LIST OF PUBLICATIONS


EFFECT OF SEX AND SHORT TERM COLD STRESS ON THE ASCORBIC ACID CONTENT IN THE KIDNEY AND THE Pancreas OF COMMON INDIAN TOAD, BUFO MELANOSTICTUS

A. K. Panda, R. P. Ruth and S. N. Padhi*  
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The importance of ascorbic acid in the metabolic activities has been reviewed(1,2). It takes part in wound-healing and regeneration(3-7), as also in collagen-biosynthesis(8). Possibly it also acts as an anti-oxidant(9) and fatigue-retardant(10). Though considerable work has been done on ascorbic acid in birds and mammals, amphibian physiology in general and ascorbic acid metabolism in particular are, by and large, are as yet to be reasonably explored. With this in view we took up the estimation of the ascorbic acid content in the kidney, the synthesising organ in the amphibians(11) and in the pancreas of the common Indian toad. The purpose of choosing these two organs is obvious. The significant part the kidney plays in homeostasis is known while the pancreas on the other hand has a key role in the secretion of important hormones like insulin and glucagon. The present study aims at finding out the effect of different environmental factors and sex on the ascorbic acid metabolism in the tissues of *Bufo melanostictus*.

Toads collected from Bhadrak locality during March, with a body weight range 31-73 gm, S-V length range 7.5-9.5 cm for females and 33-73 gm and 8.2-9.0 cm for males were subjected to experimentation. The experimental toads (one at a time) were kept in the freezer chest of a refrigerator (Kelvinator) for a period of one hour in a widemouth, 500ml capacity specimen bottle, covered with a thin cotton sheet at the open end. After one hour the animals were taken out when they were in a very inactive state. In about ten minutes time the animals get back to normalcy. Then they were killed with a blow on the head and the desired tissues were removed and transferred immediately to pre-cooled Petridish with distilled water. The tissues were cleaned and soaked in filter paper, weighed, homogenized with 5% TCA in a homogenizer (REMI) and centrifuged. Estimation was undertaken by the method of Roe(12), as modified by Tewary and Pandey(13). Control animals were killed at room temperature (31°C±2°C) on the day of collection and estimation carried on by the above method.

Hypothermia (1 hr. at 0-4°C) appears to have induced significant depletion in the ascorbic acid content of kidney in both the sexes while pancreas, apparently remained unaffected. In kidney there is a significant decrease in the absolute values (ug ascorbic acid/whole kidney) in female and the relative values (mg ascorbic acid/100 gm wet wt.) in the male. No significant alteration due to this low temperature exposure was observed in the ascorbic acid content in the pancreas (Table I). Ascorbic acid content in the kidney of female toad is higher. Sex difference in the ascorbic acid content is slightly significant in the kidney but not in pancreas.

In Toad testicular ascorbic acid synthesis undergoes a significant decrease at low temperature(14). It also decreases in the kidney of the garden lizard, *Calotes versicolor* at 9°C(15), but shows no change at 0°C—4°C (Padhi and Patnaik, unpublished data). On the other hand this shows an increase in rat(16) and in the skeletal muscles of *Calotes versicolor*(17) at low temperature. Our findings indicate that the cold stress induces signi-
significant decrease in the ascorbic acid synthesis in the kidney since the enzymes responsible for this are apparently susceptible to low temperatures. It is also in conformity with the earlier findings\(^{(18)}\) that temperature when lowered leads to a slower rate of chemical changes in amphibia. However, changes in the pancreas are rather insignificant. Rao and Patnaik\(^{(19)}\) suggest insignificant change in ascorbic acid content in the liver with regard to sex but a higher respiratory rate in the male than that of the female garden lizards\(^{(20)}\). Our results on sex difference show a higher ascorbic acid content in the kidney of female toad. This may be apparently due to the anatomical and functional differences that the kidney of female has from that of the male\(^{(21)}\), the anterior region of the kidney in the male being more or less non-renal in nature. The above findings suggest that in all likelihood ascorbic acid synthesis is species-specific, tissue-specific and is influenced by sex and temperature.

**Acknowledgement**
We are thankful to Dr. G. Sahu, Principal and Sri B. C. Rath and Sri J. K. Biswal for facilities and encouragement.

