Protection of Murine L1210 Leukemia and Bone Marrow Progenitor Cells Against Mechlorethamine and Inhibition of Choline Uptake as a Structure–Activity Relationship of 2-Dimethylaminoethanol and Its Analogues

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Abstract □ The structure–activity relationships of 2-dimethylaminoethanol and its analogues as protectors against mechlorethamine cytotoxicity and as inhibitors of choline uptake were evaluated. Of a series of inhibitors and protectors, 2-dimethylaminoethanol was the most potent inhibitor of choline uptake and the most potent protector of both hematopoietic progenitor cells and murine L1210 leukemia cells. Two analogues that exhibited both potent protection and inhibition were 1-dimethylamino-2-propanol and 2-ethylmethylaminoethanol. 2-Di-*n*-butylaminoethanol, while protecting against mechlorethamine cytotoxicity, was not an inhibitor of choline uptake. 2-*n*-Butylmethylaminoethanol, while an inhibitor of choline uptake, was not a protector against mechlorethamine cytotoxicity. Addition of 2-dimethylaminoethanol to mechlorethamine in a mole ratio of 1000:1 did not improve survival of tumor-bearing mice beyond that of mice treated with mechlorethamine alone.

Keyphrases □ Structure–activity relationships—2-dimethylaminoethanol and its analogues, protection against mechlorethamine cytotoxicity, inhibition of choline uptake □ Antitumor activity—combination chemotherapy, 2-dimethylaminoethanol and mechlorethamine, differential antitumor activity □ 2-Dimethylaminoethanol—analogues, protection against mechlorethamine cytotoxicity, inhibition of choline uptake, combination chemotherapy with mechlorethamine

The limited capacity of membrane transport carriers allows use of nutritional and appropriate pharmacological substrates to protect cells from cytotoxicity by competing with cytotoxic agents dependent, at least in part, for uptake into the cell by transport carriers (1). 2-Dimethylaminoethanol, the tertiary amine corresponding to choline, is fully protonated at physiological pH and is a substrate for the choline transport system (2, 3). Mechlorethamine(2,2′-dichloro-*N*-methyldiethylamine), a nitrogen mustard, is a cytotoxic agent transported by the choline transport carrier (4). With the view of obtaining differential protection of sensitive host tissue against mechlorethamine cytotoxicity, a systematic evaluation of the structure–activity relationships of 2-dimethylaminoethanol and its analogues as protectors of hematopoietic precursor cells and murine L1210 leukemia cells against mechlorethamine cytotoxicity and as inhibitors of choline uptake into murine L1210 leukemia cells was undertaken. Mechlorethamine cytotoxicity was used as an indicator for its transport by the choline transport system (4), and protection against mechlorethamine cytotoxicity was employed as an indicator for inhibition of mechlorethamine uptake by competitors for the choline transport system (5). Since mechlorethamine is a highly reactive alkylating agent and the transport of its hydrolyzed product, diethanolmethylamine, is only partially inhibited by choline (6), the inhibition of choline transport by 2-dimethylaminoethanol and its analogues was used to identify potential inhibitors of mechlorethamine transport. The study concluded with the *in vivo* chemotherapy of L1210 tumor-bearing mice using 2-dimethylaminoethanol and mechlorethamine in a mole ratio of 1000:1.

EXPERIMENTAL

Material—Mechlorethamine hydrochloride[1], 2-dimethylaminoethanol[1], 2-dimethylamino-1-propanol[1], 2-dimethylamino-2-methyl-1-propanol[1], 1-dimethylamino-2-propanol[1], and the higher homologues[2] were purchased from commercial sources and were used as received.

L1210 Cloning Assay—The methods for the growth and maintenance of L1210 cells have been previously described (7). The L1210 cells were maintained in RPMI medium 1630[3] plus 16% heat-inactivated fetal bovine serum[4]. They were harvested in log phase (8–12 × 10^5/mL by centrifugation at 300×*g* for 5 min, washed twice in medium composed of Dulbecco's phosphate saline[5] plus 0.1 mM bovine serum albumin (pH 7.4) supplemented with 16 mM glucose (medium I), and resuspended to an appropriate cell density as determined with a cell counter[6]. The L1210 cells at a final density of 1 × 10^5/mL were added to the incubation medium containing the compounds to be tested and incubated for 15 min at 37°C. Mechlorethamine hydrochloride was then added to a final concentration of 1.0 μM (an LD_{99} dose), and the incubation was continued for 20 min. The incubation was terminated by placing the tubes containing the mixture into an ice-water bath for 5–10 min. The cells were washed three times in growth medium composed of medium 1630, 20% fetal bovine serum, and 40 μg/mL of gentamicin. The cell density was adjusted to 1 × 10^5/mL, and cytotoxicity was assayed by clonal growth in 0.13% soft nutrient agar according to the procedure of Chu and Fischer (8) with minor modifications. The surviving fractions were estimated in triplicate after 2 weeks of incubation. They were similar whether the protector and the mechlorethamine hydrochloride were added simultaneously to the L1210 cell suspension or the L1210 cell suspension was incubated 15 min with the protector prior to the addition of mechlorethamine hydrochloride.

