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# STUDIES ON BIOTRANSFORMATION OF ELASTASE

## II. INTESTINAL ABSORPTION OF $^{131}$I-LABELED ELASTASE *IN VIVO*

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SUMMARY

Intestinal absorption of $^{131}$I-labeled elastase was studied in rats paying attention to the lymphatics and the portal vein as the route of absorption.

1. Following the intraintestinal administration of $^{131}$I-labeled elastase in rats with thoracic duct fistula, protein-bound and immunoprecipitable radioactivity were determined in both serum and lymph.

2. The amount of the absorbed immunoprecipitable radioactivity *via* lymphatics was 0.05 and 0.02 % per 1 and 5 mg dose of $^{131}$I-labeled elastase, respectively.

3. The absorption of immunoprecipitable radioactivity *via* portal vein was calculated according to the method of J. C. K. Loo and S. Riegelman (*J. Pharm. Sci.*, 57 (1968) 918) using the concentration of immunoprecipitable radioactivity in serum. The amount of absorbed immunoprecipitable radioactivity *via* both lymphatic and portal vein routes was 0.149 and 0.053 % per 1 and 5 mg dose of $^{131}$I-labeled elastase, respectively.

Absorption *via* lymphatics was 36 % of the total immunoprecipitable radioactivity absorbed *via* both routes.

The pharmacokinetic profile of $^{131}$I-labeled elastase was determined in rats following intravenous injection of $^{131}$I-labeled elastase. Serum levels declined exponentially after 1 h and the two-compartmental model was applied to analyze the time course of serum levels.

## INTRODUCTION

Our previous report[1] on the transport of $^{131}$I-labeled elastase *in vitro* has shown the possibility that elastase transfers across the intestine. The results obtained seemed to be comparable with those reported by other investigators on the intestinal absorption of $\alpha$-chymotrypsin[2] and cytochrome *c*[3].

In addition, there are a few reports stating that the intestinal lymphatics play a role in the absorption of proteins[4,5]. This paper deals with the investigations on the intestinal absorption of $^{131}$I-labeled elastase *in vivo* and on the role in the absorption of lymphatics in comparison with the portal vein.

## MATERIALS

### *$^{131}I$-labeled elastase (abbreviated as [$^{131}I$]elastase*

[$^{131}$I]Elastase (spec. act. 10–40 $\mu$Ci/mg) was prepared by the method described in the previous paper[6].

### *Antisera*

Antisera to elastase were obtained from rabbits using the procedure of McIvor and Moon[7]. Commercially prepared antisera to rabbit $\gamma$-globulin (goat sera) were obtained from Research Institute for Microbial Diseases, Osaka University.

## METHODS

### *Animal experiments*

Male rats of Wistar strain, weighing 260–290 g, were given 1 mM KI aqueous solution instead of water for 4 days *ad libitum* and fasted for 24 h prior to use. To visualize the lymph duct, 1 ml of sesame oil was administered into the stomach 2–3 h before operation. Under ether anesthesia, the thoracic duct of the rat was cannulated according to Bollman's method[8]. The operated rats were held in restraining cages[9].

For absorption studies, 1 ml of saline solution containing 1 mg (30 $\mu$Ci) or 5 mg (40 $\mu$Ci) of [$^{131}$I]elastase was injected into the upper jejunum of rat. For the control, 160 $\mu$g of K$^{131}$I (34 $\mu$Ci) instead of [$^{131}$I]elastase were administered.

Lymph was collected every 1 h in a 7-ml conical centrifuge tube and then blood was obtained by cardiac puncture. The lymph and blood samples were kept overnight at about 4 °C and then centrifuged at 3000 rev./min for 30 min. The supernatant was assayed as follows.

### *Measurement of radioactivity*

A well-type scintillation counter (Aloka JDC-207) was used for the measurement of radioactivity.

### *Immunoassay*

Immunoprecipitation of serum and lymph samples was carried out, using an excess of anti-elastase and anti-rabbit $\gamma$-globulin serum, by a modified method of $^{131}$I-labeled human growth hormone assay[10].