**Table 1**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tissue</th>
<th>(\mu g) ascorbic acid/whole tissue average±SEM</th>
<th>'P' mg. ascorbic acid/100g. wet wt. average±SEM</th>
<th>Control</th>
<th>Experimental</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Kidney</td>
<td>30.12±5.79 (5)</td>
<td>19.57±1.92 (5)</td>
<td>NS</td>
<td>9.02±0.92 (5)</td>
<td>5.29±0.50 (5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>Kidney</td>
<td>46.71±5.60 (5)</td>
<td>27.71±2.72 (5)</td>
<td>&lt;0.02</td>
<td>11.16±1.87 (5)</td>
<td>7.66±0.87 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>Pancreas</td>
<td>3.64±0.16 (4)</td>
<td>3.47±0.34 (4)</td>
<td>NS</td>
<td>4.49±1.52 (4)</td>
<td>3.22±0.79 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>Pancreas</td>
<td>3.58±1.06 (5)</td>
<td>4.83±0.63 (4)</td>
<td>NS</td>
<td>2.77±0.94 (5)</td>
<td>2.95±0.44 (4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

'P' represents comparison between control and experimental values.
P* represents comparison between male and female control values.
References


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NOTE ON THE INCIDENCE OF CUCURBIT MITE \textit{TETRANYCHUS NEOCAEDO NICUS} ANDRE (\textit{ACARINA} : \textit{TETRANYCHIDAE}) UNDER DIFFERENT ENVIRONMENTAL SET-UPS.\footnote{Abstract published in Proceedings of 68th Session Indian Sc. Congr.}

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University of Udaipur, Udaipur

Most of the published data on the population dynamics of the phytophagous mites are based on the experiments carried out on the agricultural experiment stations where conditions are pretty atypical of the situation in farmers' fields owing to mixed sowing dates, range of maturities and variable seasonal cultivars. No published data are available on the variations which might occur in different environmental set-ups.

In the present investigation the seasonal incidence of cucurbit mite, \textit{Tetranychus neocaledonicus} Andre (Acari : Tetranychidae), a polyphagous pest (Prasad, 1974) was studied on cucurbits at Udaipur at three different locations, viz. (i) the Horticulture Farm where insecticides were used more frequently, the vegetation was dense and a wide range of plants, shrubs and trees of horticultural interest were grown; (ii) the Farmers' field where insecticides were comparatively less used and the vegetation was less dense; and (iii) a small area in the College compound which was very much secluded from the farmers' fields or open atmosphere and where insecticides were not used at all and the vegetation comprised lawn grass only.

Three sets of potted plants, which were susceptible to the mite, viz. pumpkin (Local) and bottle gourd (Kalyanpur long green, summer) were placed at three different locations. Each treatment was replicated three times. The plants were about 3 months old when the first observation was taken on 6th June 1977. The next observation was recorded on 10th September 1977. The sample size used was $54 \times 2 \text{cm}^2$.

Incidence of \textit{T. neocaledonicus} Andre

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Population* per $54 \times 2\text{cm}^2$ leaf surface area on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horticulture Farm</td>
<td>6. 6. 1977</td>
<td>265.3</td>
</tr>
<tr>
<td></td>
<td>10. 10. 1977</td>
<td>361.3</td>
</tr>
<tr>
<td>College Compound</td>
<td>6. 6. 1977</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>10. 10. 1977</td>
<td>6.0</td>
</tr>
<tr>
<td>Farmers' Field</td>
<td>6. 6. 1977</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>10. 10. 1977</td>
<td>122.3</td>
</tr>
</tbody>
</table>

* Average of 3 replicates.
EFFECT OF AGE AND SEX ON THE ASCORBIC ACID CONTENT OF KIDNEY, SKELETAL MUSCLE AND PANCREAS OF COMMON INDIAN TOAD, BUFO MELANOSTICTUS

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(Received 15 July 1983)

Abstract—Ascorbic acid decreases from age-group I to II in Kidney, skeletal muscle, and increases in the Pancreas of female. There is no significant change in the Pancreas of male toad. There is an increase in the ascorbic acid content from age group II to III except in the Pancreas of females, where there is a decline of the content. Ascorbic acid remains constant in age-group IV except in the kidney of female, skeletal muscle of both sexes showing an increase and decrease of the value respectively.

