suggest the existence of the specific antibody reacted with methamphetamine moiety in the serum.

The radioimmunoassay for methamphetamine using this antibody will be described in the following communication.

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## The Radioimmunoassay for Methamphetamine[1)]

The radioimmunoassay for methamphetamine (I) in urine of man has been established by use of the $^{125}$I-labelled derivative of N-[3-(*p*-hydroxyphenylacetylamino)propyl]-methamphetamine (V) in sensitivity of 8.0 ng/100 μl.

The antigen binding capacity of the antiserum prepared from N-carboxymethyl-methamphetamine-BSA (II) was determined by a new method of immunoassay using fluorescence labelled N-(3-dansylaminopropyl)methamphetamine (IV) ("fluoroimmunoassay").

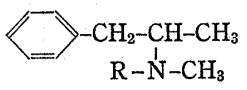
The specificity of the antibody was examined by its cross reaction with several methamphetamine analogues.

**Keywords**——radioimmunoassay; "fluoroimmunoassay"; methamphetamine; N-carboxymethylmethamphetamine-BSA conjugate; N-(3-dansylaminopropyl)methamphetamine; N-[3-(*p*-hydroxyphenylacetylamino)propyl]methamphetamine; $^{125}$I-iodination; specificity of antiserum; antihyptonic

Methamphetamine replaced in blood and urine of man is usually determined by gas– and thin–layer chromatography.[2)] These methods, however, involve a number of technical problems in the practical measurement of the compound in biological samples. Since a more rapid, highly specific and much sensitive technique in the determination has obviously been requisite for forensic and clinical purposes, an application of recent development of radioimmunoassay must be invaluable for achievement of the purpose. As far as we are aware, Cheng, *et al.* has reported the radioimmunoassay for amphetamines using $^{3}$H-labelled amphetamine.[3)] We now wish to communicate in the present paper a more convenient way of determination of methamphetamine (I) in urine of man by means of the radioimmunoassay

1) S. Inayama, Y. Tokunaga, E. Hosoya, T. Nakadate, T. Niwaguchi, K. Aoki, and S. Saito, *Chem. Pharm. Bull.* (Tokyo), **25**, 838 (1977).
2) *a*) V.P. Dole, W.K. Kim, and I. Eglitis, *J. Am. Med. Assoc.*, **198**, 115 (1966); *b*) M.L. Gastos, G.E. Kananen, R.M. Young, J.R. Monforte, and I. Sunshine, *Clin. Chem.*, **16**, 931 (1970); *c*) B.S. Finkle, E.J. Cherry, and D.M. Taylor, *J. Chromatog. Sci.*, **9**, 393 (1971).
3) L.T. Cheng, S.Y. Kim, A. Chung, and A. Castro, *FEBS Lett.*, **36**, 339 (1973).

using $^{125}$I-labelled methamphetamine. The preparation of an antiserum of methamphetamine (I) was conducted by immunization of N-carboxymethylmethamphetamine-BSA (II).[1)]



I: R=H; II: R=$CH_2CONH$-BSA;
III: R=$(CH_2)_3NH_2$; IV: R=$(CH_2)_3NHSO_2C_{10}H_6N(CH_3)_2$;
V: R=$(CH_2)_3NHCOCH_2C_6H_4OH$-*p*

Chart 1. Diagram of the Formulas of Methamphetamine Derivatives

The antigen binding capacity of the specific antibody in the serum was demonstrated by the usual Ouchterlony method and by a new method of the immunoassay by use of fluorescence labelled methamphetamine ("fluoroimmunoassay"). This method that requires no RI materials, seems to be more convenient to determine a titer of the binding capacity when compared to the conventional method.

The fluorescence labelled methamphetamine was prepared as follows. Treatment of the oily N-(3-aminopropyl)methamphetamine (III), *cf.*[3)] $C_{13}H_{22}N_2$,[4)] with dansyl chloride in the presence of $Na_2CO_3$ in dioxane-water at 60° for 1 hr afforded, in 92% of yield, N-(3-dansylaminopropyl)methamphetamine(IV) as a pale yellow oil. $C_{25}H_{33}O_2N_3S$. Fluorescence (in MeOH) $\lambda$ max: Ex. 355 nm; Em. 510 nm.

The binding capacity of IV with the serum was demonstrated by the intensity of fluorescence with the benzene extract of the precipitate, which was prepared by almost the same procedure as mentioned below. The increase of the binding capacity by use of this method is illustrated in Fig. 1.

