CHAPTER IV

EFFECT OF MINERALS ON THE CYTOCHROME C CONTENT IN SOME LEG, WING AND HEART MUSCLES OF POST-HATCHED CHICK DURING DEVELOPMENT
Factors affecting the percentage of myoglobin in muscle (Lowrie 1950) had confirmed that high concentration of this pigment in usually found in those muscles of high physiological activity. This is of particular interest in view of the strong affinity of myoglobin for oxygen which enables it to act as an intra muscular oxygen store for the tissue’s principal oxidizing catalysts, the cytochromes. (Hill 1936, Millikan 1937), cytochromes are haem proteins in which iron forms the core of the haem and it is the iron that is oxidized and reduced in the electron transport. Kiellin as early as 1925 had pointed out that all aerobic cells contains cytochromes (as determined spectroscopically) and is the highest in the most activity respiring cells. However the concentration of cytochromes has been reported both as being higher (Fujita et al. 1939) and lower (Hill 1936) in myoglobin rich or ‘red’ muscles than in the white variety.

Experimental work of the past 30 years led to the awareness that metallions are essential to a large variety of biological processes in general and in the biochemistry
of nucleic acids specifically (Einthhorn 1973). The use of organic substances and inorganic substances both metals and metal salts in the treatment of disease is well known. A number of inorganic compounds today used as medicinal agents in the modern therapeutics. Several metals though present in tissues only in minute quantities, are considered to be essential for the maintainence of life. These metals are called as essential trace elements, are required by humans as well as animals varying from 15 to 18µg per day. These are crucial to enzyme function and have significance in the synthesis and structural stabilisation of proteins and nucleic acids. In view of this we thought of studying how these metals help in synthesizing cytochrome C content in leg, wing and heart muscles of the growing chick.

MATERIAL AND METHODS:

One week old to six months age white leg horn chicks were employed in the present investigation. The birds were killed by decapitation and the required muscles were taken out by dissecting the birds and the muscles were stored at 0°C until used.
EXTRACTION AND ESTIMATION OF CYTOCHROME C

The cytochrome C content of the different leg, heart as well as breast muscles was extracted according to the procedure described by Potter and Dubois (1942). The procedure as follows:

Weighed tissues are homogenised in 5 to 20 volumes of distilled water in a Potter and Elvehjem homogenizer. The homogenate is quantitatively transferred to a beaker and the pH is adjusted to 3.5 by addition of 3 percent trichloroacetic acid. The mixture is allowed to stand for one and half hours to extract the cytochrome C. The mixture is then transferred to graduated conical tubes and centrifuged for 10 minutes at 3000 R.P.M. Since the cytochrome is distributed between the residue and supernatent, the volumes of both are recorded for subsequent calculations. The supernatent fluid is brought to pH 7.0 with 1% sodium hydroxide, and after standing 5 to 10 minutes, the supernatent fluid is poured into graduated centrifuge tubes and a quantity of 100% trichloroacetic acid solution equal to 8% of the volume of supernatent fluid is
added. The mixture is allowed to stand for 20 minutes and is then centrifuged for 10 minutes at 3000 R.P.M. The supernatant liquid is carefully removed and discarded. The precipitate containing the cytochrome C, which was then dissolved in a drop of 2 N sodium hydroxide. Excess of sodium hydroxide is neutralized by addition of 1 N hydrochloric acid. This solution is then finally diluted into a known volume with distilled water (the volume is about 2.5 ml).

To a known aliquot of the above solution 0.3 ml of 0.25 M Phosphate buffer, pH 7.4 and 0.2 ml of a kidney enzyme preparation containing both succinic dehydrogenase and cytochrome oxidase are added, giving a total volume of spectrophotometric cell and absorption is measured at 550 μm with 2.5 μm exit slit. The extinction observed is due to oxidized cytochrome plus cytochrome oxidase in the substrate.

The extinction observed is due to oxidized cytochrome plus other coloured substances present. Next 0.01 ml of 0.5 M succinate is added to the cell, after
mixing 0.03 ml. of 0.1 M neutralized cyanide is added. This cyanide stops the action of oxidases but does not combine with cytochrome C, is converted to the reduced form by the action of SDH. Again 2nd spectrophotometric reading is taken. From the two extinction values obtained the cytochrome C may be calculated with the following formula described by Potter and Dubois (1942).

RESULTS AND DISCUSSION:

The present results indicate that the minerals cupric chloride, manganese sulphate, lead acetate and zinc chloride indicated that the use of these metals showed pronounced effects on the muscle cytochrome C content. Both manganese and copper have severe effect when compared with others. (Table 1 fig 1).

In the cytochrome the natural hydrogen acceptors of the dehydrogenase system present in the cell. The best known member of the cytochrome system is cytochrome C, the soluble cytochrome. The importance of muscle function in the widest sense the determining the activity of the oxygen utilising enzyme system. The heart muscle is the most
TABLE 1: CYTOCHROME C CONTENT IN THE HEART, LEG MUSCLES AND BREAST IN MUSCLES IN THE CHICKENS FED WITH FOUR MINERALS.

VALUES EXPRESSED AS MICROGRAM/100 GM WET WEIGHT OF THE TISSUE.

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<tr>
<th>AGE</th>
<th>CONTROL</th>
<th>LEAD ACETATE</th>
<th>COPPER CHLORIDE</th>
<th>ZINC CHLORIDE</th>
<th>MANGANESE SULPHATE</th>
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<td>1250.1</td>
<td>1550.8</td>
<td>1650.92</td>
<td>1650.72</td>
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<td>1650.72</td>
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<tr>
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<td>1250.1</td>
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<td>1650.72</td>
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VALUES ± SE OF FIVE EXPERIMENTS
Cytochrome C content in the Heart, Leg, Breast Muscles in the Chickens fed with Four Minerals

Mg/gm wet weight of the Tissue

Age in Weeks
1 = Heart Muscle
2 = Sartorious
3 = Tibialis Anterio
4 = Pectoralis
continuously used muscle reflected by higher enzyme activity than skeletal muscle. The low cytochrome oxidase activity was found in pigeon breast muscle compared to horse heart. The present result exhibits that the cytochrome C contains in the muscle which are also rich in myoglobin. Myoglobin is involved in the dehydrogenation process and as such that is expected to be a correlation between myoglobin and cytochrome C content in the muscle. If more of these two components occur, such a muscle is expected to be more active than other. The results given in the table show that the leg muscle and heart muscle exhibits high amount of cytochrome C than the wing muscle. The mineral treatment though exhibited higher amount of this component, followed the same pattern. These differences have been attributed to the relative activity capacities of the leg and heart muscles. The conclusion is that the addition of minute quantities of these minerals help to attain healthy growth and higher amount of cytochrome C content in the muscles of the growing chick.