To 7-ml conical centrifuge tubes, the following solutions were added in succession: (1) 0.1 ml of serum or lymph samples to be assayed. (2) 0.1 ml of 0.5 % bovine serum albumin in 0.01 M phosphate–saline buffer (pH 7.4). (3) 0.1 ml of anti-elastase serum diluted 1/100 with 0.01 M phosphate–saline buffer. The tube was shaken and then kept at 4 °C for 24 h. (4) 0.1 ml of anti-rabbit $\gamma$-globulin. (5) 0.1 ml of 0.05 M EDTA*. After the tube was shaken and kept at 4 °C for 24 h, the precipitate separated by centrifugation was washed once by resuspension in 0.5 ml of saline. The precipitate collected by centrifugation was dissolved in 1 ml of 0.1 M NaOH and its radioactivity was counted. In order to correct for non-specific precipitation, a blank using normal rabbit serum instead of anti-elastase serum was prepared

* Ethylenediaminetetraacetic acid disodium salt.

by the same procedure as mentioned above. A calibration curve was made using serum or lymph obtained from non-treated rat and 0.5 % bovine serum albumin in 0.01 M phosphate–saline buffer containing a fixed amount of [$^{131}$I]elastase.

The linear relation observed between the radioactivity of added [$^{131}$I]elastase and that of precipitate was reproducible within 5 % error in the range of 0.002 to 0.1 $\mu$g.

*Trichloroacetic acid precipitation, dialysis and paper chromatography*

Fractionation of macromolecule-bound $^{131}$I in samples by precipitation with trichloroacetic acid, dialysis and paper chromatography were carried out as reported previously[1].

## RESULTS

*Concentrations of the radioactivity in serum and lymph after intraintestinal administration of [$^{131}$I]elastase or K$^{131}$I*

Fig. 1 presents the concentrations of radioactivity in serum and lymph *vs* time after intraintestinal administration of [$^{131}$I]elastase or K$^{131}$I. The radioactivities in both serum and lymph, after administration of 1 or 5 mg of [$^{131}$I]-elastase, reached a peak of 0.3–0.35 % of the dose within 1–2 h. In contrast, the maximal level of radioactivity in serum and lymph was over 1 % of the dose at 1 h after administration of K$^{131}$I.

The radioactivity of macromolecule-bound $^{131}$I or immunoprecipitable $^{131}$I in serum and lymph which was determined by trichloroacetic acid precipitation, dialysis, paper chromatography and immunoassay, is shown in Fig. 2. Serum levels

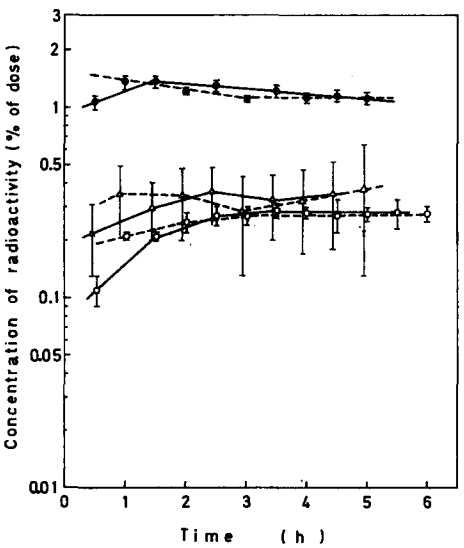


Fig. 1. Concentration curves of radioactivity in serum and lymph after intraintestinal administration. [$^{131}$I] Elastase was administered intraintestinally in doses of 1 mg (△) or 5 mg (○). As the controls, 160 $\mu$g of K$^{131}$I were administered (●). Each value was represented mean percentage to the dose in 1 ml of serum (- - - -) or lymph (———) with S.E. of 4 experiments.

of immunoprecipitable radioactivity reached a maximum of 0.06 μg/ml 3 to 4 h after admininstration of 5 mg [$^{131}$I]elastase per rat and decreased slowly. The time course of macromolecule-bound $^{131}$I concentration, which was estimated by trichloroacetic acid and paper chromatography, showed the tendency similar to that of immunoprecipitable $^{131}$I. Lymph levels of immunoprecipitable and protein-bound $^{131}$I attained a peak of 1.6 μg/ml after about 3 h, and then declined.