Sex difference shows a higher content in kidney of group IV, in skeletal muscle of group II and III and in pancreas of group II and is less in kidney of age-group II of females over their male counterparts.

INTRODUCTION

Age related studies on ascorbic acid (AA) metabolism (Patnaik, 1971) and its involvement in various functions has been discussed (Padhi, 1980). Ascorbic acid (AA) acts as an anti-aging agent (Panigrahy, 1976), takes part in wound-healing (Bartlett, Jones and Ryan, 1942; Udupa and Singh, 1964), cellular respiration (King, 1950) and declines with age in garden lizard liver (Rao and Patnaik, 1973), skeletal muscle (Haseeb and Patnaik, 1973) and in rat (Kanungo and Patnaik, 1964) has been reported. But effect of age and sex on such an important biochemical component of kidney, the synthesising organ in amphibians (Chatterjee, 1973), skeletal muscle, the tensile strength of which decreases with age and pancreas, the organ which also stores AA (Chinoy, 1977) is largely unknown in the common Indian toad. The present work aims at the colorimetric estimation of AA in kidney, skeletal muscle and pancreas of common Indian toad, Bufo melanostictus, in relation to age and sex.

MATERIALS AND METHODS

Toads of different sizes were collected from Bhadrak locality during June–November and were grouped into four age-groups taking body-weight and snout to vent (S-V) length into consideration for convenience, for statistical evaluation of data (age-group I, 0–15g; age-group II, 16–31g; age-group III, 32–47g; age-group IV, 48–onwards). That body weight and S-V length increases with age has been reported for garden lizards (Padhi

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and Patnaik, 1978) in captivity. This is the main reason why body weight and S-V length were taken as parameter in aging studies in toads as reported for lizards (Smith, 1964; Eapen and Puspendra, 1972; Tinkle and Selander, 1973; Haseeb and Patnaik, 1973), rat (Devi et al., 1963; Gerlach and Fegeler, 1973). Table 4 shows the range of body-weight, S-V length and the range of tissue weight, taken for AA estimation.

Toads were killed with a blow on their head within 24 hour of their collection. Kidney, pancreas and skeletal muscle (mostly from femoral region) were taken, adherent tissues were cleaned, weighed, homogenised in a low speed all-glass homogenizer (Remi make), and centrifuged in a centrifuge (Remi make) at 2000 rpm for ten minutes. The supernatant was taken for colorimetric estimation of AA following the method of Roe (1967) as modified by Tewary and Pandey (1964).

RESULTS

Table 1 shows the following age-related changes in kidney, skeletal muscle and pancreas of male toad, Bufo melanostictus.

In kidney AA show a significant increase with age in absolute value (μg./whole tissue) whereas it indicates a decline from age-group I to II in relative value (mg/100g. wet weight) and remained almost constant thereafter.

In skeletal muscles AA is more in age-groups III and IV over age-groups II and I respectively. There is no significant difference between age-groups I and II and III and IV toads in absolute value. Relative values exhibit a significant decrease from age-group I to II and a rise in the value from age-group II to III which remained almost constant thereafter, without showing any significant change between age-groups IV and I.

Absolute value of pancreas indicate significant increase from age-group I to III and then stabilized in Group IV showing a significant decrease from age-group IV to I. Relative values show more of AA in age-group III over group II but there is no significant difference between age-groups I and II, III and IV and IV and I. Age-related changes of AA in kidney, skeletal muscle and pancreas of female toad (Table 2) are as follows.

Kidney shows a significant rise of AA content with age in absolute value. Relative values indicate a decline of the content from age-group I to II and a steady increase thereafter. On the otherhand, there is a decrease of the content from age-group IV to I.

AA content of skeletal muscle does not show significant difference between age-group I and II but there is a sharp increase from age-group II to III and then a decline from age-group III to IV and IV to I in absolute value. Relative value shows a decline of the content from age-group I to II, III to IV and IV to I but an increase from age-group II to III.

Absolute value of pancreas does not show significant change between age group I and II but an increase from age group II to III, which remained constant thereafter showing a decline from age-group IV to I. Relative value of AA exhibits a sharp increase from age-group I to II and significant decrease from age-group II to III which remained constant thereafter.

