a mixt of product and unreacted starting material, and the free base was chromatogd on a neutral alumina column, the first 150 ml of petr ether yielding the product as a pale yellow oil (1.3 g). Treatment of the oil with Et_2O–HCl gave the 2-HCl salt as a white solid, mp 198–200° (1.4 g; 30.5%). *Anal.* ($C_{15}H_{23}BrN_2 \cdot 2HCl$) C, H, N.

1-Acetyl-2-(*N*-acetyl-*N*-isopropylaminomethyl)-7-cyano-6-methyl-1,2,3,4-tetrahydroquinoline.—A 30% soln of $NaNO_2$ in H_2O at 0° was added until just in excess to a stirred ice-cold soln of 1-acetyl-2-(*N*-acetyl-*N*-isopropylaminomethyl)-7-amino-6-methyl-1,2,3,4-tetrahydroquinoline·HCl (6.0 g) in HCl (4.3 ml) and crushed ice (17 g). This soln of the diazonium salt was neutralized by the addn of K_2CO_3 and then added to a stirred CuCN soln[35] [prepd from Cu_2SO_4 (5.28 g)] covered with a layer of PhMe, care being taken to keep the temp at 0–3°. After 0.5 hr at this temp the reaction mixt was allowed to warm up to room temp and kept overnight. The soln was then heated at 50° for 0.5 hr and cooled, and the PhMe was sepd and evapd *in vacuo*. The crude product recrystd from Et_2O–petr ether as a tan powder, mp 120° (2.9 g; 57.5%). *Anal.* ($C_{19}H_{25}N_3O_2$) N.

7-Cyano-2-(*N*-isopropylaminomethyl)-6-methyl-1,2,3,4-tetrahydroquinoline (38).—A soln of 1-acetyl-2-(*N*-acetyl-*N*-isopropylaminomethyl)-7-cyano-6-methyl-1,2,3,4-tetrahydroquinoline (1.5 g) in 5 *N* HCl (20 ml) was refluxed for 1 hr; after this time an ir anal. indicated absence of C=O absorpn but the C≡N group still remained. The reaction mixt was cooled, poured into H_2O, basified with K_2CO_3, and extd into $CHCl_3$. Evapn of the dried ($MgSO_4$) ext gave a red oil which was dissolved in EtOAc (5 ml) and treated with a soln of maleic acid (315 mg) in EtOAc (2 ml). The maleate salt had mp 190° (950 mg; 57%). *Anal.* ($C_{15}H_{21}N_3 \cdot C_4H_4O_4$) C, H, N.

7-Carbamoyl-2-(*N*-isopropylaminomethyl)-6-methyl-1,2,3,4-tetrahydroquinoline (39).—A soln of **38** (2.0 g) in 80% H_2SO_4 (15 ml) was heated on the steam bath for 1 hr. The mixt was cooled, poured onto ice, basified with K_2CO_3, and extd with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ ext was evapd and the crude solid recrystd from C_6H_6–petr ether (bp 40–60°) to yield a brown solid, mp 133° (1.6 g; 74%). *Anal.* ($C_{11}H_{23}N_3O$) C, H, N.

7-Amino-2-(*N*-isopropylaminomethyl)-6-methyl-1,2,3,4-tetrahydroquinoline (40).—A soln of **10** (0.6 g) in EtOH (150 ml) was hydrogd over Pd/C (60 mg) at an initial H_2 pressure of 7.03 kg/cm² for 3.5 hr. The catalyst was removed by filtn, and the EtOH was evapd to yield a viscous oil (0.4 g) which was dissolved in Et_2O and treated with dry HCl. The product was dried *in vacuo* over KOH and P_2O_5 for 2 days to yield a white hygroscopic powder, mp 160° dec (0.5 g; 64.5%) which analyzed as the 3-HCl salt. *Anal.* ($C_{14}H_{23}N_3 \cdot 3HCl$) C, H.

(35) J. W. Hickinbottom, "Reactions of Organic Compounds," 3rd ed, Longmans, London, 1959, p 493.

Acknowledgments.—We thank Dr. E. R. H. Jones for his encouragement and advice during the course of these investigations, Dr. R. Foster and his colleagues for the biological evaluations, Mr. R. F. Chambers, Mr. J. Graves, and Mr. P. Sherrington for excellent technical assistance, Dr. N. Scollick and his colleagues for carrying out important process improvements, and Dr. M. J. Sewell and his staff for analytical services.

