(0.10 mole) of 1-(2-diethylaminoethyl)-2-(1-phenyl-2-propyl)-hydrazine (X) in 500 ml. of 1 *N* hydrochloric acid was boiled under reflux for 15 hr. After cooling to room temperature, the orange-brown reaction mixture was extracted twice with 250 ml. portions of ether (extract A). The aqueous layer was then treated with 50 ml. of 50% sodium hydroxide solution and the alkaline mixture was again extracted twice with 250-ml. portions of ether (extract B). Subsequent addition of 200 g. of solid sodium hydroxide (cooling) and extraction gave a third ether solution (extract C). Each of the ether extracts was dried over anhydrous potassium carbonate, and the ether removed through a short Vigreux column to give residues A, B, and C.

Residue A weighed 0.5 g.; vapor phase chromatography[23] indicated that phenylacetone was the major component.

Residue B weighed 15.4 g.; vapor phase chromatography[23] indicated that amphetamine was the major constituent. A portion (14.9 g.) was distilled *in vacuo* through a short Vigreux column to give 10.3 g. (78%) of a colorless liquid, b.p. 82–87° (13 mm.). This material was shown to be identical with an authentic sample of *dl*-amphetamine by comparison of their infrared absorption spectra and their succinate salts, m.p. and mixture m.p. 161–163°.

Residue C weighed 3.3 g.; vapor phase chromatography[23] indicated that 2-diethylaminoethylamine was probably the major component. Distillation at atmospheric pressure gave 0.74 g. of a colorless liquid which was shown to be identical with an authentic sample of 2-diethylaminoethylamine by comparison of their infrared absorption spectra and their picrate salts, m.p. and mixture m.p. 209–211°.

Anal. Calcd. for $C_6H_{16}N_2 \cdot 2C_6H_3N_3O_7$: C, 37.63; H, 3.86; N, 19.51. Found: C, 37.92; H, 3.93; N, 19.51.

Treatment of 1-(2-Diethylaminoethyl)-2-(1-phenyl-2-propyl)-hydrazine (X) with Sodium Hydroxide.—A solution of 24.9 g. (0.10 mole) of 1-(2-diethylaminoethyl)-2-(1-phenyl-2-propyl)-hydrazine (X) in a mixture of 250 ml. of 1 *N* sodium hydroxide solution and 250 ml. of methanol was boiled under reflux for 16 hr. During the last 2 hr. of heating, 275 ml. of solvent was removed at atmospheric pressure. The orange-brown reaction mixture was cooled and extracted with two 250-ml. portions of ether. The combined ether extracts were dried over anhydrous potassium carbonate, the drying agent was collected, and the ether was removed through a short Vigreux column. The residue (25.2 g.) was distilled *in vacuo* to give 17.4 g. (70%) of a pale yellow liquid, b.p. 78–83° (0.08 mm.) which was identical with the starting material.

Acknowledgments.—The authors wish to express their appreciation to Dr. L. M. Long for his interest in and encouragement of this work. Further, we take this opportunity to thank Dr. G. M. Chen, Mr. C. Ensor, Dr. M. W. Fisher, Dr. J. R. McLean, Dr. P. E. Thompson, and Dr. E. E. Snell for the biological studies, and Dr. M. L. Black, Mr. C. E. Childs, Dr. F. S. Hom, Dr. D. H. Szulczewski, and Dr. J. M. Vandenbelt for microanalytical, spectral, and gas chromatography studies. We also wish to acknowledge the helpful discussions with Professor P. A. S. Smith and Professor C. L. Stevens regarding the hydrazine scission reaction.

Agents Affecting Lipid Metabolism. VIII. N,N′-Dibenzylethylenediamine, the Key to a Novel Class of Cholesterol Biosynthesis Inhibitors[1]

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A number of mevalonic acid analogs and their N,N′-dibenzylethylenediamine (DBED) salts have been tested for their effect on the incorporation of mevalonate-2-C^{14} into cholesterol by rat liver homogenates. In contrast to the mevalonic acid analogs, their DBED salts exhibited an inhibitory property which was found to reside in DBED itself. The site of action of DBED is at the level of the conversion of 7-dehydrocholesterol to cholesterol. DBED was moderately effective in lowering serum sterols in hypercholesterolemic rats. Molecular modifications of DBED have resulted in compounds of increased potency, showing activity, *in vitro*, at a concentration of 1×10^{-6} *M*.

