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).
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Table I. Effluent Solutions Components

Buffer No.	pH	Na^+(M)	Buffer System
1	5.28	0.30	0.100M Na-citrate
2	8.02	0.60	0.025M Na-borate
3	11.08	0.23	0.200M Na-salicylate

Detecting and quantitative samples of amines were prepared from putrefaction of marin animals as follows; meat of mackerel and sea bream without skin and flesh of bloody color were prepared. Tentacles of octopus without skin were prepared. Meat of crab and short-necked clam without shell and tendon was prepared. Fifty mililitres of distilled water was added to 50 g of the meats and the mixture was homogenized. To the homogeneous mixture, distilled water was added and its gross volume was 100 ml. Each sample was kept alone in an incubator at 28° during 24 hr, then it was added 1.5 times of 85% (v/v) ethanol; and the mixture was extracted for 30 min at 80° with stirring. After centrifugation (3000 rpm, 20 min), the supernatant was separated and the residue was similarly reextracted three times successively. After deproteinization with 1% picric acid, picric acid was removed by Dowex 2×8 type (chloride form) resin.[6] The treated sample was concentrated to 50 ml. Half mililitre to 2.0 ml were used in order to analyze the amines.

Results and Discussion

Quantitative analysis of amines were examined through the amino acid analyzer using ion-exchange resin by applying ninhydrin reaction; it was found that method is fully suitable for the analysis of primary amines. To obtain successful results, selection of resin and buffers used in the ion-exchange chromatography of amines was one of the most important factor. As a Hitachi KLA-2 Amino Acid Analyzer was used when buffer change is to be effected three times automatically, it was applied to this study.

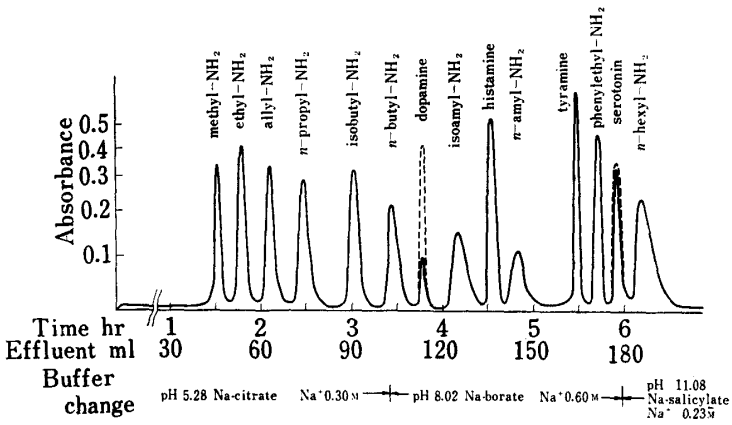

Fig. 1. Chromatogram of Primary Monoamines separated at 50° on an Aminex A_5 (0.6×10 cm) Column

——— : 570 mμ, ——— :440 mμ

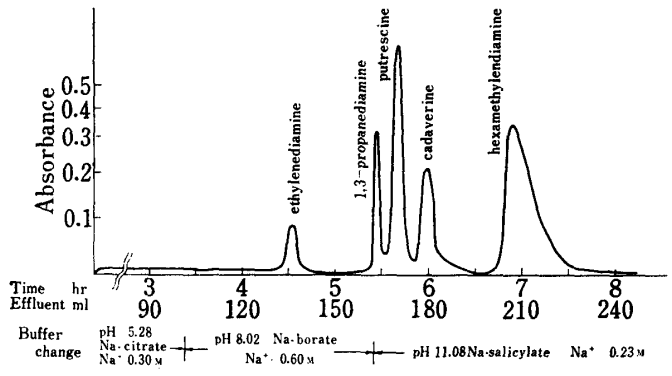

Fig. 2. Chromatogram of Primary Diamines separated at 50° on an Aminex A_5 (0.6×10cm) Column

——— : 570 mμ

Chromatograms of monoamines and diamines carried on with a 0.6×10 cm column and Aminex A_5 which has a good separating ability, are shown in Fig. 1 and Fig. 2, respectively. To analyze monoamines it takes about 7 hr, and to analyze diamines take about 8 hr. The substances separated by the citrate buffer (pH 5.28, Na^+ 0.30 M) are methylamine, ethylamine, allylamine, isobutylamine and *n*-propylamine, then the borate buffer (pH 8.02, Na^+ 0.60 M) sep-

6) W.H. Stein and S. Moore, *J. Biol. Chem.*, **211**, 915 (1954).

arates *n*-butylamine, dopamine, isoamylamine, histamine, *n*-amylamine and ethylenediamine; tyramine, *β*-phenethylamine, serotonin, *n*-hexylamine, 1,3-propanediamine, putrescine, cadaverine and hexamethylenediamine are eluted by the salicylate buffer (pH 11.08, Na^+ 0.20M). isoamylamine and *n*-amylamine which are eluted by the borate buffer, and cadaverine, *n*-hexylamine and hexamethylenediamine by the salicylate buffer occur tailing, but addition of benzyl alcohol could give normal chromatograms. As secondary and tertiary amines do not react with ninhydrin, analysis could not done by this method; on the other way among primary amines, isopropylamine and aromatic amines have a negative ninhydrin reaction. As all the aminoalcohols are concentratedly effluent around ammonia when citrate buffer is applied, analysis is difficult in this system, and more investigations are necessary. Agmatine and higher aliphatic amines (having more than 7 carbon atomes) react with ninhydrin, but as they are not eluted in this system, furthermore investigation is necessary.