Aged-related differences of AA with regard to sex (Table 3) are given below.

Absolute value of kidney shows a higher content in age-groups I and IV females over their male counter-parts. Relative values indicate less in group II and more in group IV females over their male counter-parts. Age group III females show higher content in absolute value while in relative value group II and III females indicate higher content of AA in the skeletal muscle over their male counter-parts.

AA content of pancreas does not indicate any significant difference in absolute value. The relative value show higher content in age-group II females over its male counter-part.
### Table 1. Effect of Age on the Ascorbic Acid Content of Kidney, Skeletal Muscle and Pancreas of Male Toad *Bufo melanostictus*

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Kidney</th>
<th>Skeletal Muscle</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>a</td>
<td>p'</td>
<td>b</td>
</tr>
<tr>
<td>I</td>
<td>2.42 ± 0.22</td>
<td>&lt;0.01</td>
<td>6.93 ± 0.41</td>
</tr>
<tr>
<td>II</td>
<td>10.92 ± 0.58</td>
<td>&lt;0.01</td>
<td>5.27 ± 0.47</td>
</tr>
<tr>
<td>III</td>
<td>20.10 ± 1.44</td>
<td>&lt;0.01</td>
<td>5.96 ± 0.48</td>
</tr>
<tr>
<td>IV</td>
<td>26.11 ± 1.22</td>
<td>&lt;0.001</td>
<td>6.15 ± 0.29</td>
</tr>
</tbody>
</table>

(a = μg ascorbic acid/whole tissue; b = mg ascorbic acid/100 G. net weight. Number in parentheses indicate animals used. Values are average ± SEM. Comparison 'p' between age group I & II, II & III, III & IV and IV & I).

### Table 2. Effect of Age on the Ascorbic Acid Content of Kidney, Skeletal Muscle and Pancreas of Female Toad

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Kidney</th>
<th>Skeletal Muscle</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6.51 ± 0.79</td>
<td>&lt;0.025</td>
<td>5.63 ± 0.51</td>
</tr>
<tr>
<td>II</td>
<td>9.58 ± 1.00</td>
<td>&lt;0.001</td>
<td>3.66 ± 0.40</td>
</tr>
<tr>
<td>III</td>
<td>22.93 ± 1.54</td>
<td>&lt;0.005</td>
<td>5.31 ± 0.33</td>
</tr>
<tr>
<td>IV</td>
<td>49.37 ± 4.69</td>
<td>&lt;0.005</td>
<td>7.26 ± 0.41</td>
</tr>
</tbody>
</table>

(a = μg ascorbic acid/whole tissue; b = mg ascorbic acid/100 G. wet weight. Number in parentheses indicate animals used. Values are average ± SEM. Comparison 'p' between age groups I & II, II & III, III & IV and IV & I).
**Table 3. Effect of sex on the ascorbic acid content of kidney, skeletal muscle and pancreas of common Indian toad**

<table>
<thead>
<tr>
<th>Age group/ Sex</th>
<th>Kidney</th>
<th>Skeletal Muscle</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a 'p' b 'p'</td>
<td>a 'p' b 'p'</td>
<td>a 'p' b 'p'</td>
</tr>
<tr>
<td>Male I</td>
<td>2.42 ± 0.22 (7)</td>
<td>6.93 ± 0.41 (7)</td>
<td>3.19 ± 0.63 (10)</td>
</tr>
<tr>
<td>Female I</td>
<td>6.51 ± 0.79 (11)</td>
<td>5.63 ± 0.51 (11)</td>
<td>2.94 ± 0.25 (8)</td>
</tr>
<tr>
<td>Male II</td>
<td>10.92 ± 0.98 (17)</td>
<td>5.27 ± 0.47 (17)</td>
<td>2.57 ± 0.39 (14)</td>
</tr>
<tr>
<td>Female II</td>
<td>9.58 ± 1.00 (16)</td>
<td>3.66 ± 0.40 (16)</td>
<td>2.88 ± 0.26 (10)</td>
</tr>
<tr>
<td>Male III</td>
<td>20.10 ± 1.44 (11)</td>
<td>5.96 ± 0.48 (11)</td>
<td>5.12 ± 0.74 (8)</td>
</tr>
<tr>
<td>Female III</td>
<td>22.93 ± 1.54 (10)</td>
<td>5.31 ± 0.33 (10)</td>
<td>8.22 ± 0.89 (11)</td>
</tr>
<tr>
<td>Male IV</td>
<td>26.11 ± 1.22 (10)</td>
<td>6.15 ± 0.29 (13)</td>
<td>5.55 ± 0.74 (8)</td>
</tr>
<tr>
<td>Female IV</td>
<td>49.37 ± 4.69 (9)</td>
<td>7.26 ± 0.41 (8)</td>
<td>4.36 ± 0.40 (8)</td>
</tr>
</tbody>
</table>

