The various enzyme systems within the living cell are exposed to heterogeneous environment consisting of proteins of different species with variable physical and chemical properties. The characteristics of pure enzymes observed in isolated state are subject to modifications in the heterogeneous environment. Hence, the properties of the enzymes in a living cell are always subject to the influence of the subcellular environment of which the proteins occupy a major part. Previous studies on the gastrocnemius muscle of frog (Govindappa, 1967; Muralikrishnadass, 1967) and the brain of sheep (Ramana Rao, 1968), indicated that addition of positively and negatively charged proteins and amino acids to enzyme systems resulted in variations in activities and characteristics of these enzymes, suggesting that in vitro enzyme activity is regulated by exogeneously added protein and amino acid charges.

In the present investigation, the amphibian kidney was chosen for the study of the regulation of enzyme activity and protein synthesis in relation to the charge pattern contributed by the protein and amino acids.

Among the plasma proteins, the albumins have an isoelectric pH relatively on the acid side than those of the gamma globulins. Thus, they represent acid proteins. The gamma globulins represent relatively basic proteins. These proteins were isolated from the kidney. In addition, the acidic and basic proteins were extracted from the kidney at pH values 5.0 and 8.0 respectively. Besides isolated proteins, commercial gamma globulins
and commercial bovine plasma albumins were employed. The albumins and acidic proteins provide negative environment to the enzyme systems, while the gamma globulins and basic proteins provide positive environment to the enzymes respectively. Similarly, glutamic acid and lysine provide negative and positive environments respectively.

The activity levels of succinate, lactate and glutamate dehydrogenases as a function of pH

The levels of activities of succinate, lactate and glutamate dehydrogenases showed variable influences of pH. In general, at pH values alkaline to the homogenate pH (7.0), there was an elevation of activity of these enzymes (Figs. 1, 12 and 23). At pH values less than the homogenate pH, there was a general decrease in the level of their activities (Figs. 1, 12 and 23). The homogenate pH of 7.0 probably indicates a situation which is intermediate in influencing the expression of the activity. If the pH range varying from 5.0 to 8.0 is considered, the level of activities of enzymes, in general, seems to increase with the increase in the pH value or decrease in acidity (Figs. 1, 12 and 23). More is the basic environment higher is the activity, suggesting that the groups in basic condition promote the activity of the enzymes.

These enzymes seem to exhibit peak activities at two pH values; one in the alkaline and the other in the acid region (Figs. 1, 12 and 23). In the alkaline region a pH value of 7.4 and in the acid region a pH value around 5.7 and 6.0 seem to
induce an elevated activity of the enzymes (Figs. 1, 12 and 23). Between these optimal pH values, the alkaline conditions had an upper hand in the induction of activity of the enzymes. The lesser peak activity in the acid region is probably dependent upon the ionization of the carboxyl groups (alpha and distal carboxyls) whose pKa values extend up to 4.7. Hence, at pH values of 5.7 and 6.0 in the homogenates, these carboxyl groups assume negative charge. These pH values represent a situation in the homogenate where a negative charge density begins to develop in the homogenate proteins.

The high peak activity of the enzymes observed in the alkaline region (Figs. 1, 12 and 23), indicates the approach-ment of the pKa range of alpha and distal amino groups. At 7.4 pH value, the amino groups lose their positive charge proton thereby getting neutralized, i.e., at the pH value of 7.4 and above the positive charge density in the homogenate, contributed by amino groups, starts decreasing. As the positive charge decreases, the negative charges prevail and dominate in the heterogeneous protein fraction of the homogenate.

Thus, it appears that the ionization of carboxyls and to some extent phosphate and imidazolyl groups in the acid region and the neutralization of the alpha and distal amino groups in the alkaline region seem to have a stimulating influence on the activity of these enzymes. The two pH optimal conditions observed for all the three enzymes, one in the acid and the other in the alkaline region (Figs 1, 12 and 23), suggest a
resemblance to that of the existence of a pattern comparable to isozymes. Since isozymes of succinate dehydrogenase are not known, the present observation may not suggest the existence of isozyme pattern. It is likely that the acid range of the pH values may induce secondary interactions between the pH values 5.7 and 6.0, consequently accounting for the appearance of lesser peak activity.

Of the three enzymes, the GDH seems to have a lesser peak in the acid region than the corresponding ones of the other two enzymes (Figs. 1, 12 and 23).