Bone Marrow Colony-Forming Units in Culture—The method of obtaining colony-forming units in culture from bone marrow was adapted from a previously reported work (7). The central core of the femurs from male CDF_1 mice were flushed with medium I. The bone marrow cells were washed three times with this medium and resuspended to a nucleated cell concentration of 1 × 10^5/mL. The incubation of the cells with the test compounds was performed in the same manner as described for the murine L1210 leukemia clonal growth assay. The cells were then incubated with the LD_{95} dose of 0.5 μM mechlorethamine hydrochloride and for 20 min. They were then washed three times in McCoy's 5A medium[7] supplemented with 16% fetal bovine serum (non-heat in-

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Sapon Laboratories, Division of Overlook Industries, Bloomsbury, N.J.
[3] Prepared by the NIH media unit.
[4] Flow Laboratories, McLean, Va.
[5] Miles Laboratories, Elkhart, Ind.
[6] Model ZBI; Coulter Electronics, Hialeah, Fla.
[7] Grand Island Biological Co., Grand Island, N.Y.

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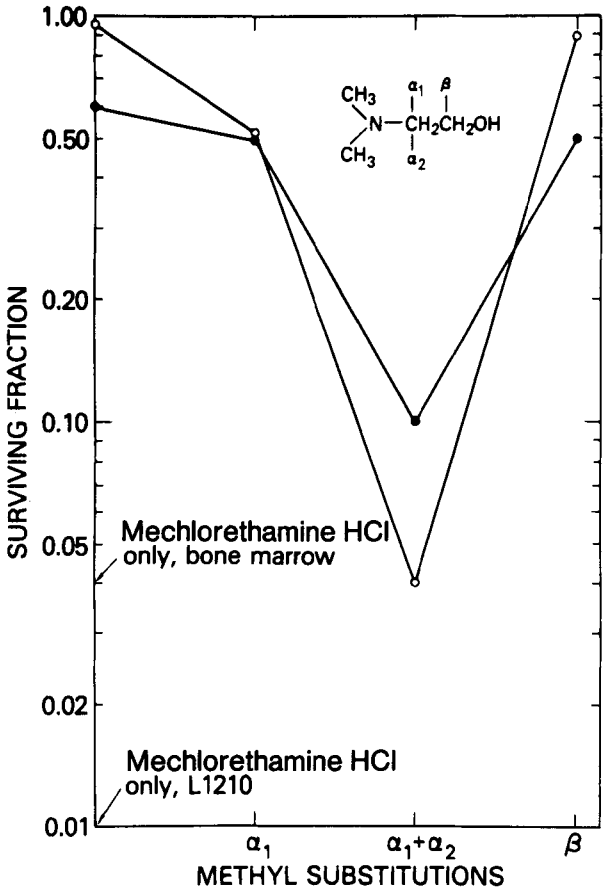

Figure 1—*Protection of murine L1210 leukemia cells and bone marrow colony-forming units by 2-dimethylaminoethanol and its α- and β-methyl derivatives against mechlorethamine cytotoxicity. The L1210 cells (○) and the bone marrow cells (●) at a density of 1 × 10⁵/mL were exposed to 200 μM 2-dimethylaminoethanol, 2-dimethylamino-1-propanol (α_1), 2-dimethylamino-2-methyl-1-propanol ($\alpha_1 + \alpha_2$), and 1-dimethylamino-2-propanol (β_1) plus 1.0 or 0.5 μM mechlorethamine hydrochloride, respectively, at 37°C. After 20 min the L1210 cells and bone marrow cells were washed and resuspended in their respective growth medium-containing agar; colonies were counted after 2 weeks and 1 week, respectively.*

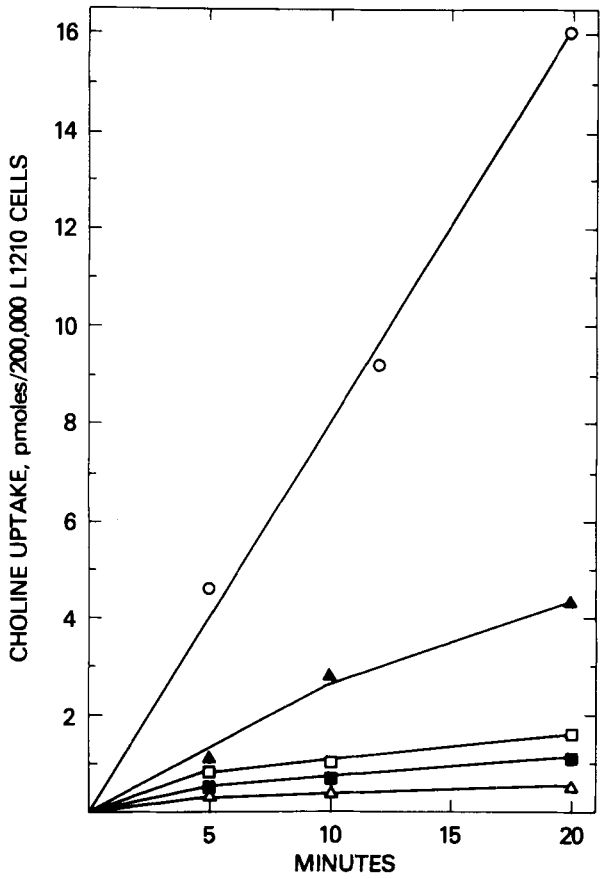

Figure 2—*Inhibition of choline uptake by 2-dimethylaminoethanol, 2-dimethylamino-1-propanol, 2-dimethylamino-2-methyl-1-propanol, and 1-dimethylamino-2-propanol. A volume of a L1210 cell suspension at a density of 2 × 10⁶/mL was added to an equal volume of transport medium at 37°C containing 1.0 μM [³H]choline (○) plus 200 μM 2-dimethylaminoethanol (△), 2-dimethylamino-1-propanol (□), 2-dimethylamino-2-methyl-1-propanol (▲), or 1-dimethylamino-2-propanol (■). At indicated time intervals triplicate 200-μL samples of the cell suspension were layered over silicone oil and centrifuged at 12,000×g. The centrifuge tips were placed in scintillation fluid and the incorporated [³H]choline was counted.*

activated), 20 U/mL of penicillin, and 20 μg/mL of streptomycin and plated in soft nutrient agar (0.3%) with colony-stimulating factor[8] such that 1 × 10⁵ nucleated cells gave 100 colonies after 7 d of growth at 37°C in a humidified, 10% carbon dioxide atmosphere. Colonies having 50 or more cells were counted in triplicate.

