CELLULAR CHARGE PATTERN IN CELLS
AND
THEIR RELATION TO METABOLISM

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by
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Preface

The present investigation was undertaken, considering the previous work in this department as a basis. The work on the gastrocnemius muscles indicated the relation between net sign of the charge and enzyme activity. Hence it was thought that similar lines of investigations could be extended to sartorius muscle as it was most suitable for subcellular electrophoretic studies.

An attempt was made in this direction, inspite of the difficulties in the procuring necessary equipment such as ultracentrifuge, isotope equipment etc. The nonavailability of certain chemicals had also limited the scope of the investigation. Inspite of all these drawbacks, possible venues of study were selected and a preliminary investigation was conducted.
Acknowledgements

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INTRODUCTION (GENERAL)
Introduction

Activity of the tissues in general, action potentials and their propagation

One of the fundamental characteristics of living cells are the electrophysiological features exhibited at their surfaces, possibly in the interior also. A potential difference exists between the interior and the exterior of most of the living cells (Grundfest, 1955; Hodgkin, 1955, 1958). Any alteration in the potential difference, produces profound effects in the physico-chemical system of the protoplasm of living cells (Heilbrunn, 1956; Tobias, 1949; Plaiz, 1947). Calcium, potassium and sodium are important for the maintenance of membrane potentials and for the development of the action potentials (Frakenhaeuser, Hodgkin and Frakenhaeuser, 1957). It has been shown that agents, viz., electric shock and electromagnetic radiations etc. cause release of bound calcium (Heilbrunn, 1955; Frank, 1963; Bianchi, 1961) which is responsible for the initiation of physiological process, such as muscular contraction and other phenomena (Shashi, 1961; Bianchi, 1961). Contractions induced by membrane depolarization were associated with influx and efflux of calcium ions, (Bianchi and Shanes, 1959; Shanes and Bianchi, 1959) which were found to play an important role in the excitation and contraction process.
(Thomas, 1960; Bianchi and Shanes, 1960; Hodgkin and Keyes, 1957). When muscle and nerve cells are stimulated a wave of action potential travels from the point of stimulation to either side. The velocity of transmission of impulse is low when compared to the conventional electronic flow (Giese, 1953). Hence, the action potential travelling across the cell is not electrical but electro-chemical in nature. The conduction of the action potentials along the surface is characterised by the influx and outflux of charges namely the Na$^+$ and K$^+$ ions respectively in the activated muscle and nerve (Giese, 1953; Hodgkin, Huxley and Katz, 1952; Hodgkin, 1945; Prosser and Brown, 1961; Shanes and Bianchi, 1960; Nachman, 1960; Cosmos and Harris, 1961; McLennan, 1961; Hodgkin, 1951; Huxley, 1954; Rothenberg, 1950; Davies, 1963). During recovery, respiratory process could provide the energy required to transport ions against their electro-chemical gradient so that original pattern of distribution of ions is restored (Keyes and Maikel, 1954). The possibility of proteins involving in the transport system was suggested by Goldacre (1952) where the folding and unfolding of the long chain polypeptides could account for the transport. Displacement of calcium by potassium (ion exchange events), movement of water into the membrane phase (Hydrokinetic
events) influence the fall in the membrane resistance followed by an increased transmembrane fluxes of sodium and potassium, which are important events of the excited state in nerves (Hodgkin, 1954). When the membrane potentials are suddenly reduced there is influx and outflux of Na+ and K+ ions moving down their electrochemical gradients and should account for the propagation of the action potentials.

Excitation, however, considerable indirect evidence for some change in the membrane structure (Lilly, 1922; Curtis and Jole, 1942; Hodgkin and Katz, 1949). A direct evidence for a change in the cytoplas in structure (electron, 1933; Goldruin, 1933 and 1943). The exact relationship between the electrophysiological events that are going on in the membranes and the events in the subcellular colloidal structure of the protoplasm is not clearly understood (Goldruin, 1933; Liberman and Chalkidion, 1955;。「リンプセカミ, 1956; Swami et al., 1952; Sibambo and Swami, 1954).

This study is to find out how the change in the membrane permeability affects the metabolism of the cell (Kaye and Van de Velde, 1953).
This ionic movement during stimulation is down the electrochemical gradient, probably affected by the change in permeability of the cell membranes. During the resting state the cell membrane offers resistance to such flux and contains a system which will make the ions move against the electro-chemical gradients actively. Conway and his colleagues (1959; 1960) suggested that the energy for ion transport in cells which leads to the development of electrochemical gradients is derived from the redox system which involves electron movements and hence these are termed as "redox pumps" by Conway (1957) and "electron cycles" by Davies (1954). Another hypothesis stated that the ATP is directly concerned with the ion transport.

