LETTER

Further Notes on Protamines[1]

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It has recently been shown by the present authors[2] that our clupein has alanine as the *N*-terminal residue of the molecule, while salmine has proline. Further investigations showed that all the specimens of our clupein (from *Clupea pallasii* obtained in Hokkaido, Japan in 1947), either prepared by the classical methods of Rasmussen[3] and Felix[4] or by the recent one of Mirsky,[5] or prepared from the material caught in another year (1951), also have only alanine as their *N*-terminal residue.

Furthermore, manometric determination by Van Slyke method of amino nitrogen gave 0.22% N for the free base of clupein (amino N found: 0.1704 mg. from 78.44 mg. clupein. M.W. of clupein calculated therefrom: 6,400), while almost none for salmine (amino N found: 0.0225 mg. from 49.52 mg. salmine, i.e. 0.046% N) in accordance with the fact that the terminal proline gives no detectable amino nitrogen by the present method. This substantiated enough our results above obtained.

The disagreement shown in the *N*-terminal residue of clupein molecule between the result above obtained in our laboratory and that described by German researchers[6,7] who asserted that proline occupied the position, seems to be attributable to the species difference of materials used. One of the latter (Felix) described that *Clupea harengus* was used. It is of interest that a slight difference in species of herrings may cause such a distinction in a part of their sperm protamine molecules. Salmine in its turn, notwithstanding its origins either from the genus *Oncorhynchus* as in Japan[2] (*Onc. keta*) and Canada[8] (*Onc. tschawytscha*?) or from the genus *Salmo* as in Europe[9] (*Salmo salar*?), has always been shown to have proline as the *N*-terminal amino acid.

As for melecular weight, repeated estimations on both protamines by various methods such as methoxyl determinations of the esters (M.W. of clupein: ca. 6,100[10] and 10,800[11], M.W. of salmine: ca. 6,100[10] and 7,300[11]), intensity measurements of absorption near

1) The present paper was read by one of the authors (C.H.) before the symposium on protein structure held on April 26, 1953 in Tokyo University.
2) T. Ando, et al., This Bulletin, **25**, 132 (1952).
3) K. E. Rasmussen, *Z. physiol. Chem.*, **224**, 97 (1934).
4) K. Felix, et al., ibid., **249**, 111 (1937).
5) A. E. Mirsky, et al., *J. Gen. Physiol.*, **30**, 101 (1946).
6) K. Felix, et al., *Z. physiol. Chem.*, **286**, 67 (1950).
7) E. Waldschmidt-Leitz, et al., *Experientia*, **7**, 183 (1951).
8) S. F. Velick, et al., *J. Biol. Chem.*, **191**, 233 (1951).
9) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).
10) The result of repeated esterifications.
11) The result of once esterification.
12) The similar result has been obtained by means of diffusion-viscosity measurements by K. Iso, T. Kitamura and I. Watanabe in this institute (unpublished).

ultra-violet region of the DNP-derivatives (the former: ca. 5,600~7,200, the latter: ca. 6,100~6,800), electrometric titrations of protamines (the former: ca. 4,600, the latter: ca. 4,400) and also manometric determination of amino nitrogen in clupein as shown above, may lead to the conclusion that both protamines should probably have the molecular weight of nearly the same order[12] (6,000~7,000).

The arginine content in clupein also was successfully determined (87.8±2%) by chromatographic method using Amberlite XE-64 and citrate buffer solution (0.2 *M*, *p*H, 6.50). Further experiments on probable fractionations of protamines and accurate determinations of constituent amino acids are yet in progress. Details will be fully reported later.

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