2-Amino-4-(4′-hydroxybutyl)amino-5-nitroso-6-hydroxypyrimidine (IIIb)——This compound was prepared by the same procedure as described for IIIa; mp 228° (decomp.); Yield 96%. *Anal.* Calcd. for $C_8H_{13}O_3N_5$: C, 42.29; H, 5.73; N, 30.84. Found: C, 42.19; H, 5.83; N, 30.76.

2-Amino-4-(4′-hydroxybutyl)amino-5-formamido-6-hydroxypyrimidine (IVb)——To a stirred solution of IIIb (5 g) in a mixture of $HCONH_2$ (30 ml) and HCOOH (15 ml), $Na_2S_2O_4$ (4 g) was added portionwise at 70°. Gradually, the color of the solution became yellow. The reaction mixture was refluxed for 15 min and then allowed to stand in a refrigerator. The crystals that precipitated were collected by filtration and recrystallized from H_2O to afford a pure sample, mp 244—245° (decomp.). Yield 3.2 g (59.3%). *Anal.* Calcd. for $C_9H_{15}O_3N_5 \cdot \frac{1}{4}H_2O$: C, 43.99; H, 6.31; N, 28.51. Found: C, 44.30; H, 6.59; N, 28.10.

9-(4′-Hydroxybutyl)-2-amino-6-hydroxypurine (Vb)——The foregoing IVb was refluxed for 3.5 hr in $HCONH_2$ (14 ml) containing HCOOH (1 ml). The reaction solution was then worked up as described for Va, giving 1 g (37.1%) of a pure product, mp 220—222° (decomp.). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254 (9700), 280 (6400); $\lambda_{max}^{PH\,13}$ mμ (ε): 255 (inf.), 269.5 (9800). *Anal.* Calcd. for $C_9H_{13}O_2N_5 \cdot \frac{1}{2}H_2O$: C, 46.55; H, 6.04; N, 30.17. Found: C, 47.00; H, 5.74; N, 30.54.

9-(2′-Hydroxyethyl)-2-amino-6-hydroxypurine 2′-Phosphate (VIa)——The compound Va (1.2 g) was phosphorylated described for the preparation of VIb and the product was purified by column chromatography on decolorizing resin. The resulting ammonium salt of VIa was converted with $Ba(OAc)_2$ to the barium salt. Yield 490 mg (18.6%). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254.5 (6100), 280 (inf.); $\lambda_{max}^{PH\,13}$ mμ (ε): 255 (inf.), 269 (9500). *Anal.* Calcd. for $C_7H_8O_5N_5PBa \cdot H_2O$: C, 19.63; H, 2.34; N, 16.36. Found: C, 19.23; H, 2.57; N, 16.26.

9-(4′-Hydroxybutyl)-2-amino-6-hydroxypurine 4′-Phosphate (VIb)——To a mixture of 85% H_3PO_4 (13 g) and P_2O_5 (10 g) was added Vb (1 g), and the mixture was stirred for 3 hr at 60—70°. Gradually, it became clear. After the reaction, 100 ml of H_2O was added and the solution was heated on a steam-bath to hydrolyze the resulting polyphosphates. An aliquot from the solution showed a single spot on a paper chromatogram. The solution was brought to pH 2 and passed through a column (2 × 60 cm) of decolorizing resin.[9] The column was washed with H_2O, and VIb was eluted with 0.5 N NH_4OH. The effluent was concentrated *in vacuo* to *ca.* 5 ml, to which one volume of EtOH was added to precipitate the ammonium salt of VIb. This was collected by filtration and taken up in 100 ml of H_2O. The pH of the solution was adjusted to 8.5, and to this was added a solution of $Ba(OAc)_2$ (909 mg) in 20 ml of H_2O. The resulting precipitate, mainly consisted of $Ba_3(PO_4)_2$, was removed. After the filtrate was concentrated to *ca.* 50 ml, addition of EtOH (50 ml) afforded a white precipitate, which was collected by centrifugation. Washing with EtOH and ether and drying *in vacuo* at 80° gave 320 mg (14.5%) of VIb. The above ammonium salt of VIb was found to be tasteless. *Rf*: 0.22, *n*-PrOH–NH_4OH(28%)–H_2O (20:12:3, v/v); 0.51, iso-PrOH–sat. $(NH_4)_2SO_4$–H_2O (2:79:19). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254 (10600), 281 (6500); $\lambda_{max}^{PH\,13}$ mμ (ε): 270 (10300). *Anal.* Calcd. for $C_9H_{12}O_5N_5PBa \cdot 2H_2O$: C, 22.79; H, 3.38; N, 14.77; P, 6.54. Found: C, 22.68; H, 3.38; N, 14.39; P, 6.90.