Synthesis of Aminoethyl Derivatives of α,ω-Alkylenediamines and Structure–Activity Relationships for the Polyamine–Bovine Plasma Amine Oxidase System[1,2]

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Growth-inhibitory activity in mammalian cell and bacterial systems, as well as phagocidal action on T-uneven phages, of the naturally occurring polyamines, spermine and spermidine, is known to be the result of conversion of the polyamine to a cytotoxic derivative by means of the enzyme bovine plasma amine oxidase (BPAO). In an attempt to define the geometry of the substrate molecule required for this conversion, a number of polyamine structural variants were examined for growth-inhibitory activity against KB cells (human epidermoid carcinoma) in culture in media supplemented with calf serum (contains BPAO). During these studies, it became necessary to assay some polyamines, other than diethylenetriamine and triethylenetetramine, with 2-aminoethylamino terminal groupings. Such compounds were prepared expeditiously by direct mono- and diaminoethylation of α,ω-alkylenediamines; the diaddition products from these reactions, however, are not of unequivocal structure and it required X-ray diffraction powder analysis to characterize the products as the desired bis-substituted derivatives. Correlation of ID_{50} values with molecular structure indicates that the terminal grouping $H_2N(CH_2)_3NH$ is essential for inhibitory activity and that the secondary amino group must be at least 3 carbon atoms removed from the next basic center. These findings suggest the existence of a hydrophobic region adjacent to the active site of BPAO. We believe that the failure of certain amines to undergo oxidative deamination in the presence of BPAO is related to their inability to bond at this hydrophobic region.

For some time, we have been engaged in a program of synthesis of analogs of the biogenetic amines spermidine (**1**, $x = 4$) and spermine (**2**, $x = 4$) as a source of potential antitumor substances. In connection with this program, we previously reported the synthesis of some homologs of spermidine and spermine;[3] these products retained the 3-aminopropyl terminal function which is present in the naturally occurring polyamines, but showed variation of the putrescine portion of the molecule from 2 through 12 methylene units. A number of these substances were found to inhibit the growth of transplantable mouse tumors *in vivo*.[3,4] The tetrahydrochloride salt of **2**, $x = 9$, was particularly effective against a broad spectrum of experimental tumor systems in mice, rats, and hamsters.[4,5] Against the murine C1498 myeloid leukemia, this agent significantly inhibited tumor growth at the implant site and prevented leukemic infiltration in distant organs.[5]

(1) This investigation was supported in part by Research Grant C6516 and Research Career Development Award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

(2) Brief accounts of this work have appeared. Chemistry: M. Israel and B. M. Wentworth, Abstracts of Papers, First Northeast Regional Meeting, American Chemical Society, Boston, Mass., Oct 1968, p 40. Structure–Activity Correlations: M. Israel and E. J. Modest, Abstracts of Papers, XXIIIrd International Congress of Pure and Applied Chemistry, Boston, Mass., July 1971, p 87.

(3) M. Israel, J. S. Rosenfield, and E. J. Modest, *J. Med. Chem.*, **7**, 710 (1964).

(4) M. Israel, C. L. Maddock, and E. J. Modest, Abstracts of Papers, Ninth International Cancer Congress, Tokyo, Japan, Oct 1966, p 320.

(5) M. Israel and E. J. Modest, Abstracts of Papers, Tenth International Cancer Congress, Houston, Texas, May 1970, p 682.

1 **2**

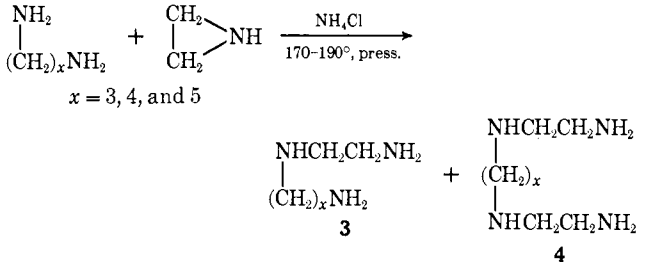
The mechanism of *in vivo* antitumor action of the synthetic polyamines is still unclear. However, from a number of *in vitro* studies[3,6–11] it is known that spermine is converted into a cytotoxic derivative by the action of an amine oxidase found in calf serum. Bovine plasma amine oxidase is one of a number of enzymes from diverse biological sources capable of effecting oxidative deamination of spermine and spermidine.[12,13]

A possible relationship between the growth-inhibitory activity of spermine *in vitro* and the antitumor activity of synthetic polyamines *in vivo* was explored in part by means of a cell culture bioassay system. The large number of polyamine structural variants available from our synthetic program were systematically examined for growth-inhibitory activity against KB cells, derived from a human nasopharyngeal carcinoma, in media supplemented with calf serum. As the cell culture studies progressed, it became apparent that, to complete a structure–activity correlation, a few polyamine derivatives incorporating a 2-aminoethyl terminal grouping would be required for bioassay. In the present paper we describe the synthesis and characterization of some 2-aminoethyl polyamines and the structure–activity relationships for the polyamine–bovine plasma amine oxidase system. The latter studies have led to a definition of the substrate requirement for the enzyme reaction.