Choline Uptake Studies—Murine L1210 leukemia cells were washed three times in transport medium composed of Dulbecco's phosphate-balanced saline, 0.1 mM bovine serum albumin, and 6.4 mM glucose at 37°C, and the final cell density was adjusted to 1 × 10⁶/mL. Incubations were initiated by the addition of a volume of an L1210 cell suspension to an equal volume of medium containing [³H]choline[9] and the compounds to be tested. The incubation was terminated by layering 200 μL of medium over silicone oil[10] in microcentrifuge tubes and pelleting the cells through the silicone oil for 1 min at 12,000×*g* in a microcentrifuge[11]. The centrifuge tube tips containing the pellet were cut off, mixed with liquid scintillation fluor, and the incorporated radioactivity estimated in a liquid scintillation counter[12]. Each experimental point was determined in duplicate or triplicate.

Chemotherapy—L1210 cells (NCI stock) were maintained in female DBA/2 mice and grown intraperitoneally for experimental work in male CDF_1 mice[13], 8–12 weeks old and weighing 22–31 g. The mice were grouped by weight, 5 or 6 per plastic cage with wood chip bedding, and were given laboratory diet *ad libitum*. The L1210 cells were harvested 7 d after the passage inoculum, 1 × 10⁵ cells were injected intraperitoneally on day 0, and intraperitoneal chemotherapy was begun on day 1.

[8] Gift of Dr. T. R. Bradley of the Cancer Institute, Melbourne, Australia and Dr. R. Knazek, NCI, Bethesda, Md.

[9] New England Nuclear, Boston, Mass.

[10] Versilube F-50, specific gravity 1.045, viscosity 70 centistokes at 25°C; Harwick Chemical Corp., Cambridge, Mass.

[11] Model 235; Fisher Scientific Co., Rockville, Md.

[12] Model LS8100; Beckman Instruments Inc., Silver Spring, Md.

[13] Charles River Laboratories, Wilmington, Mass.

RESULTS

2-Dimethylaminoethanol and Its α- or β-Methyl Analogues—The structure–activity pattern of protection by 2-dimethylaminoethanol, 2-dimethylamino-1-propanol (α_1-methyl), 2-dimethylamino-2-methyl-1-propanol (α_1- plus α_2-methyl), and 1-dimethylamino-2-propanol (β-methyl) for L1210 cells quantitatively parallels that of the protection pattern for bone marrow progenitor cells (Fig. 1). Quantitatively, there are differences. The protector–mechlorethamine ratio was 400:1 during the incubation of precursor bone marrow cells and 200:1 during the incubation of L1210 cells. Yet, the surviving fraction of L1210 cells is uniformly greater except when the protector analogue has two α-methyl groups as in 2-dimethylamino-2-methyl-1-propanol, which results in an equally poor protector. Though this poor protector results in a greater survival of bone marrow colony-forming units compared with L1210 cells, the surviving fraction differs by a factor of ~2, consistent with the difference in their protector–mechlorethamine ratio.

The potent protectors 2-dimethylaminoethanol and 1-dimethylamino-2-propanol achieve an L1210 surviving fraction of 0.95 and 0.90, while the comparable surviving fractions of bone marrow colony-forming units are 0.6 and 0.5. 2-Dimethylaminoethanol and its α- and β-methyl analogues acting as inhibitors of choline uptake reflect their qualitative pattern of protection. 2-Dimethylamino-2-methyl-1-propanol is a rela-