(a = μg ascorbic acid/whole tissue, b = mg ascorbic acid/100 G. wet weight, number in parentheses indicate animals used, values are average ± SEM. Comparison 'p' between age groups I & II, II & III, III & IV and IV & I)
DISCUSSION

Our findings that there is a fall of AA level from age-group I to II initially which rises in age-group III and stabilised thereafter in kidney and skeletal muscle of male (Table 1), whereas an increase in age-group IV in the kidney of female toad (Table 2) are in conformity with the age-related changes of AA in the garden lizard brain (Padhi and Patnaik, 1975).

The results on pancreas of the male toad (Table 1) does not signify any change between age-group I and II but a high content in age group III which remained constant thereafter. Our observations on pancreas coincides with the age-related changes of glycogen content in the skeletal muscle of Calotes versicolor (Haseeb and Patnaik, 1973).

Skeletal muscle of the female toad (Table 2) show significant decrease of AA from age group I to II and III to IV as observed for age-related changes of DNA content in the skin of male garden lizard (Mishra and Patnaik, 1974).

Age related changes of pancreas of the female toad (Table 2) show an increase in AA content from age-group I to II and then a decrease from age group II to III which remained constant thereafter in age group IV are identical with the age-related changes of Ascorbic Acid content in the kidney of male garden lizard (Padhi, 1980).

Our observations on sex difference show less content in age-group II kidney of males, whereas high content in age-groups IV, II and III and II in the kidney, skeletal muscle and pancreas of females over their male counter-parts suggest differential utilisation of AA in various tissues. This is in contrast to the observation as reported for serum calcium (Patnaik and Singh, 1964) where there is no effect of sex. But Padhi (1982) observed higher AA content in the kidney of male garden lizard, Calotes versicolor and in the kidney of female lizard, Hemidactyulus flaviviridis.

In brief, it can be said from the above that the age-related changes in poikilo-thermic vertebrates so far studied follow almost similar trend with some minor deviations.

Acknowledgements—We thank Dr. G. Sahu, Principal, Bhadrak College, Dr. J.N. Patnaik, Principal, Talcher College and UGC for facilities and financial assistance.
REFERENCES


Padhi, S.N. (1982), Unpublished data.


EFFECT OF SEX AND SHORT TERM COLD STRESS ON THE REGIONAL DISTRIBUTION OF ASCORBIC ACID IN THE BRAIN OF COMMON INDIAN TOAD, *BUFO MELANOSTICTUS*

R. P. Ruth, A. K. Panda and S. N. Padhi

Department of Zoology, Bhadrak College, Bhadrak-765 100

The study of ascorbic acid metabolism on fishes and amphibians is rather rare (1). Its importance in metabolic activities has been recently reviewed (1). Brain, being the coordinating centre of the body, may be susceptible to cold stress and may exert influence on the different biochemical contents of tissues. The purpose of the present study is to estimate the ascorbic acid content of the different regions of the brain in both male and female toads with regard to short term cold stress under laboratory conditions to test the physiological adaptations of the animal to environmental factors (2).

Toads collected from Bhadrak locality during March with a body weight range 33—77 gm., S-V length range 7.2—9.0 cm. for males and 33—73 gm. and 7.5—9.5 cm. for females were studied. The toads collected were grouped into controls and experimentals. On the day of collection the control-animals were killed, with a blow on the head, for estimation. The experimentals were kept in the freezer chest of a refrigerator (Kelvinator) for one hour in a wide mouth bottle of 500 ml. capacity and the open end of the bottle was covered with thin cotton sheet to facilitate the entry of air into it. After the stipulated time the animals were taken out in an inactive state and were killed with a blow on the head. After they regained normalcy, which they do sometimes in less than ten minutes. The brain was removed immediately into a precooled Petridish with distilled water. The adherent tissues were cleaned. It was soaked in Whatman filter paper, different regions separated with a fine scalpel, weighed and homogenized with 5% TCA in an all-glass low speed homogenizer (1200 rpm, Remi make) for ten minutes, centrifuged and estimation was done by the method of Roe (3) as modified by Tewary and Pandey (4).