Proteins of gamma globulin and albumin nature in the kidney of frog

Fractions 1, 2 and 3 (Fig. 85) obtained with salt fractionation were more in the kidney than the other fractions. These fractions represent majorly of gamma globulins and other globulins, while the other two fractions represent the albumin content which was considerably low in level. These observations indicate that the globulin level, that too, the gamma globulin level is considerably high in the amphibian kidney than the albumins (Fig. 85). The investigations using the technique of the isoelectric precipitation of proteins at different pH values substantiate the present findings (Fig. 84). More of the proteins were precipitated at pH values between 4.8 and 5.6 which represent the globulins than the proteins precipitated at pH values less than 4.8 which majorly constitute the albumins.
The investigations with salt precipitation and isoelectric precipitation agree very well and suggest that the globulin level is considerably high in the amphibian kidney. Consequently, the alkaline region elicited higher peak activities of the enzymes.

**Effect of protein charges on succinate dehydrogenase activity**

The present investigation reveals a relationship between the type of charge density and the enzyme activity. The different charge densities are contributed by different proteins at a given pH value. To get a more realistic view of such relationship, it was thought necessary to use purified preparations of proteins varying in their isoelectric points and then study the enzyme activity patterns in their presence. In the gastrocnemius muscle, the activation of SDH is under the influence of the albumin content in the homogenate (Anandaratnam, unpublished data), while in the amphibian kidney it is under the influence of the globulin content in the homogenate. The SDH enzyme seems to pose a different type of activity pattern in the amphibian kidney when compared to the amphibian gastrocnemius muscle. If the elevated level of globulin is capable of eliciting greater stimulation of the enzyme, addition of globulins should elevate the activity further. The addition of albumins should decrease the level of activity of the enzyme. In the present study it was found to be the case (Figs. 1 and 3). Addition of commercial gamma globulins as well as the isolated gamma globulins elevated the levels of activity of the SDH throughout the pH
range (Figs. 1 and 3). On the other hand, the commercial albumins and the isolated albumins decreased the level of activity of the enzyme especially at pH values in the alkaline region (Figs. 1 and 3). It is likely that the character of stimulatory influence by the globulins on the enzymes is knocked out in the alkaline region by the albumins. To be more precise, the abolition of the accelerating influence of the gamma globulins by the albumins was achieved at pH values on the alkaline side of 6.0. Since the gamma globulins have isoelectric points at this pH value, it is likely that the ionization pattern induced by the gamma globulins on the alkaline side of their isoelectric points may be exerting an accelerating influence on the expression of the enzyme activity. Albumins, being relatively acidic to the gamma globulins, are probably capable of neutralizing the influence of the relatively basic gamma globulins in regulating the enzyme activity at these pH values. At pH values less than 6.0, the situation seems to be different. The albumins seem to elevate the activity of the enzyme at a time when the gamma globulin influence is completely abolished.

The mitochondria of the amphibian kidney and the amphibian gastrocnemius muscle seem to have different sensitivities to the proteins such as the albumins and globulins. The amphibian kidney mitochondria probably provide a stimulating influence on the contained SDH enzyme in the presence of gamma globulins, while the amphibian muscle mitochondria provide an accelerating influence on the contained SDH enzyme in the presence of albumins.
Superficially it appears that the mitochondria of amphibian kidney and muscle are different in terms of their sensitivity toward different proteins. Since isolated and purified mitochondria from different tissues are known to conduct the oxido-reduction reactions in a similar pattern, it is possible that the original characters of mitochondria are similar in all tissues. The present observation, namely, the differential sensitivity of the mitochondria to the proteins in the amphibian kidney and muscle could possibly be due to some type of specific associations. In the amphibian kidney it is likely that the gamma globulin, because of its high concentration in cell is probably forming a complex with the mitochondrial protein, thus contributing its own characters and influence in the expression of the level of activity of the enzyme.

It is known that particles suspended in a soluble protein get a fine coating of the surrounding protein (Abramson et al., 1942). Thus, different types of particles with different charges are known to assume identical charge pattern because of the surface protein films and thus manifest physical properties identical to those proteins in the solvent phase. The dominating soluble protein contributes its own properties to the different types of particles suspended in it by means of the surface films formed around the particles. In the amphibian kidney it is likely that the major soluble protein fraction, namely, the gamma globulin content form surface layers around the mitochondria and contribute its properties to the mechanism of activation of the contained enzymes. In the amphibian muscle,
the dominating protein content, namely, the albumins form surface film around the mitochondria and contribute the albumin characteristics to the mitochondria. Thus, it is likely that the protein association with the mitochondria could be responsible for the differential sensitivity of the proteins to the mitochondria in the amphibian kidney and the gastrocnemius muscle.