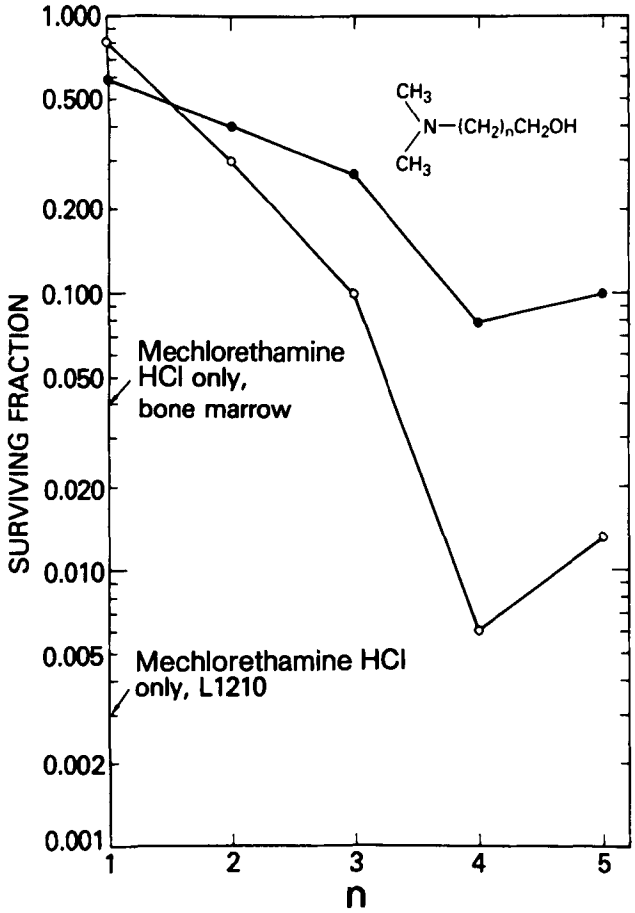

Figure 3—*Protection of murine L1210 leukemia cells and bone marrow colony-forming units by 2-dimethylaminoethanol and its higher homologues of the ethanol moiety against mechlorethamine cytotoxicity. The L1210 cells (○) and the bone marrow progenitor cells (●) at a density of 1 × 10^5/mL were exposed to 200 μM 2-dimethylaminoethanol (n = 1), 3-dimethylaminopropanol (n = 2), 4-dimethylaminobutanol (n = 3), 5-dimethylaminopentanol (n = 4), and 6-dimethylaminohexanol (n = 5) plus 1.0 or 0.5 μM mechlorethamine hydrochloride, respectively, at 37°C. The cells were processed in the same manner as described in the legend under Fig. 1.*

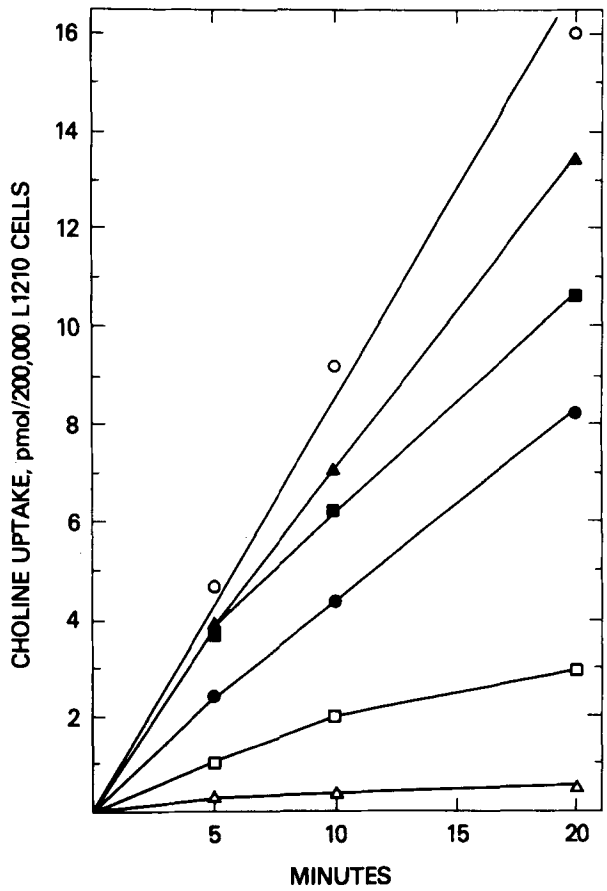

Figure 4—*Inhibition of choline uptake by 2-dimethylaminoethanol and its higher homologues of the ethanol moiety. A volume of a L1210 cell suspension at a density of 2 × 10^6/mL was added to an equal volume of transport medium at 37°C containing 1.0 μM [^{3}H]choline (○) plus 200 μM 2-dimethylaminoethanol (△), 3-dimethylaminopropanol (□), 4-dimethylaminobutanol (●), 5-dimethylaminopentanol (▲), or 6-dimethylaminohexanol (■). The cell suspension was sampled and the incorporated [^{3}H]choline was counted as described in the legend under Fig. 2.*

tively poor inhibitor compared with the other three in this series (Fig. 2). To compare choline with 2-dimethylaminoethanol, a β-methyl addition onto the choline molecule results in a compound with decreased inhibition of choline uptake, whereas a single α-methyl substitution slightly decreases the K_m of the compound for the choline transport carrier (2, 9, 10). However, a single methyl addition onto the β-position of 2-dimethylaminoethanol does not result in diminished protection against mechlorethamine cytotoxicity nor in decreased inhibition of choline uptake (Figs. 1 and 2).

2-Dimethylaminoethanol and Its Higher Methylene Homologues—With each successive increase in the number of methylene groups between the nitrogen and the hydroxyl group of 2-dimethylaminoethanol, generated higher homologues show decreased protection of both bone marrow and L1210 cells against mechlorethamine cytotoxicity (Fig. 3). Comparison of the surviving fractions of bone marrow with that of L1210 cells shows preferential protection of the bone marrow colony-forming units. A greater decrease in the surviving fraction of the L1210 cells occurs with each protector of the higher homologue than in that of the bone marrow cells. Protection by 5-dimethylaminopentanol results in a differential surviving fraction of the bone marrow cells, with the difference between these and L1210 surviving fractions being greater than an order of magnitude. This finding may describe differential protection of bone marrow progenitor cells or may be accounted for by the particular sensitivity of the L1210 cells, resulting in a survival fraction of 0.003 for mechlorethamine alone (Fig. 3). The pattern of choline uptake into L1210 cells produced by 2-dimethylaminoethanol and its higher homologues as competitors of choline is decreased inhibition of choline uptake by each higher homologue (Fig. 4). This structure–activity pattern of tertiary alkylamino alcohols is unlike that seen with the quaternary ammonium ions with the structure of alkyltrimethylammonium, which shows increased inhibition of choline uptake into erythrocytes with each methylene addition to the alkyl branch (11).

