

19. Goso, Y. and Hotta, K.: Types of oligosaccharide sulphation, depending on mucus glycoprotein source, corpus or antral, in rat stomach.
Biochem. J., 264, 805-812 (1989).
20. Sellers, L.A., Allen, A., Morris, E.R. and Ross-Murphy, S.B.: Submaxillary mucins.
Biochem. J., 256, 599-607 (1988).
21. Mantle, M., Mantle, D. and Allen, A.: Polymeric structure of pig small-intestinal mucus glycoprotein.
Biochem. J., 195, 277-285 (1981).
22. Smits, H.L. and Kramer, M.F.: Human duodenal gland (Brunner's gland) mucus glycoprotein analysis.
Arch. Biochem. Biophys., 228, 64-70 (1984).
23. Pearson, J.P., Kaura, R., Taylor, W. and Allen, A.: The composition and polymeric structure of mucus glycoprotein from human gallbladder bile.
Biochim. Biophys. Acta, 706, 221-228 (1982).
24. Roberts, G.P.: The role of disulfide bonds in maintaining the gel structure of bronchial mucus.
Arch. Biochem. Biophys., 173, 528-537 (1976).
25. Creeth, J.M., Bhaskar, K.R. and Horton J.R.: The separation and characterization of bronchial glycoproteins by density-gradient methods.
Biochem. J., 167, 557-569 (1977).

26. Rose, M.C., Lynn, W.S. and Kafman, B.: Resolution of the major components of human lung mucosal gel and their capabilities for reaggregation and gel formation. *Biochemistry*, 18, 4030-4037 (1979).
27. Meyer, F.A.: Polymeric structure of a high-molecular-weight glycoprotein from bovine cervical mucus. *Biochem. J.*, 215, 701-704 (1983).
28. Carlstedt, I., Lindgren, H., Sheehan, J.K., Ulmsten, U. and Wingerup, L.: Isolation and characterization of human cervical-mucus glycoproteins. *Biochem. J.*, 211, 13-22 (1983).
29. Carlstedt, I., Lindgren, H. and Sheehan, J.K.: The macromolecular structure of human cervical-mucus glyco proteins. *Biochem. J.*, 213, 427-435 (1983).

LEGENDS

Fig. 1. Flow diagram of procedures for purification of human colonic mucus glycoprotein.

Fig. 2. Initial chromatography on Sepharose CL-4B. Each sample was applied to a column (2.2 x 100 cm) of Sepharose CL-4B in 4 M Gu-HCl/50 mM Tris-HCl buffer (pH 7.4) containing protease inhibitors. Fractions (5 ml) eluted at a flow rate of 10 ml/h were collected and analyzed for hexose at 490 nm (●) and for protein at 280 nm (○). As shown by horizontal bars, fractions 25-40 (—) were pooled.

Fig. 3. a, b and c. Equilibrium density gradient centrifugation. All ultracentrifugations were carried out in a CsCl density gradient at an initial density 1.38 g/ml at 20°C at 156,000 x g for 72 h. The first and second ultracentrifugations were in 4 M Gu-HCl/50 mM Tris-HCl buffer (pH 7.4) containing the protease inhibitors. The third ultracentrifugation was in CsCl with 0.2 M Gu-HCl. Fractions were collected from the top of the tubes and analyzed for hexose at 490 nm (●) and protein at 280 nm (○), and for density (■). Fractions were pooled as shown by horizontal bars (—).

Fig. 4. Second Chromatography on Sepharose CL-4B. Sepharose CL-4B chromatography was carried out under the same conditions as those described in Fig. 2. As shown by horizontal bars, fractions 25-40 (I—) were pooled.

Fig. 5. Sepharose CL-2B chromatogram. A column (1.5 x 150 cm) of Sepharose CL-2B was equilibrated with 4 M Gu-HCl/50 mM Tris-HCl buffer (pH 7.4) and 30 mg of the mucus glycoprotein fraction was applied to the column and eluted with the same buffer at a flow rate of 6 ml/h at 20°C. Fractions (3 ml) were collected and analyzed for hexose at 490 nm (●) and for protein at 280 nm (○). The eluate was fractionated into five subfractions: Fr. I, tube numbers 35-39; Fr. II, tube numbers 40-44; Fr. III, tube numbers 45-49; Fr. IV, tube numbers 50-54; Fr. V, tube numbers 55-64. Frs. I, III and V were designated H-GP, M-GP and L-GP, respectively and recovered.

Fig. 6 SDS-PAGE of mucus glycoproteins. SDS-PAGE was performed through 10% gel, and the gel was stained with Coomassie brilliant blue R-250. Lanes 1 and 8, molecular weight standards; lane 2, crude mucin fraction; lane 3, initial Sepharose CL-4B step (Fig. 2); lane 4, first ultracentrifugation step (Fig. 3a); lane 5, second ultracentrifugation step (Fig. 3b); lane 6, third ultracentrifugation step (Fig. 3c); lane 7, second Sepharose CL-4B step (mucus glycoprotein fraction) (Fig. 4).

Fig. 7 HPLC of mucus glycoproteins. HPLC was carried out using a Hitachi L-6000. A Shodex OHpak KB-805 column (0.8 x 30 cm) was used and eluted with 0.2 M NaCl at a flow rate of 0.5 ml/min at 30°C. The detector used was a refractive index monitor (Hitachi L-3300 RI Monitor). Numbering of Frs. I - V was based on the Sepharose CL-2B chromatogram in Fig. 5.

Fig. 8 Calibration curve of molecular weight. Molecular weight was determined using HPLC under the same conditions as those described in Fig. 7. Dextrans (2,000 K, 400 K and 70 K) and glycogen (400 K) were used as standards.