In the amphibian kidney around pH value 6.0 which is very close to the isoelectric point of the gamma globulins, these proteins are least soluble because of the isoelectric conditions and thus probably dissociate from the complex they have been entered into with the mitochondria. When such association is disrupted, the mitochondria are free from the influence of the gamma globulins. Addition of albumins at pH values less than 6.0 could have an uninterrupted influence by the albumins since the intervention of the gamma globulins in the activity pattern does not exist. In the present investigation, it was actually found to be the case. At pH values less than 6.0, the albumins elevated the activity of the enzyme (SDH) because of the abolition of the gamma globulin effect (Figs. 1 and 7). The amphibian kidney mitochondria at these pH values acquired properties identical to that of the amphibian gastrocnemius muscle.

The estimations of levels of activities of SDH with the acidic and basic proteins indicate similar relationship suggesting that the pattern of activation and inactivation of SDH is actually dependent upon the ionization pattern of the contained proteins (Fig. 2).
Protein charge effects on the activity levels of glutamate dehydrogenase

The pattern of activity of GDH in the presence of commercial as well as isolated gamma globulins and albumins, acidic and basic proteins indicate similarity to the pattern of regulation of enzyme activity of SDH with slight modifications. Globulins and basic proteins, in general, elevated the activity of the enzyme throughout the pH range except at the optimal condition, i.e., at pH 7.4 (Figs. 23, 24 and 25). There was inhibition of activity by albumins at pH optimal condition which was more with the gamma globulins (Figs. 23 and 25). Albumins and acidic proteins, in general, suppressed the activity of the enzyme (Figs. 23, 24 and 25). The pattern of enzyme regulatory mechanism at pH optimal condition and the patterns at other pH values are contradictory in function. Though, the regulatory mechanisms operating on this enzyme seem to bear a relationship to that of SDH, the fundamental characteristics seem to differ. The sensitivity of the enzyme at pH optimal conditions to exogeneously added proteins seems to be very high, while it is not so at other pH values. At the optimal condition, the enzyme is ionized maximally so that the molecules may have maximum number of active centers. Since the isoelectric point of the enzyme, in general, lies very close to that of the pH optimal condition, it is likely that the enzyme has least solubility. The greater sensitivity in terms of inhibition by acidic and basic proteins suggest that the activity of the enzyme required specific proportion of positive and negative charges. Either
increase in positive or decrease in the negative charges in the environment will bring about inhibition of activity. Unlike SDH, a balance between the positive and negative charge density regulates the activity of the enzyme.

The comparison of the pH optimal conditions of SDH and GDH shows that the SDH level was increased considerably by the gamma globulin protein, while the GDH activity was decreased (Figs. 1, 3, 23 and 25). The albumins decreased the activity of SDH as well as GDH (Figs. 1, 3, 23 and 25). Hence, there is a differential regulation of enzyme activity by the gamma globulins.

**Influence of protein charges on lactate dehydrogenase activity levels**

The regulatory pattern of LDH in the presence of the albumins and gamma globulins seems to take a different pattern. The effect of albumins in the region acidic to the pH optimal condition seems to elevate the activity, while the gamma globulins could also elevate the activity but not to the same extent as the albumins (Figs. 12 and 14). At the optimal pH condition, which will be generally close to the isoelectric point, the exposure of the active sites will be greater than at other pH values. On the acid side of the pH optimal condition there will be increased emphasis on the positive charge density. Since albumins are more acidic than the globulins they may probably increase the emphasis on the positive charge density.
and consequently this could be a factor responsible for the elevated activities in the acid range. The gamma globulins also contribute positive charge density at pH values acidic to the pH optimal condition on the enzyme molecule. But the effect will be more by the albumins since they are more acidic than the gamma globulins. Hence, it appears that the regulation of LDH activity is dependent more upon the development of positive charges on the enzyme molecule unlike the other two enzymes where negative charges contribute to the elevation of activity. Hence, the pattern of activation of LDH is entirely different from that of the pattern observed with the other two enzymes. LDH is a soluble enzyme known to be located in the high centrifuge supernatant, while the SDR and GDH are located in the particulate fraction. Even this differential localization of enzymes might be responsible for differential regulation of their activities.