Chemistry.—Direct aminoethylation of α,ω-alkylenediamines with ethylenimine (Scheme I) appeared to offer a possible one-step synthesis of triamines **3** and tetramines **4** containing terminal aminoethyl functions. The addition of ethylenimine to unsubstituted alkylenediamines has not been previously reported. We based our approach, however, upon the known addition of ethylenimine to NH_3 and aliphatic amines. Coleman and Callen[14] prepared a number of N-substituted ethylenediamines by treatment of various primary and secondary amines with ethylenimine in refluxing hydrocarbon solvent; $AlCl_3$ catalyst was found to facilitate the reaction but necessitated the use of H_2O during the work-up of the products. Clapp[15] later reported that the use of H_2O could be avoided in this reaction by heating the amine and ethylenimine under pressure in the presence of NH_4Cl, a weak acid catalyst. The latter conditions were employed in the present study.

One equiv of the appropriate alkylenediamine (1,3-propanediamine, 1,4-butanediamine, and 1,5-pentanediamine were used in this investigation) and 2 equiv of ethylenimine were heated with internal agitation in a stainless steel autoclave at 170–190° in the presence of

SCHEME I

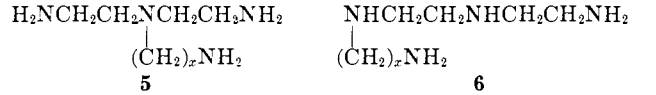


$x = 3, 4,$ and 5

3 **4**

NH_4Cl; reaction pressures rarely exceeded 10.5 kg/cm² (150 psi). High vacuum distillation of the crude reaction mixtures afforded a low boiling fraction (unchanged diamine), a middle fraction (monoaminoethyl derivative), and a high boiling fraction. Microchemical analysis of the high boiling material was found, in each instance, to be consistent with the addition of 2 molecules of ethylenimine to the original diamine. Analytical values (Table I) were obtained on center distillation cuts of the mono- and diaminoethyl products and on solid salt derivatives of each base. Yields of the mono- and disubstituted products were somewhat variable but generally of the order of 25% for the monosubstituted derivatives and 40–50% for the disubstituted derivatives; unchanged starting material and polymeric products accounted for the remainder of the material balance.

It is evident from Scheme I that the monoaminoethyl products, **3**, are of unambiguous structure and their characterization required little more than confirmatory analytical data.[3,16] The diaminoethyl products from these reactions, however, are not of unequivocal nature. Addition of ethylenimine at each end of the starting diamine would result in the desired products, **4**. However, the possible intermediacy of **3** could also permit ethylenimine to add at either the newly formed secondary amine to give **5** or at the newly formed terminal amine to afford **6**. Thus, 3 possible isomeric structures had to be considered as a result of the uncertainty of the position of attack of the second ethylenimine molecule.



5 **6**

Differentiation of the isomeric structures **4** and **6** from **5** was accomplished by means of functional group analysis. Isomers **4** and **6** are both linear and are thus required to have 2 primary amino groups; **5** is a branched isomer and must contain 3 primary amino groups. Samples of the disubstituted products, analytically pure and homogeneous by paper chromatography, were examined for primary amino N content *via* the Van Slyke method and, as indicated in Table I, were found, in each instance, to possess only 2 primary amino groups.

Discrimination between **4** and **6**, which differ only in the transposition of a single CH_2, was a more formidable task. In order to assign correct structures to the diaminoethyl products, we elected to compare a sample of the disubstituted derivative from the reaction of ethylenimine and 1,3-propanediamine with samples of **4** ($x = 3$) and **6** ($x = 3$), prepared by unambiguous syn-

(6) J. G. Hirsch and R. J. Dubos, *J. Exp. Med.*, **95**, 919 (1952); J. G. Hirsch, *ibid.*, **97**, 323 (1953).

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(14) G. H. Coleman and J. E. Callen, *J. Amer. Chem. Soc.*, **68**, 2006 (1946).

(15) L. B. Clapp, *ibid.*, **70**, 184 (1948).

(16) It should be noted that samples of *N*-(2-aminoethyl)propane-1,3-diamine (**3**, $x = 3$) prepd from 1,3-propanediamine and ethylenimine were identical with samples of **1**, $x = 2$, previously prepd in these laboratories by cyanoethylation of ethylenediamine, followed by catalytic reduction of the resulting nitrile.[3]

