Communications to the Editor

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Autoradiographic Study on the Distribution of ^{125}I-Labeled Lysozyme after Intravenous Injection in Rats

Autoradiograms of rats following intravenous injection of ^{125}I-labeled lysozyme can be taken to support indirectly the effectiveness and usefulness of lysozyme as a therapeutic agent for chronic sinuitis.

Keywords——^{125}I-labeled hen egg-white lysozyme; rat; intravenous injection; autoradiogram; tissue distribution; chronic sinuitis

Although many enzyme proteins such as lysozyme and chymotrypsin have been used as anti-inflammatory drugs, the definite pharmacological mechanism of these enzymes remains to be solved. The intestinal absorption and biotransformation of hen egg-white lysozyme-hydrochloride (abbreviated as lysozyme) in rats were reported previously.[1] It has been shown that the most part of the radioactivity dosed was absorbed from the intestine and excreted in urine, but only 2% of the dose was absorbed as immunoprecipitable and trichloroacetic acid-precipitable radioactivity. This paper, therefore, deals with autoradiographic study on the distribution of ^{125}I-labeled lysozyme (abbreviated as ^{125}I-lysozyme) injected intravenously to rats, especially on the distribution in the nasal sinuses in relation to the clinical effectiveness of lysozyme administered orally.

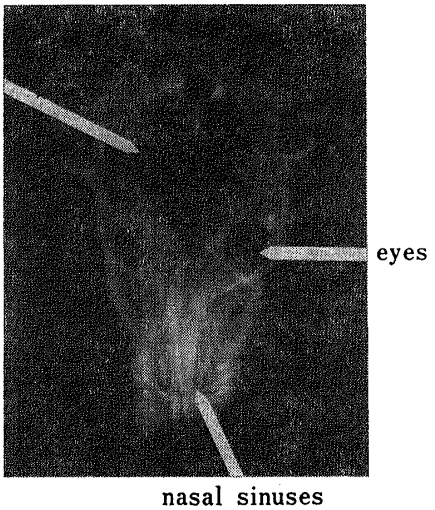

Fig. 1. Autoradiogram of Head Involving Nasal Sinuses of the Rat 5 min after Intravenous Injection of 0.4 mg ^{125}I-Lysozyme per kg

^{125}I-lysozyme with the specific radioactivity of 857 μCi/mg was prepared by the method of ^{131}I-labeling reported previously.[1a] Male rats (200—220 g) of Wistar strain were fasted for 24 hr prior to experiment. The rat was injected into the femoral vein with a dose of 0.4 mg of ^{125}I-lysozyme per kg, and the rat after the injection was treated with the usual manner for whole-body autoradiogram. The dried sections cut at 20 μ were contacted with X-ray film (Sakura, Type N) and exposed for 2 days.

In the preliminary experiment, most of the radioactivity in serum, liver and kidney during the first 30 min after the injection was noticed to originate from the intact lysozyme molecules.

Fig. 1 shows the autoradiogram of head involving nasal sinuses of the rat 5 min after the injection.

As shown in Fig. 1, the radioactivity was intensively localized in nasal sinuses but not in eyes and brain. This result indicated that ^{125}I-lysozyme injected transferred to nasal sinuses. This finding was in good agreement with the result reported by Narabayashi.[2] In his study on the lysozyme labeled with fluorescence which was orally administered to rabbits, it was shown that the fluorescence was detected intensively in tunica mucosa nasi.

1) *a*) T. Yuzuriha, K. Katayama, and T. Fujita, *Chem. Pharm. Bull.* (Tokyo), **23**, 1309 (1975); *b*) *Idem*, *ibid.*, **23**, 1315 (1975).
2) S. Narabayashi, *Jap. J. Otol.* (Tokyo), **73**, 473 (1970).

The therapeutic effect of lysozyme for chronic sinuitis has been reported by numerous investigators.[3)] It has been clinically ascertained by means of the double blind test that the effectiveness depended on the dose levels of lysozyme.[3a)] Imamura, *et al.*[3b)] reported that the viscosity of pus in patients with chronic sinuitis decreased without damage of tunica mucosa nasi following the treatment of lysozyme. In comparative studies on the therapeutic effects against chronic sinuitis using α-chymotrypsin, streptokynase, streptodornase, pronase-P, bromelin and lysozyme, Kagitomi[3c, 3d)] reported that the effect of lysozyme was more remarkable than that of the proteolytic enzymes on the cure of inflammation at the nasal sinuses based on the observation of the clinical and histopathological aspects. Additionally, lysozyme has bactericidal[4a)] and anti-virus[4b)] action. Therefore, the result shown in Fig. 1 may be taken to support that the enzyme has the therapeutic effect against chronic sinuitis.

Fig. 2 shows the whole-body autoradiogram of the rat 30 min after the injection.

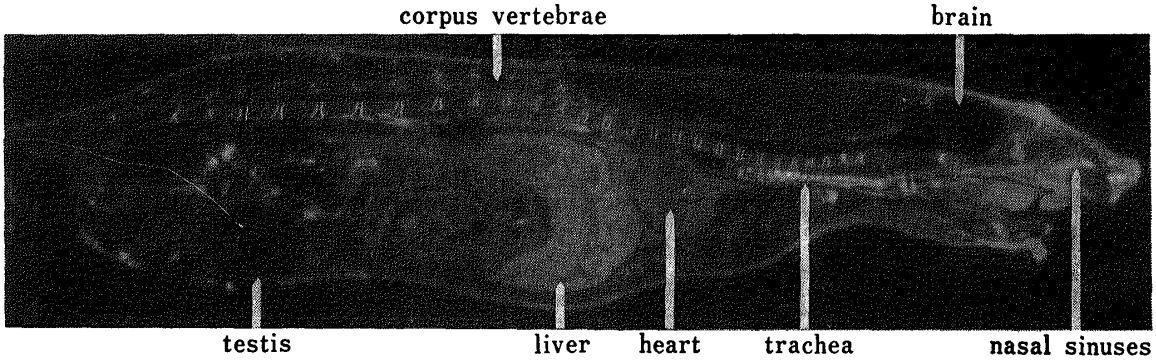

Fig. 2. Whole-Body Autoradiogram of the Rat 30 min after Intravenous Injection of 0.4 mg ^{125}I-Lysozyme per kg