Furthermore, it may be suggested that the positive charge density contributed by the exogeneously added gamma globulins and basic proteins might have exerted a regulative influence on the groups prevailing in the active center possibly by protein-protein interaction thereby modifying the configuration of the enzyme molecule.

When the level of activities of the three enzymes at homogenate pH (7.0) is considered (Fig. 38), it appears that the albumins have an inhibitory effect on the levels of activities of the two aerobic enzymes, while LDH is elevated in activity
posing a condition similar to that of aerobic glycolysis. On the other hand, the gamma globulins seemed to elevate the activity of SDH and GDH, while there is a slight suppression of activity with LDH indicating a situation similar to Pasteur effect (Fig. 38).

When percentage deviation of the three enzymes is considered (Figs. 75, 76 and 77), the GDH seems to have undergone inhibition of activity relatively with the albumins and acidic proteins, while the basic proteins and gamma globulins elevated the activities considerably in the acid region (Fig. 77). In the case of LDH, all the proteins, in general, seem to elevate the activity of the enzyme in the acid pH values (Fig. 76). In the case of SDH, the situation seems to be intermediate (Fig. 75). Thus, it appears as if the pattern of activation and inactivation of these enzymes seems to vary widely in their fundamental characteristics.

The sensitivity of the proteins to the level of activity of the enzyme at pH optimal condition seems to be less when compared at other pH values. This pH optimal condition seems to be similar in all the three enzymes (Figs. 1, 12 and 23). In the case of SDH, a pH value of 5.4; in the LDH, a pH value of 5.7 and in the GDH, a pH value of 6.0 in the acid regions seem to induce maximum sensitivity of the enzyme to the exogenously added proteins in the acid region of the optimal pH condition (Figs. 1, 12 and 23). Since the sensitivity of SDH,
LDH and GDH in the acid region were found at different pH values, namely, 5.4, 5.7 and 6.0 the fundamental characteristics governing the enzyme are probably under the influence of different charge pattern specific to each.

Kwon and Clecott (1965) as cited by Atkinson (1966) reported that glyceraldehyde-3-phosphate dehydrogenase can augment the activity of crystalline aldolase. The enhancement of aldolase activity is apparently due to protein-protein interaction. It is likely that the augmentation of SDH, GDH and LDH activities by various proteins may be due to such protein-protein interactions.

**Amino acid charge effects on succinate, lactate and glutamate dehydrogenases**

Glutamic acid and lysine have been selected since they provide negative and positive charges to the homogenate. The results obtained with these amino acids are more or less in conformity with the results obtained with the protein fractions. Glutamic acid seems to inhibit the activities of SDH and GDH at all pH values (Figs. 41 and 43) suggesting that negative charge density will suppress the activities of these two enzymes. The inhibition of GDH by glutamic acid may also be due to substrate inhibition. Furthermore, the reasons for inhibition of these oxidative enzymes might be the same as given hitherto. Glutamic acid inhibited the LDH activity at the acidic pH values of the homogenate pH value and elevated toward the alkaline side.
of the homogenate pH (Fig. 42). Conversely, lysine elevated the LDH activity at the acidic pH values of the homogenate pH and inhibited towards the alkaline side of the homogenate pH value (Fig. 42), suggesting differential regulation of the enzyme activity by different charge densities.

**Protein charges and pH optima of enzymes**

The investigations on SDH, LDH and GDH indicated a shift in the pH optima of the enzymes in the homogenates containing different proteins and amino acids.

**SDH:** Addition of the albumins seem to shift the pH optimum toward the acid side, i.e., from 7.4 to 7.0. Addition of the gamma globulins and basic proteins seem to shift the pH optimum toward the alkaline side, i.e., at pH value higher than the pH optimum (Figs. 1, 2 and 3).

**LDH:** The situation seems to be somewhat similar to that of SDH as far as the albumin effect is concerned, i.e., the pH optimum tends to shift toward the acid side, while the gamma globulins and basic proteins, in general, do not seem to have an effect on the pH optimum in terms of the shift (Figs. 12, 13 and 14).