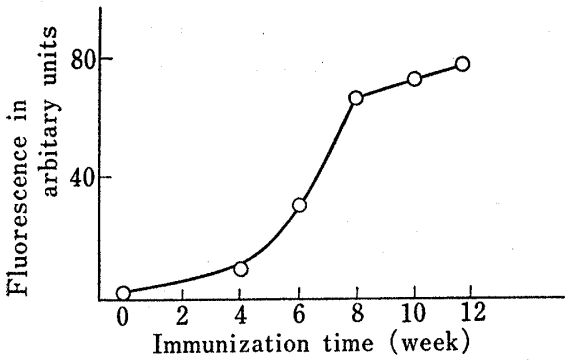



Fig. 1. Antigen Binding Capacity of Antiserum for Methamphetamine (I) by "Fluoroimmunoassay" using N-(Dansylaminopropyl)-methamphetamine (V)

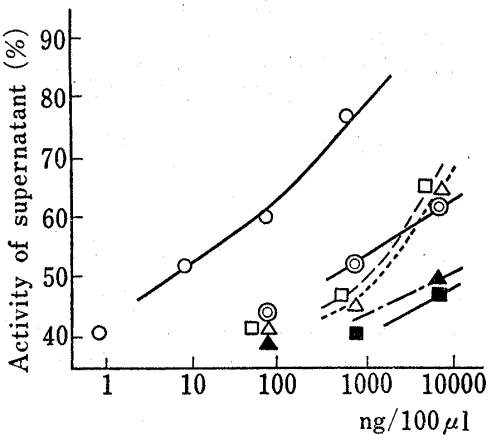



Fig. 2. Standard Curve of Methamphetamine (I) and Inhibition Curves by Several Phenethylamines

—○—○—: methamphetamine (I)
—◎—◎—: chloroephedrine
---△---△---: ephedrine
—▲—▲—: *p*-hydroxymethamphetamine
—□—□—: amphetamine
—■—■—: methoxyphenamine

The RI labelled methamphetamine for the immunoassay was prepared as follows. A solution of III in tetrahydrofuran was allowed to stand overnight at room temperature with *p*-acetoxyphenylacetyl chloride in the presence of anhydrous $Na_2CO_3$. Hydrolysis of the product with 5% KOH-MeOH for 10 hr at 25° gave, in 66% of yield, N-[3-(*p*-hydroxyphenylacetylamino)propyl]methamphetamine (V) as a colorless oil. $C_{21}H_{28}O_2N_2$. $^{125}$I-iodina-

4) Satisfactory elemental analyses and spectral data were obtained for all the new compounds described in this paper.

tion of V was executed, according to Greenwood, *et al.*,[5] and followed by purification through SP-Sephadex C25 column.

The radioimmunoassay procedure is as follows. 100 μl of urine or Tris-HCl buffered saline(0.05 M, pH 7.4), containing various amounts of unlabelled methamphetamine, was submitted to incubation at 37° for 1 hr with 300 μl of the antiserum diluted 900 times by the same buffered saline as above, which contains a fixed amount of the $^{125}$I-labelled compound (*ca.* 50000 cpm). An equal volume of satd. $(NH_4)_2SO_4$ solution was added after the incubation to all reaction mixtures. After centrifugation at 3000 rpm for 15 min at 5°, the activity of the supernatant containing the unbound labelled compound was conuted as usual. The sensitivity of the present immunoassay proves to be 8.0 ng/100 μl. To examine the specificity of the antiserum several kinds of substituted phenethylamines were tested for their abilities of inhibition of the binding of the labelled compound with the antibody. The results are exhibited in Fig. 2 together with the standard curve of I.

An illegal and non-medical consumption of more than 10 mg of I have been prevailing in a certain community indulged with I by its abuse. It was reported that the most abundunt excrement into urine in man is the unchanged I, and the rate of the excretion is calculated approximately 20% per 24 hr.[6] Therefore, the concentration of I in the urine collected within 24 hr (totally 1—2 liter) seems to be estimated about 1.0—2.0 μg/ml. In conclusion, since this new method for determination of I has revealed the sensitivity of 8.0 ng/ 100 μl, it may be expected for application in the field of forensic sciences.

A further study on multiplication of sensitivity of the method is now under progress.

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5) F. Greenwood, W. Hunter, and J. Glover, *Biochem. J.*, **89**, 114 (1963).
6) J. Caldwell, L.G. Dring, and R.T. Williams, *Biochem. J.*, **129**, 11 (1972).

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## A New Synthesis of Dihydromancunine

Dihydromancunine, a model for a proposed indole alkaloid biosynthetic intermediate, mancunine, was synthesized using a modified Polonovski reaction of desmethylhirsutine N-oxide, which was derived from the indole alkaloid hirsutine.

**Keywords**——indole alkaloid; biosynthetic intermediate; biomimetic synthesis; Polonovski reaction; N-oxide

In 1974, Brown and co-workers[1] reported the synthesis of dihydromancunine (**4**), a model for a proposed indole alkaloid biosynthetic intermediate, mancunine, from dihydro-

1) R.T. Brown, C.L. Chapple, and A.A. Charalambids, *Chem. Commun.*, **1974**, 756.