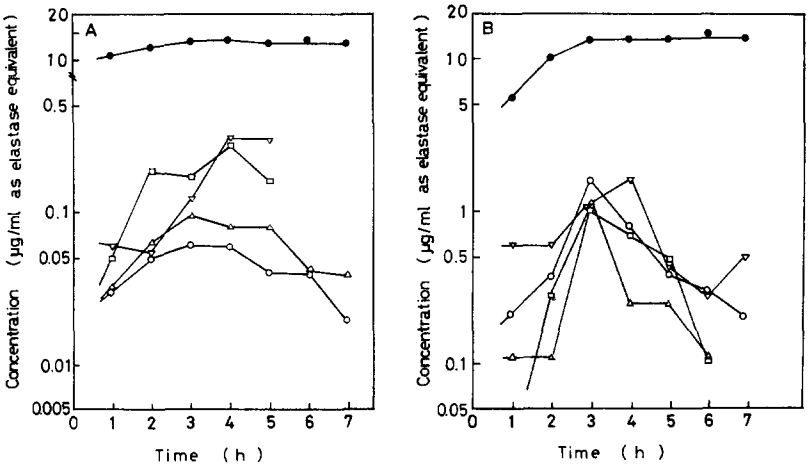


Fig. 2. Concentration of protein-bound and immunoprecipitable radioactivity after intraintestinal administration of 5 mg [$^{131}$I]elastase per rat. Concentrations of radioactivity in serum (A) or lymph (B) were represented as elastase equiv μg/ml. ●, radioactivity; ▽, non-dialysable radioactivity; △, trichloroacetic acid precipitable radioactivity; □, radioactivity at $R_F$ −0.05 to +0.05 on paper chromatogram; ○, immunoprecipitable radioactivity. Each value was represented as mean of 4 experiments.

*Effect of doses of [$^{131}$I]elastase on the concentrations of immunoprecipitable radioactivity in serum and lymph after intraintestinal administration*

The concentrations of immunoprecipitable radioactivity in serum and lymph after intraintestinal administration at doses of 1 or 5 mg of [$^{131}$I]elastase are shown in Fig. 3. The levels in serum following the administration of 1 or 5 mg reached a maximum of 0.03 μg/ml at 2h and 0.06 μg/ml at 3 h, respectively, and then declined slowly. Also, the lymph levels attained a peak of 0.34 and 1.58 μg/ml at almost the same time as each peak time in serum, respectively, and thereafter decreased. The ratios of concentration in lymph to that in serum at each time during the first 7 h following the administration of 1 or 5 mg were 10–11 and 7–26, respectively. At the dose of 5 mg, the concentration in lymph at the peak time was 26 times higher than that in serum.

*Concentrations of the radioactivity in serum and lymph after intravenous administration of [$^{131}$I]elastase*

The time course of the concentrations of radioactivity in serum and lymph after intravenous injection of [$^{131}$I]elastase (0.1 mg/kg of body weight) is shown in Fig. 4. The concentrations of total radioactivity in serum were almost equal to those of radioactivity fractionated by trichloroacetic acid precipitation and immuno-

assay, but those of total radioactivity in lymph were a little higher than the fractionated radioactivity. The lymph levels of radioactivity attained a maximum after 2 h and disappeared at a rate similar to that of serum.

Since the immunoassay for the determination of protein-bound radioactivity was found to be the more suitable of the methods used in the present study in respect

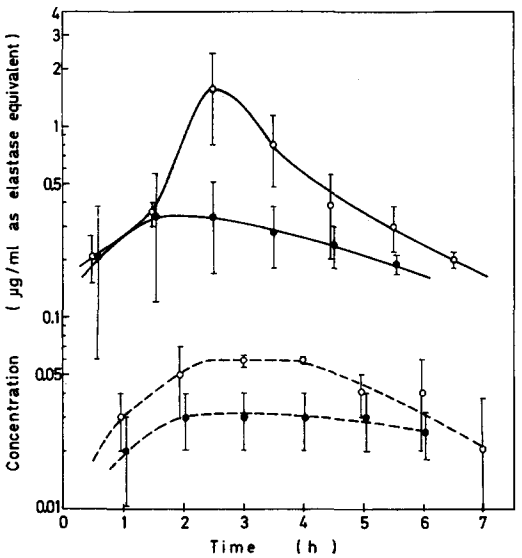


Fig. 3. Concentration curves of immunoprecipitable radioactivity in serum and lymph after intraintestinal administration of [$^{131}$I]elastase. Concentrations of immunoprecipitable radioactivity in serum (- - - - -) and lymph (——) after administration of 1 mg/rat (●) or 5 mg/rat (○) were shown as elastase equiv μg/ml, and were represented as mean with S.E. of 4 experiments. The levels in serum and lymph after 5 mg dosage were the same values as those in Fig. 2.