Acknowledgement The authors wish to thank Professor M. Ikehara of the Osaka University for his helpful suggestion, and Drs. H. Oeda, T. Takenishi, and I. Kumashiro of Ajinomoto Co., Inc., for their continued encouragement. The authors are also indebted to Messrs. M. Okutsu and M. Akiyama for their technical assistance.

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Bis(guanidinoethyl)amine Derivatives

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Recently, we have synthesized derivatives of 2-guanidinoethylamine[2] (I), 3-guanidinopropylamine[3] (II) and bis(3-guanidinopropyl)amine[4] (III), in order to find pharmacolo-

1) Location: *Shirokane-Sankocho, Shiba, Minatoku, Tokyo.*
2) T. Ueda and S. Akihama, unpublished.
3) T. Ueda and K. Ishizaki, *Chem. Pharm. Bull.* (Tokyo) **15**, 228 (1967).
4) T. Ueda and S. Watanabe, Japan. Patent 470947 (1966).

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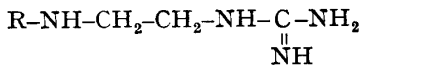
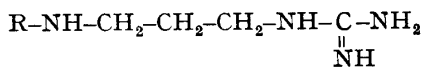
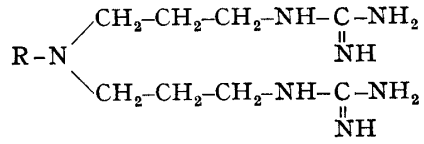
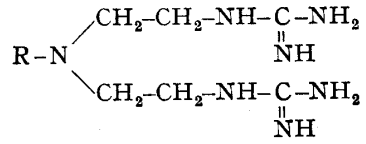
gically active agents. Among these compounds, there existed some of hypertentive, analgesic and muscle relaxant agents.


(I)


(II)


(III)


(IV)

Related to these derivatives, the authors have newly obtained several derivatives of bis-(2-guanidinoethyl)amine (IV), any of which has not been revealed in literature to date.

To synthesize objective compounds, it was considered to guanidinize the corresponding bis(2-aminoethyl)amine with sulfate of methyl isothiourea. Attempts, thus, were made to prepare bis(2-aminoethyl)amine derivatives as the intermediates.

Mann, *et al.*[5] synthesized bis (2-aminoethyl) amine (V) and bis(2-aminoethyl)methylamine (VI) by employing diethanolamine as the starting material, as illustrated in Chart 1.

