The thioazlinone ring made from the first amino acid residue is opened easily with TEA–CO_2 solution (pH 9.0) at room temperature and turned to DNTC–amino acid, which had fluorescence maximum at 450 mμ (exciting 340 mμ). Above mentioned condition was applied to Leu–Gly–Gly. In the first round, 75% production of Gly-Gly and in the second, 100% of Gly were determined with ninhydrin. DNTC–Leu, DNTC–Gly, DNTC–Leu–Gly–Gly and DNTC–Gly–Gly produced were identified on TLC plate, detection limit was about 10^{-10} mole and the fluorescence was stable in a dark place for a few days. The method is much more sensitive (about 100 times) than phenylisothiocyanate method, and has another merit that DNTC–peptide produced is also fluorescent and easily purified by thin-layer chromatography. Details of this experiment will be reported in the near future.

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Detection of Catecholamines after N-Dansylation on the Surface of Alumina

Catecholamines (CA), *i.e.* dopamine, norepinephrine and epinephrine are biologically important amines and determined by fluorometry (THI method and ED method) and gas chromatography. The THI method[1,2] is most popular, however the separate determination of norepinephrine and epinephrine is difficult on account of the similarity of those fluorofores. The ED method[3] is cumbersome because it requires the separation of each amines before the reaction. The gas chromatographic separation and detection have been examined by trimethylsilyl, acetyl, trifluoroacetyl and heptafluorobutyryl derivatives and Kawai, *et al.* succeeded in the determination of CA in urine and tumor by gas chromatography.[4] The method is sensitive and selective for CA, however it is difficult to treat many samples simultaneously in the case of clinical use. Now we investigated dansylation of CA with 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS–Cl) and detection of those fluorescent derivatives on a thin layer chromatogram.

Seiler, *et al.*[5] and Creveling, *et al.*[6] have studied dansylation of CA in the presence of sodium bicarbonate to gain some fluorescent products which are probably the mixture of N-mono-DNS, N,O-di-DNS and N,O,O-tri-DNS derivatives. To overcome this difficulty complete dansylation was examined by Diliberto, Jr., *et al.*[7] On the contrary, we have attained N-mono-dansylation of CA by protecting the pyrocatechol with alumina.

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2) R.J. Merrills, *Nature*, **193**, 988 (1962); *idem. Anal. Biochem.*, **6**, 272 (1963).
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5) N. Seiler and M. Wiechmann, *Experientia*, **21**, 203 (1965); *idem, Z. Anal. Chem.*, **220**, 109 (1966); *idem, J. Chromatog.*, **28**, 351 (1967).
6) C.R. Creveling, K. Kondo and J.W. Daly, *Clin. Chem.*, **14**, 302 (1968).
7) E.J. Diliberto, Jr. and V. DiStefano, *Anal. Biochem.*, **32**, 281 (1969).

The procedure is as follows;[8] 1) adsorption of CA (6×10^{-9} mole) on alumina (0.1 g) at pH 8.8 under shaking for 5 min, 2) washing of alumina with 0.25M triethylamine–carbonate buffer (pH 8.8), 3) dansylation of CA on the surface of alumina with DNS–Cl (3.7×10^{-6} mole in 0.2 ml of dioxane) in 0.25M triethylamine–carbonate buffer (0.2 ml) at pH 8.8 for 5 min, 4) degradation of the reagent with the addition of one drop of pyridine, 5) washing of alumina with the buffer, methanol and dist. water, 6) elution of DNS derivatives from alumina with 0.2N acetic acid (10 ml), 7) evaporation of the eluate under reduced pressure, 8) thin–layer chromatography of the residue on silica gel with benzene–dioxane–acetic acid (90:25:4) as a developing solvent, 9) detection of DNS–CA under ultraviolet (UV) light (360 mμ). The limit of detection is about 0.1 μg of CA.

The same treatment was taken to the hydrolysed urine of normal persons (1 ml) and that of a pheochromocytoma patient (0.1 ml) and the chromatogram thus obtained is shown in Fig. 1.

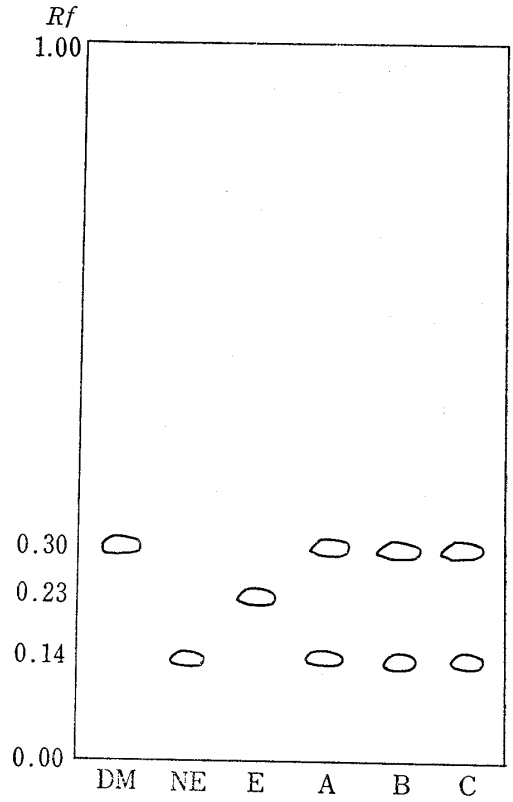

Fig. 1. Thin–Layer Chromatogram of N-Dansylated Catecholamines

DM: dopamine, NE: norepinephrine, E: epinephrine, A and B: urinary CA of normal persons, C: urinary CA of pheochromocytoma patient

This study is under continuation, and the details and applications of the method will be published in a near future.

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8) Triethylamine was refluxed with phthalic anhydride for 10 hr and distilled. The same treatment was taken again. Dioxane was refluxed with 1N HCl for 7 hr, and after dehydration with NaOH distilled with metalic sodium. All glass apparatuses were siliconized with dichlorodimethylsilane in toluene.