In a previous publication[2] we reported the synthesis of a series of mevalonic acid analogs. Most of these analogs were oils and it was found convenient to characterize them as solid derivatives. For the purification and characterization of synthetic mevalonic acid itself,[3] the salt with N,N′-dibenzylethylenediamine (DBED) was employed and, consequently, this diamine was also our choice for the preparation of crystalline salts of our mevalonic acid analogs.

In due course, it was discovered that, in contrast to the mevalonic acid analogs, their salts with DBED inhibited the incorporation of mevalonate-2-C^{14} into cholesterol by rat liver homogenates and, quite unexpectedly, we found that the inhibitory activity resided in DBED itself. These results led us to investigate various analogs, homologs, and derivatives of DBED for their ability to inhibit cholesterol biosynthesis. DBED itself has also been studied *in vivo* with respect to its effects on rat serum sterol levels.

The discovery of the inhibitory effect of DBED on hepatic cholesterogenesis has eventually led to the synthesis of *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride,[4] a representative of a novel class of cholesterol biosynthesis inhibitors[5] which acts by interfering with the conversion of 7-dehydrocholesterol to cholesterol.[6] Thus, we have also studied the effect *in vitro* of DBED on the metabolism of 7-dehydrocholesterol. These results form the basis of this report.

(1) (a) Presented before the Division of Medicinal Chemistry, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 6–10, 1964; (b) part VII: D. Dvornik, M. Kraml, and J. Dubuc, *Proc. Soc. Exptl. Biol. Med.*, **113** (1964), in press; for part VI, see ref. 5.

(2) L. G. Humber, M. Kraml, J. Dubuc, and R. Gaudry, *J. Med. Chem.*, **6**, 210 (1963).

(3) C. H. Hoffman, A. F. Wagner, A. N. Wilson, E. Walton, C. Shunk, D. E. Wolf, F. W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, **79**, 2316 (1957).

(4) L. G. Humber, Belgian Patent 627,610 (1963); Derwent Belgian Patent Report 30 (1963).

(5) C. Chappel, J. Dubuc, D. Dvornik, M. Givner, L. Humber, M. Kraml, K. Voith, and R. Gaudry, *Nature*, **201**, 497 (1964).

(6) D. Dvornik, M. Kraml, J. Dubuc, M. Givner, and R. Gaudry, *J. Am. Chem. Soc.*, **85**, 3309 (1963).

Experimental[7]

The compounds investigated are listed in Tables I to IV. Some of these were prepared by methods previously described, others were obtained through the courtesy of Dr. A. Langis of these laboratories and of Dr. W. F. Bruce, Wyeth Laboratories, Philadelphia, Pa., as indicated by footnotes to the tables. The syntheses of the new compounds are described below.

N-Benzoyl-N′-benzylideneethylenediamine.—N-Benzoylethylenediamine[8] (10.0 g.) and benzaldehyde (8.16 g.) were refluxed in benzene (300 ml.) until the theoretical volume of water had been collected in a Dean–Stark trap. The benzene solution was concentrated to 100 ml. and the product was obtained as colorless crystals, m.p. 132–134° (14.0 g.).

Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.15; H, 6.39; N, 11.10. Found: C, 75.77; H, 6.51; N, 10.58.

N-Benzoyl-N′-benzylethylenediamine.—N-Benzoyl-N′-benzylideneethylenediamine (4.3 g.), dissolved in ethanol (80 ml.), was hydrogenated at atmospheric pressure and at room temperature with a platinum oxide catalyst (100 mg.). The theoretical amount of hydrogen was consumed in 2 hr. and the product was isolated in the usual manner. A hydrochloride salt was prepared and crystallized from 2-propanol, m.p. 206–208°.

Anal. Calcd. for $C_{16}H_{19}ClN_2O$: Cl, 12.19; N, 9.63. Found: Cl, 12.04; N, 9.55.