Methylene Additions onto a Single Alkyl Branch of 2-Dimethylaminoethanol—The analogues in the series from 2-dimethylaminoethanol to 2-methyl-*n*-propylaminoethanol show minimal differences in their protection of bone marrow and L1210 cells against mechlorethamine (Fig. 5). 2-*n*-Butylmethylaminoethanol is different from the rest of the series, showing a marked decrease in the ability to protect both bone marrow progenitor and L1210 cells. The entire series protected L1210 cells better than bone marrow cells. As inhibitors of choline uptake into L1210 cells, they are equally potent (Fig. 6). Compared with the pattern of inhibition of choline uptake by this series, the affinity for the choline transport carrier by quaternary ammonium analogues of choline, which were generated by increasing the number of methylene groups in a single alkyl branch, shows a biphasic pattern with maximum decrease occurring with the *n*-propyl and *n*-butyl derivatives and a progressive increase in affinity with *n*-pentyl and higher homologues (12). The alkyltrimethylammonium ions, containing no alcohol group, show a similar transition in affinity for the choline transport carrier occurring with the *n*-propyl to *n*-pentyl derivatives (11).

Methylene Additions into Both Alkyl Branches of 2-Dimethylaminoethanol—Protection of bone marrow progenitor and L1210 cells by 2-dimethylaminoethanol and its analogues, which are generated by methylene additions to both alkyl branches, shows a biphasic pattern (Fig. 7). Within the series the most potent protector is 2-dimethylaminoethanol and the least potent protector is 2-di-*n*-propylaminoethanol; at the transition to a potent protector is 2-di-*n*-butylaminoethanol.

2-Di-*n*-butylaminoethanol may detoxify mechlorethamine by func-

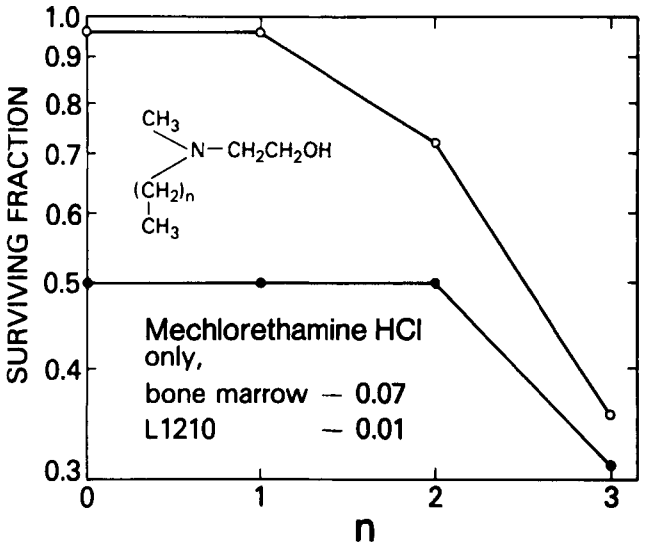

Figure 5—*Protection of murine L1210 leukemia cells and bone marrow colony-forming units by 2-dimethylaminoethanol and its analogues generated by methylene additions into a single alkyl branch. The L1210 cells (○) and the bone marrow progenitor cells (●) at a density of 1 × 10^5/mL were exposed to 200 μM 2-dimethylaminoethanol (n = 0), 2-ethylmethylaminoethanol (n = 1), 2-methyl-n-propylaminoethanol (n = 2), and 2-n-butylmethylaminoethanol (n = 3) plus 1.0 or 0.5 μM hydrochloride, respectively, at 37°C. The cells were processed in the same manner as described in the legend under Fig. 1.*

tioning as a potent nucleophile in the manner of 2-dimethylaminoethanol (13). In solution, mechlorethamine exists in various molecular forms (14) and is transported by a two-component transport system (6). Since it is a poor inhibitor of choline uptake (Fig. 8), 2-di-*n*-butylaminoethanol may protect against a larger number of mechlorethamine moieties which are not transported by the transport carrier inhibited by choline or its analogues. The small differential protection of bone marrow cells by 2-diethylaminoethanol and 2-di-*n*-propylaminoethanol may be due to the higher ratio of protector to mechlorethamine in its culture system (400:1 compared with 200:1 in the L1210 culture system).

2-Dimethylaminoethanol and 2-diethylaminoethanol are approximately equipotent inhibitors of choline uptake (Fig. 8). Their quaternary ammonium congeners, choline and diethylcholine, were found to show the same affinity for the high-affinity choline uptake system, but to differ in their affinity for the low-affinity system (15).

Treatment of L1210 Tumor-Bearing Mice with a Combination of 2-Dimethylaminoethanol and Mechlorethamine Hydrochloride—The *in vivo* treatment combination of 2-dimethylaminoethanol plus mechlorethamine hydrochloride in a mole ratio of 1000:1 decreased survival of the combination treatment group compared with the mechlorethamine hydrochloride only treatment group (Table I). This finding may be secondary to the detoxification of mechlorethamine by its ability to react with 2-dimethylaminoethanol (13). This point is possibly illustrated by the survival data, which show that the mean survival time of the group of animals which received mechlorethamine hydrochloride prior to the protector was longer (11.2 d) than the group which received the protector prior to mechlorethamine hydrochloride (9.8–10.5 d). The large doses of 2-dimethylaminoethanol produced minimal toxicity, such as salivation and shivering. The use of choline in high doses has been limited by its neurotoxicity, with a mole ratio of 30:1 of choline to mechlorethamine hydrochloride not protecting rats from a lethal dose of mechlorethamine hydrochloride (16).