Fig. 9 β -Mercaptoethanol treatment of mucus glycoprotein. Each 1 mg of mucus glycoprotein, (H-GP, M-GP and L-GP), was reduced in 1 ml of 4 M Gu-HCl/50 mM Tris-HCl buffer (pH 8.0) containing 5 mM ethylenediamine tetraacetate and 0.5 M β -mercaptoethanol. At 12 h, 24 h, 48 h and 120 h, each sample was applied to the HPLC under the same conditions as those described in Fig. 7, except that 0.2 M NaCl containing 0.1 M β -mercaptoethanol was used as a solvent.

Fig. 10 Actinase E digestion of mucus glycoproteins. Each 1 mg of mucus glycoproteins, (H-GP, M-GP and L-GP) was dissolved in 0.5 ml of ion-exchanged distilled water, and boiled in a water bath for 5 min. An equal volume of 0.1 M Tris-HCl buffer (pH 8.0, containing 20 mM CaCl_2) and 100 ug of actinase E was added to each sample. The digestion was then continued until 120 h. HPLC was performed under the same conditions as those described in Fig. 7. Each arrow shows 780 K (▼) and 260 K (▽), respectively.

Fig. 10. Acetone & digestion of sugar derivatives.
Each 1 cc of sugar derivative (E-C, H-W, and I-G) was
dissolved in 2.5 ml of anhydrous distilled water, and
boiled in a water bath for 5 min. The sugar solution (2.5 ml)
was added to 100 ml of 5% acetic acid (pH 5.0, containing 10 ml NaCl) and 100 ml
of acetone E was added to each sample. The digestion was
then continued until pH 5.0. The pH was determined after the
same conditions as those described in Fig. 1. The results
showed 750 E (V) and 750 X (V) respectively.

Molecular Structure of Mucus Glycoprotein in Human Colon
(Tables & Figures)

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Table 1. Amino acid composition of colonic mucus glycoprotein.

Values are expressed as residues per 1,000 residues of amino acids.

	Before Sepharose CL-2B Chromatography	After Sepharose CL-2B Chromatography		
	[Mucus Glyco- protein Fraction]	[H-GP]	[M-GP]	[L-GP]
Lys	3	6	17	81
His	23	20	12	21
Arg	20	54	15	12
Hyp	0	0	0	0
Asp	21	52	26	38
Thr	260	184	206	124
Ser	202	135	155	186
Glu	86	57	58	44
Pro	125	174	116	82
Gly	54	84	192	196
Ala	55	46	51	83
Cys	0	0	0	0
Val	37	27	30	30
Met	16	16	15	16
Ile	32	37	24	27
Leu	32	28	23	17
Thr	9	6	6	7
Phe	23	74	54	36

Table 2. Carbohydrate composition of colonic mucin glycoprotein.

Data are expressed as molar ratio to N-acetylgalactosamine residue. Each monosaccharide was determined as pyridylaminated derivatives. N-acetylneuraminic acid was determined by the method of Warren.

	Before Sepharose	After Sepharose		
	CL-2B chromatography	CL-2B chromatography		
	[Mucus Glyco-protein Fraction]	[H-GP]	[M-GP]	[L-GP]
N-Acetylgalactosamine	1.0	1.0	1.0	1.0
N-Acetylglucosamine	3.7	3.6	3.3	3.3
Galactose	3.9	4.0	4.4	3.6
Fucose	2.2	2.0	2.8	1.7
N-Acetylneuraminic acid	1.8	2.2	2.2	1.8

Fr. I (H-GP)

Fr. II

Fr. III (M-GP)

Fr. IV

Fr. V (L-GP)