N,N′-Di(*p*-phenylbenzyl)ethylenediamine.—*p*-Phenylbenzaldehyde[9] (9.1 g.) and ethylenediamine (1.5 g.) were converted to the corresponding Schiff base by refluxing in benzene (80 ml.) for 3 hr., with continuous removal of the liberated water. It was dissolved in a methanol–benzene (2:1) mixture (100 ml.) and refluxed for 16 hr. with sodium borohydride (1.9 g.). The solvents were removed *in vacuo* and the residue distributed between chloroform and water. The chloroform phase yielded the product (8.7 g.), m.p. 150–151° (from acetone).

Anal. Calcd. for $C_{28}H_{28}N_2$: C, 85.65, H, 7.19; N, 7.14. Found: C, 85.33, H, 7.40; N, 7.01.

N,N′-Bis(2-hydroxyethyl)-N,N′-dibenzylethylenediamine.—N,N′-Dibenzylethylenediamine (7.1 g.) and 2-chloroethanol (9.66 g.) were combined in benzene (80 ml.) and refluxed for 5 hr. The mixture was made basic with 10% aqueous sodium hydroxide and the benzene phase was dried and distilled to yield 3.2 g. of product, b.p. 180° (0.15 mm.). The dihydrochloride salt was crystallized from an aqueous ethanol solution and melted at 187–191°.

Anal. Calcd. for $C_{20}H_{30}Cl_2N_2O_2$: Cl, 17.66. Found: Cl, 17.75.

N-(2-Benzylaminoethyl)-1,2,3,4-tetrahydroisoquinoline.—N-(2-Chloroethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (23.2 g.), prepared by the action of thionyl chloride on the corresponding alcohol,[10] and benzylamine (21.4 g.) were combined and heated at 100° for 12 hr. The reaction mixture was made alkaline with 15% aqueous sodium carbonate and extracted with benzene. Distillation yielded the title compound, b.p. 165–170° (0.3 mm.); dihydrochloride salt, m.p. 249–251° (from methanol–ether).

Anal. Calcd. for $C_{18}H_{24}Cl_2N_2$: Cl, 20.90; N, 8.26. Found: Cl, 20.75; N, 8.18.

N-Benzyl-N′-(1-naphthyl)ethylenediamine.—N-(1-Naphthyl)-ethylenediamine (9.3 g., 0.05 mole) and benzaldehyde (5.3 g., 0.05 mole) were converted to the Schiff base in benzene (100 ml.) in the usual manner, followed by reduction with sodium borohydride (2.15 g.) in ethanol to yield the title product, **hydrochloride** m.p. 210–211° (from ethanol).

Anal. Calcd. for $C_{19}H_{21}ClN_2$: Cl, 11.33; N, 8.95. Found: Cl, 11.44; N, 8.90.

N,N′-Dibenzyl-1,4-butanediamine.—1,2-Dibromobutane (21.6 g.) and benzylamine (21.4 g.) were combined without solvent and heated at 100° for 2 hr. The mixture was filtered and the filtrate distilled to yield the title product, b.p. 152° (0.2 mm.), **dihydrochloride** m.p. 223–224° (from methanol–ether).

Anal. Calcd. for $C_{18}H_{26}Cl_2N_2$: Cl, 20.78; N, 8.18. Found: Cl, 20.92; N, 8.37.

N,N′-Dibenzyl-*trans*-1,4-cyclohexanebis(methylamine).—*trans*-1,4-Cyclohexanebis(methylamine) (28.4 g., 0.2 mole) and benzaldehyde (42.4 g., 0.4 mole) in benzene (175 ml.) were converted to the Schiff base which was reduced with sodium borohydride (15.4 g., 0.4 mole) in methanol (150 ml.) in the usual manner to yield the title product as an oil in 75% yield. The **dihydrochloride** was crystallized from dilute ethanol and melted at 358° dec.

Anal. Calcd. for $C_{22}H_{32}Cl_2N_2$: Cl, 17.93; N, 7.08. Found: Cl, 18.05; N, 7.12.

N,N′-Dibenzyl-1,7-heptanediamine.—1,7-Heptanediamine (5.2 g.) and benzaldehyde (7.0 gm.) in benzene (100 ml.) were converted to the Schiff base, which was reduced with sodium borohydride (3.7 gm.) in methanol to yield the title compound as an oil. The **dihydrochloride** melted at 292–293° (from ethanol).