**GDH:** The albumin effect in shifting the pH optimum is not significant, while the gamma globulins seem to shift the pH optimum toward the acid side (Figs. 23, 24 and 25).
pH optimum and characteristics of enzymes

The three enzymes studied in the present investigation showed differential response to the exogenously added proteins in terms of the shift in the pH optimum. The SDH and GDH seem to have contradictory sensitivity to the albumins and gamma globulins, while the LDH seems to be sensitive only for the albumins and not for the gamma globulins (Figs. 1, 2, 3, 12, 13, 14, 23, 24 and 25).

There is a body of evidence suggesting shifts in the pH optimum of an enzyme in the presence of a protein. Thus, Rodwell et al. (1956, 1957) observed a shift in the optimum pH of 3-phosphoglycerate mutase in the presence of enolase. The activity of muscle phosphofructokinase is increased multifold on the addition of crystalline myogen A (Gulij, 1960).

Since a shift in the pH optimum indicates a change in the basic and fundamental characteristics of the enzyme, it is likely that the addition of proteins to the homogenate actually affect the enzyme properties.

The present investigation suggests that the characteristics of the enzyme are dependent upon the environmental proteins. Either elevation or suppression in the level of activity of the enzymes are actually due to variations in the characteristics of the enzymes, but not due to changes in the enzyme concentration. Hence, "the activation and inactivation of enzymes are often be induced by the environmental factors, probably related to the
conformational changes and they are known to play most important role in the regulation of enzyme activity and its manifestation in the biological phenomena" (Grisolia, 1964). The extent of variation in the level of activity of the enzymes in a given environmental protein is different. That is, any one of the protein will not bring about simultaneous elevation of activity or decrease of activity of all the enzymes. The given protein may activate some enzymes, inactivate some other enzymes and with no effect on another set of enzymes. The differential regulation of activity of enzymes could thus be achieved by providing protein environment of suitable charge pattern.

Albumins decreased the level of activity of SDH and to some extent that of GDH, while the activity of LDH was elevated at the homogenate pH value (Figs. 1, 3, 12, 14, 23 and 25). The gamma globulins seem to have a reverse effect. The gamma globulins seem to elevate the activity of SDH with apparently slight activation of GDH and inactivation of LDH at the homogenate pH value (Figs. 1, 3, 12, 14, 23 and 25). Since it is known that LDH increases (Hill, 1955; Rees and Huggins, 1960), and the oxidative enzyme activity decreases in malignant tissues (Wenner, 1967) suggesting a condition of a Crab-tree effect, the observations in the present investigation suggest that an increased level of albumins induce a situation very similar to it, while the increased level of gamma globulins show a reverse effect.

Hence it is reasonable to assume that if the albumin level in the kidney function increases it may lead to some abnormality
in the kidney function. Under these circumstances, the kidney should promote the synthesis of protein which is generally known to be a gamma globulin, thus neutralizing the albumin effect. Such a type of stimulation of gamma globulin synthesis by the albumin will correct abnormal metabolism, thus restoring the normal condition of the kidney. To verify this aspect, it is very important that the protein synthetic potentiality should be studied in relation to the albumins and gamma globulins. If a normal kidney tissue has to maintain normal protein ratios, addition of the albumins should elevate the rate of protein synthesis, while the addition of the gamma globulins may or may not have any effect. If at all they have any effect it should be toward the inhibition of the rate of protein synthesis.

Regulation of protein synthesis

In the present investigation, the rate of protein synthesis was studied in terms of the uptake of leucine-1-$^{14}$C into the proteins of the kidney. It was actually observed that the addition of albumins and acidic proteins elevated the protein synthetic activity, while the gamma globulins and basic proteins decreased the protein synthetic activity (Fig. 83). Albumins stimulated protein synthesis, in other terms, stimulated the gamma globulin synthesis. The gamma globulins inhibited the gamma globulin synthesis by the kidney tissue. This inhibition is probably a product inhibition since the turnover rate of protein has to be maintained. These observations are in conformity with the previous presumptions, namely, the elevated albumin level increases
the gamma globulin synthesis, while the elevated gamma globulins decrease the protein synthetic potentiality

**Protease activity**

When a tissue is involved in elaborate protein synthesis, cathepsins are known to increase in their activity. The proteolytic activity increases so that the proteins remain in a dynamic state and the level is regulated. In the present investigation, the level of proteolytic activity was also studied. Albumins and acidic proteins, in general, seem to elevate the level of activity of proteases, while the gamma globulins and basic proteins decrease the level of activity (Fig. 79). Thus, the exogenously added proteins showed an effect similar to that of protein synthetic potential. Increased level of albumins elevated protein synthesis (Fig. 83) as well as protein degradation (Fig. 79) suggesting a high turnover of proteins in the kidney. The gamma globulins decrease the protein turnover rate. The elevated turnover of proteins by the albumins is expected to be associated with an elevated synthesis of messenger RNA and the increased synthesis of messenger RNA should be associated with an elevated synthetic activity of DNA (Taylor, 1963). In future, it is proposed to study the RNA and DNA metabolism in the presence of these proteins so that the direct relationships with the proteins may be established.