Fr. VI

Fig. 1

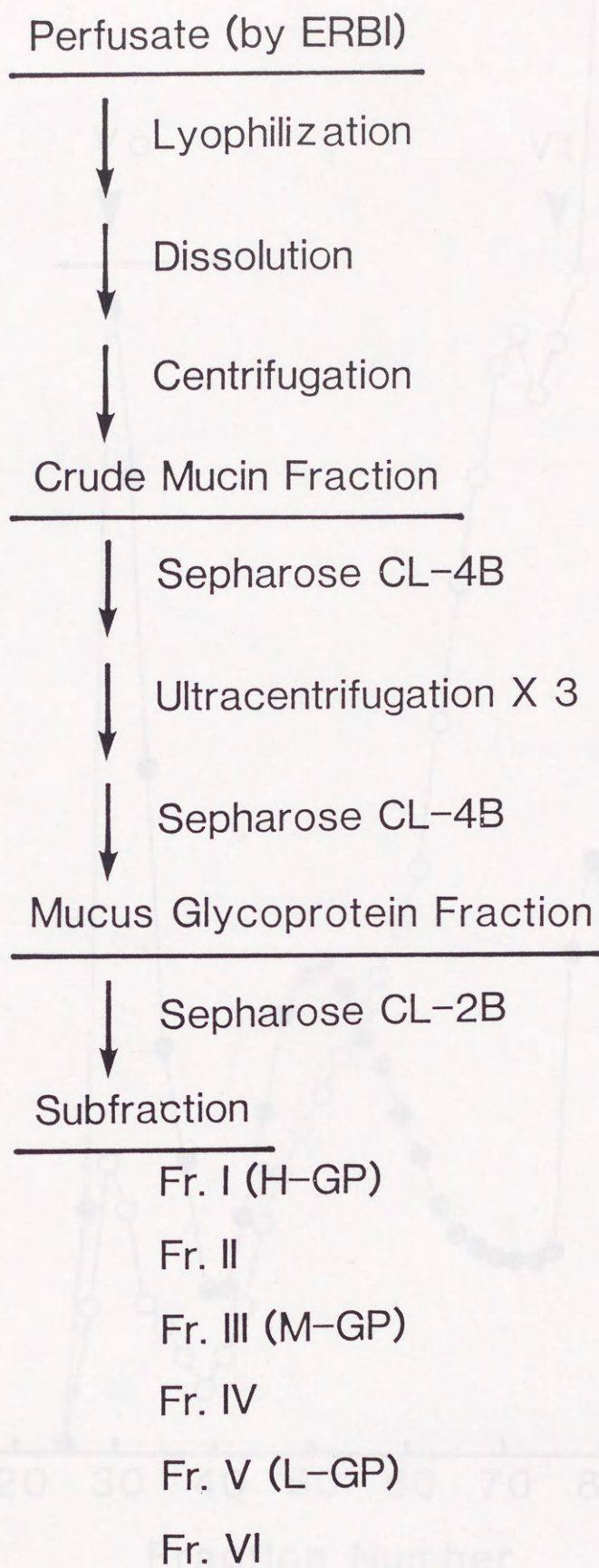


Fig. 1

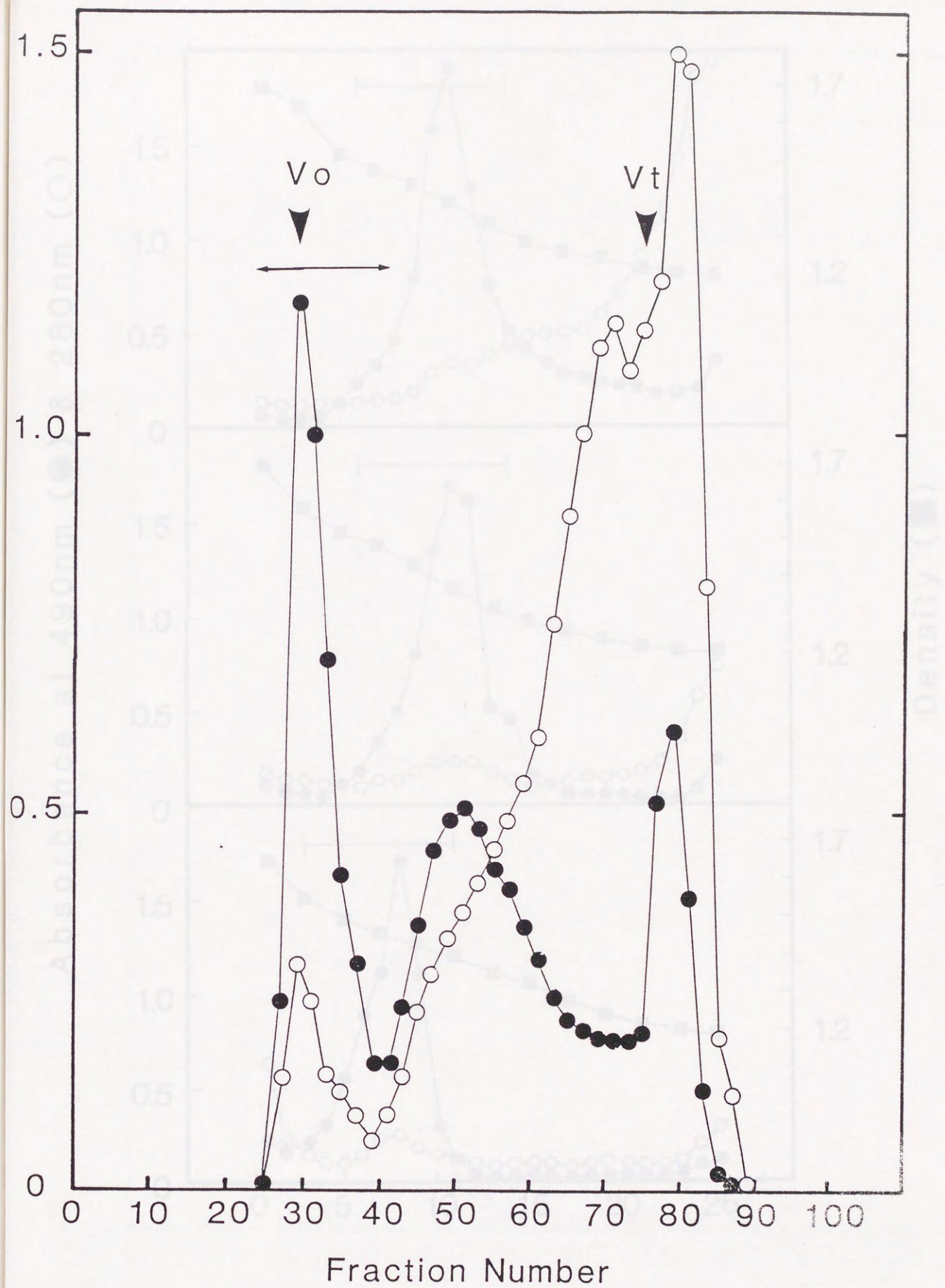