Anal. Calcd. for $C_{21}H_{32}Cl_2N_2$: Cl, 18.49; N, 7.31. Found: Cl, 18.51; N, 7.14.

Methods. A. Effect on Hepatic Cholesterogenesis *in Vitro*.—The effect of the test compounds on the incorporation of mevalonate-2-C^{14} into cholesterol by rat liver homogenates was estimated as previously described.[11] The results are presented in Tables I–IV.

B. Antihypercholesterolemic Effect in Nephrotic Rats.—Rats were rendered nephrotic and hypercholesterolemic by subcutaneous administration of puromycin aminonucleoside.[12] The ability of DBED to antagonize this hypercholesterolemia formed the basis of the *in vivo* test.[13] Total sterol levels[14] expressed as cholesterol are presented in Table V.

C. Site of Inhibition.—Experiments were undertaken to localize the site of inhibition of cholesterol biogenesis by *in vitro* techniques. Inasmuch as *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane has been shown to inhibit the enzyme 7-dehydrocholesterol-Δ^7-reductase,[6] the effect of DBED on this enzyme was also examined. The Δ^7-reductase activity was assayed by the procedure of Kandutsch,[15] which consists in measuring the disappearance of 7-dehydrocholesterol in the presence of liver enzymes and suitable cofactors. The medium was as follows: 2.9 ml. of rat liver homogenate (10 g. of fresh liver homogenized in 15 ml. of Tris buffer (0.1 *M*, pH 7.4), centrifuged at 3000 r.p.m. for 10 min.), glucose 6-phosphate dehydrogenase (1 unit in 0.1 ml. of water; 1 unit will reduce 1 μmole of TPN/min. at pH 7.4 and 25°; obtained from Sigma Chemical Co., St. Louis, Missouri), 80 μmoles of niacinamide, 4 μmoles of TPN, 44 μmoles of glucose 6-phosphate, and 2.4 μmoles of 7-dehydrocholesterol in a final volume of 4.0 ml. Incubations were run for 90 min. in a Dubnoff metabolic shaker, with air as the gas phase and terminated by the addition of 7 pellets of potassium hydroxide. After the proteins dissolved, 1.0-ml. aliquots were pipetted into stoppered tubes followed by 1.5 ml. of water, 2.5 ml. of ethanol, and 10 ml. of hexane. The residual 7-dehydrocholesterol was estimated in the hexane extract at 281.5 mμ[16,17] using a Beckman DK-1 spectrophotometer. The effects of DBED on Δ^7-reductase and on the incorporation of mevalonate-2-C^{14} into cholesterol are compared in Table VI.

Results and Discussion.—The effects of the compounds tested on hepatic cholesterogenesis from mevalonate-2-C^{14} *in vitro* are presented in Tables I to IV. In contrast to the mevalonic acid analogs, their N,N′-dibenzylethylenediammonium salts depressed the incorporation of mevalonate into cholesterol (Table I), thus suggesting that DBED was the inhibitor. This was confirmed by the demonstration that at a final concentration of 1×10^{-3} *M*, DBED dihydrochloride (**7**) completely blocked the formation of cholesterol from mevalonate. The discovery of such an inhibitory property in DBED appeared to be unique and has

(7) All melting points were determined on a Fisher-Johns apparatus and are corrected.

(8) F. C. Schaeffer, *J. Am. Chem. Soc.*, **77**, 5922 (1955).

(9) G. Cavallini and E. Mazzarani, Italian Patent 600,214 (1959); *Chem. Abstr.*, **55**, 16486 (1961).

(10) B. Belleau, *J. Med. Pharm. Chem.*, **1**, 343 (1959) (p. 350).

(11) L. G. Humber, M. Kraml, and J. Dubuc, *Biochem. Pharmacol.*, **11**, 755 (1962).

(12) S. Frenk, I. Antonowicz, J. M. Craig, and J. Metcoff, *Proc. Soc. Exptl. Biol. Med.*, **89**, 424 (1955).

(13) P. Morand, J. Bagli, M. Kraml, and J. Dubuc, *J. Med. Chem.*, **7**, 504 (1964). This test was performed under the direction of Dr. C. Chappel.