Table I—Survival of Murine L1210 Leukemia-Bearing Mice Following Treatment with Mechlorethamine Hydrochloride or 2-Dimethylaminoethanol and Mechlorethane Hydrochloride

Treatment[a]	Mean Survival Time, d ± *SEM*	T/C, %
None or 5000 μmol/kg of 2-dimethylaminoethanol	7.7 ± 0.7	100
5 μmol/kg of mechlorethamine hydrochloride (days 1–5)	12.0 ± 0.9	156
5 μmol/kg of mechlorethamine hydrochloride and 5000 μmol/kg of 2-dimethylaminoethanol (0.25 hr, days 1–5)	11.2 ± 1.2[b]	146
5000 μmol/kg of 2-dimethylaminoethanol and 5 μmol/kg of mechlorethamine hydrochloride	9.8 ± 1.8[c]	114
0.25 h, days 1–5		
0.5 h, days 1–5	9.5 ± 1.1[d]	123
1 h, days 1–5	10.5 ± 0.8[e]	121

[a] L1210 cells, 1 × 10^5, were injected on day 0, and intraperitoneal chemotherapy was begun on day 1 and continued through day 5. In the combination treatment groups, the time indicates the interval between the two treatments. There were six mice per group. [b] No significant difference from mechlorethamine hydrochloride treatment only group at $p > 0.05$, as determined by the one-sided Student's *t* test. [c] Significantly different from the mechlorethamine hydrochloride treatment only group, $p < 0.02$. [d] Significantly different from the mechlorethamine hydrochloride treatment only group, $p < 0.01$. [e] Significantly different from the mechlorethamine hydrochloride treatment only group, $p < 0.05$.

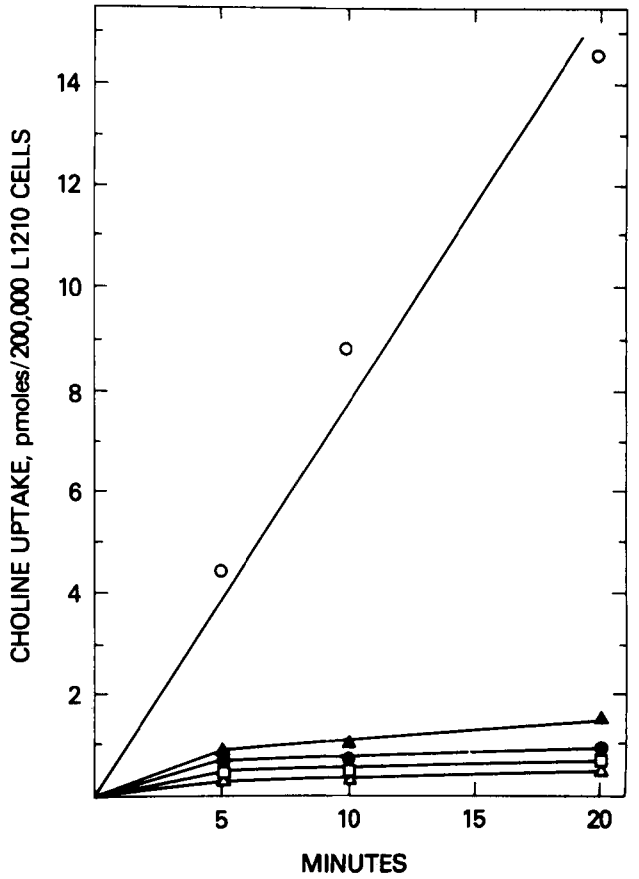

Figure 6—*Inhibition of choline uptake by 2-dimethylaminoethanol and its analogues generated by methylene additions into a single alkyl branch. A volume of a L1210 cell suspension at a density of 2 × 10^6/mL was added to an equal volume of transport medium at 37°C containing 1.0 μM [^{3}H]choline (○) plus 200 μM 2-dimethylaminoethanol (△), 2-ethylmethylaminoethanol (□), 2-methyl-n-propylaminoethanol (●), or 2-n-butylmethylaminoethanol (▲). The cell suspension was sampled and the incorporated [^{3}H]choline was counted as described in the legend under Fig. 2.*

DISCUSSION

With the possible exception of 5-dimethylaminopentanol (Fig. 3), the structure–activity relationships of 2-dimethylaminoethanol and its analogues to protection of bone marrow colony-forming units and L1210 cells did not identify a differential protector of bone marrow progenitor cells. During treatment with the protectors, the maximum achieved surviving fraction was observed with L1210 cells, unlike the plasmacytoma cell–bone marrow precursor cell culture system which showed preferential survival of bone marrow cells because of the differential sensitivity of the plasmacytoma cells to mechlorethamine (17). The maximum surviving fraction of bone marrow cells appears consistently fixed between 0.5 and 0.6 when they were exposed to 2-dimethylaminoethanol and mechlorethamine hydrochloride. The protector alone at