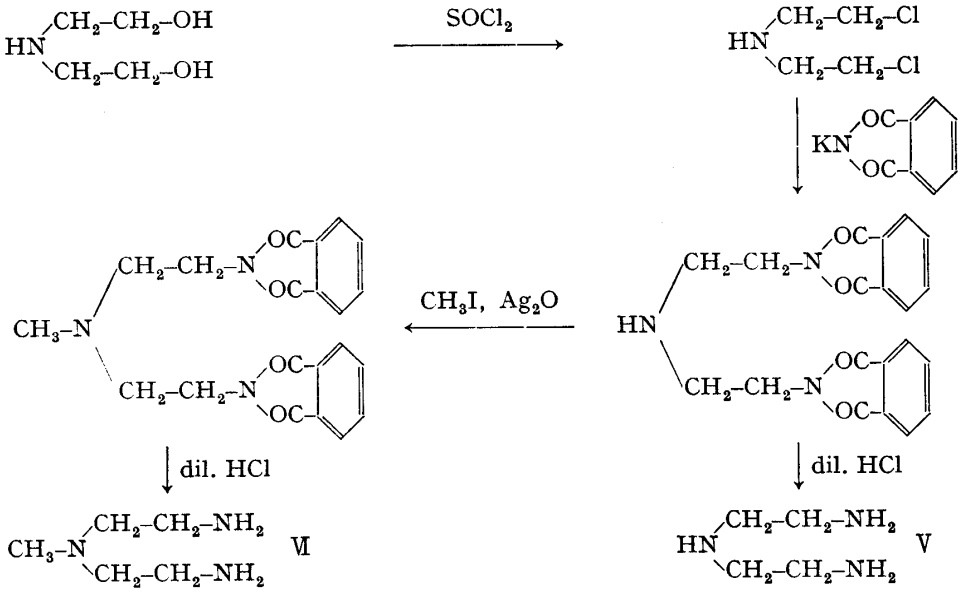


Chart 1

According to the experimental results reexamined by the authors, it was found that the both steps of the fusion with potassium phthalimide and the methylation with methyl iodide took place with difficulty. This synthetic method of Mann, therefore, afforded very poor yield of the final product. However, the authors found that the reaction of bis(2-chloroethyl)amine with potassium phthalimide was enhanced by using dimethylformamide as the reaction solvent instead of the fusion with potassium phthalimide. Further, Mann's method

5) Frederick G. Mann, *J. Chem. Soc.*, **1934**, 461.

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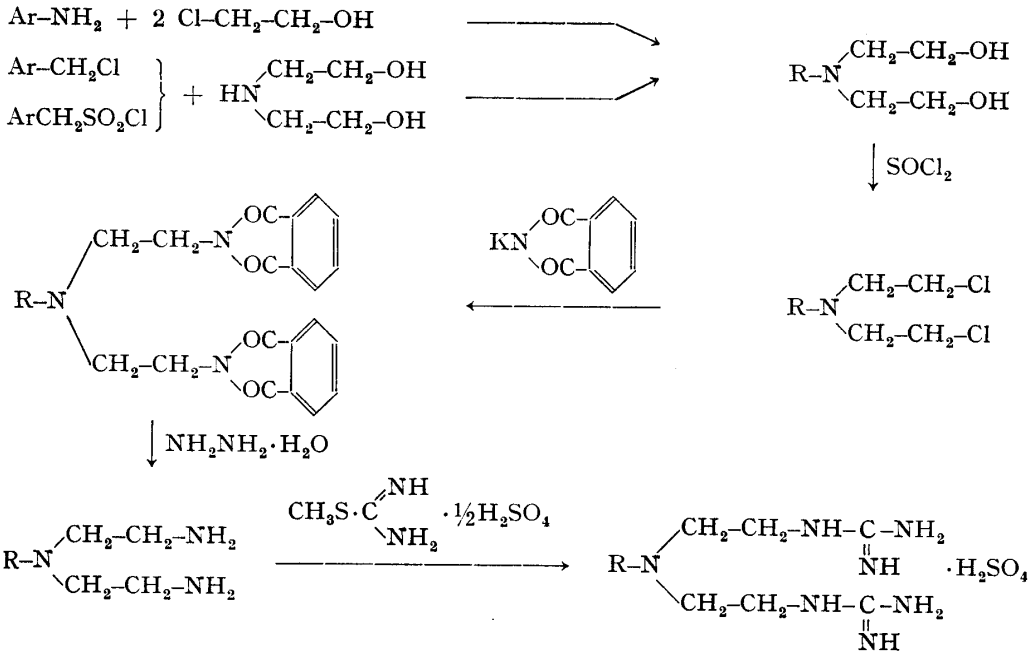
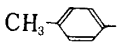
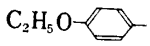
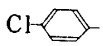
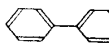
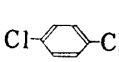
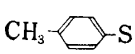