TABLE I

2-AMINOETHYL DERIVATIVES *via* AMINOETHYLATION OF ALKYLENEDIAMINES

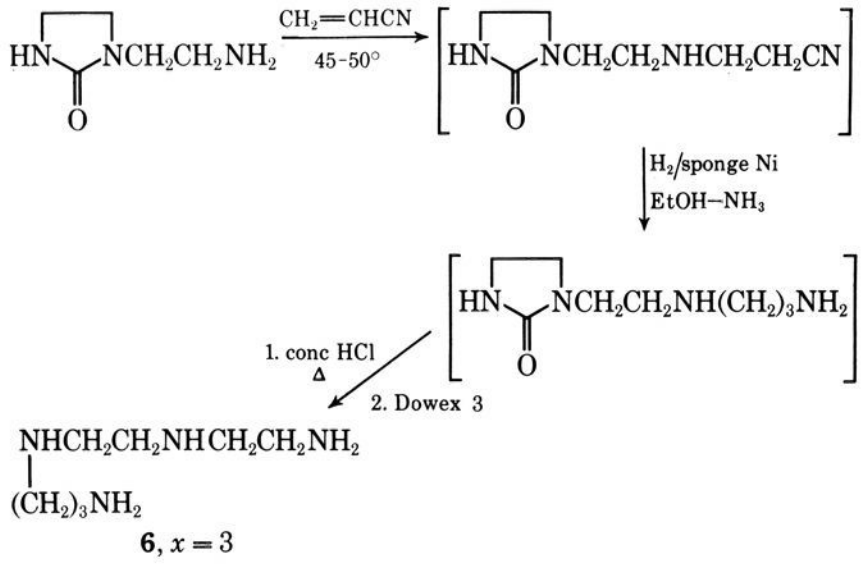
Compd	Base: Bp (mm), °C[a]	Base: Formula	Base: Analysis[b]	Hydrochloride: Mp, °C[c]	Hydrochloride: Analysis[b]
3, $x = 3$[d]	66 (0.1)	$C_5H_{15}N_3$		225–228[e]	
3, $x = 4$	52–54 (0.08)	$C_6H_{17}N_3$	C, H, N	235 dec	C, H, Cl, N
3, $x = 5$	85 (0.35)	$C_7H_{19}N_3$	C, H, N	224.5 dec	C, H, Cl, N
4, $x = 3$	105–108 (0.25)	$C_7H_{20}N_4$	C, H, N	275 dec	C, H, Cl, N[f]
4, $x = 4$	68.5 (0.006)	$C_8H_{22}N_4$	C, H, N	211.5 dec[g]	C, H, N[f,g]
4, $x = 5$	135–136 (0.1)	$C_9H_{24}N_4$	C, H, N	240 dec	C, H, Cl, N[f]

[a] The boiling points listed are those of the center distn fraction from which the sample was withdrawn for analysis. [b] Where analyses are indicated by symbols, the elements or functions were within ±0.4% of the calcd values. [c] HCl salts were cryst from aq EtOH and dried at 70° *in vacuo* for 17 hr. [d] This product was identical with material previously obtained by cyanoethylation of ethylenediamine, followed by catalytic reduction.[3] [e] Lit.[3] mp 223–228°. [f] Total N and primary amino N. [g] Tetraoxalate salt; prepd by addn of an EtOH soln of the base to a satd soln of oxalic acid in EtOH, followed by crystn from aq EtOH.

thesis. As will become apparent shortly, a comparison involving an authentic sample of only 1 of the 2 possible isomers would have been insufficient for characterization.

The sample of **4**, $x = 3$, was prepared according to the procedure of vanAlphen[17] by reaction of 1,3-dibromopropane with excess ethylenediamine in EtOH.[18] Compound **6**, $x = 3$, was obtained by the sequence shown in Scheme II. Reaction of *N*-(2-aminoethyl)-

SCHEME II



imidazolidone with acrylonitrile afforded a cyanoethyl derivative, which was not purified. Hydrogenation of the nitrile in the presence of sponge Ni catalyst and NH_3, followed by acid hydrolysis of the imidazolidone moiety, gave the desired product. Each authentic polyamine sample was characterized both as the free base and the tetrahydrochloride salt.

We have previously noted[3] that the ir spectra of homologous polyamines show little difference from compound to compound, either as the free base or as the HCl salt. Similarly, the ir spectra of the isomeric bases **4** and **6**, $x = 3$, were essentially identical; the spectra of the tetrahydrochloride salts initially showed some differences in intensity of certain absorption signals, but, upon further examination, most of these were attributable to differences in the preparation of the KCl disks. The two bases gave the same nmr spectrum and they had the same retention time on vpc. The tetrahydrochloride salts both sintered at 247° and melted into black tars at about 260°; a mixture of the 2 salts showed no alteration of behavior on decomposition. To establish an abs characterization of our ambiguous reaction product, we therefore sought an analytical method which would clearly differentiate the 2 isomers. In this regard, examination of the tetrahydrochloride salts of **4** and **6**, $x = 3$, by X-ray diffraction powder analysis was expected to be definitive on the basis of symmetry arguments: isomer **4**, regardless of x, possesses a point of symmetry in the molecule, whereas **6** is inherently unsymmetrical. Although there was a remote possibility that the X-ray powder patterns might be similar due to the similarity of H-bonded dimers in the crystal state, actually the diffraction patterns for the tetrahydrochloride salts of **4** and **6**, $x = 3$, were clearly different (Figure 1). The diffraction pattern given by