Fig. 2

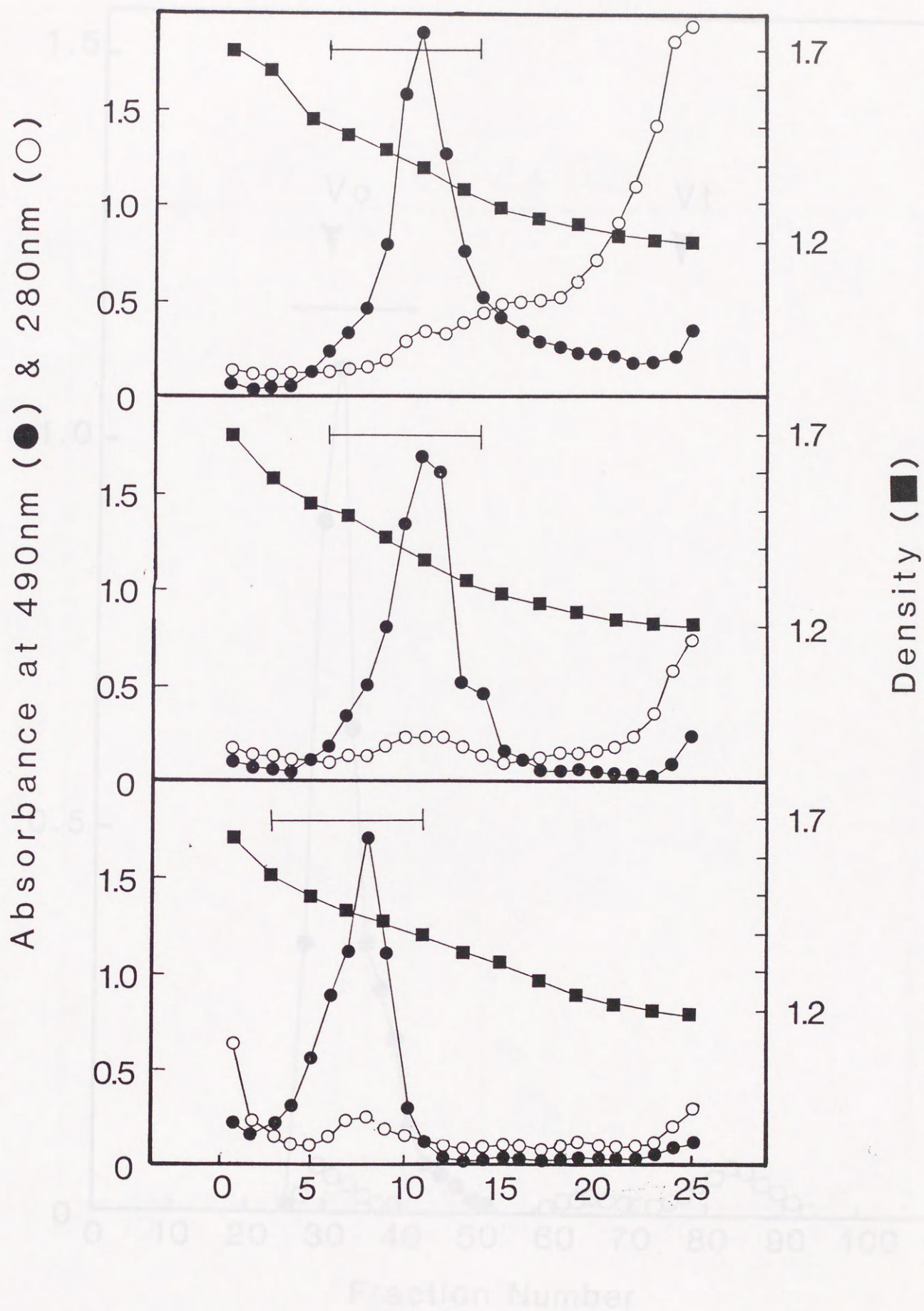


Fig. 3