The present observations suggest that the activity patterns of SDH and proteases in the presence of different protein charges
are quite different and as such these results are in agreement with the previous findings of Muralikrishnadass (1967).

Energy requirements

Since protein synthesis requires energy, an increase the protein synthetic potential should require an increase in the energy producing efficiency. In the present study cytochrome oxidase was considered an index of the energy yielding oxido-reduction reactions associated with mitochondria. An increased activity of cytochrome oxidase should normally mean an elevated oxido-reduction reactions leading to a pronounced generation of triphosphates. If albumins increase the protein turnover they should also increase the level of activity of cytochrome oxidase. In the present study it was actually found to be the case. Albumins as well as acid proteins elevated the level of activity of cytochrome oxidase, while the gamma globulins decreased the level of activity when compared to the albumins (Fig. 80). Hence, it is likely that the energy producing system is also stepped up by the albumins.

However, when the level of activities of SDH and GDH were considered in the presence of albumins the situation was contradictory. The level of activity of these two enzymes decreased with the increase in the albumin level in the kidney homogenate suggesting a drop in the oxidative enzyme activity (Figs. 1, 3, 23, and 25) However, the cytochrome oxidase being very closely associated with phosphorylation reactions than the SDH and GDH, it is likely that the energy is probably derived in the
oxido-reduction reactions associated with enzymes other than the SDH and GDH. In the present investigation, it was found that the LDH activity increases in the presence of albumins (Figs 12 and 14). Hence, it is likely that the substrates contributing to oxido-reduction reactions leading to the intervention of the cytochrome oxidase are probably derived from the glycolytic activity. It is likely that the LDH could contribute to the oxido-reduction reactions leading to the phosphorylation mechanisms.

An increased level of albumin in the kidney homogenate shifts the emphasis on the anaerobic segment of the carbohydrate metabolism deriving energy from it. This energy is utilized for the protein turnover. When enough gamma globulin is synthesized the emphasis is shifted to aerobic segment of the carbohydrate metabolism. Thus, the inherent regulatory pattern in the kidney in terms of its protein synthetic potentiality corrects the abnormal tendencies and restore normal pattern of metabolism.

It is known that degenerate kidney is unable to check up itself on the effects of the albumin. If the albumin content continues to increase, the kidney loses its capacity to regulate its own metabolism and efficiency. Hence, it may be unable to check the albumin excretion, possibly leading to diseases such as albuminurea. If at all the protein synthetic potentiality of the kidney is restored, the defect can be rectified and the degeneracy of the kidney could be arrested. This could be achieved by decreasing the albumin effect either by stepping
up the gamma globulin synthesis in other tissues or by increasing the plasma protein breakdown in the digestive gut or by lowering the activity of the liver leading to the decrease of the albumin synthetic potential.

The estimations of the level of acid phosphatase activity suggest that the albumins and acidic proteins enhance the activity of the enzyme, while the gamma globulins and basic proteins decrease the enzyme activity (Fig. 82). Since the phosphatases are known to involve in the supply of energy, it is likely that the phosphorylation and phosphorylisis are stepped up by the albumins thereby satisfying the energy requirements for the elevated protein synthesis.

The estimation of alkaline phosphatase activity suggests considerable inhibition of activity by both albumins and gamma globulins, while the acidic and basic proteins show a limited inhibition (Fig. 81). These observations are in agreement with the observations of Bodansky (1948) on alkaline phosphatase and Ulrich (1964) on mitochondrial adenosine triphosphatase. Since alkaline phosphatase is known to involve in the cellular ossifications, they may not probably have a general effect on the general metabolism of the kidney. They show a trend which does not seem to bear any relation in the present context. Though there is an effect of protein on the level of activity, the physiological significance in the light of the present observation is not known. However, the acidic and basic proteins show a pattern identical to that of the pattern obtained with acid
phosphatase. Hence, it may be generally inferred that the phosphatase activity is involved in the regulation of metabolism by the albumins and gamma globulins.