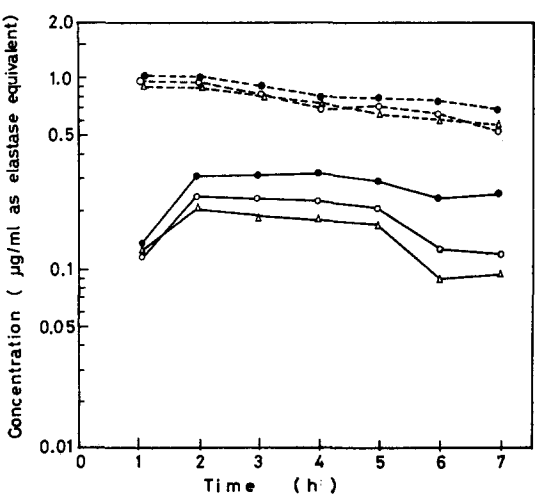


Fig. 4. Concentrations of protein-bound and immunoprecipitable radioactivity after intravenous administration of 0.1 mg [$^{131}$I]elastase per kg of body weight. Concentrations of radioactivity in serum (- - - - -) and lymph (——) were represented as elastase equiv μg/ml. ●, radioactivity; ○, immunoprecipitable radioactivity; △, trichloroacetic acid-precipitable radioactivity.

of the specificity to [$^{131}$I]elastase, further studies were performed using the method of immunoassay.

Fig. 5 shows the semilogarithmic plots of immunoprecipitable radioactivity in serum and lymph *vs* time after intravenous administration of [$^{131}$I]elastase (0.1 mg/kg). The serum levels declined with two different half-lives of 13.6 min and 5.5 h, respectively.

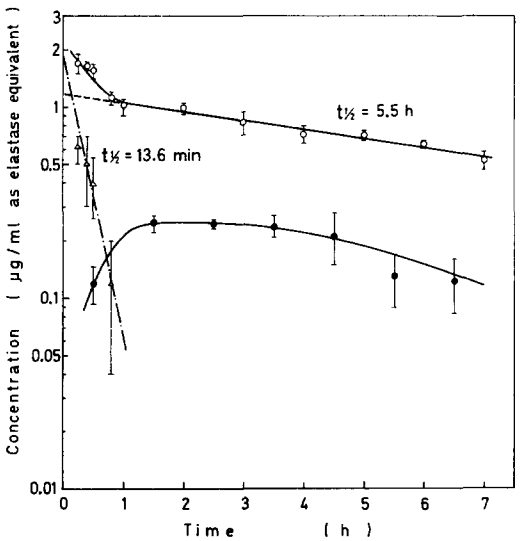


Fig. 5. Serum and lymph levels of immunoprecipitable radioactivity after intravenous administration of 0.1 mg [$^{131}$I]elastase per kg. Mean concentrations of 4 experiments in serum (○) and lymph (●) with S. E. were represented as elastase equiv $\mu$g/ml. Plots of (△) were the difference between the observed values and the values of $B \exp(-\beta t)$ in Table I.

