acetate buffer (pH 4.5, 2 ml) and partially purified RNase T_2 (1500 units)[5] was added. The mixture was incubated at 25° for 2 hr and then chromatographed on a column (1.55×25 cm) of DEAE-Sephadex A-25 (Cl^-), which was eluted with a linear gradient using 0.003N HCl and 0.15M LiCl in 0.003N HCl (each 1.5 liters). The main peak containing V was adjusted to pH 4.5 with LiOH and evaporated to dryness *in vacuo*. LiCl was removed by dissolving the residue in MeOH and precipitating with acetone repeatedly. Drying over P_2O_5 *in vacuo* gave the trilithium salt of Va(b,c).

2) The peak V obtained by chromatography of acid hydrolyzate described above was worked-up in the same manner as described for Peak IV to give the calicium salt of Va or IVb. Yields and analytical data are summarized in Table I.

Alkaline hydrolysis (1N NaOH, 100°, 90 min) of IV and V thus obtained gave adenosine 2′,5′- and 3′,5′-bisphosphate, respectively, which were detected by paper chromatography using solvent III.

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Selective and Sensitive Method for Determination of Vanillylmandelic Acid (VMA) in Urine

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The major metabolite, vanillylmandelic acid (VMA), of norepinephrine and epinephrine in human urine has been measured by colorimetric[2,3] and gas chromatographic[4–6] methods. Those methods have some faults as follows: 1) The colorimetric methods are not selective for VMA on account of the obstruction by other urinary phenolic compounds, such as *p*-hydroxymandelic acid,[2] *etc.*,[3] and sensitivity is low. 2) The gas chromatography by ionization detector[4,5] is not sensitive enough for measurement of VMA in urine of normal person. 3) Wilk, *et al.* converted VMA to vanillin and analysed it as trifluoroacetyl derivative by gas chromatography equipped with electron capture detector,[6] however, the accuracy of their methods is low because of the absence of an internal standard in gas chromatographic analysis and the recovery is about fifty percent. 4) The common separation procedure of those methods depends on extraction by organic solvent such as ethyl acetate or ether from acidified urine and it is the reason why the prepared sample is contaminated with other substances to lower the selectivity of them.

Now we investigated the separation with adsorbents and gas chromatographic analysis with electron capture detector without conversion of VMA to vanillin. The established method consists of the separation of VMA by adsorption on Amberlite XAD-2 from urine at pH 1 and gas chromatography of VMA after methylation in ethanol in a short period and trifluoroacetylation.[7]

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This method is selective and sensitive for VMA since the measurement of VMA in urine of normal person is possible as shown in Fig. 1.

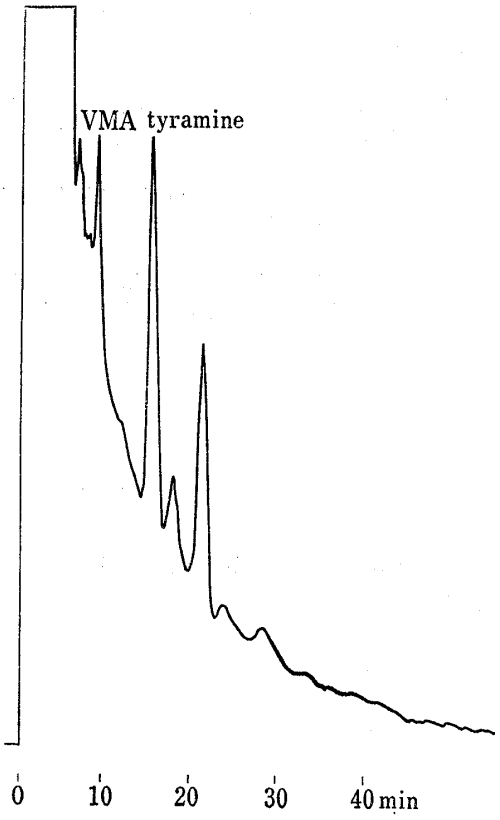

Fig. 1. Gas Chromatogram of XAD-2 Extract of Normal Person's Urine

condition: 2% GE-XF 1105 on Gas-Chrom P (80—60 mesh); glass column, 140×0.4 cm; 120° and about 80 ml of nitrogen per min; detector: an electron capture detector