(14) S. Pearson, S. Stern, and T. H. McGavack, *Anal. Chem.*, **25**, 813 (1953).

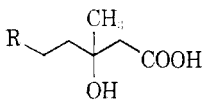
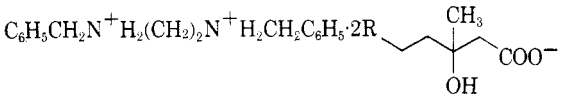
(15) A. A. Kandutsch, *J. Biol. Chem.*, **237**, 358 (1962).

(16) L. Dorfman, *Chem. Rev.*, **53**, 47 (1953).

(17) E. I. Mercer and J. Glover, *Biochem. J.*, **80**, 552 (1961).

TABLE I

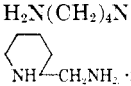
EFFECT OF DBED, MEVALONIC ACID ANALOGS, AND DBED SALTS ON HEPATIC CHOLESTEROGENESIS

No.	Compound	% inhibition (10^{-3} M)
		
1	R = OCH_3[a]	0
2	R = OC_2H_5[a]	0
3	R = CH_2OH[a]	0
		
4	R = OCH_3[a]	100
5	R = OC_2H_5[a]	100
6	R = CH_2OH[a]	99
7	$C_6H_5CH_2NH(CH_2)_2NHCH_2C_6H_5 \cdot 2HCl$[b]	93

[a] See ref. 2. [b] J. L. Szabo, C. D. Edwards, and W. F. Bruce, *Antibiot. Chemotherapy*, **1**, 499 (1951).

TABLE II

EFFECT OF UNSUBSTITUTED AND MONOSUBSTITUTED ALKYLENEDIAMINES ON HEPATIC CHOLESTEROGENESIS

No.	Compound	% inhibition (10^{-3} M)
8	$H_2N(CH_2)_2NH_2 \cdot 2HCl$[a]	0
9	$H_2N(CH_2)_3NH_2 \cdot 2HCl$[a]	0
10	$H_2N(CH_2)_4NH_2 \cdot 2HCl$[a]	0
11	 $\cdot 2HCl$[b]	21
12	$C_6H_5NH(CH_2)_2NH_2 \cdot HCl$[c]	0
13	$C_6H_5CH_2NH(CH_2)_2NH_2 \cdot 2HCl$[c]	0
14	$C_6H_5CONH(CH_2)_2NH_2 \cdot HCl$[d]	0
15	$2\text{-}ClC_6H_4CH_2NH(CH_2)_2NH_2 \cdot 2HCl$[b]	52
16	$2\text{-}ClC_6H_4CH_2NH(CH_2)_3NH_2 \cdot 2HCl$[b]	50
17	$C_6H_5CH_2NHCH(CH_3)CH_2NH_2 \cdot 2HCl$[b]	0
18	$C_6H_5CH_2CH_2NH(CH_2)_3NH_2 \cdot 2HCl$[b]	0
19	$C_6H_5(CH_2)_2NHCH(CH_3)CH_2NH_2 \cdot 2HCl$[b]	29
20	$3,4\text{-}(CH_3O)_2C_6H_3(CH_2)_2NHCH(CH_3)CH_2NH_2 \cdot 2HCl$[b]	0

[a] Commercially available. [b] Obtained through the courtesy of Dr. A. Langis, Ayerst Research Laboratories. [c] S. C. Dickerman and A. J. Besozzi, *J. Org. Chem.*, **19**, 1855 (1954). [d] See ref. 8.

prompted us to investigate the essential structural features associated with this type of activity so as to enable us to design a compound of increased activity.

The compounds listed in Table II comprise a series of unsubstituted or monosubstituted alkylenediamines. Compounds containing two primary amino groups (the unsubstituted alkylenediamines **8**, **9**, and **10**) are inactive at 1×10^{-3} *M*. Some of the compounds with one primary and one secondary amino group are active, others inactive at 1×10^{-3} *M*. None of the compounds listed in Table II approaches the level of activity found for DBED. The ability of an alkylenediamine to retain as high an effect on hepatic cholesterogenesis as obtained with DBED, appears to be associated with the presence of at least one substituent on each nitrogen.