There is some evidence in favour of ATP linked ion transport. The extrusion of sodium during recovery after the active state in living cells is dependant on the presence of ATP. Injection of ATP and arginine phosphate into the axons of Loligo was adequate to initiate sodium extrusion (Caldwell, Hodgkin, Keynes and Shaw, 1960; Caldwell, 1960). Similar ATP linked sodium transport mechanism has been demonstrated in human red cells and brain cortex (Hoffman, 1961; Whittman, 1953; Sollwain, 1961). Since ATP content depends on the metabolic activity, the ion transport comes under the influence of metabolism (Ussing, 1946; Whittman, 1961, 1962a).
Next problem will be to determine the actual role of ATP, present in the protoplasm of living cells, in the active transport of ions. It has been shown that the enzyme system present in the crab and red cells can break down ATP to ADP and inorganic phosphate in the presence of magnesium and sodium. Post and his colleagues (1960) believed that these enzyme systems present in the red cells are responsible partly for the sodium transport. An interesting work of Whittman (1962) in red cells, Skou (1962) in kidney and brain and Jarnestad (1962a, b) in brain microsomes indicated that the enzyme ATPase in the presence of sodium, potassium and magnesium is responsible for the breakdown of ATP, and thus aids the liberation of energy utilized for ion pump.

In the resting state of cells, acetylcholine is present in an inactive form bound to proteins (Nachmannssohn, 1955). On stimulating the membrane, acetylcholine is released from the bound form and made free. The free acetylcholine acts on the membrane receptors, affecting the membrane structure; this in turn brings about changes in ionic permeability, thereby increasing sodium conductance, which is responsible for the generation of action potentials (Nachmannssohn, 1959). However the precise relationship between the acetylcholinesterase action and the development and propagation of action potential is not clearly understood.
In most of the cases involving the transport of ions, necessary enzymatic machinery should exist in the membrane as well as within the cytoplasm whose activation and inactivation should be able to stuck a balanced metabolism. Hence the phenomenon of activation and inactivation of enzyme proteins should play a prominent role in the cellular activity and recovery from the active state. Hence it will be worthwhile to study the relation between enzyme activity and the pattern of organization of the cytoplasmic components.

Activity of enzymes

It was found that the net sign of the charge existing in the cell fractions dictates the activity of enzymes. The greater the density of the positive charges the greater was the activity of the enzymes such as succinic dehydrogenase, cathepsin, catalase and carbonic anhydrase in the gastrocnemius muscle of frog, *Rana hexadactyla* (Krishnamoorthy, 1963; Krishnamoorthy and Swami, 1964a; Swami and Krishnamoorthy, 1964). The more the density of the negative charges so much less was their activity. But activation of xanthine oxidase was dependent on the net negativity of the charge. Decrease in the density of the negative charge relieves the inhibitory control,
resulting in the rapid increase of this enzyme activity (Swami and Govindappa, 1964). Increase of the net negative charge density accelerates the activity of proteases (Indira, 1964). Hence it is possible to infer that the enzyme activity, in general, is under the influence of the intensity of the net positive or negative sign of the charges. A precise knowledge of the intracellular electric properties of cells is essential for a clear understanding of the phenomenon of enzyme activation in relation to the net sign of the charge in cells.

Investigations carried out in the past had shown that the net positive sign of the charge exists on the subcellular particulate fraction of amoeba (Swami, 1960) *Paramacium multi-miciro.nucleatum* (Swami, 1960) gastrocnemius muscle of *Rana hexadactyla* (Krishnamoorthy, 1963; Govindappa, 1964; Indira, 1964; Swami and Indira, 1964), axon of Lobster (Swami, 1959) and *Elodesa* (Hollburn and Daugherty, 1939). In a few cases net negative sign of the charge has been observed (Tobias and Solomon, 1959a).

When isolated axons of crayfish and lobster were exposed to a field of direct current along the long axis, number of physical events were initiated. There was cathodal swelling and decreased opacity and anodal contraction followed by an increased opacity in nerve and
muscle cells (Solomon and Tobias, 1950b; Swami, 1959; Krishnamoorthy, 1963; Friede, 1964). This swelling at the cathode was due to the electro-osmotic movement of water (Solomon and Tobias, 1950) in the axon of crayfish and due to the accumulation of water soluble substances in the gastrocnemius muscle of frog, Rana hexadactyla (Krishnamoorthy and Swami, 1964a, b, c; Krishnamoorthy, 1963).

**Hydrogen ion concentration on enzyme activity**

The existence of net positive or negative sign of the charge of the protoplasmic colloid of the cells is due to the polar groups present in their protein and phospholipid constituency (Arachet, 1957; Fruton and Simonds, 1960). These groups ionize under suitable conditions of hydrogen ion concentration (Bayliss, 1957). If the particulate fraction containing protein with ionizable groups is exposed to a pH value on the acid side of their isoelectric point, they acquire positive sign of the charge, and on the application of direct current field they migrate towards the cathode. If the same protein fraction is exposed to a pH value on the alkaline side of the isoelectric point, a net negative
charge results on the protein fraction with consequent migration towards the anode in an static electric field. The activity of the enzyme is dependant on the ionization of the polar groups of the protein molecule (Bayliss, 1959; Amudavalli, 1954; Krishnamoorthy and Swami, 1964a) so that the sign of the charge on the cell constituents, is determined by the hydrogen ion concentration. hence it is essential to have precise knowledge about the intracellular pH of the living cells, so that the true nature of the metabolism prevailing in the living cells may be demonstrated.

