formed was filtered off. The dried product (47 mg, m.p. 68-70°), homogeneous on t.l.c., was identified as 3,3-dimethylacrylic acid by comparison (i.r., n.m.r.) with an authentic sample. Spec. act. 3.1×10^8 dpm/mmole.

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Preparation of ^{131}I-labelled elastase

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Elastase is a pancreatic proteolytic enzyme with the action to solubilize elastic fibres [1, 2]. For the studies relating to its intestinal absorption and metabolic fate, ^{131}I-labelled elastase was required.

Our first attempts to prepare tritiated elastase by the Wilzbach technique were unsuccessful, which is based upon Steinberg's method for the tritiation of lysozyme [3]; a large extent of decomposition of the compound was observed during the labelling process. Then Greenwood's [4] method was applied to prepare ^{131}I-labelled elastase. The present paper is concerned with the ^{131}I-labelling of elastase and the purity of the preparation.

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Twenty mg of electrophoretically homogeneous elastase (106 elastase units/mg) [5], isolated from hog pancreas according to Loeven's method [6], was dissolved in 0.5 ml of aqueous solution of ^{131}I-sodium iodide (nearly carrier free, 0.5 mCi) [7] in a 10 ml test tube. 0.05 ml of chloramine-T solution (2 mg/ml of 0.05 M phosphate buffer, pH 7.5) was added to the elastase —$Na^{131}I$ solution at room temperature. After stirring this solution for about 20 sec., each 0.05 ml of sodium metabisulfite solution (2.4 mg/ml) and potassium iodide solution (10 mg/ml) was added to the reaction mixture in succession.

The ^{131}I-labelled elastase was fractionated from the resultant mixture by gel filtration through a 1.5 × 30 cm column of Sephadex G-50 using 0.05 M ammonium acetate buffer (pH 8.0) at 6 ml/hr of flow rate in a cold chamber (4° C). The measurements of the optical density at 280 mμ (Hitachi spectrophotometer model 124) and the radioactivity (Aloka well-type scintillation counter TDC-5) in each fraction tube afforded the elution diagram of Figure 1. By lyophilization ^{131}I-labelled elastase was isolated from the protein fractions of tube numbers 11, 12, and 13 in Figure 1.

^{131}I-labelled elastase was identical with the starting material in enzymatic activity and the specific radioactivity was 14 μCi/mg. The radiochemical purity analysed by paper-electrophoresis and paper-chromatography was 97.7 % and 96.1 % respectively (Fig. 2 and 3).

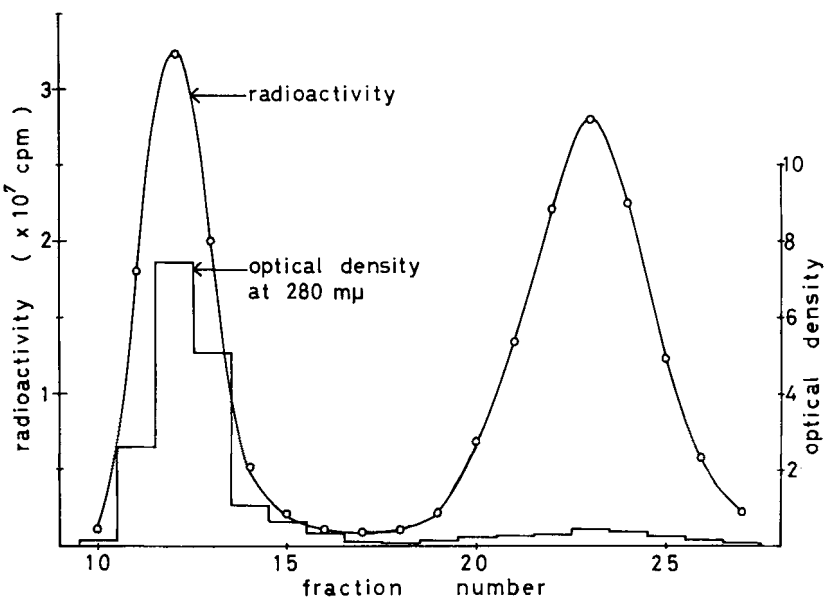

FIG. 1. Fractionation of ^{131}I-elastase on Sephadex G-50.

