PHOSPHAGEN IN PRAWN MUSCLE*

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Researches of the last two decades have demonstrated the importance of organophosphate intermediates in muscle biochemistry. A study of literature reveals a large volume of work done on the nature of the different types of phosphorus compounds (Eggleton and Eggleton, 1929; Kernot and Speer, 1933; Kurtz and Luck, 1937 and Goettsch, Lonstein and Hutchinson, 1939). Needham and Needham (1932) have investigated the nature of phosphorus compounds in the muscle of invertebrate animals belonging to different phyla. In their investigation the presence of phosphoarginine was established.

It has been shown by Appanna and Devadatta (1942) that the prawns, like fish, have protein of high biological value and digestibility coefficient and that there exists in the edible portion of prawn five different varieties of phosphorus compounds (1947). This investigation has an important bearing on the diet as this article of food is consumed as a cheap source of animal protein by majority of people in Bombay and other western coastal towns. Besides local consumption large quantities are despatched inland, the consumption in Bombay alone being 13,00,000 pounds.

This work was undertaken to study the nature of the phosphorus compounds appearing in the barium soluble portion in the acid soluble fraction of the prawn muscle as phosphagen.

METHOD AND MATERIALS

Preparation of Trichloracetic acid extract. Four varieties of prawns were selected for the study. The prawns immediately after catch were quickly dropped into freezing mixture after the removal of the exoskeleton. The frozen tissue was powdered in a chilled mortar. 1 to 2 g of the powdered muscle was then transferred to a tared centrifuge tube containing 7 percent trichloracetic acid and the tube reweighed. Immediately thereafter enough trichloracetic acid or water was added to make the final concentration approximately 4 percent. The tube was shaken for a few minutes and centrifuged. After centrifugation the supernatant liquid was transferred to a cooled 50 ml. flask. The trichloracetic acid precipitate was

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washed once with 10 ml. of 4 percent trichloracetic acid. The number of washing was reduced to minimum to get consistent results. The volume of the flask was made up to the mark and filtered so as to remove the particles which were not centrifuged down. All operations were done at low temperature.

Acid Soluble Phosphates with Barium Hydroxide. The method of separation of phosphates of muscle was essentially that based on the procedure of Eggleton and Eggleton (*loc. cit.*) for separating the muscle phosphates. A barium precipitate was obtained by adding crystalline barium hydroxide to the turning point of phenolphthalein, as described by Cori and Cori (1931). Although glycogen was high enough to interfere with the fractionation, the extract was clear and did not warrant the removal of glycogen by adsorption on mercuric sulphide. The precipitate resulting from treatment with barium hydroxide was centrifuged off and dissolved in a minimum quantity of 4 percent trichloracetic acid and again precipitated by neutralization with barium hydroxide. After centrifugation, the supernatants from barium hydroxide-precipitate were combined, made up to a known volume and used as soluble barium fraction (A) for the estimation of phosphates and arginine. The neutralization of trichloracetic acid extract was carried out at low temperature.

A number of phosphate compounds are known to be barium soluble like esters of phosphoric acid with carbohydrates, glycerophosphates and phosphoarginine. One aliquot from the acid soluble fraction and another from this fraction (barium soluble) were used for total phosphate determination by digestion with 10 N sulphuric acid and hydrogen peroxide. After digestion the liberated inorganic phosphate was determined colorimetrically by the method of Fiske and Subbarow (1925). The readings were all taken in Klett Summerson Colorimeter.

Another aliquot of the fraction from (A) was used immediatly after separation from barium precipitate (to avoid hydrolysis of esters) for estimation of phosphate as directly determinable phosphate.

A third aliquot was used for the estimation of arginine by the Sakaguchi method (1925) colorimetrically. Creatine was not detected qualitatively and so was not estimated.

Independent analyses of all the muscle samples were made for the estimation of arginine by Block's microadaptation of Kossel's procedure (1934, 1937 and 1940). The principle involved the separation of arginine from histidine as organic arginine silver and estimation of nitrogen by micro-Kjeldahl's technique.