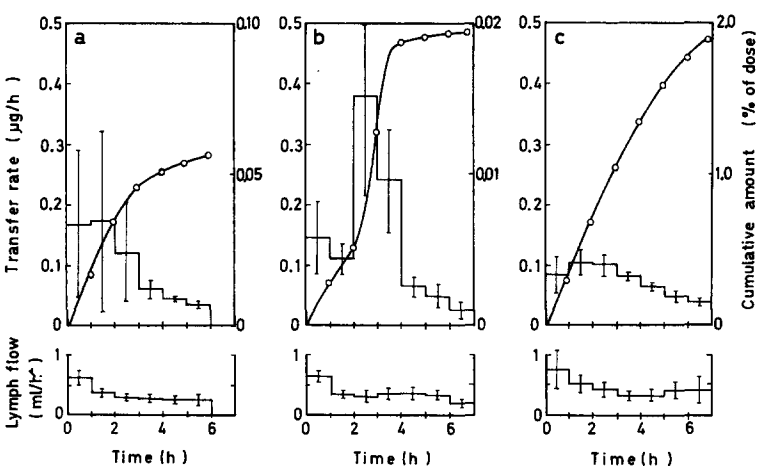


Fig. 6. Transfer rate and cumulative amount of immunoprecipitable radioactivity in thoracic duct lymph after administration of [$^{131}$I]elastase. (a) 1 mg/rat administered intraintestinally, (b) 5 mg/rat administered intraintestinally, (c) 0.1 mg/kg administered intravenously. Bars over or under horizontal line are transfer rates ($\mu$g/h) or flow rates of lymph (ml/h), respectively. Curves (○—○) are cumulative amount of immunoprecipitable radioactivity (percentages of dose). Each value is represented as mean with S.E. of 4 experiments.

*Absorption via lymphatics and transfer rate to lymph from blood*

Fig. 6 presents the transfer rate and cumulative amount of immunoprecipitable radioactivity in lymph of the thoracic duct after intraintestinal and intravenous administration of [$^{131}$I]elastase. As shown in Fig. 6c, the transfer rate to lymph across the arterial capillary wall after intravenous injection is extremely slow and the cumulative amount in lymph was only 1.9 ± 0.3 % of the dose.

## DISCUSSION

Following intraintestinal administration, protein-bound and immunoprecipitable $^{131}$I in serum and lymph were characterized by trichloroacetic acid precipitation, dialysis, paper chromatography and immunoassay. Comparing with the results of K$^{131}$I administration, the fractionated radioactivity might be protein-bound $^{131}$I originated from [$^{131}$I]elastase administered. In order to calculate the amount of absorption, parameters were obtained from the data of serum concentration of immunoprecipitable $^{131}$I after intravenous administration of [$^{131}$I]elastase. As shown in Fig. 5, a biexponential serum-level curve was obtained, so a two-compartment open system model[11] to fit the observed changes of immunoprecipitable $^{131}$I in serum was chosen on the basis of the following assumptions; (1) breakdown and elimination of [$^{131}$I]elastase take place exclusively in the central compartment, or at least in the spaces closely connected with it, (2) transfer of immunoprecipitable $^{131}$I from blood to lymph has a negligible rate, as shown in Fig. 6c. In order to obtain the parameters for the two-compartment model consisting of central and peripheral compartments, the serum levels shown in Fig. 5 were fitted to the biexponential equation by the least-square method;

$$C_p = A \exp(-\alpha t) + B \exp(-\beta t)$$

where $C_p$ is the concentration of immunoprecipitable radioactivity as elastase equiv in the central compartment. The mean values ± S.E. of $A$ and $B$ were 1.718 ± 0.164 and 1.197 ± 0.095, respectively. Immunoprecipitable radioactivity had a fast disposition rate constant, $\alpha$, of 3.068 ± 0.201, corresponding to a half-life of 13.6 ± 0.9 min. The slow disposition rate constant, $\beta$, of 0.127 ± 0.011, which was derived from the slope of the linear segment of the serum level curve, reflected the apparent elimination rate of immunoprecipitable radioactivity from the body, corresponding to a half-life of 5.5 ± 0.5 h. $Vc$, the distribution volume in the central compartment, was found to be 10.1 ± 1.2 ml.

As shown in Fig. 3, the concentration of immunoprecipitable radioactivity in lymph was 10 times higher than that in serum during the first 7 h after intraintestinal administration. In contrast, the reverse results were observed in experiments of intravenous administration (Fig. 5). These facts seem to indicate that immunoprecipitable radioactivity in lymph following intraintestinal administration is mainly due to a direct absorption *via* lymphatics. The amount of absorbed immunoprecipitable radioactivity *via* the portal vein was calculated with the parameters according to the method of Loo and Riegelman[12]. The amounts of absorption *via* lymphatics and the portal vein, and the percentages of absorption *via* lymphatics to the total absorbed immunoprecipitable radioactivity are shown in Table I.