The effects of a series of additional structural modifications in a group of ethylenediamines containing at least one substituent on each nitrogen were investigated next. These compounds are shown in Table III. When the substituents are small alkyl groups (**21**, **22**, and **23**), the compounds are inactive at 1×10^{-3} *M*; when they are large aralkyl groups, *e.g.*, benzhydryl and *p*-phenylbenzyl (**24** and **25**), the resulting compounds are less active than DBED itself.

TABLE III

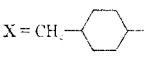
EFFECT OF POLYSUBSTITUTED ALKYLENEDIAMINES ON HEPATIC CHOLESTEROGENESIS

No.	Compound	% inhibition (final concn.)
21	$CH_3NH(CH_2)_2NHCH_3 \cdot 2HCl$[a]	0 (10^{-3} M)
22	$(CH_3)_2N(CH_2)_2N(CH_3)_2 \cdot 2HCl$[b]	0 (10^{-3} M)
23	$(CH_3)_2CHCH_2NH(CH_2)_2NHCH_2CH(CH_3)_2 \cdot 2HCl$[c]	0 (10^{-3} M)
24	$(C_6H_5)_2CHNH(CH_2)_2NHCH(C_6H_5)_2 \cdot 2HCl$[d]	57 (10^{-3} M)
25	$4\text{-}(C_6H_5)C_6H_4CH_2NH(CH_2)_2NHCH_2C_6H_4\text{-}4\text{-}(C_6H_5)$[e]	0 (10^{-3} M)
26	$C_6H_5NH(CH_2)_2NHCH_2C_6H_5 \cdot HCl$[f]	98 (10^{-3} M)
27	$C_{10}H_7NH(CH_2)_2NHCH_2C_6H_5 \cdot HCl$[g,a]	17 (10^{-3} M)
28	$C_6H_5CONH(CH_2)_2NHCH_2C_6H_5 \cdot HCl$[c]	30 (10^{-3} M)
29	$C_6H_5CH{=}N(CH_2)_2N{=}CHC_6H_5$[h]	0 (10^{-3} M)
30	$C_6H_5CONH(CH_2)_2NHCOC_6H_5$[i]	20 (10^{-3} M)
31	$C_6H_5CONH(CH_2)_2N{=}CHC_6H_5$[e]	23 (10^{-3} M)
32	$C_6H_5CH(CH_3)NH(CH_2)_2NHCH(CH_3)C_6H_5 \cdot 2HCl$[f]	15 (10^{-3} M)
33	$C_6H_5CH_2NHCH(C_2H_5)CH_2NHCH_2C_6H_5 \cdot 2HCl$[e]	33 (10^{-3} M)
34	$C_6H_5CH_2N(CH_2)_2NCH_2C_6H_5 \cdot 2HCl$[k]	0 (10^{-3} M)
	$CH_3 \quad CH_3$	
35	$C_9H_{10}N(CH_2)_2NHCH_2C_6H_5 \cdot 2HCl$[c,l]	23 (10^{-3} M)
36	$C_6H_5CH_2 \quad CH_2C_6H_5$ $N(CH_2)_2N$ $HO(CH_2)_2 \quad (CH_2)_2OH \cdot 2HCl$[c]	0 (10^{-3} M)

[a] K. Abe, *J. Pharm. Soc. Japan*, **75,** 153 (1955); *Chem. Abstr.*, **50,** 1778 (1956). [b] H. H. Fox and W. Wenner, *J. Org. Chem.*, **16,** 225 (1951). [c] J. Rameau, *Rec. trav. chim.*, **57,** 194 (1938). [d] The free base of this compound is reported by J. Van Alphen and J. L. Robert, *ibid.*, **54,** 361 (1935), but the dihydrochloride has not been reported. It has m.p. 347–350°. *Anal.* Calcd. for $C_{28}H_{30}Cl_2N_2$: Cl, 15.23. Found: Cl, 15.78. [e] See Experimental section. [f] K. Miescher and W. Klarer, U. S. Patent 2,505,133 (April 25, 1950); *Chem. Abstr.*, **44,** 6880 (1950). [g] $C_{10}H_7$ = 1-naphthyl. [h] See Table I, footnote *b*. [i] S. R. Aspinall, *J. Org. Chem.*, **6,** 895 (1941). [j] Obtained through the courtesy of Wyeth Laboratories. [k] The free base reported by H. T. Clarke, *J. Chem. Soc.*, **99,** 1927 (1911); the dihydrochloride has m.p. 266° dec. *Anal.* Calcd. for $C_{18}H_{26}Cl_2N_2$: Cl, 20.77. Found: Cl, 20.59. [l] $C_9H_{10}N$ = 1,2,3,4-tetrahydroisoquinolino.

