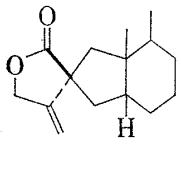
α,β-unsaturated aldehyde (**14**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1700, 1630; NMR δ: 3.51 (3H, singlet), 5.90 (1H, multiplet), 6.21 (1H, multiplet), 9.42 (1H, singlet); UV λ_{max}^{EtOH} nm(ε): 219 (6970), 333 (660), in 57% yield. Reduction of the aldehyde with $NaBH_4$ yielded **1** in 47% yield. The synthesized **1**, mp 79.0—80.5°, $[\alpha]_D$ +18° (c=0.56, MeOH), showed the following spectroscopic data: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1675, 905; NMR (in $CDCl_3$) δ: 0.84 (3H, doublet, J=6 Hz), 0.96 (3H, singlet), 4.75 (2H, multiplet), 5.02 (1H, multiplet), 5.09 (1H, multiplet); Mass Spectrum m/e: 234 (M^+), 124, 123, 111, 109 (base peak). These data completely agreed with those of natural bakkenolide A[8] and no depression of mp occurred by mixed fusion.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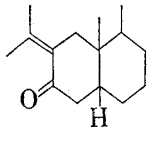
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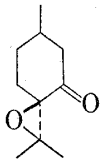
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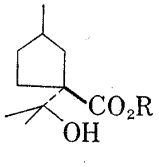
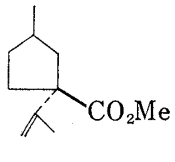

4 : R=H
5 : R=Me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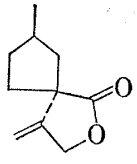
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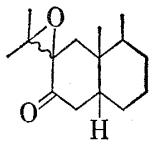
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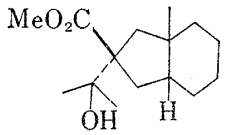
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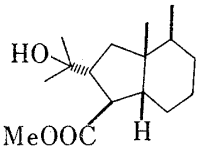
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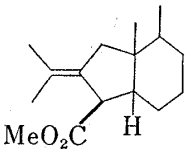
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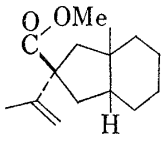
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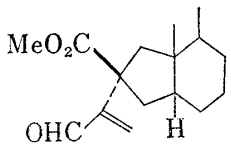
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Absorption Route of ^{131}I-Labeled Lysozyme in Rats

It has recently been suggested that a small amount of enzyme proteins are absorbed from the mammalian small intestine.[1] However, the route in intestinal absorption of enzyme proteins has not yet been clarified. This paper deals with investigations on the intestinal absorption of ^{131}I-labeled hen egg white lysozyme (abbreviated as ^{131}I-EWL) and on the role in the absorption of lymphatics in comparison with the portal vein in rats.

^{131}I–EWL was prepared by the modified method of Hansen, *et al.*[2] The specific radioactivity of ^{131}I–EWL was 97—123 μCi/mg. The radiochemical purity checked by electro-

1) *a*) R.S. Comline, H.E. Roberts and D.A. Aitchen, *Nature*, **167**, 561 (1951); *b*) A.L. Warshaw, W.A. Walker, R. Cornell and K.J. Isselbacher, *Lab. Invest.*, **25**, 675 (1971); *c*) K. Katayama and T. Fujita, *Biochim. Biophys. Acta*, **288**, 181 (1972); *d*) R.H. Engle and S.J. Riggi, *Proc. Soc. Exptl. Biol. Med.*, **130**, 870 (1969); *e*) A.E. Pierce, P.C. Risdall and B. Shaw, *J. Physiol.*, **171**, 203 (1964).
2) N.E. Hansen, H. Karle and V. Andersen, *J. Clin. Invest.* **50**, 1473 (1971).

phoresis and paper chromatography was found to be more than 99.8%. The enzyme activity and immunological behavior observed by immunoelectrophoresis of ^{131}I–EWL were identical with those of the starting material.

Male rats (230—290 g) of Wistar strain were given 1 mM KI aqueous solution, fasted for 24 hr and then the thoracic duct was cannulated as described previously.[1c] Following the injection of ^{131}I–EWL 2 mg/kg into the upper jejunum of the rat, lymph was collected at predetermined intervals during the first 6 hr and then blood was obtained by cardiac puncture. In order to examine the disappearance of EWL from blood and transfer rate from blood to lymph, ^{131}I–EWL 0.4 mg/kg was administered intravenously. The lymph and blood samples collected were centrifuged at 3000 rpm for 30 min and the supernatants were assayed as follows. (I) Radioactivity determination and measurement of trichloroacetic acid (TCA) precipitable radioactivity in lymph or serum samples were carried out as described previously.[3] (II) Enzyme activity of EWL was determined with *Micrococcus lysodeikticus* as substrate based on Gorin's method.[4] (III) Immunoprecipitation was carried out as follows. To conical centrifuge tube, the following solutions were added in succession; (1) 0.1 ml of serum or lymph sample. (2) 0.1 ml of guinea pig anti-EWL serum diluted 1/100. (3) 0.1 ml of rabbit anti-guinea pig IgG serum. After the tube was shaken and then kept at 4° for 24 hr, the precipitate separated by centrifugation was washed 3 times with 0.5 ml of saline. The precipitate collected was dissolved in 0.5 ml of 0.1 M NaOH and its radioactivity was counted.