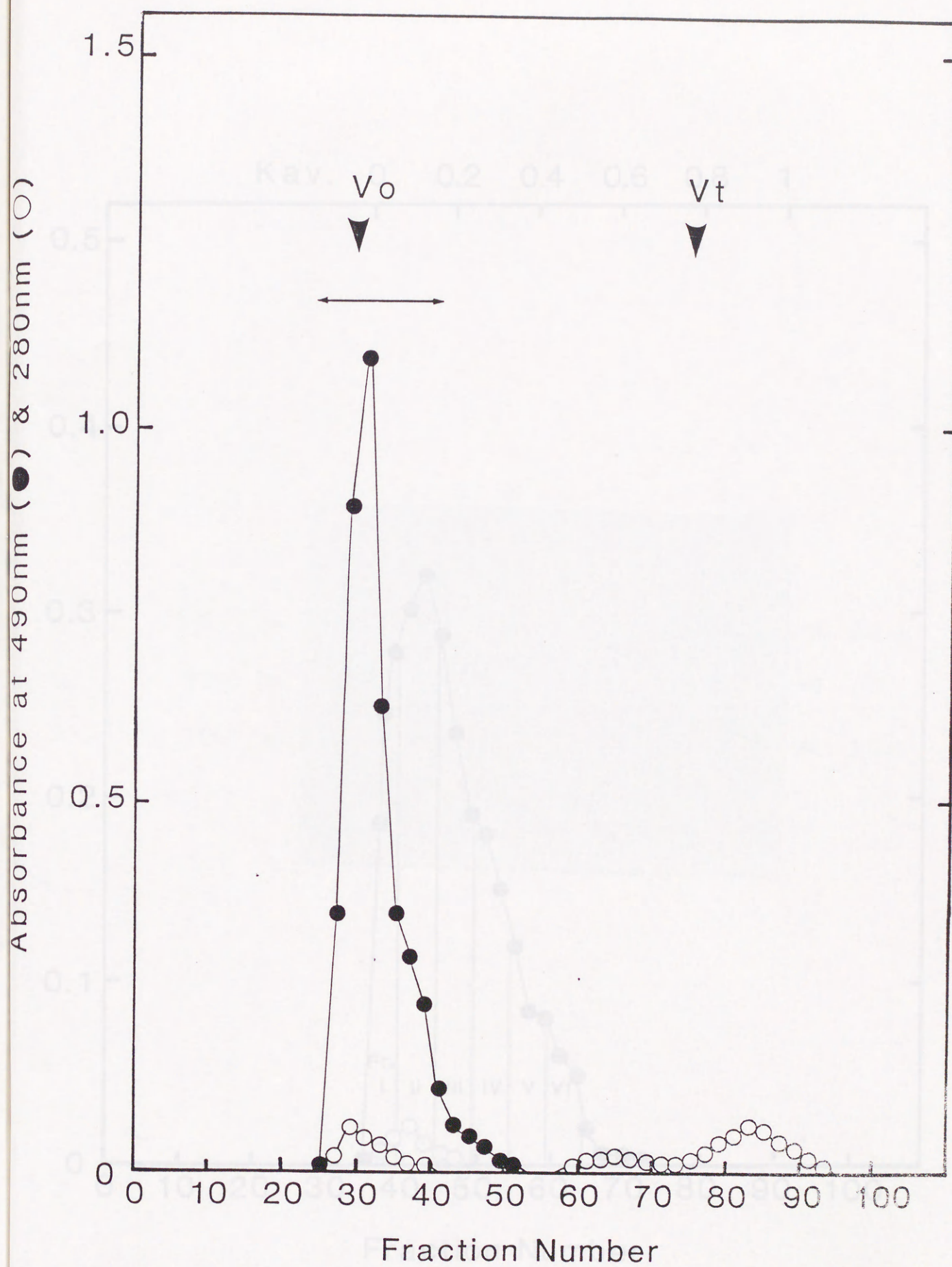


Fig. 4

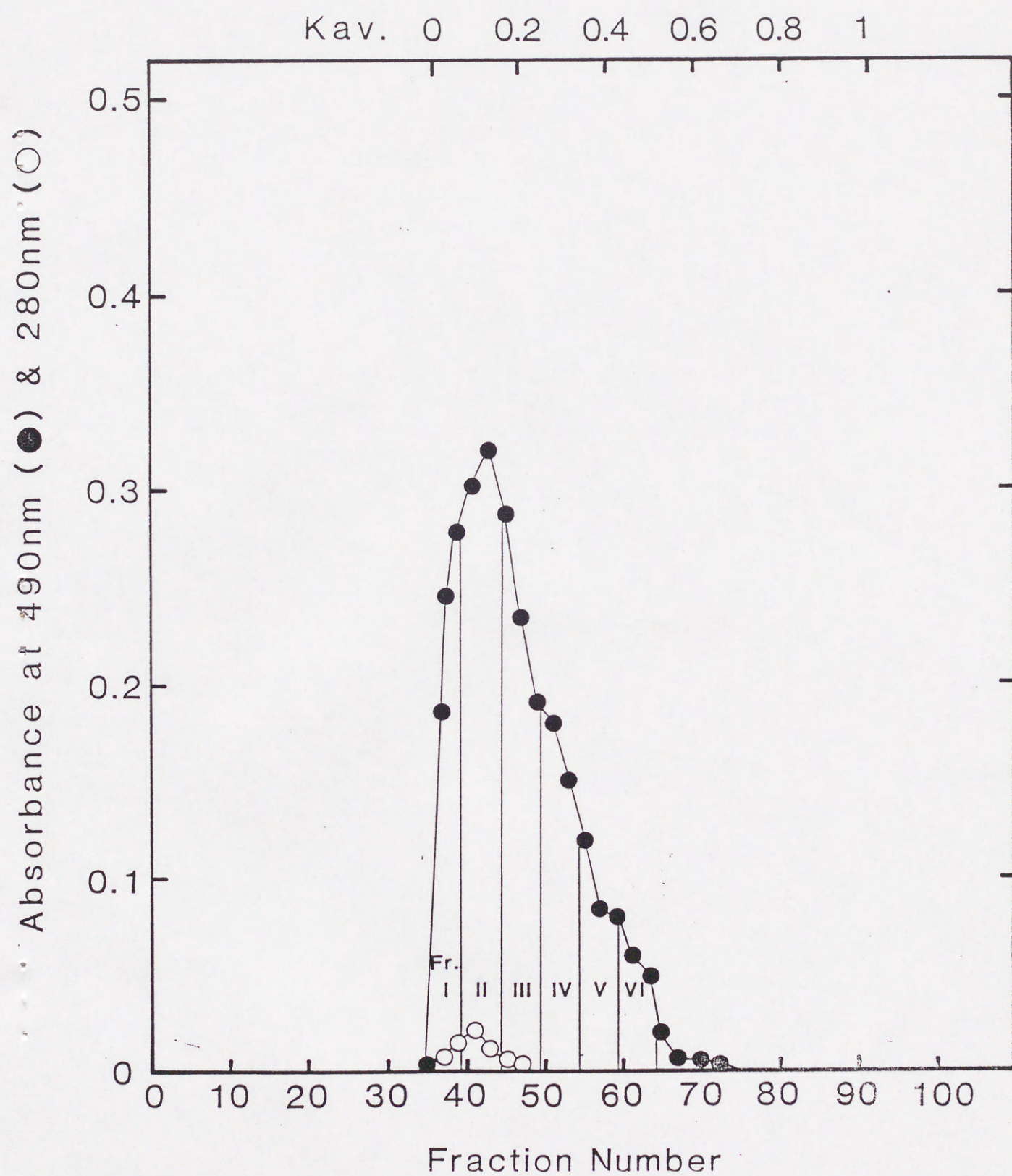


Fig. 5

