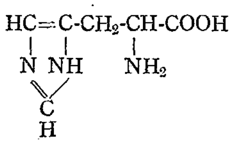
STUDIES ON WHALE BLOOD. I.

On the separation of histidine from whale blood.

Tadashi Tawara

Histidine is an amino acid of the following formula:



This amino acid was discovered in 1896 by Kossel and is found in various proteins, being most abundant in hemoglobin which contains about 11% histidine.

Histidine is one of the most important amino acids for nutrition and it is contained comparatively in a large amount in fish meat. Putrefaction of meat turns histidine into a poisonous histamine. Histidine is used for gastric ulcer.

Two methods of synthesis have been worked out by Pymann.1)2) The most common material now being employed for hislidine manufacture is blood. There are two principal manufacturing methods, one by precipitating the silver salt in alkaline medium,3)4)5) and the other using the mercuric salt.6)7)8)

The author used dried blood of a sperm whale (*Physeter catodon L.*) as material for his experiments.

Cystin, methionine, ornithin, tryptophane and histidine are some of amino acids that precipitate by corrosive sublimate (mercuric chloride). Cystine almost precipitate out in neutral media, while ornitine and tryptophane are not obtained by hydrolysis with hydrochloric acid. Methionine precipitates almost wholly in saturation of sodium chloride around pH 6.0. The author, therefore, undertook the experiments with a special consideration for the removal of methionine.

ICR
一般財団法人 日本鯨類研究所
THE INSTITUTE OF CETACEAN RESEARCH

Experimental

1 kg. Dried blood of sperm whale was hydrolysed with 4 L. 30% hydrochloric acid for 20 hours. After cooling, pigment that precipitated

out (melanine) was removed by filtration and the filtrate was neutralized to pH 2.5 with concentrated sodium hydroxide solution. After standing overnight, the solution was filtered, and the precipitate was washed with a small amount of water. The filtrate and washings were neutralized to pH 6.0 with sodium hydroxide solution and concentrated untill sodium chloride precipitated out. The mixture was cooled and filtered. Methionine can be obtained from this precipitate.

A hot saturated solution of 400 g mercuric chloride was added to the filtrate with stirring and after being left overnight, was filtered. A concentrated solution of sodum carbonate was added slowly, with stirring, until the pH of the solution reached 7.2. After stirring overnight, the supernatant liquid was decanted and the flask filled with water to the original volume. This procedure was repeated several times, and the mixture was finally filtered by a Buchner funnel. From the washings, mercury can be recovered by passing hydrogen sulfide gas.

This precipitate (mercuric salt of histidine) was suspended in 1 L. of water and decomposed by passing hidrogen sulfid gas. This was filtered to remove mercuric sulfide, which was then boiled with water several time to recover histidine adsorbed in the precipitate.

The filtrate and washings from mercuric sulfide were concentrated to about 150 cc, mixed with 300 cc 95% alcohol and stood overnight in an ice chamber. Crude crytstals of histidine monohydrochloride were thus obtained.

The crude product was dissolved in five times its weight of water, and then diluted with twice its volume of 95% alcohol. The yield of recrystallized histidine monohydrochloride was 39 g. This compound melts at 252°C with decomposition.

一般財団法人 日本鯨類研究所
THE INSTITUTE OF CETACEAN RESEARCH

In conclusion, the author wishes to express his sincere thanks to Dr. T. Maruyama, Mr. H. Okuda and Mr. T. Nakai for their kind guid and advice in this study.

His thnks are also due to prof. Dr. S. Akiya and prof. Dr. T. Ogawa, Tokyo University, for their valuable suggestions.

He is also indebted to Mr. R. Fukazawa for kind assistance during the work.

References

1) Pyman: J. Chen. Soc., 99, 668, 1386 (1911).

2) Pyman: J. Chen. Soc., **103**, 159 (1941).
3) A. Kossel F. Kutscher: Z. physiol. Chem., **31**, 165 (1900—1).
4) H. B. Vickery, Leavenworth: J. Biol. Chem., **72**, 403 (1927); **75**, 115 (1927); **76**, 707 (1928); **79**. 377 (1928).
5) H. B. Vickery, K. J. Block: J. Biol. Chem., **93**, 105 (1931).
6) Org. Syntheses: **18**, 43 (1938).
7) Brigl: Z. physiol. Chem., **64**, 337 (1910).
8) M. T. Hanke, K. K. Koessler: J. Biol. Chem., **43**, 521 (1920).

ICR
一般財団法人 日本鯨類研究所
THE INSTITUTE OF CETACEAN RESEARCH