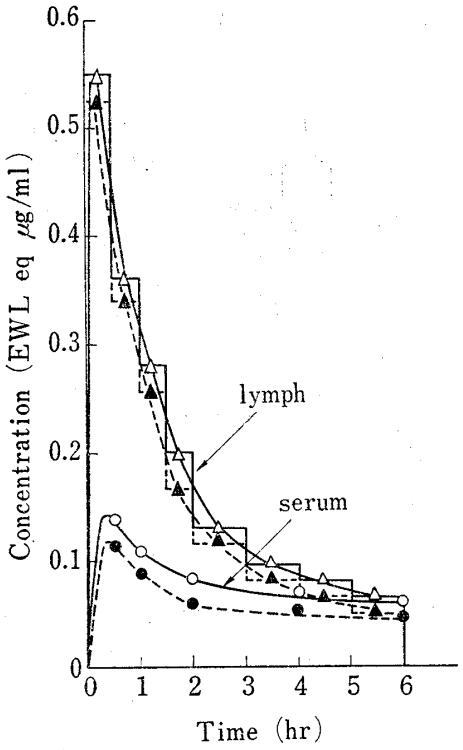

Fig. 1. Serum and Lymph Levels of Immunoprecipitable ^{131}I (▲, ●) or TCA Precipitable ^{131}I (△, ○) following Intraintestinal Administration of ^{131}I-EWL (2 mg/kg)

Data are expressed as mean (n=4).

Fig. 1 shows the time course of TCA precipitable and immunoprecipitable ^{131}I as EWL eq μg per ml of serum or lymph after intraintestinal administration of ^{131}I–EWL 2 mg/kg.

As shown in Fig. 1, the concentration of immunoprecipitable ^{131}I at each time was in good accordance with that of TCA precipitable ^{131}I. The maximum level observed at 30 min in lymph, 0.529±0.106 μg/ml, was approximately 5 times higher than that in serum.

After intravenous administration of ^{131}I–EWL, the serum concentration of immunoprecipitable ^{131}I was declined biexponentially. Then the serum levels (Cp) were fitted to the equation, $Cp=A\exp(-\alpha t)+B\exp(-\beta t)$, by the least-square method. The parameters of A, B, α and β were 6.57±0.32, 1.10±0.22 μg/ml, 5.225±0.138 and 0.236±0.017 hr^{-1}, respectively. The transfer rate of immunoprecipitable ^{131}I to lymph after intravenous injection of ^{131}I–EWL was only 1% of the dose during the first 6 hr. This finding demonstrates that the transfer of ^{131}I–EWL from blood to lymph is negligible. The amount of immunoprecipitable ^{131}I absorbed *via* portal vein was calculated with the parameters according to the method of Loo and Riegelman.[5] The amounts of absorption *via* lymphatics and the portal vein, and the percentages of absorption *via* lymphatics to the total immunoprecipitable ^{131}I absorbed are shown in Table I.

3) K. Katayama and T. Fujita, *Biochim. Biophys. Acta*, **288**, 172 (1972).
4) G. Gorin, S.F. Wang and L. Papapavlou, *Anal. Biochem.*, **39**, 113 (1971).
5) J.C.K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918 (1968).

Table I. Absorption of Immunoprecipitable ^{131}I *via* Portal Vein and Lymphatics after Intraintestinal Administration of ^{131}I-EWL 2 mg/kg in Rats

Absorption	EWL eq μg	% of dose
Amount *via* portal vein (A)	10.61±2.40	1.99±0.41
Amount *via* lymphatics (B)	0.37±0.18	0.07±0.04
Amount *via* both routes (A+B)	10.98±2.45	2.06±0.43
Percentage of lymphatics [100×(B)/(A+B)]	3.24±1.69	

Each value is represented as mean with S.E. of 4 experiments.

Table I indicates that the main route in intestinal absorption of ^{131}I–EWL is the portal vein, not lymphatics. This result seems to be in accordance with the results of heparin[1d] and insulin.[1e] However, as compared with the result that 36% of the total amount of elastase absorbed was *via* lymphatics,[1c] the percentage of lymphatics absorption of ^{131}I–EWL is extremely small. Thus, further studies on the relationship between the physicochemical properties of macromolecules and the absorption rate *via* lymphatics will be necessary. In addition, whether or not the immunoprecipitable and protein-bound ^{131}I in serum and lymph originates from intact ^{131}I–EWL administered remains to be investigated.

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Wave Length Dependent Photolysis of Phenanthrene Oxide

Though the chemistry of monocyclic arene oxides has been investigated,[1] studies on polycyclic arene oxides are very poor.[2] The evidece, however, is growing that arene oxides are the active carcinogens formed from polycyclic aromatic hydrocarbons.[3] The stereochemistry of polycyclic arene oxides is dreamy: the epoxide ring is nearly perpendicular to the aromatic ring(s) from investigation by a Dreiding Model. This may cause an extensive conjugation between the pi-bonds and the C–O sigma-bonds.[4] These situations prompted us to investigate the chemical behaviour of compounds of this kind. In this communication

1) J.W. Daly, D.M. Jerina and B. Witkop, *Experientia*, **15**, 1129 (1972).
2) D.M. Jerina and D.R. Boyd, *Tetrahedron letters*, **1970**, 457; M.S. Newman and S. Blum, *J. Am. Chem. Soc.*, **86**, 5559 (1964).
3) for example, J.K. Selkirk, H. Huberman and C. Heiderberger, *Biochem. Biophys. Res. Commun.*, **43**, 1010 (1971).
4) T.G. Traylor, W. Hanstein, H.J. Berwin, N.A. Clinton and R.S. Brown, *J. Am. Chem. Soc.*, **93**, 5715 (1971).