triphosphatase measurements conducted at daily intervals on the spleens and thymus glands are summarized in Fig. 2 in which each value on the curves is an average for at least 4 animals. At 3 days after administration of 5 and 10 mg/kg of HN2 hydrochloride the adenosine triphosphatase activity of the spleens of mice increased to 165% and 186% of normal respectively. Similar increases were noted in the thymus glands. The respective mean values and ranges for the spleens of mice receiving the 2 doses of HN2 were 18.2 (15.7-19.9) and 20.5 (17.4-23.6) adenosine triphosphatase units. Statistical analysis of the difference between these values yielded a t value of 2 indicating that the difference is of doubtful significance. Thus a dose of 5 mg/kg produced the maximum increase in the adenosine triphosphatase activity which can be induced by HN2 in the spleens and thymus glands of mice. A similar effect was observed in rats in that doses of 2.5 and 5 mg/kg of HN2 hydrochloride caused essentially the same amount of increase in the adenosine triphosphatase activity of the hematopoietic tissues. Dose-dependent increases in phosphatase activity are, therefore, observed mainly in the sublethal dosage range after administration of the nitrogen mustards or exposure to x-ray(3). Another series of mice received 2 mg/kg of HN2 hydrochloride which is sublethal for this species. The adenosine triphosphatase activity of the spleen increased to an average value of 156%

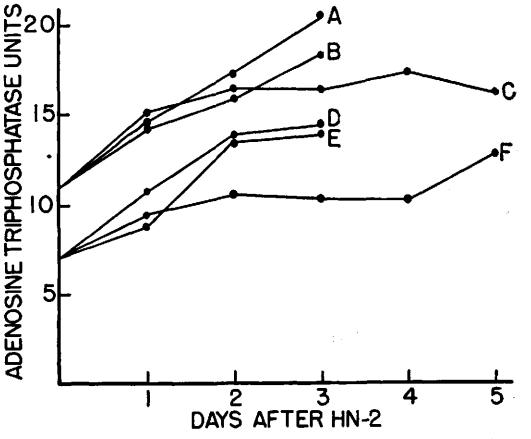

FIG. 2. Influence of methyl *bis*(2-chlorethyl)amine (HN2) hydrochloride on adenosine triphosphatase activity of spleens and thymus glands of mice. A, spleen 10 mg/kg; B, spleen 5 mg/kg; C, spleen 2 mg/kg; D, thymus glands 10 mg/kg; E, thymus glands 5 mg/kg; F, thymus glands 2 mg/kg.

of normal in 4 days after which time a gradual reversal toward normal levels occurred.

Measurements of the influence of HN2 on the 5-nucleotidase activity of the spleens and thymus glands of rats were also conducted. For this experiment a series of rats was given 0.5 mg/kg of HN2 hydrochloride intraperitoneally and groups each containing 4 animals were sacrificed at daily intervals for 5 days. The 5-nucleotidase activity of the spleens increased to 153% of normal in 4 days and an increase to 167% of normal was noted in the thymus glands at 3 days after administration of the nitrogen mustard. Gradual return of the enzyme activity to normal levels followed the initial increase in activity. The intraperitoneal administration of 0.75 mg/kg of HN2 hydrochloride caused an increase in the 5-nucleotidase activity of the spleen to 173% of normal and an increase in the enzyme activity of the thymus glands to 149% of normal.

Discussion. Results of this investigation demonstrated that sublethal doses of the nitrogen mustards produce increases in the adenosine triphosphatase and 5-nucleotidase activity of the spleens and thymus glands. The amount and duration of the increase in enzyme activity was dependent upon the dose of the nitrogen mustards. Higher doses of HN2 were necessary to produce increases in the adenosine triphosphatase activity of the hematopoietic tissues of mice than rats in agreement with the higher toxicity of this nitrogen mustard to rats. At equivalent dosage levels HN1 produced a greater increase in adenosine triphosphatase activity than HN2 thus indicating a relationship between the toxicity of various nitrogen mustards and their actions on phosphatase activity *in vivo*. After sublethal doses of the nitrogen mustards the increase in enzyme activity was reversible. The effects of the nitrogen mustards on the phosphatase activity of the spleens and thymus glands resembled qualitatively the effects of x-ray on the phosphatases of these tissues. This relationship was evident with respect to time of onset, magnitude and duration of the increase in enzyme activity. However, from a quantitative standpoint x-ray was more effective in terms of the LD_{50} values than the nitrogen mustards in increasing the phosphatase activity of hematopoietic tissues.