There is a significant decrease in the ascorbic acid content in the forebrain and the midbrain but not in the hind brain of the male toads exposed to cold. There was no change in values between the control and the experimental female toads.

Results on regional distribution indicate a higher ascorbic acid content in the forebrain in comparison to other regions in both the control and experimental animals of both the sexes. There is a significant difference in ascorbic acid content in the different brain regions of males. In the females, some difference in the ascorbic acid content was observed, though it is insignificant. But in all cases there is a sharp difference in the ascorbic acid content between forebrain and hind brain. Sex difference in the ascorbic acid content in different regions of the brain is insignificant.

Our present results on cold stress suggest a depletion in ascorbic acid metabolism at low temperature in the brain. It is similar to the observations of Roychoudhury and Mukherji (5) on testicular ascorbic acid.

The results on sex difference in ascorbic acid content is insignificant.
**Regional Distribution of Ascorbic Acid Content and Effect of Sex and Temperature in the Brain of Common Indian Toad.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tissue</th>
<th>µg ascorbic acid/tissue 'P'</th>
<th>mg ascorbic acid/100g. wt. 'P'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td>M</td>
<td>Fore brain</td>
<td>20.26±1.33</td>
<td>12.31±1.62</td>
</tr>
<tr>
<td>A</td>
<td>'p'</td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td>L</td>
<td>Mid brain</td>
<td>9.63±0.69</td>
<td>4.88±0.26</td>
</tr>
<tr>
<td>E</td>
<td>'P'</td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>Hind brain</td>
<td>6.23±1.17</td>
<td>6.91±1.44</td>
</tr>
<tr>
<td>E</td>
<td>'P'</td>
<td>(3)</td>
<td>(5)</td>
</tr>
<tr>
<td>F</td>
<td>Fore brain</td>
<td>16.99±2.65</td>
<td>*NS20.69±4.41</td>
</tr>
<tr>
<td>E</td>
<td>'P'</td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>M</td>
<td>Mid brain</td>
<td>11.11±4.15</td>
<td>*NS 7.73±0.68</td>
</tr>
<tr>
<td>L</td>
<td>'P'</td>
<td>(5)</td>
<td>(3)</td>
</tr>
<tr>
<td>E</td>
<td>Hind brain</td>
<td>3.99±1.49</td>
<td>*NS 5.16±1.17</td>
</tr>
<tr>
<td>E</td>
<td>'P'</td>
<td>(4)</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>'P'</td>
<td>(c&amp;b)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P* horizontally represents comparison between different regions of the brain.

*P* vertically represents comparison between control and experimental values.

*P* values between male and female.

like most other biochemical parameters such as protein (6), ascorbic acid (7) and serum calcium level (8).

Regional distribution of ascorbic acid (9) and protein (6) in garden lizard has been observed. The findings that in fishes the ascorbic acid content is very high in cerebral hemispheres (10) correspond with our findings in the brain of toad. This leads us to believe that perhaps specific brain parts are involved separately to withstand the cold stress and that the cerebral hemispheres in toad is more active in its functions. Hence the higher concentration of ascorbic acid in this part.

In conclusion it may be said that most likely the ascorbic acid metabolism in brain regions is proportional to their activity. The decrease in the ascorbic acid content at 0-4°C in male only and not in female may be due to temperature variance of different sexes. Interestingly there is no sex difference in the ascorbic acid content in different parts of the brain of toads.

**Acknowledgement**

The authors are thankful to Dr. G. Parida, Principal, Bhadrak College for facilities; to Sri J. K. Biswal and Sri B. C. Rath for their help.