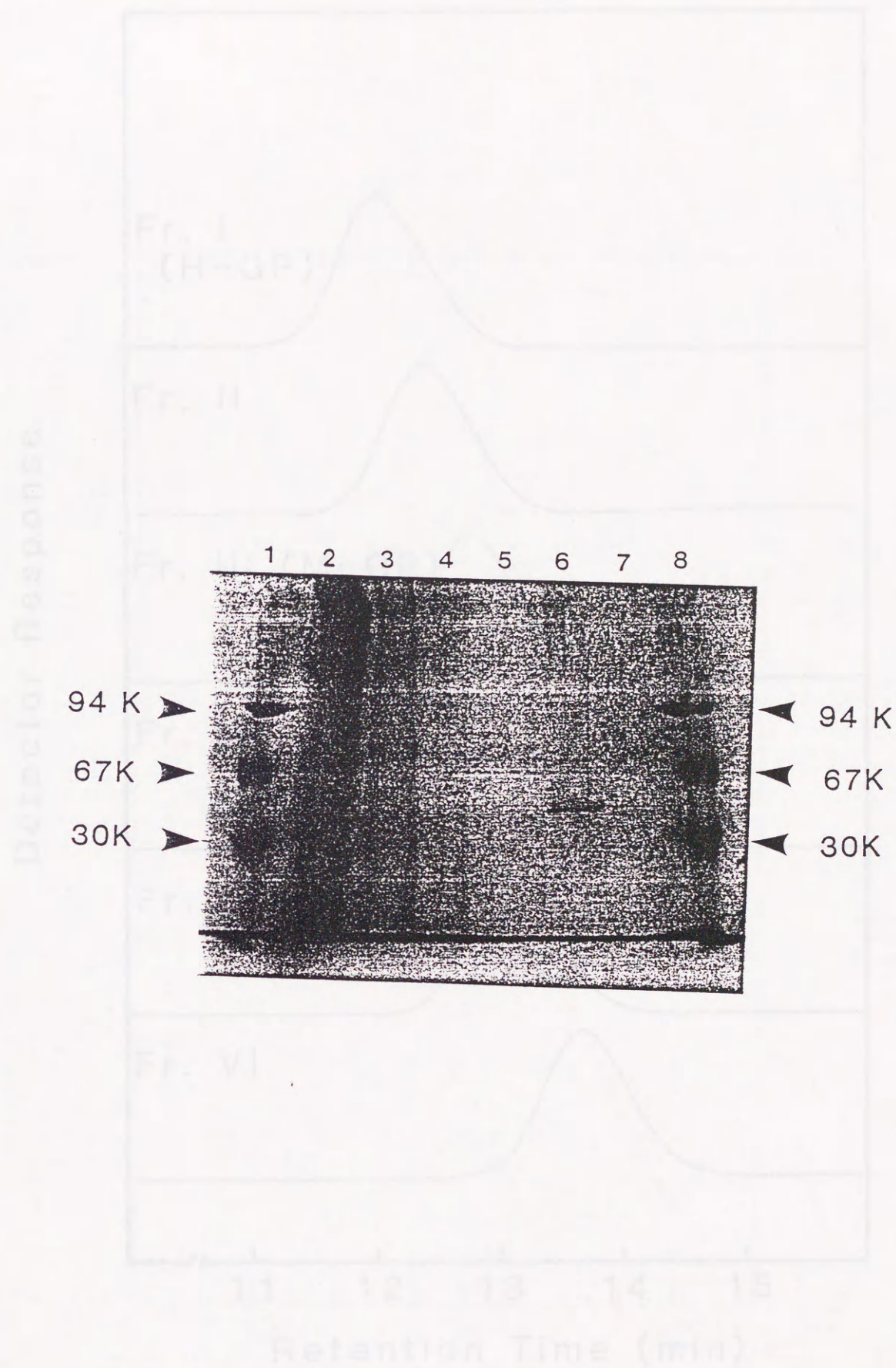


Fig. 6

Detector Response

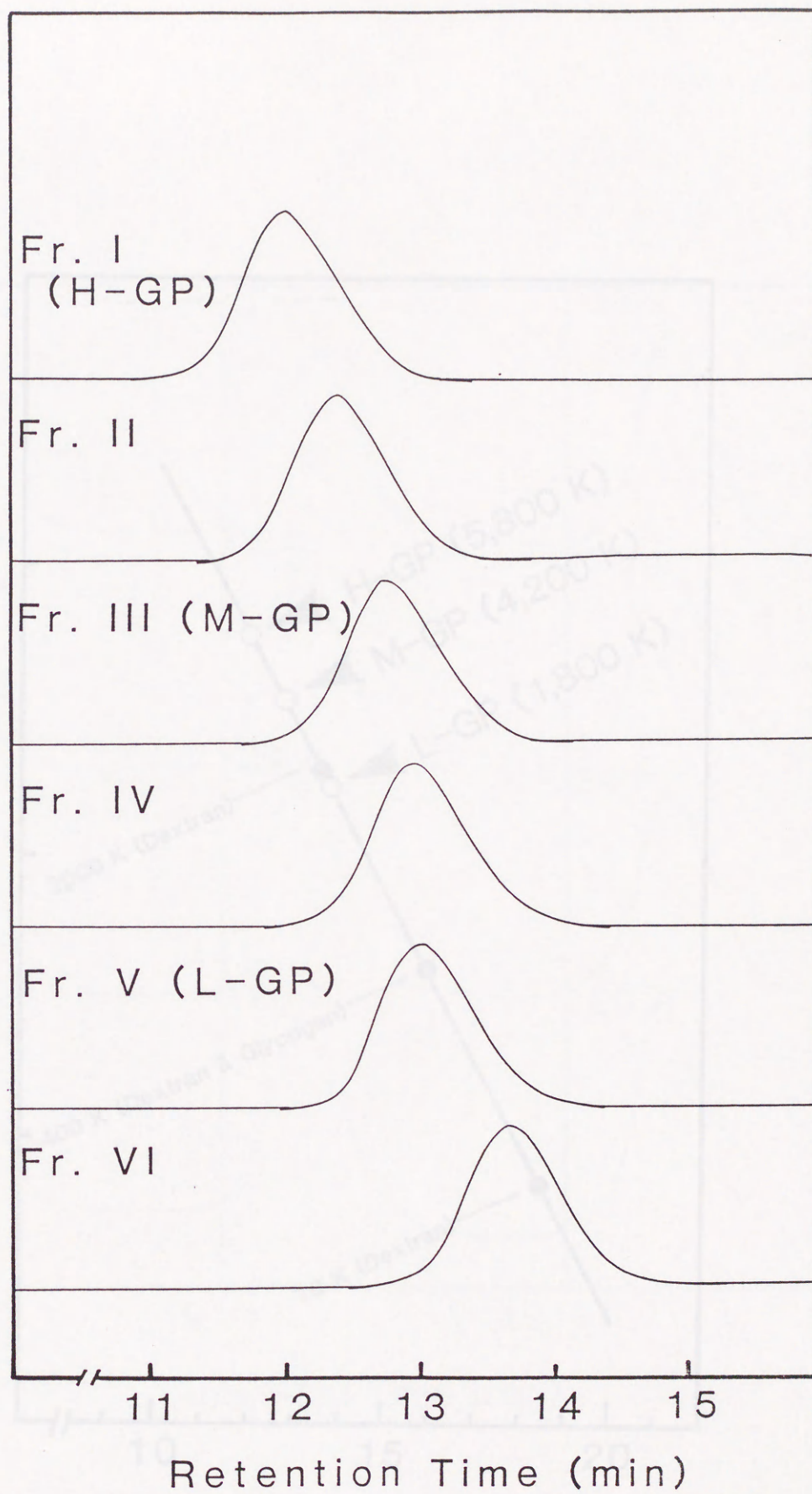