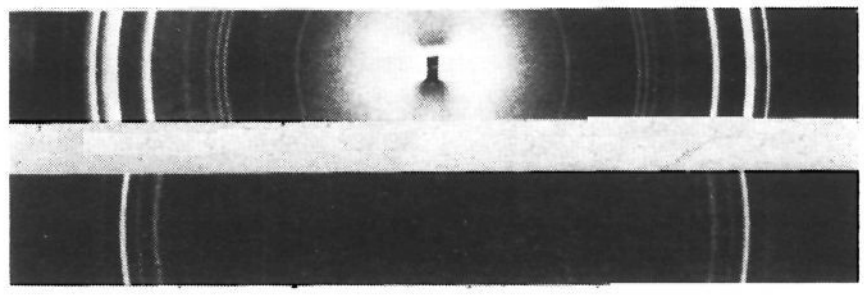


Figure 1.—X-ray diffraction powder patterns of **4**, $x = 3$, tetra hydrochloride (upper) and **6**, $x = 3$, tetrahydrochloride (lower)-prepared by unambiguous syntheses. The tetrahydrochloride salt of the ethylenimine-1,3-propanediamine diaddition product gave a diffraction pattern which was completely superimposable on that of **4**, $x = 3$, tetrahydrochloride.

the salt of the disubstituted propanediamine product was identical with that of authentic **4**, $x = 3$, tetrahydrochloride; the X-ray diagrams were obtained on samples which had all been crystallized in exactly the same manner and dried under identical conditions.

These findings thus established that the addition of ethylenimine had occurred at each end of the diamine to give the desired bis-substituted derivative.

Structure–Activity Studies.—The distribution, biochemistry, and pharmacology of spermine and spermi-

(17) J. vanAlphen, *Recl. Trav. Chim. Pays-Bas*, **55**, 835 (1936).

(18) The product, *N*,*N*′-bis(2-aminoethyl)propane-1,3-diamine, is available from Eastman Organic Chemicals, Rochester, N. Y. We thank Mr. R. C. Hapeman, Eastman Organic Chemicals Department, Eastman Kodak Company, for technical information and for a sample of their material.

dine have been described in a review.[19] Of interest here is the fact that, *in vitro*, these polyamines have been shown to stabilize nucleic acids, to complex with ribosomes and other subcellular components, and to affect cell membrane stability.[19–21] Yet, under certain experimental conditions spermine and, to a lesser extent, spermidine, cause significant inhibition in the growth of bacteria,[6,7] chick embryo fibroblasts,[10] mammalian spermatozoa,[7] and a variety of mammalian cell lines in culture.[3,8,9,11] This growth-inhibitory activity is known to be due to the action of the enzyme–bovine plasma amine oxidase [amine: O_2 oxido-reductase (deaminating)] on the polyamine; the enzyme is a normal component of calf serum,[12] which is used to supplement the bioassay media. It has been suggested[22,23] that oxidative deamination of the polyamine by the enzyme results in the formation of cytotoxic β-aminoaldehyde derivatives, although Alarcon[24] ascribes the cytotoxic effect to acrolein rather than to the initial enzymic product. "Oxidized spermine" has also been shown to inactivate various bacterial, plant, and animal viruses.[25–30]

The availability of a variety of amine derivatives from our synthetic program provided us with an opportunity to delimit the structural feature of a polyamine required by bovine plasma amine oxidase for formation of the cytotoxic derivative. The bioassay procedure involved determination of the amount of agent necessary to inhibit by 50% the growth of KB cells (human epidermoid carcinoma) in a culture system containing calf serum;[31] as with murine leukemic cells,[9] substitution in this system of horse serum, which lacks an enzyme with spermine specificity, for calf serum resulted in loss of growth inhibition. At the beginning of our studies, little was known of the substrate specificity of bovine plasma amine oxidase, other than its high rate of oxidative attack on spermine and spermidine and its lack of significant oxidation of typical monoamines (*n*-$PrNH_2$ and $Ph(CH_2)_2NH_2$, for example) and short-chain diamines (putrescine, cadaverine, histamine) and of diethylenetriamine and triethylenetetramine.[12,13,32] However, in addition to the spermine and spermidine specificity, bovine plasma amine oxidase is reportedly able to function as a typical monoamine oxidase with such substrates as 10-aminodecanoic acid and 1,10-diaminodecane.[12,33,34]

In a previous report,[3] we described the synthesis of some homologs of spermidine and spermine. The compounds were prepared by mono- and bis-cyanoethylation of α,ω-alkylenediamines, followed by reduction to the appropriate triamine **1** and tetramine **2**, where x = 2, 3, 4, 5, 6, 9, 10, and 12. The monocyanoethyl derivatives, **7**, may be considered as a series of substituted amines containing terminal aminoalkyl functions of varying chain length attached at a secondary amino group. As shown in Table II, of the monocyanoethyl compounds derived from short-chain diamines, only **7**, x = 3, was active in the range of concn comparable to spermidine (**1**, x = 4) and spermine (**2**, x = 4). This agent possesses, in common with spermidine and spermine, a 3-aminopropyl terminal grouping attached at a secondary amine; cyanoethyl derivatives containing 2-aminoethyl, 4-aminobutyl, 5-aminopentyl, and 6-aminohexyl terminal functions were not growth inhibitory in the presence of calf serum. The growth-inhibitory activity of **7**, x = 3, and the compound's structural resemblance to the naturally occurring polyamines suggested that the 3-aminopropyl chain attached at a secondary amine might be the structural feature which, under the influence of the enzyme, gives rise to cytotoxicity.