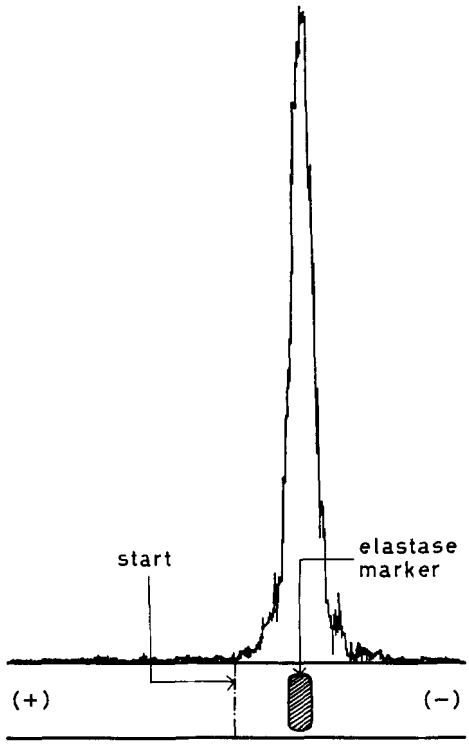

FIG. 2. Electrophoretic pattern of ^{131}I-labelled elastase.
Electrophoresis : cellulose acetate strip, 5 × 9 cm; buffer, 0.1 M glycine-sodium hydroxide (pH 10.0); room temp.; 2 hr; 0.6 mA/cm.
Radio-scanning : speed, 12.5 mm/min; time const., 1 sec; slit-width, 1 mm with a thin window gas-flow counting system (Aloka thinlayer chromatogram scanner model TLC-2B).

Biochemical experiments with this labelled material will be reported elsewhere.

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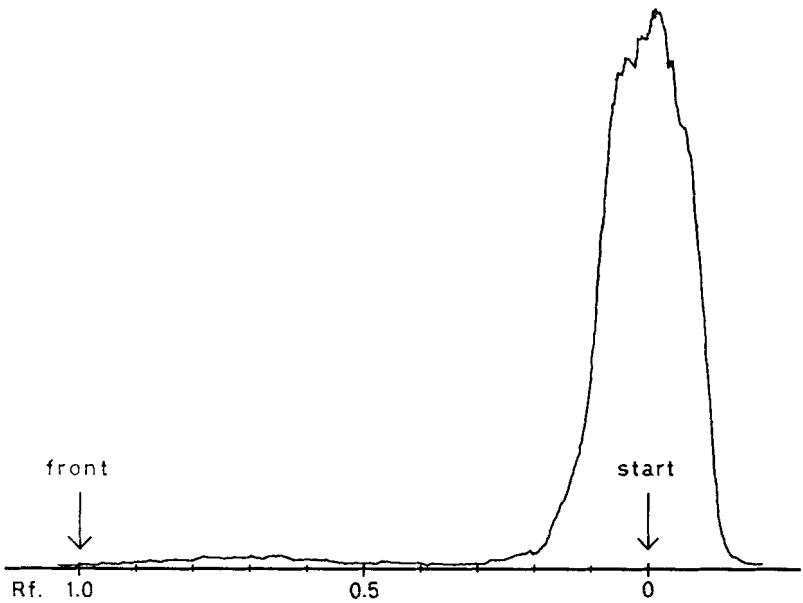

FIG. 3. Radio-paper-chromatogram of ^{131}I-labelled elastase.

Paper-chromatography : Toyo roshi No. 51; solvent, 95 % ethanol : 2 N ammonia (9 : 1).

Radio-scanning : speed, 12.5 mm/min; time const., 1 sec; slit-width, 1.5 mm with a windowless gas-flow counting system (Aloka paper chromatogram scanner model TRM-1B).

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Synthesis of 7-chloro-1-[cyclopropylmethyl-(methylene-^{14}C)]-5-phenyl-1H-1,4-benzodiazepin-2 (3H)-one and 7-chloro-1-(cyclopropylmethyl)-5-phenyl-1H-1,4-benzodiazepin-2 (3H)-one-5-^{14}C

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In order to more easily study the absorption, excretion, and distribution patterns of 7-chloro-1-(cyclopropylmethyl)-5-phenyl-1H-1,4-benzodiazepin-