Table II. Quantitative Data of Some Amines

Amines	Absorption ratio 640/570	Absorption ratio 440/570	H×W at 570 mμ per 1 μmole (Deviation%)	H×W (Ethylamine 1.00) Average
Methylamine	.449	.338	6.891 (2)	1.38
Ethylamine	.446	.308	5.332 (1)	1.00
Allylamine	.449	.358	4.762 (4)	.90
n-Propylamine	.447	.359	2.288 (4)	.43
Isobutylamine	.457	.307	5.064 (3)	.99
n-Butylamine	.473	.302	1.680 (4)	.33
Dopamine	.459	5.770	2.847[a] (5)	.50
Isoamylamine	.468	.294	1.481 (5)	.27
Histamine	.481	.515	2.773 (3)	.51
n-Amylamine	.459	.326	.996 (6)	.18
Tyramine	.459	.306	11.088 (3)	2.19
Phenethylamine	.429	.322	13.108 (5)	2.49
Serotonin	.535	1.094	3.295[a] (6)	.66
n-Hexylamine	.475	.289	2.469 (5)	.51
Ethylenediamine	.473	.378	2.149 (5)	.42
1,3-Propanediamine	.518	.535	6.500 (6)	1.25
Putrescine	.505	.458	17.989 (5)	3.55
Cadaverine	.526	.446	7.657 (7)	1.42
Hexamethylenediamine	.477	.469	23.458 (2)	4.66

a) H×W at 440 mμ

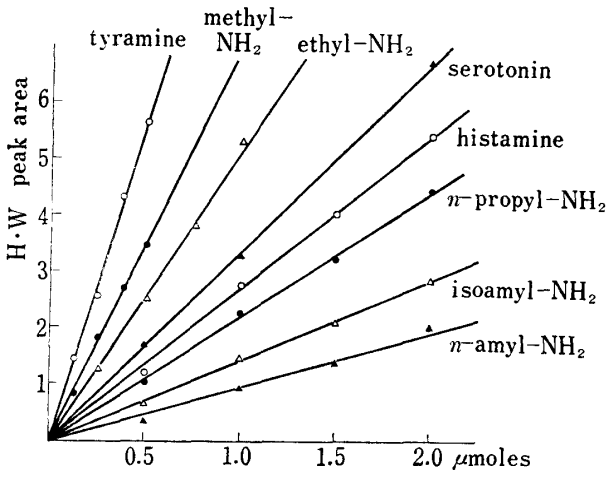

Fig.3. Ralationships of Peak Area (H×W) to Amount of Amine

Absorption ratio of the ninhydrin color of each amine at wavelength of 640, 570 and 440 mμ on peak of chromatogram and the quantitative data of the amines by the chromatograms are summarized in Table II and Fig. 3. As it is shown in Table II, absorption ratios of each amine is constant. Therefore, as it is possible not only by checking the effluent position but also the absorption ratio. As result of these data, the accuracy in the amines ordinarily is less than 5%.

Using above mentioned procedure, amines produced by putrefaction of

marin animals were analyzed. The amines which had not been fully investigated. The quantitative data obtained here are shown in Table III.

Table III. Amines produced by Putrefaction of Marine Animals

Amines \ Kinds	Mackerel	Sea bream	Octopus	Crab	Short-necked clam	Shark	Carp	
hr	24	24	24	24	24	24	18	24
Histamine	9.26		.15	1.41			.94	3.91
Tyramine	1.77	.23	.10	2.58	.47	.31	.04	.27
Putrescine	1.08	.41	4.43	13.70	1.06	.30	.31	.81
Cadaverine	5.72	1.22	2.27	3.27	2.15	.85	2.56	4.59

μmoles/meat 1 g

In this method effluent speed of the developer is 30 ml/hr, but if effluent speed is increased, analytical times should be shortened. Developer were effluent on three times of stepped change, but effluent on graded change or connection of stepped and graded change, it will be successful furthermore.

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The Reimer-Tiemann Reaction of *m*-Halophenols. III.[1] Formation of Dialdehydes[2]

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The preceding paper[1] reported that in addition to the three isomers, 2-bromo-4-hydroxybenzaldehyde and 4- and 6-bromosalicylaldehydes, a new dialdehyde was isolated in the Reimer–Tiemann reaction with *m*-bromophenol.

There have been reports about the isolation of dialdehydes in the Reimer–Tiemann reaction with resorcinol,[4] with resorcinol methyl ether,[5] with thymol,[6] and with orcin.[7] A dialdehyde obtained from resorcinol was confirmed to be 2,4-dihydroxyisophthalaldehyde on the basis of chemical evidence[8] and the structure was supported by its nuclear magnetic resonance (NMR) spectrum.[9] In the case of orcin, two isomeric dialdehydes isolated were

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3) Location: *No.* 78, *Sho-machi-1-chome, Tokushima.*
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