The hypothesis was initially beclouded, however, by the observation that mono- and dicyanoethyl derivatives of longer chain diamines (**7** and **8**, x = 9, 10, 12, resp) showed a gradual increase in inhibitory activity. We now believe this activity to be unrelated to polyamine specificity, but rather to be an indication of the enzyme's ability to function, in the presence of particular substrates, as a typical intracellular monoamine oxidase or by some other, as yet undefined, mechanism.[34]

The various triamines **1** and tetramines **2** listed in Table II all have the 3-aminopropylamino end unit. In line with the hypothesis, all the synthetic polyamines were found to be inhibitory in the same range of concentration as spermine, *except*, curiously, for **1**, x = 2, and **2**, x = 2, those products derived from ethylenediamine, which were totally inactive.

Compounds **1** and **2**, x = 2, although containing 3-aminopropylamino residues, have another basic N function located just 2 atoms removed from the secondary amine point of attachment. The lack of inhibitory activity of these products led us to suggest earlier that the presence of another basic group in close proximity to the secondary amine attachment interfered with enzymatic oxidation of the 3-aminopropylamine system.[11]

Hirsch[32] observed that diethylenetriamine and triethylenetetramine in the presence of bovine plasma amine oxidase failed to inhibit the growth of *Mycobacterium tuberculosis*. Alarcon[35] made a similar observation with murine sarcoma (S-180) cells in culture and we have confirmed these findings against KB cells in the present investigation. However, in view of our observation with **1** and **2**, x = 2, it was conceivable that the lack of inhibitory activity of diethylenetriamine and triethylenetetramine might be due, not to the 2-C aminoethyl terminal group, but rather to the close proximity of the two secondary amino functions. Put in another way, we wondered if compounds with terminal aminoethylamino groups might be active in the

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(34) H. Blaschko, S. M. Kirpekar, and S. D. Phipps, *Arch. Int. Pharmacodyn.*, **139**, 120 (1962).
(35) R. A. Alarcon, *Arch. Biochem. Biophys.*, **106**, 240 (1964).

TABLE II

INHIBITORY ACTIVITY (ID_{50} VALUES) OF SOME AMINE DERIVATIVES AGAINST KB CELLS IN CULTURE IN THE PRESENCE OF BOVINE PLASMA AMINE OXIDASE[a,b]

	$NHCH_2CH_2CN$ | $(CH_2)_xNH_2$		$NHCH_2CH_2CN$ | $(CH_2)_xNHCH_2CH_2CN$		$NH(CH_2)_3NH_2$ | $(CH_2)_xNH_2$		$NH(CH_2)_3NH_2$ | $(CH_2)_xNH(CH_2)_3NH_2$	
	7		**8**		**1**		**2**	
x	μg/ml	mmoles[c]/ml	μg/ml	mmoles[c]/ml	μg/ml	mmoles[c]/ml	μg/ml	mmoles[c]/ml
2	>1000	>537	>1000	>418	>1000	>441	>1000	>312
3	3.0	1.5	>1000	>395	7.3	3.03	4.5	1.35
4	>100	>46.7	>100	>374	5.0	1.96	5.8	1.67
5	>100	>43.8			2.8	1.04	4.8	1.32
6	>100	>41.3	95	32.2	14	4.95	2.2	0.82
9	>100	>35.2	82	24.3	4.0	1.23	4.3	1.03
10	52	17.4	52	14.8	3.8	1.12		
12	23	7.1	5.0	1.32	5.0	1.36		

[a] Serially propagated cell line (human epidermoid carcinoma) in media supplemented with 5% whole calf serum (contains spermine-specific plasma amine oxidase—see text). Compds are considered highly active in this system if a dose of less than 5×10^{-5} mmole/ml (usually less than 10 μg/ml) inhibits the growth of the culture by 50%. [b] Compds were assayed in the form of their HCl salts. [c] mmoles $\times 10^5$.

polyamine–bovine plasma amine oxidase system if the next basic center was separated by a distance greater than 2 CH_2 units. The lack of availability of compounds fitting the structural requirements for bioassay necessitated the synthesis of the polyamine derivatives described in the first part of this report. In all instances, compounds containing 2-aminoethylamino terminal residues were found to be inactive in the KB assay systems in the presence of calf serum regardless of the distance of the next amino function.