Attempts have been made in the past to determine the intracellular pH of living cells. The difficulties involved in the determination of intracellular pH were discussed by Heilbrunn (1956), Hercinski (1955), Caldwell (1956) and Swami (1960). Some of the techniques possibly give reliable values of hydrogen ion concentration, however a new method was employed in the present investigation. It involves the electrophoretic characteristics and ionization pattern of the cytoplasmic constituents of the muscle.
Intracellular pH and the cell metabolism

Since hydrogen ion concentration influences the activity of the enzymes, any alteration of hydrogen ion activity in the environment will affect the enzymatic activity. Under these conditions, enzymes having optimum pH value close to that of the intracellular pH will exhibit their activity while others remain inactive. Living cells have a mechanism within them which offers protection to enzymes having pH optimum different from the intracellular pH so that they may be able to manifest their activity.

Alteration in the hydrogen ion concentration may have profound effect on the cell metabolism since enzyme activity is affected (Heilbrunn, 1956). The hydrogen ion activity of cytoplasm is dependent on the number of subcellular charged particles present in it. If the charged macromolecules are more in number, they interfere with the free movement of hydrogen ions and consequently alter pH value in the environment. In living cells, charged molecules are present having their own

neutralized intracellular pH different from that of the immediate substance. This phenomenon of "suspension
effects prevents free diffusion of hydrogen ions through
the interface (Overbeek, 1956) (between localized area
and the ground substance) and do not obey the Donnan
effect (Caldwell, 1956), thereby giving relatively stable
hydrogen ion environment in the localized area, imparting
optimum pH conditions for the enzymes lodged in them
(Danielli, 1944). If all the enzymes were exposed to
the same pH value, for example the intracellular pH value,
then most of them would be inactive, unless their pH
optimum were the same as that of the intracellular pH.
'Suspension effect' protects the enzyme molecules from
such inhibition by intracellular pH and provides an
optimum pH condition conducive to the expression of
their enzyme activity. Hence the activity of these
enzymes is dependent on the subcellular localization of
charged macromolecules which avoid the inhibitory influ-
ence of intracellular pH by effectively providing a
barrier at the interface.

The stable hydrogen ion environment in the ground
substance could be advantageous during the transport of
the enzymes to different localities, from the site of
their synthesis to the site of their action. During
the transport from one place to the other of enzyme molecules, having pH optimum different from that of the intracellular pH, their enzymatic activity would be low due to the unfavourable intracellular pH. When once they arrive at their respective localized areas of subcellular charged macromolecules, optimum pH conditions are provided in this localized area by the phenomenon of 'Suspension effect'. The transport of enzyme molecules having optimum pH identical with the intracellular pH could be different. It is possible that they are transported in an inactive form through the ground substance such that when once they reach the site of action they are converted into active form by some other mechanism.

During metabolic processes the concentration of hydrogen ions may change and enzyme molecules bathed in an environment having a pH value close to their optimum pH are affected, and may be inactivated. When an excess of hydrogen ions is removed from the enzyme molecules, original activity is restored. The excess of hydrogen ions cannot be balanced out as in the interference by 'Suspension effect', but is transported in the form of bicarbonate ions (Cadwell, 1956). The bicarbonate ions are formed by the
dissociation of carbonic acid which in its turn is formed as the carbon dioxide dissolved in water, and the water being formed in the reduction reactions involving oxygen causing the elimination of hydrogen ions (barring at the general intake of water). The sequence of steps involved in the transport of hydrogen ions suggests that an active process may exist which requires expenditure of energy. A similar mechanism has been shown to exist in the oxyntic cells of gastric mucosa (Davies, 1950) which requires consumption of substrates.

Present approach

In the light of these considerations it is obvious that the intracellular pH of living cells is having a profound influence on the cell metabolism. Even the slightest alteration will have marked influence on the enzyme systems of the living cells. It is clear that the general metabolism of the living cells depends on hydrogen ion concentration. Any excessive increase or decrease of the hydrogen ion concentration of living cells will cause acidosis or alkalosis respectively, leading to the death of the cell. Hence, it is essential to determine the intracellular pH of living cells since it helps us to understand the cell's metabolism.
In the present investigation the subcellular electrical properties, electrophoretic characteristics and ionization pattern of the protein constituents of sarcoplasm of sartorius muscle of frog, Rana hexadactyla, were investigated in the hope that they may throw some light regarding these aspects of cell biology.