Selected samples were subjected to repeated fat extraction in the Soxhlet. The fat-free muscle of known quantity was treated with 25 ml. 8 N sulphuric acid and refluxed in round bottom flask for 16 hours. After the completion of hydrolysis, the protein hydrolysate was adjusted to pH 7.4 by 50 percent silver nitrate to give a blue colour with bromothymol blue. Copious histidine silver was thrown down and separated from supernatant which was recovered quantitatively. The supernatant was treated with hot baryta until dark red to phenolphthalein (pH 13.0 to 14.0). Arginine silver was quantitatively precipitated, allowed to settle and washed several times with cold baryta. The precipitate was then dissolved in dilute sulphuric acid, and the solution was adjusted to a blue colour to congo. The arginine silver was recovered quantitatively and decomposed by hydogen sulphide. The excess of hydrogen sulphide was removed by a stream of air and the solution was treated with barium hydroxide and adjusted to black to congo. This was centrifuged to remove the barium as sulphate and the filtrate containing arginine silver was reduced in volume under reduced pressure. The nitrogen in arginine was estimated by the micro-Kjeldahl's technique.

RESULTS AND DISCUSSION

The method of Eggleton and Eggleton (loc. cit.) was followed to great advantage for the separation of the phosphates in the acid soluble barium soluble fraction. The values compare favourably with those obtained by Needham and Needham (loc. cit.). It was found that when the trichloracetic acid precipitate was left in contact with acid, a progressive increase of phosphate in the acid soluble fraction occurred. It is possible that some of the esters of phosphoric acid with glycerol and hexose might get hydrolysed and contribute to the increment. In the table are given the results of the analyses of four varieties of prawn muscle and quantities of total phosphorus and phosphorus combined with arginine. The nitrogen content of arginine in this fraction is also shown. The amount of total phosphorus in the barium soluble fraction is far in excess of that combined with arginine. Prawn muscle is rich in glycogen (figure not recorded in table) and it is possible that esters of carbohydrate with phosphoric acid may be formed with the help of enzymes like phosphorylases. McCollum et al. (1946) state that "there are numerous evidence to demonstrate the ability of the body to synthesize nucleoproteins, hexosephosphates and glycerophosphates using inorganic phosphate and the appropriate organic substance as starting materials". Possible existence of hexose esters with phosphoric acid is indicated herein. The values for these have not been included in the table for want of reproducible results.

The arginine fraction obtained both by the Block's indirect method and Sakaguchi's procedure agree within limits of experimental error.

TABLE *

Distribution of Phosphorus and Nitrogen in the Acid Soluble Barium Soluble Fraction of Prawn Muscle.
(The measure of variability is the standard error of the mean)

Local Name and Scientific Name	Total acid soluble phosphorus mg. percent	Total acid soluble barium soluble phosphorus mg percent	Phosphorus used up in phosphoarginine mg. percent	Nitrogen in Arginine mg.	Corresponding Arginine mg. percent (Block)	Arginine mg. percent (Sakaguchi)
Sode I. Parapaeneopsis Sculptilus.	161.00 ± 5.1	78.65 ± 1.9	5.75 ± 1.1	10.04 ± 1.0	39.80 ± 0.69	37.50 ± 1.0
Sode II. Parapaeneopsis Uncta.	154.20 ± 4.6	72.50 ± 2.1	5.67 ± 1.0	10.31 ± 1.2	33.60 ± 1.6	31.50 ± 1.2
Tendli. Metapeneus Monoceros.	140.40 ± 3.1	62.23 ± 1.6	5.66 ± 1.2	10 24 ± 1.1	38.40 ± 0 89	34.50 ± 0.80
Golim. Leander Styliferus.	130.00 ± 2.6	58.50 ± 1.9	5.66 ± 1.2	10.20 ± 1.09	33.40 ± 1.20	33.00 ± 1.1

*Average of 10 samples in each variety.

SUMMARY

Using barium hydroxide as a reagent at pH 9 it is possible to detect and estimate phosphagen (phosphoarginine) in the acid soluble barium fraction. A method for the separation of phosphagen in the invertebrate prawn is given. Experiments have been carried out to determine the arginine both by direct and by indirect methods. The presence of phosphoarginine is established in prawn muscle while phosphocreatine is not detected in the barium fraction.

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