TABLE IV

EFFECT OF N,N'-DIBENZYLALKYLENEDIAMINES ON HEPATIC CHOLESTEROGENESIS

No.	Compound	N–N distance, Å.[a]	% inhibition (final concn.)
	$C_6H_5CH_2NH\text{—}X\text{—}NHCH_2C_6H_5 \cdot 2HCl$		
7	X = $(CH_2)_2$	3.74	32 (10^{-4} M)
37	X = $(CH_2)_3$[b]	4.84	11 (10^{-4} M)
38	X = $(CH_2)_4$[c]	6.01	49 (10^{-4} M)
39	X = $(CH_2)_6$[d]	7.11	81 (10^{-4} M)
40	X = $(CH_2)_8$[c]	8.20	85 (10^{-4} M)
41	X = $(CH_2)_{12}$[e]	9.25	67 (10^{-4} M)
42	X = 	7.42	45 (10^{-4} M)

[a] Measured on Dreiding models. [b] Obtained through the courtesy of Wyeth Laboratories; this compound was tested as the diacetate salt. [c] The free base has been previously reported: S. Yabuta and H. Ikeda, Japanese Patent 3480 (1954); *Chem. Abstr.*, **50,** 1075 (1956). The dihydrochloride has m.p. 312–314°. *Anal.* Calcd. for $C_{18}H_{26}Cl_2N_2$: Cl, 20.77. Found: Cl, 20.70. [d] The diacetate salt has been previously reported (see footnote *e*.) The dihydrochloride has m.p. 322–323°. *Anal.* Calcd. for $C_{19}H_{28}Cl_2N_2$: Cl, 19.96. Found: Cl, 19.82. [e] J. L. Szabo and W. F. Bruce, U. S. Patent 2,739,981 (March 27, 1956); *Chem. Abstr.*, **50,** 16850 (1956). [f] See Experimental section.

The effect of reducing the basicity of the nitrogen in a series of N,N′-disubstituted ethylenediamines was also investigated. When one or both nitrogens are rendered nonbasic, *e.g.*, in an amide or Schiff base linkage, the resultant compounds (**28**–**31**) are considerably less active than DBED. However, when the basicity of only one of the nitrogens is moderately reduced by conjugation with an aromatic ring, the activity of the resultant compounds (**26** and **27**) is not significantly affected.

Alkyl substitution of the methylene groups adjacent to the nitrogen (**32** and **33**) or rendering one or both of the nitrogens tertiary (**34**–**36**) does not enhance the activity with respect to DBED.

The influence of the internitrogen distance in a series of N,N′-dibenzylalkylenediamines was investigated next, and the activities of these compounds are compared in Table IV. A moderate increase in activity is obtained when the ethylene moiety of DBED is homologated. With compound **42**, containing the *trans*-cyclohexanebis(methylamine) moiety a dramatic increase in activity is observed (45% inhibition at 1×10^{-6} *M*), being about 100 times more potent than DBED itself. Subsequent extensive modification of this compound has led to the development of *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane.[4-6]

We do not assign the singular activity of compound **42** solely to its unique internitrogen distance of 7.42 Å. The figures shown in Table IV refer to *maximum* internitrogen distances; those obtained with the most elongated conformations. Compound **42**, however, contains a cyclohexane ring of restricted conformational flexibility and, as a result, also possesses a *minimum* internitrogen distance of 6.90 Å. Some of the flexible acyclic analogs, *e.g.*, **39**–**41**, should be capable of assuming, at an enzyme surface, conformations with internitrogen distances within the range of 6.90–7.42 Å. However, the relative inactivity of these acyclic analogs with respect to **42** suggests either that they are incapable of assuming such conformations or, more likely, that there is some other feature of **42** which accounts for its striking activity.

