The chief objective of the present study was to assess the influence of some physiological factors like temperature, starvation, partial hepatectomy, season, circadian rhythm, and age on ascorbic acid (AA) contents of kidney (organ of AA synthesis) and skeletal muscle (one of the sites of AA storage) of common Indian toad, Bufo melanostictus Schneider.

Morphometric correlation studies were made and morphometric data were employed to classify the animals into different age groups. This was done to study the effects of age and to sort out the adult toads for assessing the effects of other factors, on AA contents. Both sexes were chosen to find out if sex has some bearing on AA metabolism. Tissue AA concentrations in the organs selected were also compared.

It was thought that selection of these aspects concerning age as well as AA metabolism was worthwhile in view of the immense dearth of work in these areas, in particular, pertaining to toad. It was believed that the findings from this study would be useful in developing some understanding concerning age and AA metabolism of this species in conditions of stress, during different periods of the
year and the day, and in animals of different age groups. Conclusions arrived at from these may be applicable to other anurans with similar niche requirements, habits and ways of life. It was further visualized that, it would help in comparing these trends with observations already made on other vertebrates to see if patterns obtained were comparable. Since the basic physiology (including age and ageing processes) of this species has not been explored to a reasonable extent, the present study was confined to quantitative evaluation of AA in relation to different factors, and to age grouping based on morphometric considerations.

The findings and conclusions are summarized below:

9.1: MORPHOMETRIC CORRELATIONS

Significant positive correlations were observed between body weight and snout-to-vent length as well as organ weights. Since these parameters increase with time these could be successfully employed as indirect markers of chronological age, as in reptiles and mammals, in assorting toads into different age groups. The age groups so made are, Gr I (Juvenile), Gr II (Immature), Gr III (Adult) and Gr IV (Fully mature and older). The exact age, however, cannot be determined by this and so laboratory culture data as well skeletochronological techniques have to be relied
upon to assess the validity of this indirect method.

9.2 : TEMPERATURE STRESS

Short term cold stress (hypothermia) induced a significant depletion of AA contents in kidney of both sexes. Such depletion has also been observed in some other tissues of toad and in some tissues of other poikilotherms. In poikilotherms including toad response to cold stress appears to be similar, lowered temperature presumably depressing the rate of AA synthesis. A trend of increase in AA contents has been reported for some homeotherms. Possibly toad as well as other poikilotherms and the homeotherms respond differently to cold stress. Muscular AA levels, on the contrary increased during hypothermia. Both kidney and muscular AA levels, in general, showed a rise during hyperthermia. This rise may be due to enhanced synthesis and turnover due to increased temperature. The differential response of kidney and skeletal muscle to cold stress underlies some sort of tissue-specificity. The alterations in AA concentrations during temperature stress appear to be a consequence of a sort of "alarm reaction" to stress. By and large, the trends of response by the two sexes were similar.
9.3: STARVATION STRESS

Starvation induced a, more or less, progressive fall in kidney and muscular AA levels in males. In females the trend was almost similar, but for a rise in kidney and a fall in muscle at 1 wk. The depletion appears to be the consequence of lowering of supply of substrates for AA synthesis. This trend of depletion in toad is broadly similar to those reported for some other poikilotherms, but is unlike the tissue-specific response observed in some homeotherms.

9.4: PARTIAL HEPATECTOMY

A significant increase of AA at 4 hr and a decline at 24 hr of partial hepatectomy occurred in kidney of both sexes and in skeletal muscle of females. In skeletal muscle of males a steady decline was observed from the beginning suggesting a small degree of sex-difference in the response. The initial enhanced and the subsequent lowered levels of AA appear to reflect higher synthesis followed by greater utilization of AA in wound healing. This is in conformity with role of AA in wound healing and regeneration. It also indicates a link between these processes and AA synthesis.

9.5: SEASON

AA levels in both tissues of both sexes were lowest during July-October (Rainy season) - a period largely coinciding
with the breeding season. This indicates a possible greater utilisation of AA during the active period. The March - June (Summer) levels were high in kidney of both sexes, in conformity with higher rate of synthesis in summer reported for this species earlier. High winter (November - February) levels observed also in kidney of females; and in skeletal muscle of both sexes, appear to be due to lesser utilisation during the cold season. In females higher AA levels were observed during the rainy season and winter. The summer values did not differ significantly, but females had a higher annual average in both tissues.

9.6: CIRCADIAN RHYTHM

Nocturnal (2200 hrs) AA levels were highest in both organs of both sexes. The daytime values were low. High nocturnal values indicate a possible higher rate of synthesis in keeping with the higher tempo of activity during the period. Low daytime levels may be due to lowered synthesis during the inactive period - a possible mechanism of conservation of energy as reported for reptiles. No sex-difference was observed in the nocturnal values and only a small difference was observed in the daytime levels between the sexes. The daily averages also did not differ significantly.

9.7: AGE

AA contents in Gr I (Juvenile) toads of both sexes were high in both organs. In Gr II (Immature) toads the levels
were the lowest. Thereafter there was a, more or less, progressive rise in AA levels occurred but for a fall in muscular levels of Gr IV female toads. Apart from a fall at Gr II stage, no progressive decline with age, of AA contents was observed. Some degree of sex-related difference in AA contents was also observed, with females of Gr II and III having higher muscular levels and of Gr IV higher kidney levels. Males of Gr I and II had a slightly higher level of AA in kidney. Males also showed a higher overall average in kidney, but in females this value was higher in skeletal muscle.

9.8: SEX AND TISSUE DIFFERENCES

It appears that sex has some bearing on AA contents in this species. Forty eight different situations involving the two organs of control and experimental animals during stress tests, animals collected during the different periods of the year and the day, and animals of different age groups, were compared. Males showed a significantly higher level in kidney in seven and females in six cases. In the skeletal muscle females had significantly higher levels in nine cases and males in none. Moreover, values observed during different seasons indicate higher AA levels in females except during summer. A higher annual average was also observed in females. Therefore, it is likely that females have a higher rate of synthesis and turnover of AA
compared to males.

Muscular AA concentrations were almost invariably lower in comparison to kidney levels. Of the forty eight situations compared, in forty seven cases kidney levels were higher, the difference being significant in forty cases. Therefore, it appears that in toad lower muscular AA level is the rule. Reports of similar lower muscular AA contents are available for other vertebrates (both poikilotherms and homeotherms). So lower AA contents in muscle appear to be the common pattern. These differences in toad may be due to functional differences between the organs in relation to AA. The lower AA contents in skeletal muscle further indicate that this tissue is rather inconsequential as a site of AA storage.

9.9: APPLICABILITY OF THE FINDINGS

The present study relates to one of the amphibian species, *Bufo melanostictus*. Though it is a fairly widely distributed tropical anuran, it could hardly be taken as a representative amphibian. The generalisations made and the findings may not be true for amphibians as a whole. These may hold good for other *Bufo* species (*Bufo stomaticus* and *Bufo andersoni*) and other tropical anurans with similar niche requirements, habits and ways of life. These findings may not be applicable for *Rana* species or
Rhacophorus species of the tropics which have different ways of life etc.

Therefore, it is necessary to extend such studies to other anurans and amphibians in order to make generalizations concerning the principles involved and factors operative in AA metabolism in relation to stress and other conditions including age. It is also necessary to extend studies relating to age determination by morphometric data to other anuran species and to assess the validity of this method by skeletochronological techniques and by laboratory culture methods in this species and other anurans.