**Titrmetric analysis of the kidney homogenate with different proteins and amino acids**

The kidney homogenate was found to have approximately equal number of titratable groups on the acid and basic regions of the homogenate pH. However, there is a slightly more of the basic groups than the acidic groups (Fig. 96). Addition of the albumins and gamma globulins increased both the titratable groups (Figs. 97, 98, 101 and 102). Albumins seem to induce more of the groups ionizable in the acid region than the gamma globulins, while this effect is less with the latter. A detailed sub-analysis of the titratable groups indicates that the gamma globulins, in general, seem to ionize more of the groups of 'd' type (Figs. 97 and 101), while the albumins seem to increase 'c' type groups in addition to their ionization in the acid region (Figs. 98 and 102). Hence, it is likely that the gamma globulins are, in general, responsible for the ionization of basic groups such as guanidine, phenolic omega amino and to some extent alpha amino groups. Previously it was also found that the dehydrogenase activity in the amphibian kidney homogenate was stimulated to higher levels of activity by the basic groups. It was found that the gamma globulins, in general, elevated the level of activity of the dehydrogenases due to their basic nature.
The present titrimetric analysis also indicates in the same direction. The gamma globulins induce ionization of the basic groups and consequently induce greater basic condition, thus becoming responsible for the elevated activity of these enzymes. Albumins effect groups which are relatively acidic and consequently they decrease the basic nature of the homogenate, thus leading to decrease in the level of activity of the dehydrogenases. The protein synthetic mechanism in the amphibian kidney homogenate is under the influence of the regulatory mechanism having opposing characters. This mechanism requires a decrease in the basic nature of the homogenate.

The titrimetric analysis of the amphibian kidney homogenate with glutamic acid and lysine indicates a similar relationship (Figs. 103 and 104). When the total titratable range on the acid and basic region is considered, lysine was found to ionize more of the groups in the basic region (Fig. 104). A detailed sub-analysis suggests that lysine induces ionization of 'c' type groups (Fig. 104). The glutamic acid induces ionization or more of 'a' and 'b' type (Fig 103). Thus, lysine shows an emphasis on the ionization of basic groups, while the glutamic acid shows emphasis on the ionization of acidic groups. The effect of lysine and glutamic acid are found to be similar to the gamma globulins and albumins respectively.

The titrimetric analysis of the kidney homogenate with acidic and basic proteins also indicates a relationship in the similar direction (Figs. 99 and 100). When the titratable range
on the acidic and basic region is considered, the basic proteins
induced greater ionization in the alkaline region (Fig 100)
The sub-group analysis also suggests that the basic proteins
induce ionization of the more of 'c' and 'd' groups than the
acidic proteins thus suggesting induction of greater basicity
(Fig 100). The regulation of dehydrogenases activity, in
general, and the protein synthetic activity indicate similarity
to a large extent with that of the gamma globulins and lysine.
The acidic proteins also induced greater ionization in the
alkaline region when the whole titratable range is considered
(Fig. 99). Perhaps this might be the cause for the elevated
activity of the acid phosphatase by both the acidic and basic
proteins (Fig. 82).

Thus, the levels of activity of enzymes seem to be regula-
ted by these proteins through the intervention of their ioniza-
tion properties of the amphibian kidney homogenate

Conclusion

It is surmised that the proteins and amino acids (albumins
and glutamic acid) which decrease oxidative enzyme activity
increase the protein synthesis. The proteins and amino acids
(gamma globulins and lysine) which increase the oxidative enzyme
activity decrease the protein synthesis. Thus, it appears that
the factors which regulate the oxidative enzyme activity and
protein synthetic activity have opposing influences in the
kidney homogenate. The abnormality in one regulating factor
is corrected by the other regulating factor
Since the proteins and amino acids are found to exert influences similar to these regulating mechanisms and since the proteins and amino acids are known to contribute charge pattern, it is reasonable to presume that the sub cellular regulating mechanisms in the amphibian kidney, probably in other tissues also, are under the influence of the charge pattern manifested by the heterogeneous protein environment.

The author feels that in vitro effect of protein and amino acid charges on purified enzymes would be of more use to substantiate his observations. He could not study these aspects as he is faced with limited availability of chemicals.