Chart 2

Table I. $RN\left(CH_2CH_2N\begin{matrix}CO\\CO\end{matrix}C_6H_4\right)_2$

R	Yield (%)	mp (°C)	Appearance	Formula	Analysis N (%) (calcd./found)
	91	209—210.5	yellow plates	$C_{26}H_{21}O_4N_3$	9.56/9.64
	91	149—151	yellow needles	$C_{27}H_{23}O_4N_3$	9.27/9.42
	47	203—205	yellow plates	$C_{27}H_{23}O_4N_3$	9.27/9.26
	92	169—171	yellow powder	$C_{27}H_{23}O_5N_3$	8.95/8.83
	96	126—127	yellow powder	$C_{28}H_{25}O_5N_3$	8.69/8.53
	71	131—133	orange needles	$C_{28}H_{23}O_5N_3$	8.69/8.78
	83	219—221	yellow plates	$C_{26}H_{20}O_4N_3Cl$	8.87/8.77
	73	148—149	yellow plates	$C_{30}H_{23}O_4N_3$	8.58/8.65
	83	213—214	yellow plates	$C_{32}H_{25}O_4N_3$	8.15/7.93
	89	130—132.5	colorless plates	$C_{27}H_{23}O_4N_3$	9.27/9.37
	74	138—139	colorless plates	$C_{27}H_{22}O_4N_3Cl$	8.61/8.70
	87	139—141	colorless needles	$C_{27}H_{22}O_4N_3Cl$	8.61/8.40
	62	187—190	colorless plates	$C_{27}H_{23}O_6N_3S$	8.12/8.45
	53	201—202	colorless needles	$C_{27}H_{23}O_6N_3S$	8.12/7.81

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was found unsuitable for the preparation of N-aryl derivatives, because aryl iodide did not react with any of bis(2-phthalimidoethyl)amine.

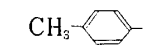
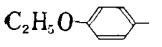
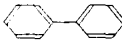
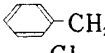
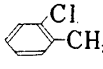
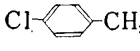
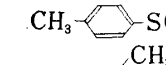
The authors conceived to conduct the N-substitution at the beginning of the overall synthetic process. Thus, N-substituted diethanolamine was prepared by reacting chloroethanol with an arylamine in the presence of calcium carbonate according to the method of Ross,[6] while N-aralkyl or arylsulfonamide derivatives synthesized by reacting diethanolamine with aralkyl chloride or arylsulfonyl chloride in the presence of alkali carbonate.[7]

N-Substituted ethanolamine was converted to N-substituted bis(2-chloroethyl)amine by the chlorination with thionyl chloride. This product gave N-substitute of bis(phthalimidoethyl)amine by the reaction with potassium phthalimide in dimethylformamide solution. The resulting product was hydrolysed with hydrazine hydrate to yield N-substitute of bis (2-aminoethyl)amine. The guanidination of the latter was succeeded in reacting with methyl isothiourea. The whole synthetic process furnished by us is shown in Chart 2.

The synthesized compounds of bis(2-phthalimidoethyl)amine, bis(2-aminoethyl)amine and bis(2-guanidinoethyl)amine are shown in Table I, II and III respectively.

The derivatives of bis(2-guanidinoethyl)amine were characterized by data of elementary analysis and infrared absorptions.