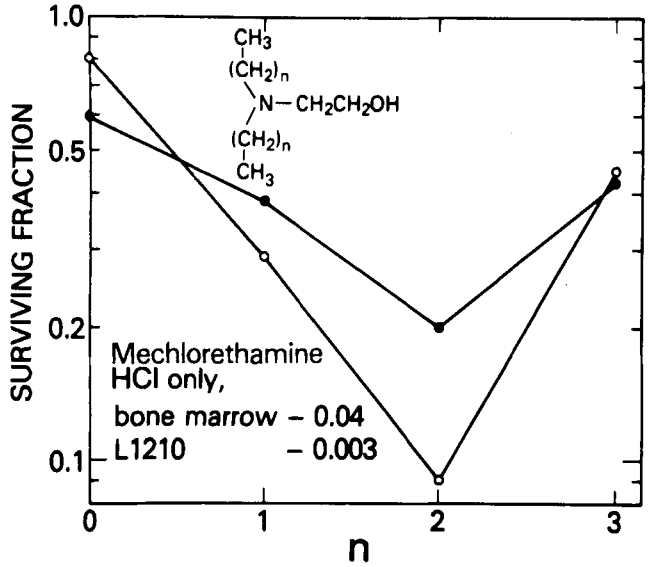

Figure 7—*Protection of murine L1210 leukemia cells and bone marrow colony-forming units by 2-dimethylaminoethanol and its analogues generated by methylene additions into the dialkyl branches. The L1210 cells (○) and the bone marrow progenitor cells (●) at a density of 1 × 10^5/mL were exposed to 200 μM 2-dimethylaminoethanol (n = 0), 2-diethylaminoethanol (n = 1), 2-di-n-propylaminoethanol (n = 2), and 2-di-n-butylaminoethanol (n = 3) plus 1.0 or 0.5 μM mechlorethamine hydrochloride, respectively, at 37°C. The cells were processed in the same manner as described in the legend under Fig. 1.*

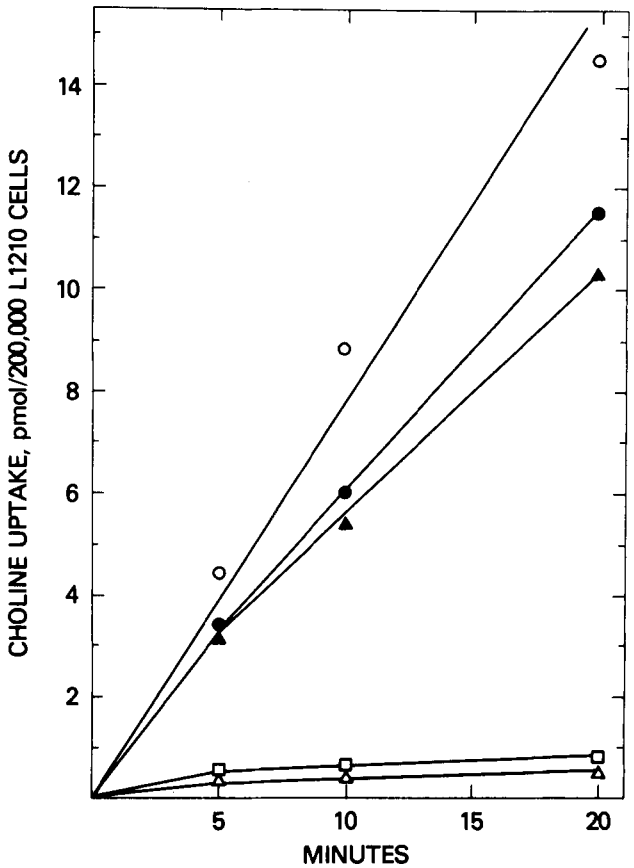

Figure 8—*Inhibition of choline uptake by 2-dimethylaminoethanol and its analogues generated by methylene additions into the dialkyl branches. A volume of a L1210 cell suspension at a density of 2 × 10^6/mL was added to an equal volume of transport medium at 37°C containing 1.0 μM [^{3}H]choline (○) plus 200 μM 2-dimethylaminoethanol (△), 2-diethylaminoethanol (□), 2-di-n-propylaminoethanol (●), or 2-di-n-butylaminoethanol (▲). The cell suspension was sampled and the incorporated [^{3}H]choline was counted as described in the legend under Fig. 2.*

the concentration used was not toxic to the bone marrow cells. Though an explanation for this persistent feature is not known, bone marrow precursor cells may have an increased number of choline transport sites or these sites may have a greater affinity for the various transformation products of mechlorethamine. In the milieu of the incubating bone marrow progenitor cells, the molar ratio of protector to mechlorethamine is 400:1, compared with 200:1 in that of the L1210 cells. Therefore, a counterflow phenomenon may exist (18). The increased intracellular concentration of protector may result in a preferential influx of mechlorethamine into the bone marrow progenitor cells.

The molecular transformations of mechlorethamine vary according to pH, and these products have different activities (13, 14). 2-Methyl-*n*-propylaminoethanol is an isostere of a hydrolysis product of mechlorethamine, 2-chloroethyl-2-hydroxyethylmethylamine. The isostere and its higher homologue, 2-*n*-butylmethylaminoethanol, are poor protectors against mechlorethamine cytotoxicity (Fig. 5); yet, both are potent inhibitors of choline uptake into the L1210 cells (Fig. 6). Another nonparallel finding between protection and inhibition is the potent protection against mechlorethamine cytotoxicity and the poor inhibition of choline uptake by 2-di-*n*-butylaminoethanol (Figs. 7 and 8). Using protection as an indicator of transport would have failed to identify an inhibitor of choline uptake in the former case (Figs. 5 and 6), while using inhibition of choline uptake as an indicator of protection would have failed to identify a protector in the latter case (Figs. 7 and 8). There may not be a direct correlation between protection against mechlorethamine cytotoxicity and inhibition of choline uptake by a choline analogue. Preincubation of the L1210 cell suspension with the compound to be tested rather than simultaneous addition of the compound and mechlorethamine hydrochloride to the cell suspension was selected because this approach would most closely represent the *in vivo* situation of maximizing protection of the sensitive host tissues.