Fig. 7

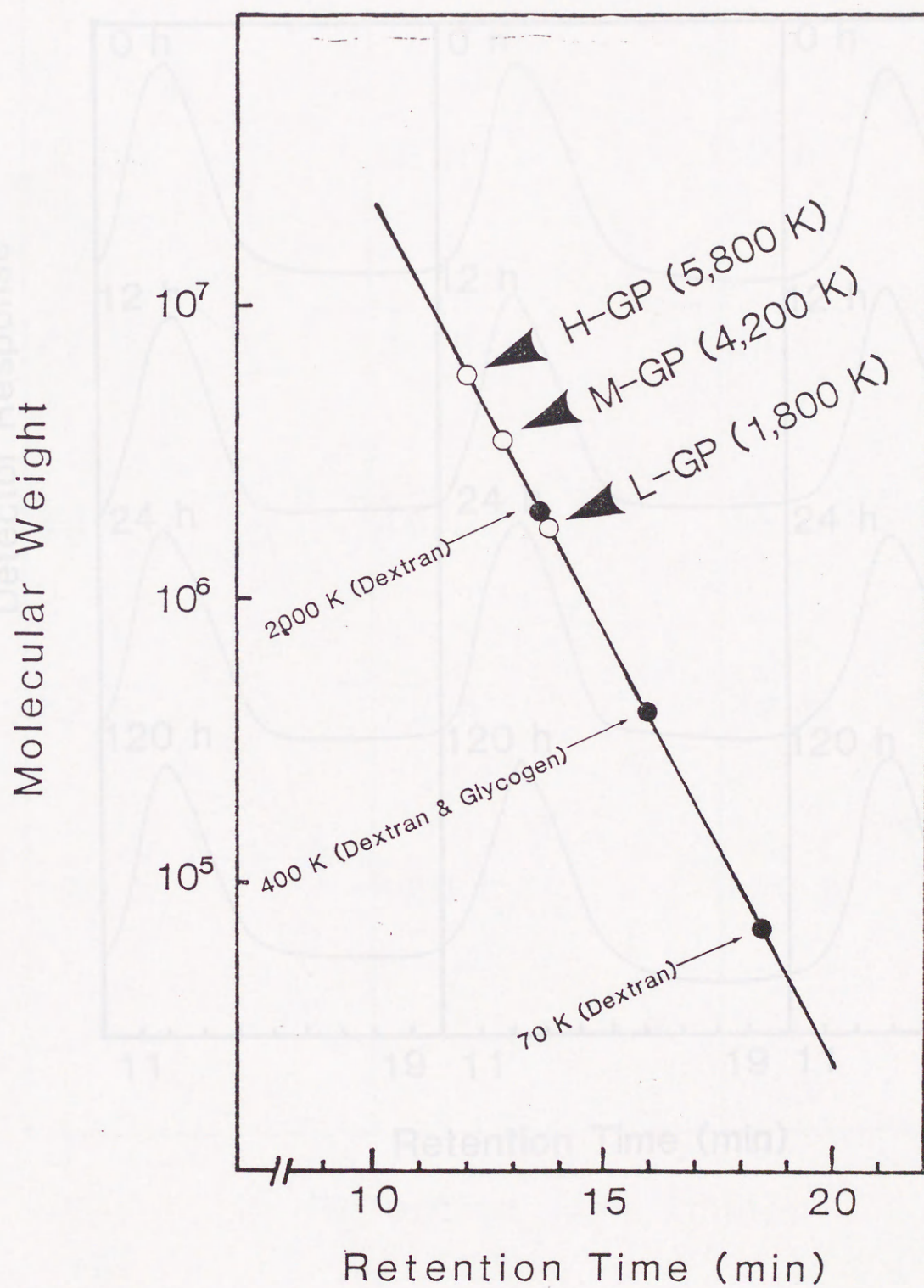


Fig. 8

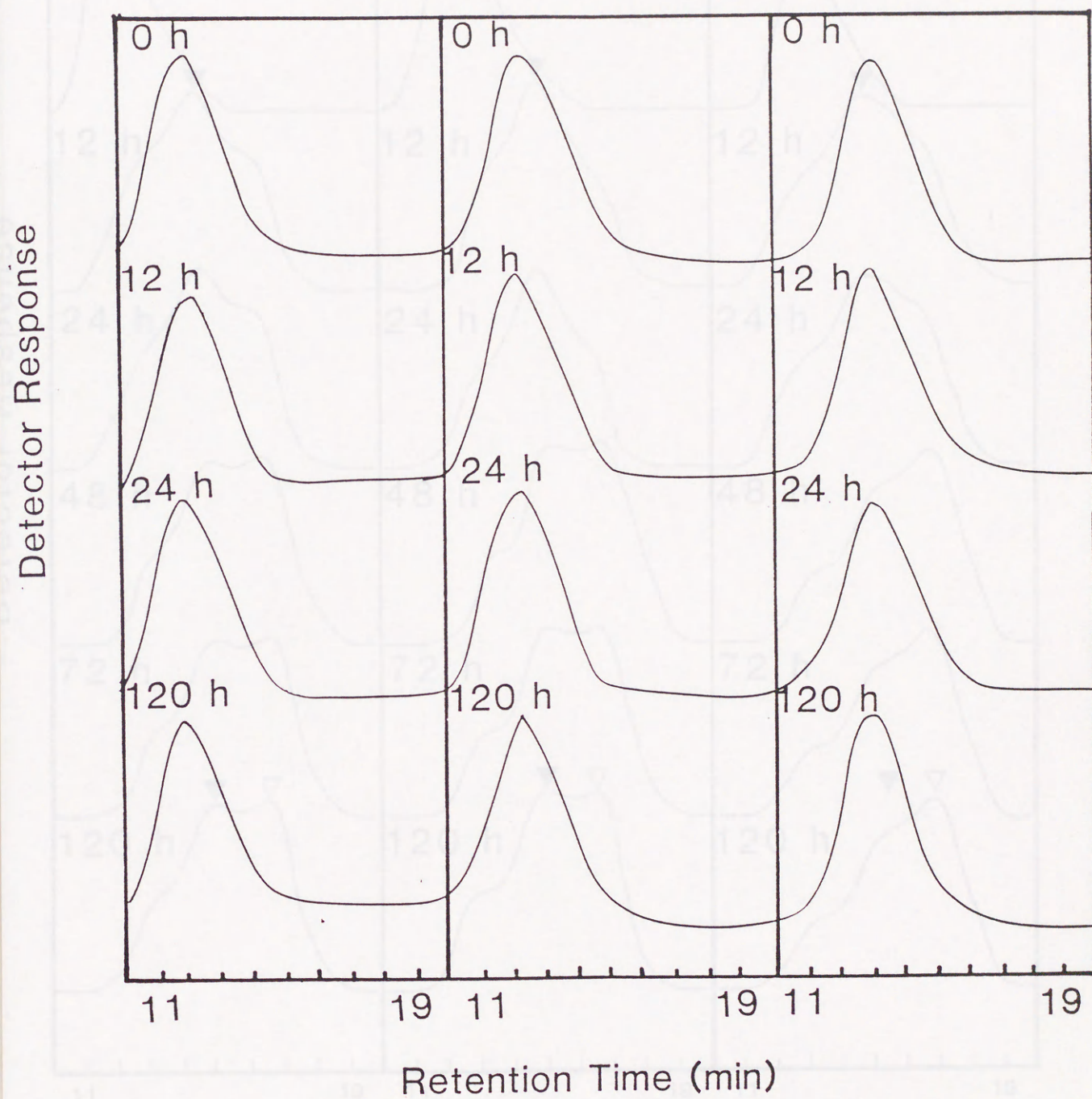