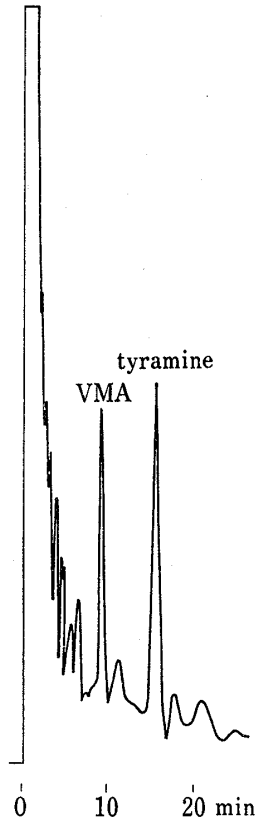

Fig. 2. Gas Chromatogram of XAD-2 Extract of Urine of The Patient with Pheochromocytoma

condition and detector: the same as in Fig. 1

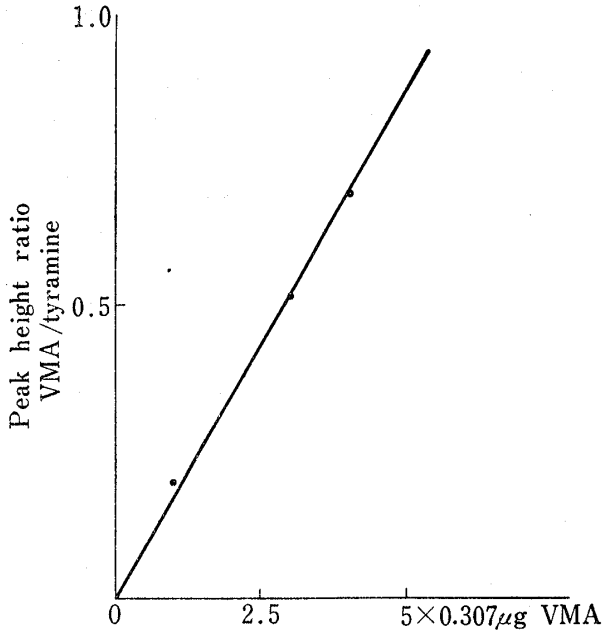

Fig. 3. Working Curve for VMA using Tyramine as an Internal Standard

Gas chromatographic conditions are the same as in Fig. 1.

Method and Results

An aliquot of urine was acidified to pH 2 with 1N hydrochloric acid and diluted to 10 volumes with dist. water. Five ml of the solution was passed through SE-Sephadex column (1.5 ml, H form) to remove the urinary pigments and the column was washed with 4 ml of dist. water. The effluent and the washings were combined and, after adjustment of its pH to 1 with N hydrochloric acid, passed through Amberlite XAD-2 column (2 ml, 200—400 mesh, conditioned with 0.1N hydrochloric acid) and the column was washed with 2 ml of 0.1N hydrochloric acid. The adsorbed VMA was eluted with 4 ml of dist. water and the successive 2 ml of 10% methanol in water. An aliquot of the combined eluate was concentrated under reduced

pressure and dried over calcium chloride for 5 minutes *in vacuo* at room temperature. The residue was dissolved in 0.5 ml of ethanol, and one drop of freshly prepared ethereal diazomethane (28 mg of diazomethane per ml of ether) was added. After 10 seconds, the reaction was stopped with a few drops of 0.1 N acetic acid and to the mixture 0.1 ml of tyramine solution in 0.1 N acetic acid (containing 0.723 μg of tyramine hydrochloride) was added. The solution was evaporated to dryness under reduced pressure and dried over calcium chloride for 5 minutes. The residue was trifluoroacetylated with one drop of ethyl acetate and 10 μl of trifluoroacetic anhydride for 5 minutes, then diluted with 0.5 ml of *n*-hexane. One μl of the solution was injected to a gas chromatograph equipped with electron capture detector (Shimadzu GC 4APE, 1.4 m glass column packed with 2% GE–XF 1105 on Gas Chrom P, column temperature, 120°).

The chromatogram thus obtained was shown in Fig. 1 and 2, in which the peak of VMA was identified by its retention time and by addition treatment of the authentic compound. The chromatogram in Fig. 1 corresponds to 400 mμl of the urine of normal person, while that in Fig. 2 to 6 mμl of the urine of the patient with pheochromocytoma. The linearity between the peak height ratio of VMA to tyramine and the amount of VMA was attained as shown in Fig. 3. The average recovery percent of added VMA to urine was 103% as shown in Table I.

Table I. Recovery Percents of Added VMA (2.77 μg) to Urine of Normal Person (0.5 ml)

No.	Recovery %	No.	Recovery %
1	101	6	109
2	106	7	101
3	105	av.	103
4	99.4	st. dev.	4.02
5	97.8		

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Two Methyl Ethers of 4-Aminotropolone and Their Bromination

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Until present time, many reactions of various aminotropone derivatives have been investigated.[2]

The pK_a values of aminotropones have also been measured in order to study the correlation of their reactivities and pK_a values.[3] In the present work, two methyl ethers of 4-

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