In vivo experiments of interest would be the use of 2-di-*n*-butylaminoethanol as a protector against mechlorethamine host toxicity, with the possibility of avoiding the dose-limiting complications of neurotoxicity imposed by a substrate of the choline transport carrier, and use of 5-dimethylaminopentanol as a possible differential protector of the bone marrow progenitor cells. In the series of protectors that were tested, 2-dimethylaminoethanol consistently showed maximum protection of bone marrow and L1210 cells and was used in large doses as a protector against mechlorethamine toxicity *in vivo* with no benefit in survival to the tumor-bearing animal. There may be a role for 2-dimethylaminoethanol as a potent detoxifying agent, as with thiosulfate (19).

The correlation of the structure–activity relationship of choline and its congeners that are used in other disciplines (2, 9–12) to that of the murine L1210 leukemia cell is striking. Though exceptions have been found (15), such substrates can be used to predict similar structure–activity relationships in tumor systems.

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Simple Rapid Method for the Preparation of Enteric-Coated Microspheres

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Abstract □ A method is presented for encapsulating high molecular weight biological materials such as viral antigen, concanavalin A, and other proteins with cellulose acetate phthalate. The method is simple, inexpensive, and rapid; the process takes ~15 min. Capsules generated by this method are produced as microspheres 1–3 mm in diameter. They are stable for at least 6 h in simulated gastric conditions, but disintegrate rapidly under simulated intestinal conditions. Encapsulation had no effect on the activity of the biological materials. The method has potentially wide application for encapsulation of drugs and other substances.

Keyphrases □ Microspheres—enteric-coated, method for rapid preparation, encapsulation, cellulose acetate phthalate □ Encapsulation—method for the rapid preparation of enteric-coated microspheres, cellulose acetate phthalate □ Cellulose acetate phthalate—method for the preparation of enteric-coated microspheres, encapsulation □ Delivery systems—enteric-coated microspheres, cellulose acetate phthalate, method for rapid preparation

Cellulose acetate phthalate (I) has been used extensively as an enteric coating. Due to the presence of ionizable phthalate groups, the polymer is insoluble in acid media ≤pH 5, but is soluble when the pH is ≥6 (1). Since it is also remarkably inert *in vivo* (2), it is used to coat material for the release of drugs and other substances in the intestine. In recent years, I-coating technologies have been applied to the encapsulation of many biologically active materials, ranging from low molecular weight drugs [*e.g.*, sodium salicylate and phenacetin (3, 4)] to microorganisms [*e.g.*, viruses and bacteria (5–7)].

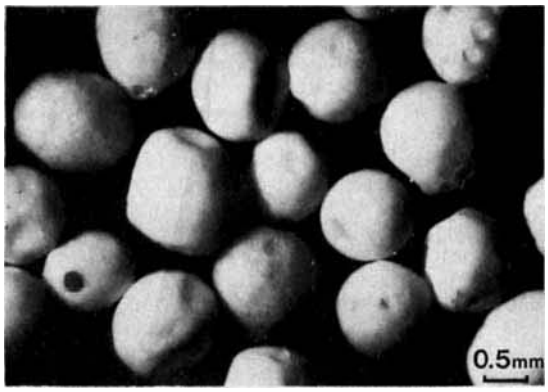

Figure 1—*Sucrose microspheres prepared as detailed in the text (formation time: 5 min).*

This report describes the development of an enteric coating for an oral vaccine used to protect wildlife against rabies. Studies on the vaccine itself will be reported elsewhere. The present report describes the principles of a method for encapsulation of the vaccine in the form of quasi-spherical particles ~1–3 mm in diameter (microspheres). The method is simple, rapid, and can be used to encapsulate a wide variety of materials. Therefore it has potential applications other than vaccine encapsulation.

EXPERIMENTAL

Materials—Core materials (*i.e.*, high molecular weight materials) that were encapsulated included rabies antigen (ERA-H strain of virus grown in BHK-21 cells and inactivated with β-propiolactone[1]), concanavalin A[1], and bovine serum albumin[1]. Radiolabeling of these materials with iodine-125 was carried out essentially as described by Thorell and Larson (8). Before use, the labeled preparations were passed through columns of Sephadex G-25[2] and extensively dialyzed against phosphate-buffered

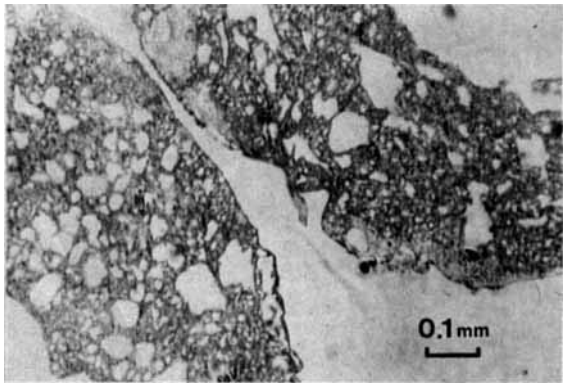

Figure 2—*Paraffin section (hematoxylin–eosin stain) of part of two sucrose microspheres showing the I matrix and the randomly distributed pockets that contained microparticles of the sucrose/core material. Hollow interiors of the microspheres are at the top right and bottom left of the photomicrograph.*

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Pharmacia, Uppsala, Sweden.

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