Correlation of ID_{50} values with the molecular structure of a large number of amines, including synthetic products not yet reported from these laboratories and various commercially available substances, has led to a generalization regarding the polyamine structural geometry required by bovine plasma amine oxidase. The requisite feature (Figure 2) which results in cytotoxicity

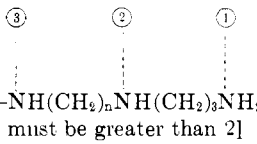

Figure 2.—Substrate requirement for the polyamine–bovine plasma amine oxidase system. The presence of another basic function (N^3) 2 C atoms removed from N^2 apparently interferes with enzyme–substrate complex formation.

appears to be a primary amino group (N^1) separated from a secondary amine (N^2) by 3 CH_2 units. Alarcon, in a parallel investigation, has arrived at a similar conclusion based upon a limited and less systematic selection of agents.[35] Our generalization, however, requires the further restriction that the next basic center (N^3) be separated from N^2 by 3 or more CH_2 units. We previously suggested this substrate requirement on the basis of preliminary data.[11] The results just described thus confirm our earlier hypothesis.

In terms of the enzyme, we believe our results indicate the presence of a hydrophobic region on bovine plasma amine oxidase near the active site. It appears that the active site reaction, which has been shown to be oxidative deamination of primary amino groups in spermine[22,23] and long-chain amine derivatives,[12,33,34] must be preceded or accompanied by hydrophobic bonding between enzyme and substrate molecules in the region near the active site. Thus, the location of an additional polar function (N^3) in **1** and **2**, $x = 2$, does not allow for hydrophobic bonding; cytotoxicity is not seen with these compounds even though the requisite 3-aminopropylamino grouping is present in the molecule. The hypothesis of a hydrophobic bonding requirement for bovine plasma amine oxidase is consistent with the findings of Blaschko, *et al.*,[34] who demonstrated the decreasing rate of oxidation of ω-aminoalkanoic acids with decreasing chain length by goat plasma amine oxidase, which, like the bovine enzyme, is derived from the plasma of a ruminant and is highly spermine specific. The carboxylic acid function in 6-aminohexanoic acid approximates the location of N^3 in **1** and **2**, $x = 2$; this compound is not significantly oxidized, although 8-aminooctanoic and 10-aminodecanoic acids are oxidized at a high rate.[34]

The presence in various mammalian tissues of enzymes capable of effecting the oxidative deamination of spermine[12,13] suggests that the *in vitro* antitumor effects of compounds containing terminal 3-aminopropylamino functions may very well be related to the cytotoxic effects of such agents in microbiological, viral, and cell culture systems; we are continuing to explore this possibility.

Experimental Section[36]

Ir spectra were obtained by means of a Perkin-Elmer Model 137B spectrophotometer; spectra of the free bases were detd in $CHCl_3$ or as thin films, and those of the salt derivatives as KCl disks. Melting points were taken by the capillary method in a modified Wagner-Meyer melting point apparatus[37] at a heating rate of 1°/min within 10° of the mp and are corrected.

Apparatus.—High efficiency distn equipment was required for the work-up of the ethyleneimine reactions. Crude reaction mixts were first distd under vacuum with the aid of a Nester/Faust Model NF-130 lab size spinning band distn assembly.[38] Purification of all fractions was accomplished by means of high vac distn with a Nester/Faust Model NF-117 semimicro spinning band distn assembly equipped with an automatic reflux ratio control timer set for a ratio of 60:2 sec. Both units were

(36) Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Werby Laboratories, Inc., Boston, Mass. Primary amino nitrogen analyses (Van Slyke) were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Where analyses are indicated by symbols, the elements or functions were within ±0.4% of the calculated values.

(37) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1938).

(38) The distillation equipment was purchased from the Nester/Faust Manufacturing Company, Newark, Del.

equipped with thermistor-controlled relay systems for maintenance of precise pot and column temps.

2-Aminoethylalkylenediamine Derivatives.—A mixt composed of 1 equiv of the appropriate α,ω-alkylenediamine, 2 equiv of cold ethylenimine, and 2 equiv of NH_4Cl was placed in a 1-l. stainless steel Magne-Dash autoclave (Autoclave Engineers, Inc., Erie, Pa.). It was heated for 48 hr at 170–190° with continuous internal agitation. After cooling, the slight residual pressure was vented and the contents of the bomb were transferred to a round-bottom flask with the aid of abs EtOH. The major portion of EtOH was removed on a rotary evaporator at water pump pressure. The remaining viscous liquid was distd under high vacuum on a spinning band apparatus. A low-boiling fraction consisting of unchanged diamine distd first, followed successively by the monoaminoethyl product and the diaminoethyl derivative. The products were purified further by careful fractionation under high vacuum; center distn cuts were taken for the analytical samples. HCl salts were prepd by saturating an EtOH soln of the base with dry HCl; Et_2O was added on occasion to completely ppt the salt. An oxalate salt of **4**, $x = 4$, was prepd in place of the HCl derivative, which could not be isolated.

