guinea-pig cerebral cortex. It will therefore be of considerable interest to characterize the sialic acid-containing constituents of the mitochondrial and microsomal membranes of rat liver. Preliminary experiments indicate that the sialic acid in these fractions is not extractable into organic solvents, even following digestion of the membranes with trypsin.

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The chemical structure of one component of clupeine*

Clupeine from Pacific herring (*Clupea pallasii*) has been fractionated on a preparative scale in two main fractions, Y and Z, by elution chromatography from alumina[1]. By means of counter-current distribution, the Y fraction was further resolved into two fractions, YI (amino acid composition: Arg, Pro, Ala, Ser, Thr, Ileu, and Gly; N-terminus: Ala $\gg$ Pro) and YII (composition: Arg, Pro, Ala, Ser, Val, and Thr; N-terminus: Pro $\gg$ Ala), while in contrast no further ıractionation was achieved with Z fraction (composition: Arg, Pro, Ala, Ser, and Val; N-terminus: Ala only). This fraction, which is probably homogeneous, has now been used for structural studies.

Amino acid analyses of clupeine Z using both the dinitrophenyl method and an automatic analyzer, gave the molecular formula $Ala_3Ser_3Pro_2Val_2Arg_{21}$. Only alanine was found at the N-terminus by isolation as the dinitrophenyl derivative and the measurement of the ratio of absorbancies at 390 mμ and 360 mμ of the dinitrophenyl-protein[2]. The molecular weight estimated from the amount of dinitrophenyl introduced (about 5000 as hydrochloride) was consistent with that calculated from the above formula (4929 as hydrochloride). Using a procedure practically the same as

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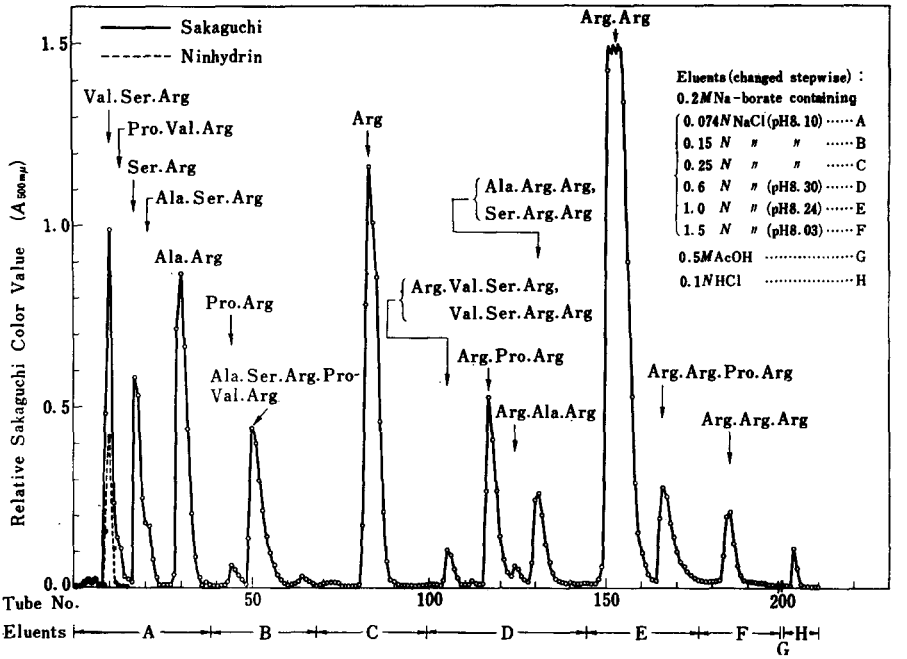

Fig. 1. Chromatographic separation and identification of peptides in a tryptic digest of clupeine Z. Digestion: 70.1 mg clupeine Z·HCl in 3.5 ml 0.067 *M* phosphate (pH 7.8) with 0.393 mg trypsin (Nutritional Biochem. Corp., twice recrystallized), for 20 h at 30°. Elution chromatography: 3 ml digestion mixture (corresponding to about 10 μmoles of clupeine Z) on an Amberlite CG-50 column (1.0 × 30 cm) in equilibrium with the starting eluent, using eluents described in the figure. The effluent was collected in 2.99-ml fractions with a flow rate of about 4 ml/h at 30°. Recovered color value was 96% (tube No. 1–210).