**References**

AGE-RELATED CHANGES OF ASCORBIC ACID CONTENT
OF LIVER AND BRAIN OF COMMON INDIAN TOAD,
BUFO MELANOSTICTUS

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Abstract—Ascorbic acid content reaches a peak level in liver in group III male toads and then declines significantly. But there is no significant age-related change in ascorbic acid in the brain of male toads or in liver and brain of female toads. Sex comparison indicates that in females the content declines in the liver of age-group III and increases in age-group II; and there is an increase in the brain of age-group III females over their male counterparts.

INTRODUCTION

That ascorbic acid declines with age in kidney (Padhi*, 1980), liver (Rao and Patnaik, 1973), skeletal muscle (Haseeb and Patnaik, 1973) of male garden lizards and in rats (Kanungo and Patnaik, 1964) is known. Its involvement in various metabolic functions has been discussed (Patnaik, 1971; Padhi, 1980). The present study aims at estimating the ascorbic acid content in liver and brain of the common Indian toad, Bufo melanostictus.

MATERIALS AND METHODS

Wild toads of different sizes of both sexes and of four different age-groups were collected.

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Range of body wt. (G) male/female</th>
<th>Range of snout-vent length (cm) male/female</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4-14/6-15</td>
<td>3.4-6.0/4.0-6.2</td>
</tr>
<tr>
<td>II</td>
<td>17-30/16-34</td>
<td>5.8-7.7/6.0-7.7</td>
</tr>
<tr>
<td>III</td>
<td>35-44/36-47</td>
<td>7.5-8.5/7.3-9.1</td>
</tr>
<tr>
<td>IV</td>
<td>47-81/49-110</td>
<td>8.0-10.0/8.0-11.5</td>
</tr>
</tbody>
</table>

The animals were killed with a blow on the head, livers and brains were removed and transferred to an ice-cold petri dish with distilled water, cleaned, weighed, homogenized with 5% Trichloroacetic acid in a low speed all-glass homogenizer (Remi) and centrifuged in a Remi centrifuge at 2000 rpm for 10 minutes. The supernatant was taken for estimation following the method of Roe (1967) and modified by Tewary and Pandey (1964).

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RESULTS

Our results on male toads (Table 1) show that there is a significant increase of ascorbic acid content in liver in age-group III. It then declines, whereas there is no such significant change in brain. Females do not exhibit any age-related change of ascorbic acid content in liver or in brain.

Sex comparison on liver (Table 1) indicates that the content is high in group II and low in group III females where as in brain group III females show higher content than males.

DISCUSSION

That the ascorbic acid metabolism declines with age in mammals has been reviewed (Patnaik, 1971). In garden lizards, ascorbic acid declines with age in liver (Rao and Patnaik, 1973), skeletal muscle (Haseeb and Patnaik, 1973) and kidney (Padhi, 1980), but in brain it remained almost constant. Our results (Table 1) in toads show an almost similar trend. Such age-related changes were thought to be due to changes in the rate of synthesis in the kidney and in the rate of utilization in the respective tissues. Therefore, it may be suggested that a basically common pattern of aging changes occurs in these animals.

So far we are aware, there is no report on age related changes in relation to sex. Our results indicating a significant difference in ascorbic acid level in liver and brain might be due to its rate of utilization in those tissues.

Table 1. Effect of age on the ascorbic acid (AA) content of liver and brain of common Indian toad, Bufo melanostictus (values are average ± SEM, number in parentheses indicates animals used. (‘p’ between age group I & II, II & III, III & IV & I)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age group</th>
<th>Liver Mg AA/100g. wet wt.</th>
<th>'p'</th>
<th>Brain Mg AA/100g. wet wt.</th>
<th>'p'</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>I</td>
<td>11.78 ± 1.54</td>
<td>NS</td>
<td>30.77 ± 3.80</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>II</td>
<td>9.63 ± 0.75</td>
<td>0.001</td>
<td>25.03 ± 2.23</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12)</td>
<td></td>
<td>(17)</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>III</td>
<td>16.94 ± 0.76</td>
<td>0.02</td>
<td>20.83 ± 2.30 &lt;0.05 NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9)</td>
<td></td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>IV</td>
<td>13.79 ± 0.90</td>
<td>NS</td>
<td>29.94 ± 3.36</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
<td>(11)</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of male and female toads with respective age-groups.

*Not Significant.
*Significance at 0.05 level.
*Significance at 0.01 level.
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