Analytical values for the free bases and their salt derivatives are given in Table I.

***N,N'*-Bis(2-aminoethyl)propane-1,3-diamine (4, $x = 3$) by Unambiguous Synthesis.**—To a soln of 16.5 g (0.33 mole) of 1,2-ethanediamine in 50 ml of abs EtOH heated to 70° was added dropwise with stirring a soln of 14.2 g (0.07 mole) of 1,3-dibromopropane; a temp of 70–75° was maintained during the addn, after which time the reaction mixt was heated at reflux for 2 hr. The mixt was allowed to cool to 40° and KOH pellets (8.3 g) were added slowly. The mixt was allowed to stand at room temp overnight. The ppt of KBr was sepd and the EtOH was removed on the rotary evaporator. Distn under vacuum gave a forerun of unchanged 1,2-ethanediamine and a fraction (6.7 g, 60%), bp 122.5–126.5° (0.2 mm). This fraction was redistd and a cut boiling at 126° (0.2 mm) was collected for analysis and for conversion to the 4 HCl salt [lit.[17] bp 185–186° (35 mm)].

A sample of the anal. pure material was dissolved in anhyd EtOH, and the soln was satd with dry HCl. The product was crystd several times from aq EtOH for analysis; mp 260° dec. *Anal.* Calcd for $C_7H_{24}Cl_4N_4$: C, H, Cl, N

For the X-ray diffraction powder pattern, a sample of 4 HCl salt (100 mg) was recrystd from 1.3 ml of H_2O and 5 ml of EtOH. The hot soln was allowed to cool gradually to room temp, then was placed in the freezer for 4 hr. The crystals were collected, washed with EtOH and Et_2O, and dried in a drying pistol overnight at 38°.

1,4,7,11-Tetraazaundecane (6, $x = 3$).—Practical grade 1-(β-aminoethyl)-2-imidazolidone (Aldrich Chemical Company) was purified as its HCl salt by elution from a Dowex 50W-X4 column with 1 *N* HCl, evapn to dryness, and crystn from 95% EtOH. A sample of 1-(β-aminoethyl)-2-imidazolidone·HCl (20.0 g, 0.121 mole) was neutralized by passage through a Dowex 3 column. The eluate, collected until the pH became neutral, was taken to dryness on the rotary evaporator and the pale yellow oil was thoroughly dried, first by several azeotropic dists with EtOH, then with Na_2SO_4 after dissn in $CHCl_3$. The $CHCl_3$ was evapd and the yellow oil was warmed to 43°. To it was added dropwise with stirring 8.0 ml of acrylonitrile (6.4 g, 0.121 mole). After complete addn, the mixt was warmed at 45–50° for 2.5 hr. The pale green viscous liquid was dissolved in 50 ml of abs EtOH satd with NH_3, and the mixt was shaken under H_2 [initial pressure 3.37 kg/cm^2 (48 psi)] in the presence of Davison sponge Ni catalyst.[3,39] After reduction, the catalyst was sepd and the EtOH was removed under reduced pressure. To the viscous orange oil was added 250 ml of concd HCl, and the soln was refluxed for 24 hr. It was concd, treated with charcoal, and passed through a Dowex 3 column to neutralize remaining HCl. The eluate was taken to dryness and distd on the spinning band apparatus. The first fraction, bp 38–65° (0.05 mm), consisted mostly of diethylenetriamine; the next fraction, bp 70–81° (0.05 mm), was crude product. Redistn of this material gave a sample of **6**, $x = 3$, in anal. purity: bp 71–73° (0.03 mm). *Anal.* Calcd for $C_7H_{20}N_4$: C, H, N (total), N (primary amino).

A sample of the 4HCl salt was prepd in the usual fashion; mp 264° dec. *Anal.* Calcd for $C_7H_{24}Cl_4N_4$: C, H, Cl, N (total), N (primary amino).

X-Ray Diffraction Powder Patterns.—The X-ray source was a Jarrell-Ash small-focus tube with a Cu anode. The camera consisted of a bent and ground (Johansson type) quartz crystal, which focussed the Cu Kα radiation into a convergent beam whose vertical height was limited to about 0.5 mm by means of slits. The powdered samples were packed into quartz capillaries about 1 mm in diameter, and photographs were taken at a specimen to film distance of 5.0 cm.

Cell Culture Studies.—The various amine derivatives were examined for inhibitory activity against KB cells a serially propagated cell line derived from a human epidermoid carcinoma of the nasopharynx, in a minimal essential medium supplemented with 5% whole calf serum. The bioassay system has been described previously.[31] Compds contg terminal 3-aminopropylamino groups generally inhibited the growth of cells by 50% in this system at a concn range of $1–3 \times 10^{-5}$ mmole/ml (Table II).

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(39) H. N. Schlein, M. Israel, S. Chatterjee, and E. J. Modest, *Chem. Ind. (London)*, 418 (1964).