Table II. $RN(CH_2CH_2NH_2)_2$

R	Yield (%)	bp (°C/mmHg)	Hydrochloride mp(°C)	Formula	Analysis N (%) (calcd./found)
	20	146/2	300—301 (d.)	$C_{10}H_{19}N_3Cl_2$	14.61/14.95
	25	146—148/2	195—196.5	$C_{11}H_{21}N_3Cl_2$	15.79/15.77
	36	147—149/2	231—232	$C_{11}H_{21}N_3Cl_2$	15.79/15.39
	43	133—135/1	256—257 (d.)	$C_{11}H_{21}ON_3Cl_2$	14.89/14.89
	58	144—146/2	266—267 (d.)	$C_{12}H_{23}ON_3Cl_2$	14.19/14.41
	19	153—155/1	227—228	$C_{12}H_{23}ON_3Cl_2$	14.19/13.87
	52	178—180/3	266 (d.)	$C_{10}H_{18}N_3Cl_3$	14.66/14.66
	52	173—176/6	244—246	$C_{14}H_{21}N_3Cl_2$	13.90/13.88
	28	221—224/3	264—266	$C_{16}H_{23}N_3Cl_2$	12.80/12.55
	42	156—158/10	239—240	$C_{11}H_{21}N_3Cl_2$	15.79/15.61
	52	153—157/3	206—207	$C_{11}H_{20}N_3Cl_3$	13.98/13.80
	35	143—146/2	218—219 (d.)	$C_{11}H_{20}N_3Cl_3$	13.98/13.76
	34		265—267 (d.)	$C_{11}H_{21}O_2N_3Cl_2S$	12.72/12.50
	12		222—225	$C_{11}H_{21}O_2N_3Cl_2S$	12.72/12.66

(d.) : decomp.

6) W.C.J. Ross, *J. Chem. Soc.*, **1949**, 183.
7) Y. Sakurai and M. Izumi, *Pharm. Bull.* (Japan), **1**, 297 (1953); J, Kloubek and A. Marhoul, *Collection Czech. Chem. Commun.*, **28**, 1016 (1963).

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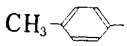
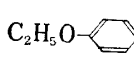
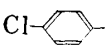
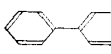
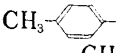
For the identification of the compounds synthesized, their infrared absorption spectra were inspected as for guanidino grouping. These compounds absorbed commonly in the regions of 1650—1700 cm^{-1}, 1600—1650 cm^{-1} and 3100—3300 cm^{-1} as shown in Table IV.

The first region may be assigned to the grouping >C=N, which was established by Randall[8] and Lieber, *et al.*[9] It is difficult to assign the second at the present. Guanidine salt and the related also absorb in this region, in addition of the first. The both absorptions were found to combine with each other into a broad band in some of the compounds. The third regions may be assigned to N-H linkage which was established by Bellamy, *et al.*[10]

The finding with the infrared absorptions of these compounds, thus, suggested that they were objective showing the existence of guanidino group. Compared with compounds of 3-aminopropylguanidine,[3] they also absorbed more clearly at 1600—1650 cm^{-1} and 1650—1700 cm^{-1}, as shown in Table IV.

Table III. $RN(CH_2CH_2NH\text{-}\underset{\underset{NH}{\|}}{C}\text{-}NH_2)_2 \cdot H_2SO_4$

R	Yield (%)	mp (°C) (decomp.)	Appearance	Formula	Analysis (calcd./found) (%) C	H	N
	3	256—257.5	needles	$C_{12}H_{23}O_4N_7S$	39.88/40.03	6.42/6.01	27.14/27.40
	56	294—295	prisms	$C_{13}H_{25}O_4N_7S$	41.67/41.29	6.66/6.77	26.13/25.99
	52	272—273	prisms	$C_{13}H_{25}O_4N_7S$	41.60/41.44	6.66/6.64	26.13/25.95
	52	272—273	prisms	$C_{13}H_{25}O_5N_7S$	39.89/39.91	6.44/6.28	25.05/25.05
	54	286—287	prisms	$C_{14}H_{27}O_5N_7S$	41.47/41.80	6.71/6.90	24.19/24.46
	8	266—268	needles	$C_{14}H_{27}O_5N_7S$	41.47/41.40	6.71/6.66	24.19/23.95
	55	286	prisms	$C_{12}H_{22}O_4N_7ClS$	36.41/36.19	5.60/5.43	24.77/24.97
	43	308—309	prisms	$C_{16}H_{25}O_4N_7S$	46.70/46.75	6.12/6.09	23.83/23.89
	10	291—292	needles	$C_{18}H_{29}O_4N_7S$	49.41/49.89	6.22/5.94	22.41/22.10
	60	275—276	prisms	$C_{13}H_{25}O_4N_7S$	41.59/41.43	6.71/6.32	26.12/26.46
	66	284—286	needles	$C_{13}H_{24}O_4N_7ClS$	38.10/38.15	5.90/6.03	23.93/23.72
	73	303—305	plates	$C_{13}H_{24}O_4N_7ClS$	38.10/38.41	5.90/6.10	23.93/23.87
	41	275—276	prisms	$C_{13}H_{25}O_6N_7S_2$	35.52/35.39	5.73/5.85	22.31/21.84
	23	254—256	prisms	$C_{13}H_{25}O_6N_7S_2$	35.52/35.14	5.73/5.91	22.31/21.28