The absorption amounts *via* the portal vein during the first 7 h after intraintestinal administration at doses of 1 and 5 mg are 0.95 and 1.70 μg as elastase equiv, respectively. The absorption amounts *via* lymphatics at these doses were 0.54 and 0.94 μg, respectively. Therefore, the total amount absorbed *via* both routes was 0.149 and 0.053 % of the doses after the intraintestinal administration of 1 and 5 mg of [$^{131}$I]elastase. The absorption percentages of [$^{131}$I]elastase seem to be relatively lower than those of α-chymotrypsin (0.94 %)[13] and insulin (3 %)[14]. The percentages of absorption *via* lymphatics to the total absorbed immunoprecipitable radioactivity are found to be 36 % for both doses.

TABLE I

ABSORPTION OF IMMUNOPRECIPITABLE RADIOACTIVITY *via* PORTAL VEIN AND LYMPHATICS AFTER INTRAINTESTINAL ADMINISTRATION

Absorption amounts of immunoprecipitable radioactivity by both the portal vein and lymphatic route were calculated with the data in Fig. 3 as elastase equiv. Each value is represented as mean with S. E. of 4 experiments.

| *Absorption* | *Dose (mg/rat)*: 1 | 5 |
|---|---|---|
| Amount (μg) *via* portal vein (*A*) | 0.95 ± 0.21 | 1.70 ± 0.19 |
| Amount (μg) *via* lymphatics (*B*) | 0.54 ± 0.37 | 0.94 ± 0.20 |
| Amount (μg) *via* both routes (*A* + *B*) | 1.49 ± 0.42 | 2.64 ± 0.27 |
| Rate (%) (100 × (*A* + *B*)/dose) | 0.149 ± 0.042 | 0.053 ± 0.005 |
| Percent of lymphatics (100 × (*B*)/(*A* + *B*)) | 36 ± 11 | 36 ± 7 |

As for a route of absorption of proteins, Comline *et al.*[5], Payne and Marsh[4] suggested that the lymphatics in newborn animals played a critical role in the absorption of proteins. The possibility of lymphatic absorption of macromolecules has also been suggested in the review of Ballard[15], in which the drainage through lymph channels after intramuscular and subcutaneous injection could be of relatively great importance for the absorption of large molecules compared to smaller ones. Comline *et al.*[5] have reported that unchanged globulins of the colostrum do not enter the portal circulation in any appreciable amounts but are carried from the lymph to the peripheral blood. The results shown in Fig. 2 and Table I are suggestive of the significant role of lymphatics in the intestinal absorption of [$^{131}$I]-elastase. This suggestion was further strengthened by the fact that the major source of immunoprecipitable radioactivity in lymph after intraintestinal administration was not from the arterial blood but intestinal lumen (Fig. 6). However, it has been reported that most of absorbed insulin[16] or heparin[17] passed directly into the blood, not *via* lymph. So further studies on the relationship between the physicochemical properties of macromolecules and the role of lymphatics will be necessary.

The difference between the concentrations of immunoprecipitable radioactivity in lymph after intraintestinal administration and after intravenous administration may be explained as follows. In the case of intraintestinal administration, elastase which is absorbed across the intestinal epithelium moves through interstitial spaces and passes the lymph capillary wall. In contrast, in the case of intravenous injection, elastase passes across arterial capillary wall at first, and then moves through inter-

stitial spaces. According to Nakamura *et al.*[18], the intra- and extravascular distribution of proteins were related to the molecular size of the proteins; as the size of a molecule increased, the extravascular distribution decreased. In addition, the molecular size of elastase in blood should be increased by binding with inhibitors found in serum[19]. Hence, it is supposed that the permeability of immunoprecipitable radioactivity across the arterial capillary wall is a limiting factor of the transfer to lymph in the case of intravenous administration. Furthermore, in the case of intraintestinal administration, it is very interesting to decide whether the binding of elastase with inhibitors would take place before or after passing across capillary wall in the intestine.

This study is suggestive of the possibility of intestinal absorption of elastase and shows the significant role of lymphatics, but whether or not the immunoprecipitable and protein-bound radioactivity in serum and lymph originates in intact [$^{131}$I]elastase administered remains to be solved.

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