Fig. 9

Detector Response

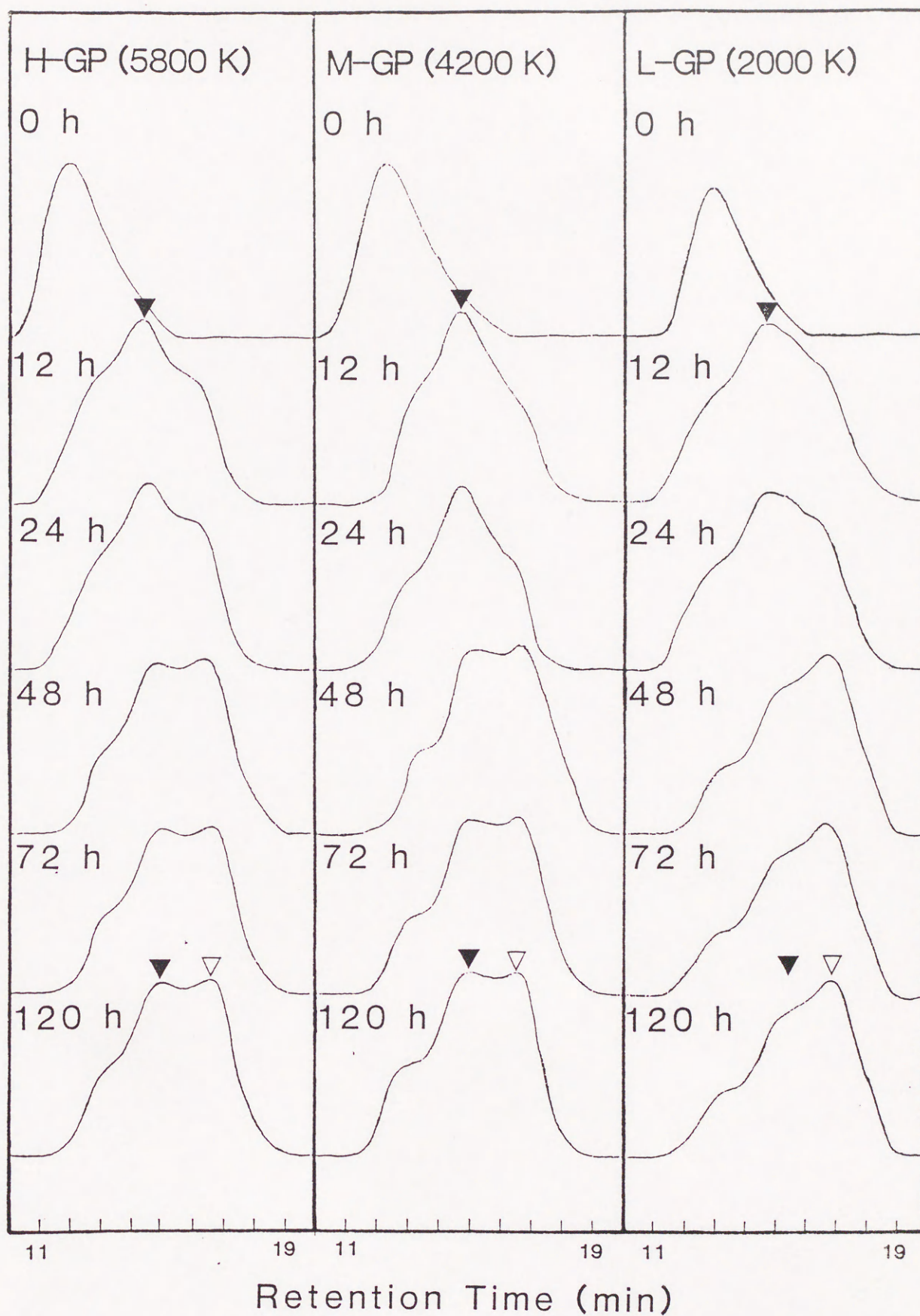


Fig. 10