8) H. Randall, N. Fowler, N. Fuson and N. Dangl, "Infrared Determination of Organic Structures," 1949.
9) E. Lieber, D.R. Levering and L.J. Patterson, *Anal. Chem.*, **23**, 1594 (1951); P.L. Pickard and G.W. Polly, *J. Chem. Soc.*, **1954**, 5169; N.J. Leonard and V.W. Gash, *J. Am. Chem. Soc.*, **76**, 2781 (1954).
10) L.J. Bellamy and L. Beecher, *J. Chem. Soc.*, **1952**, 1701; I.R. Beattic and H.J.V. Tyrrell, *J. Chem. Soc.*, **1956**, 2849; D.B. Powell and N. Sheppard, *J. Chem. Soc.*, **1956**, 3108; J. Chatt, L.A. Duncanson and L.M. Venanzi, *J. Chem. Soc.*, **1955**, 4461.

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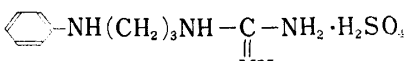
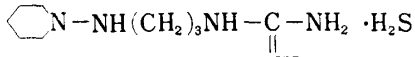
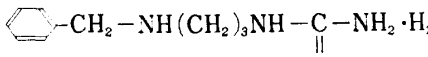
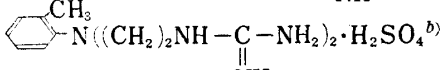
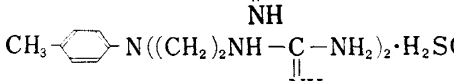
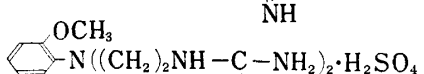
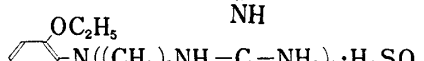
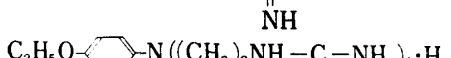
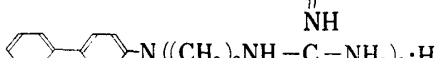
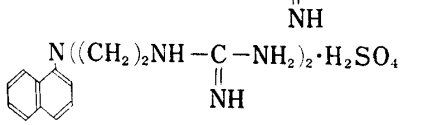
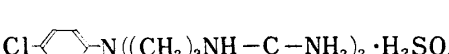
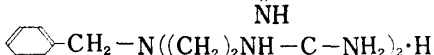
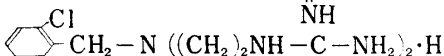
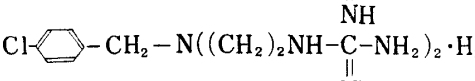
Among the compounds synthesized, N,N-diguanidinoethyl-*o*-toluidine sulfate was found to have a muscle relaxant activity. Works on this line will be reported in a medical journal in the future.