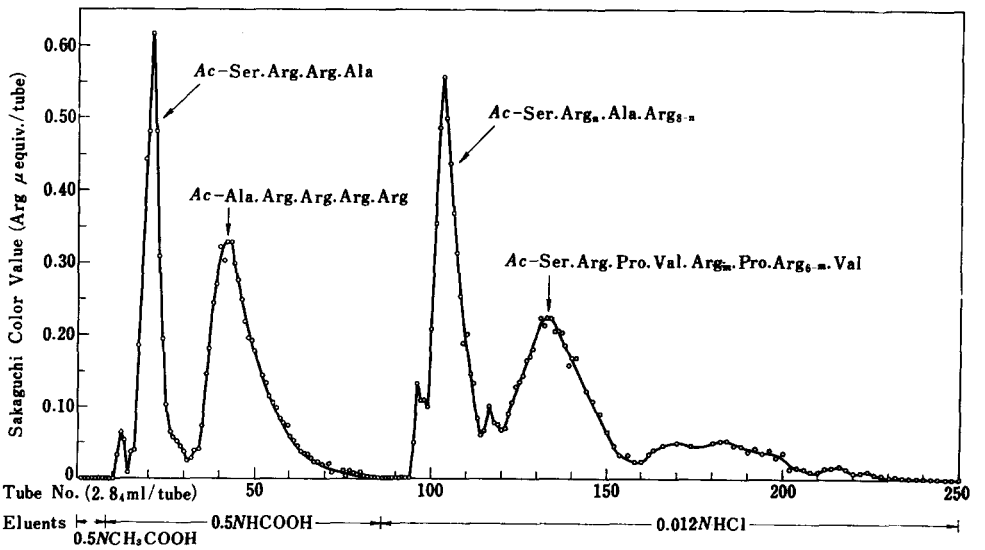

Fig. 2. Chromatographic separation and identification of acetyl oligopeptides in selective degradation products of N → O rearranged clupeine Z followed by acetylation. Elution chromatography: Fission products (conditions in the text) from 2.2 μmoles of clupeine Z on an Amberlite CG-50 column (0.95 × 30 cm) using eluents described in the figure at a flow rate of 5.5 ml/h at 30°. Recovered color value was 90% (tube No. 1–250).

that used for the study of the structure of an unfractionated clupeine[3], 16 peptides and arginine were identified and determined nearly quantitatively in a tryptic digest of clupeine Z as shown in Fig. 1. Taking the amounts of peptides into consideration, the following sequences involving monoamino acids must be present in clupeine Z molecule: one mole each of Ala–Arg (N-terminal)[4], Arg–Ser–Arg, Arg–Ala–Ser–Arg–Pro–Val–Arg, Arg–Pro–Arg, Arg–Val–Ser–Arg, and Arg–Ala–Arg. Sequences of several arginine residues must also be included, if any transpeptidation occured durring digestion.

Longer peptide fragments with intact sequences of arginine residues were obtained by application of $N \rightarrow O$-acyl rearrangement reaction at the serine residues by treatment with conc. H_2SO_4 (ref. 5) at 20° for 4 days, followed by selective chemical cleavage of the chain at the resultant ester bonds with acid (6 N HCl at 20° for 16 h) or weak alkali after acetylation[6] (0.3 N Na_2CO_3 at 30° for 4 h). The degradation products were satisfactorily fractionated by stepwise-elution chromatography on an Amberlite CG-50 column to yield four main peaks as expected, the structures of which were determined as indicated in Fig. 2 by determination of amino acid composition, N- and C-terminals, as well as by the stepwise degradation with carboxypeptidases-A and -B.

Consideration of these results together with those obtained after tryptic hydrolysis led us to the following total chemical structure for clupeine Z: H–Ala–$(Arg)_4$–Ser–$(Arg)_2$–Ala–Ser–Arg–Pro–Val–$(Arg)_m$–Pro–$(Arg)_{6-m}$–Val–Ser–$(Arg)_n$–Ala–$(Arg)_{8-n}$–OH, in which most probably $m = 4$ and $n = 4$*. The structure was also supported by the results obtained by the action of leucine aminopeptidase and carboxypeptidases upon the protamine. It has peculiar sequences which would not be expected from repeating structures hitherto suggested for clupeine[7–10], and hence the mode of combination of this protamine component with DNA *in vivo* and *in vitro* will be of much interest.

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* *Note added in proof*: Both m and n have now been determined with certainty to be 4 by the digestion experiments with carboxypeptidases. (Received January 4th, 1961)