As shown in Fig. 2, the radioactivity was found to be localized on the joint surfaces of vertebrae, and in the trachea, nasal sinuses and liver. The distribution of radioactivity in the other tissues was in good agreement with the findings reported previously.[1b)]

It was clinically found that lysozyme relieved rheumatic fever and pain,[5a)] and had a therapeutic effect on rheumatoid arthritis.[5b)] The autoradiogram shown in Fig. 2 reveals transferring of ^{125}I-lysozyme to the hyaline cartilage, and may be suggestive of the usefulness of this enzyme as a therapeutic agent for arthral diseases.

The findings of autoradiographic study reported herein may be taken to support indirectly such therapeutic effects of lysozyme as described above, although the peculiarity of lysozyme as compared with other proteins in the localization to nasal sinuses remains to be studied.

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3) *a*) R. Takahashi, M. Yoshimi, K. Yano, Y. Yasu, M. Hamada, K. Seki, S. Minami, A. Yokoyama, T. Ohto, M. Takayanagi, H. Moriyama, M. Tsutsumi, Y. Kasahara, K. Nagakura, Y. Shimizu, and T. Takatsu, *Oto-Rhino-Laryngology* (Tokyo), **17**, 731 (1974); *b*) T. Imamura, H. Saito, T. Kawamoto, and M. Suzuki, *Pract. Otol.* (Kyoto), **61**, 748 (1968); *c*) T. Kagitomi, *Otologia* (Tokyo), **38**, 61 (1966); *d*) T. Kagitomi and K. Shouzuka, *Pract. Otol.* (Kyoto), **60**, 478 (1967).

4) *a*) K. Inoue, Y. Tanigawa, M. Takubo, M. Satani, and T. Amano, *Biken J.*, **2**, 1 (1959); S. Nakazawa, A. Yamamoto, Y. Yokota, T. Mitsutake, H. Miyoshi, and Y. Yamashita, *Jap. J. Antibiotics*, **21**, 10 (1968); *b*) Z.V. Ermolèeva, *Antibiotiki*, **8**, 39 (1963).

5) *a*) M. Yamamoto and T. Hoshino, *Jap. J. Clin. Exp. Med.*, **44**, 2432 (1967); *b*) A.B. Sannelli and A. Trirasacchi, "3 rd International Symposium on Fleming's Lysozyme," Museo della Scienza e della Tecnica, Millan, 1964, p. 150.

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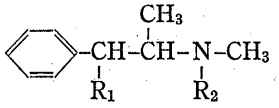
Preparation of a Specific Antibody to Methamphetamine

N-Carboxymethylmethamphetamine(III) has been synthesized directly from methamphetamine(I) and through a new route starting from ephedrine(IV). This new hapten was conjugated with BSA and the antiserum for I was prepared by immunization of rabbits with the conjugate (VIII). The production of the antibody for I was confirmed by the ring test and Ouchterlony method.

Keywords——radioimmunoassay; methamphetamine; new synthetic route; N-carboxymethylmethamphetamine; N-carboxymethylmethamphetamine-BSA; specific antibody; antiserum; ouchterlony method

Methamphetamine is widely noted for the distinctive antihypnotic activity. Since this medicine has often caused a serious social problem owing to its dangerous abuse, the preparation, storage and consumption of the antihypnotics have always been under a severe legal restriction. This particular situation has led us first to device a new synthetic method of N-carboxymethylmethamphetamine (III) being derived from readily available ephedrine(IV) without use of methamphetamine (I), which is also prepared directly from I. The present paper concerns the preparation of III, the conjugate of the hapten with bovine serum albumin (BSA) (VIII) and the specific antibody being utilized for the radioimmunoassay for I, which will be described in the following paper.



I: R_1=H, R_2=H
II: R_1=H, R_2=$CH_2CO_2CH_2CH_3$
III: R_1=H, R_2=CH_2CO_2H
IV: R_1=OH, R_2=H
V: R_1=Cl, R_2=H
VI: R_1=SC_6H_5, R_2=H
VII: R_1=SC_6H_5, R_2=$CH_2CO_2CH_2CH_3$
VIII: R_1=H, R_2=CH_2CONH-BSA
IX: R_1=H, R_2=CH_2CONH-EA[a]

a) EA=egg albumin

Chart 1. Diagram of the Formulas of Methamphetamine Derivatives

Treatment of I in benzene with ethyl bromoacetate in the presence of anhydrous sodium carbonate (Na_2CO_3) at room temperature overnight afforded, in 71% of yield, 1-phenyl-2-(N-ethoxycarbonylmethyl-N-methyl)aminopropane(II) as a colorless oil. $C_{14}H_{21}O_2N$.[1] Mass Spectrum *m/e*: 236 (QM^+).[2] bp 151—154° (5 mm Hg). IR ν_{max}^{KBr}: 1753 cm^{-1} (C=O). PMR

1) Satisfactory elemental analyses were obtained for all the new compounds described in this paper.
2) Quasi molecular ion observed by the chemical ionization mass spectrometry using methane as a reagent gas.