Table IV. IR Spectra of Guanidine Derivatives

Guanidine derivatives	ν_{N-H} (cm^{-1})		$\nu_{C=N}$ (cm^{-1})	δ_{N-H} (cm^{-1})
$(CH_3)_2CH\ NH(CH_2)_3NH-C(=NH)-NH_2\cdot H_2SO_4$ [a]			1690	1625
			1710	1650
			1690	1650
			1690	1630
 [b]	3320	3100	1665	1625
	3350	3160	1670	1643
	3280	3120	1655	1620
	3280	3120	1650	1626
	3280	3100	1650	1640
	3280	3080	1665	1610
	3240	3000	1656	1628
	3300	3120	1663	1638
	3280	3130	1655	1613
	3240	3110	1645	1620
	3280	3120	1650	1623

a) Nujol mull b) KBr disk

Experimental

General Procedure for Synthesis of N-substituted 2,2′-Diphthalimidodiethylamine——A mixture of N-substituted 2,2′-dichlorodiethylamine (0.1 mole), potassium phthalimide (0.2 mole) and 100 ml of D.M.F. was heated 2—3 hr under reflux with stirring. After cooling, the reaction mixture was filtered and washed with water. The diphthalimide derivatives were submitted to subsequent reaction without further purification, except the sample for elementary analysis which was recrystallized from EtOH. The compounds synthesized were listed in Table I.

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General Procedure for Synthesis of N-substituted 2,2′-Diaminodiethylamine——To the diphthalimido derivatives (0.1 mole), 200 ml of 95% EtOH and 100% hydrazine hydrate (0.2 mole) were added, and refluxed for 2 hr on the water bath. After cooling, the mixture was made strongly acidic to congo-red paper with conc. HCl. The voluminous precipitate was filtered off and washed with 95% EtOH. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was treated with a cold 40% NaOH under stirring. The resulting oil separated was extracted with $CHCl_3$, and dried with Na_2SO_4. After removal of $CHCl_3$, the oily residue was distilled under reduced pressure. The compounds synthesized were listed in Table II.

General Procedure for Synthesis of N-Substituted Bis(2-guanidinoethyl)amine Sulfate——A solution of 0.1 mole of N-substituted 2,2′-diaminodiethylamine and 0.2 mole of S-methylisothiourea sulfate in 100 ml of water was warmed for 3—5 hr until MeSH was finished to evolve. The mixture was concentrated and a suitable amount of acetone was added. The resulting crystals were collected by suction and recrystallized from water. The compounds synthesized were listed in Table III.

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Quantitative Analysis of Primary Amines by Ion-exchange Chromatography[1)]

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Certain biogenic amines have been known, for some time now, to be of physiological importance.

In order to elucidate these phenomena quantitatively it is necessary to establish a systematic quantitative analysis of these amines. Formaly, paper chromatography had been used for this purpose, but it is not a reproducible and quantitative method. Lately, several investigators have developed ion-exchange chromatographic techniques for analysis of some amines.[3,4)]

As primary amines have generally a positive ninhydrin reaction, we have investigated the factors affecting the systematic analysis by an amino acid analyzer.

Experimental and Methods

Most of the authentic amines used in this investigation were obtained from commercial sources.

The quantitative determination of amines was then examined by reaction with ninhydrin on the Hitachi KLA-2 Amino Acid Analyzer by means of the procedure of Moore, *et al.*[5)] Color produced by ninhydrin reactive amines was determined at 440, 570 and 640 $m\mu$. Ion-exchange resin Aminex A_5 was used for chromatography of amines, the resin column was 0.6×10 cm an equilibrated at 50°. Flow rates of developer and ninhydrin solution were 30 ml/hr and 15 ml/hr respectively.

Three effluent buffers were prepared. Their compositions are shown in Table I.

1) A part of this research was presented at the 88th Annual Meeting of the Pharmaceutical Society of Japan in Tokyo (April, 1968).
2) Location: 5 *Nakauchicho, Misasagi, Yamashina, Higashiyama, Kyoto.*
3) T. Miyagi and S. Ando, *Annual Rep. Inst. Food Microbiology*, **6**, 93 (1953).
4) T.L. Perry and W.A. Schroeder, *J. Chromato.*, **12**, 358 (1963).
5) S. Moore, D.H. Spackman and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

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