Previous studies in this laboratory have demonstrated(1,2) a qualitative similarity between the ability of the nitrogen mustards and x-ray to inhibit citrate synthesis in hematopoietic tissues of rats. On the basis of the available evidence it is not possible to state whether the defect in citric acid synthesis is related to the alteration in phosphatase activity. Both of these biochemical changes occur at essentially the same time and persist for approximately equal periods after treatment of animals with x-ray or a nitrogen mustard. The exact mechanism involved in the production of increased phosphatase activity in the hematopoietic tissues produced by these toxicants is unknown. On the basis of histochemical findings Ackerman *et al.*(7) suggested that the radiation-induced increase in phosphatase activity of the spleen is due to a relative increase in the red pulp and clasmatocytes as well as an actual increase in the phosphatase of the clasmatocytes. The similarity between the nitrogen mustards and x-ray which was demonstrated in the present investigation indicates that either of these agents may be employed in future studies on the exact mechanism responsible for the increased phosphatase activity of hematopoietic tissues.

Summary. The effects of nitrogen mustards *in vivo* on the phosphatase activity of the spleens and thymus glands of rats and mice were studied. The results of this study indicated that sublethal doses of HN2 produce dose-dependent increases in the adenosine triphosphatase activity and the 5-nucleotidase activity of the spleens and thymus glands with the maximum increase being observed at 3 or 4 days after intraperitoneal administration of the compound. Reversal of the increase in enzyme activity was noted after sublethal doses of HN2. At equivalent dosage levels HN1 was more toxic and more effective than HN2 in increasing the adenosine triphosphatase activity of the spleens and thymus glands of rats.

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Biotin Derivatives in Human Urine. (22227)

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The nature of the biotin derivatives that occur in urine has been the subject of a number of studies since Snell *et al.* (1) first showed that urine is a relatively rich source of the vitamin. Oppel(2) concluded that there exist in urine two forms of biotin, one avidin combinable and one avidin non-combinable. Burk and Winzler(3) postulated the existence in natural material (including urine) of a number of biotin derivatives (miotin, tiotin, and rhiotin) distinguishable by differences in microbiological activity, chemical stability, or avidin combinability. Chu and Williams(4), on the other hand, could not detect the presence in any natural material other than urine of avidin non-combinable forms of biotin. They even seriously questioned the real validity of their own data concerning the existence in urine of small amounts of such a derivative(s).

The above described studies were carried out primarily with *Saccharomyces cerevisiae* as the assay organism. Since yeast growth is notoriously influenced by a variety of stimulatory and inhibitory materials of unknown nature, the specificity of the responses described above with respect to any real relationship to biotin may be questioned. Significant to this discussion also is the fact that none of the compounds whose existence was postulated in the references cited has ever been obtained in the pure or even highly concentrated state. On the contrary, a number of other biotin derivatives have been derived from natural material or products derived from natural material and have been adequately characterized. These derivatives are biocytin (ε-N-biotinyl-L-lysine)(5), biotin d-sulfoxide(6-8), and biotin l-sulfoxide(9-11). A number of other biotin derivatives including biotinamide(12,13), oxybiotin(14), and N-biotinyl derivatives(12,13) of glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-leucine, and p-aminobenzoic acid have been synthesized in the laboratory and studied microbiologically. Differential microbiological assays with a high order of specificity in some instances are now available for studies with these compounds. In combination with the bioautographic technic, such differential microbiological asays can yield considerable information concerning the nature of the biotin derivatives in a given sample. One study(15) employed butanol-acetic acid-water as the developing system and *Neurospora crassa* as the indicating organism. The butanol-acetic acid-water system is remarkably free of complications from salts and other components of natural material. The organism *Neurospora crassa* is extremely versatile as far as response to biotin derivatives is concerned. The following compounds are essentially equally active: Biotin, oxybiotin, biotinamide, desthiobiotin, biotin d-sulfoxide, biotin l-sulfoxide, biocytin, biotinyl β-alanine, biotinyl glycine, biotinyl aspartic acid, and biotinyl leucine(10,12,13,16). In addition, R_F data obtained with *Neurospora crassa* are available for the mixed sulfoxides or the individual d- and l-sulfoxides in some