We consider that the activity of this series of compounds is due to the formation of an enzyme-inhibitor complex involving ion-pair formation between the nitrogens and anionic sites on an enzyme surface (or surfaces). While the distance between these anionic sites is undoubtedly unique and therefore the internitrogen distance of the inhibitor will be of considerable importance, we suggest that a most important part of the binding force between enzyme and inhibitor is provided by van der Waals attraction between the hydrocarbon moiety of the internitrogen area of the inhibitor and a complementary lipophilic region on the enzyme surface; such van der Waals forces are seen as being much stronger with **42** which contains a bulky cyclohexane ring, than with the acyclic analogs.

Although the results *in vitro* show that DBED and many of its close structural analogs are moderately potent inhibitors of hepatic cholesterogenesis, it cannot be taken for granted that these agents will be effective in lowering serum sterols in intact animals.[18] Poor absorption, rapid excretion, and rapid catabolism to inactive metabolites are factors which singly, or in combination, can act to keep DBED levels below those required for effective cholesterol biosynthesis inhibition and the subsequent lowering effect on serum cholesterol. In rats rendered nephrotic and hypercholesterolemic by administration of the aminonucleoside of puromycin, DBED caused a significant reduction (*cf.* Table V) in serum sterol levels at the 60 and 80 mg./kg. doses, these being 20 and 39%, respectively. Inasmuch as the LD_{50} of DBED is about 110 mg./kg.,[19] the doses causing reductions of serum sterols are rather high. Even at this high dosage schedule no hypocholesterolemic effect was observed in normal rats.

TABLE V

EFFECT OF DBED IN NEPHROTIC HYPERCHOLESTEROLEMIC RATS

Dose of DBED, mg./kg., s.c.[a]	Total sterols, mg./100 ml. serum ± S.E. Controls	Treated	% reduction	Significance
40	696 ± 46	616 ± 26	11	None
60	732 ± 58	587 ± 57	20	$0.05 < P < 0.10$
80	605 ± 56	369 ± 45	39	$P < 0.10$

[a] Eight animals were used in each test group.

TABLE VI

COMPARISON OF THE EFFECT OF DBED ON CHOLESTEROL BIOSYNTHESIS FROM MEVALONATE-2-C^{14} AND ITS EFFECT ON THE ENZYME Δ^7-REDUCTASE

DBED, final concn.	% inhibition: Mevalonate-2-C^{14}	7-Dehydrocholesterol
1.0×10^{-3} *M*	93	100
1.0×10^{-4} *M*	32	40

While DBED has been the key to the development of *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane, a very potent, orally active inhibitor of cholesterol biosynthesis that prevents the conversion of 7-dehydrocholesterol to cholesterol, it remained to show that DBED itself belongs to the same class of inhibitors. Experimental data presented in Table VI indicate that the degree of inhibition of cholesterol formation from mevalonate-2-C^{14}, caused by DBED, parallels very closely its blockage of the hepatic Δ^7-reductase enzyme. Thus, it appears that the over-all inhibitory effect of DBED on hepatic cholesterogenesis from mevalonate is accounted for by its interference with the conversion of 7-dehydrocholesterol to cholesterol.

Under our experimental conditions the substrate concentration was 4×10^{-4} *M* final and had we employed the DBED salt of mevalonate-2-C^{14} our controls would have been inhibited to the extent of about 80%. Most workers employing the commercially available DBED salt of mevalonate-2-C^{14} convert it to the potassium salt prior to incubation. Thus, an earlier discovery of the inhibitory effect of DBED on the biosynthesis of cholesterol was not forthcoming. On the other hand, at doses of 1×10^{-4} *M* final, or less, the DBED salt of mevalonate-2-C^{14} could be used directly in the incubation medium and the slight inhibition resulting would most likely escape detection.

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(18) D. Steinberg in "Advances in Pharmacology," Vol. I, S. Garrattini and P. A. Shore, Ed., Academic Press Inc., New York, N. Y., 1962, p. 85.

(19) J. Seifter, J. M. Glassman, A. J. Begany, and A. Blumenthal, *Antibiot. Chemotherapy*, **1**, 504 (1951).