CHAPTER IV

PARTICIPATION OF ASCORBIC ACID FOR THE INDUCTION OF HEPATIC DRUG OXIDATION DURING STARVATION

Introduction:

Dixon et al. (1) observed that the activities of drug metabolizing enzymes have been shown to be depressed during starvation in male mice. However, Kato et al. (2) reported a marked increase in the activities of aminopyrine N-demethylase and aniline hydroxylase in liver microsomes of fasted female rats. Kato and Gillette (3,4) later showed that starvation impaired those microsomal enzymes which are markedly sex dependent but not those which are less sex dependent. Aniline hydroxylase activity is higher in starved male rats (5). Female rats have higher enzyme activities towards all substrates after starvation. Species dependence of the effect of starvation on hepatic mixed function oxidase system was shown by Furner and Feller (6). Induction of mixed function oxidase system by phenobarbital was shown to be greater in starved animals (7). The mechanism by which starvation exerts its effect on hepatic mixed function oxidase system is not known. Brodeur and Lambert (8) suggested that stress associated hormonal changes may be an important factor in increasing microsomal enzyme activities during fasting.

Starvation induces alteration in the pathways of various
Fig. A: Effects of starvation on serum and tissues levels of ascorbic acid.

Fig. B: Effects of starvation on the activities of drug metabolizing enzymes and microsomal protein.

Fig. C: Effects of starvation on hepatic microsomal electron transport components.
metabolism at several junctions. Independent effects and precise mechanisms associated with this type of imbalance are particularly difficult to evaluate.

Stubbs and Griffin (9) have shown that activities of ascorbic acid synthesizing enzymes in rat liver were altered during starvation. Therefore, it will be interesting to study the interaction of ascorbic acid and activities of drug metabolizing enzymes during starvation. Data pertaining to this aspect is lacking. Hence the present investigations were designed to examine the correlation between the ascorbic acid metabolism and activities of hepatic drug metabolizing enzymes during progressive fasting period.

Materials and Methods:

Young Hindustan Antibiotics male rats initially weighing 80-90 g were obtained from Hindustan Antibiotics Ltd., Pune. The animals were housed in individual cages in an air-conditioned room and supplied with standard rat pellets and water ad libitum for 15-20 days prior to the initiation of the experiments. Male rats, six in each group, were starved for 12 hours, 24 hours, 36 hours, 60 hours and 84 hours. During fasting, rats were allowed to drink water ad libitum. The control group of animals were fed on rat pellets supplied by Hindustan Lever, Bombay. Animals were sacrificed at the end of each starving period. The control group of animals were
sacrificed along with animals starved for 60 hours. Blood was collected from each animal as soon as possible. Liver, kidney and adrenals were isolated and kidney and adrenals were chilled at -4°C. The livers were perfused with 0.9% ice-cold saline, weighed, a part of them were homogenized (1:4 w/v) in ice-cold 50mM tris-HCl buffer pH 7.4, containing 1.15% KCl. The microsomes were isolated as described under 'Materials and Methods' in Chapter-I. The microsomal pellets were resuspended on 0.25M sucrose. Microsomal proteins were estimated according to the biuret method (10), using crystalline bovine serum albumin as the standard.

Ascorbic acid content in the second portion of liver, kidney, adrenals and serum was determined as described under 'Materials and Methods' in Chapter-I.

The drug metabolizing enzymes are assayed as described in Chapter-I using microsome as an enzyme source. The method of Nash (11) was used to estimate formaldehyde formed during the N-demethylation of aminopyrine. The p-OH acetonilide formed, was used as a measure for the acetonilide hydroxylase activity (12).

The levels of microsomal NADPH cytochrome c reductase, cytochrome b5 and cytochrome P-450 were determined as described under 'Materials and Methods' of Chapter-I.
Statistical analyses were carried out according to the modified students 't' test (13).

RESULTS

Effect of starvation on the liver and serum ascorbic acid content (Fig. 1):

At the end of 12 hours fasting period, liver, kidney and serum ascorbic acid content remained unaltered while adrenal ascorbic acid content showed a noticeable decrease (15%). Liver and serum ascorbic acid were slightly elevated in animals fasted for 24 hours. The per cent increase in liver and serum ascorbic acid at the end of 24 hours' fasting was 12 and 16 and at the end of 36 hours' fasting was 19 and 38 respectively. However, after 60 and 84 hours' fasting liver ascorbic acid content was lowered by 20%, and 30% respectively as compared to the corresponding value for the well-fed animals. Serum ascorbic acid content was declined to normal level after 60 hours of fasting. However, extension of the fast to a period of 84 hours, resulted 10% decrease in serum ascorbic acid content. Starvation lasting 36 hours and 84 hours produced 3% and 10% increase respectively in adrenal ascorbic acid content as compared with fed group of rats.

Starvation up to 84 hours has no profound effect at all on kidney ascorbic acid content.
Effect of starvation on hepatic microsomal protein content:

During the early period of starvation, microsomal protein content showed a significant increase in male rats as compared to fed-rats. The percentage of increase was 14% at the end of 12 hours' fasting period. It continued to increase up to 38% till the fasting period of 60 hours. However, further extension of the fast to a period of 84 hours resulted in liver microsomal protein content, 14% below the corresponding values for the fed animals.

Effect of starvation on the activities of hepatic drug metabolizing enzymes:

The activities of aminopyrine N-demethylase and acetonilide hydroxylase started showing an increase during the early hours of fasting period and it continued to show further increase till the 60 hours of fasting period. However, decrease in the activities was observed at the end of 84 hours' fasting period. The percentage of increase in aminopyrine N-demethylase and acetonilide hydroxylase activity during various periods of fasting was as follows:

<table>
<thead>
<tr>
<th>Fasting period (in hours)</th>
<th>Aminopyrine N-demethylase</th>
<th>Acetonilide hydroxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>36</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

However, the percentage of decrease in the activity of
aminopyrine N-demethylase and acetonilide hydroxylase was 36 and 22 respectively as compared to the well fed animals.

**Effect of starvation on the levels of microsomal electron transport components:**

The initiation of the starvation showed an increase in cytochrome c reductase activity and continued to increase with an increase in starvation period up to 36 hours. However, at 60 hours of fasting, cytochrome c reductase started declining. Starvation lasting 84 hours caused 6% decrease in cytochrome c reductase activity as compared with fed group of male rats. The percentage of increase in cytochrome c reductase in male rats fasted for various period of time was as follows:

<table>
<thead>
<tr>
<th>Fasting period (in hours)</th>
<th>Cytochrome c reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td>36</td>
<td>110</td>
</tr>
</tbody>
</table>

During the first 60 hours following food withdrawal, there was a progressive increase in the levels of cytochrome b$_5$ and cytochrome P-450 in the male rats. However, in the male rats fasted for 84 hours there was a marginal decline in the levels of cytochrome b$_5$ (5%) and cytochrome P-450 (8%).

The percentage of increase in cytochrome b$_5$ and cytochrome P-450 levels in varying period of fasted rats was as follows:
DISCUSSION

Metabolic responses induced by short term fasting differ substantially from those induced by long term fasting. The result presented here indicates that the starvation caused a biphasic effect on the tissue level of ascorbic acid and activity of drug metabolizing enzymes. During the early period of fasting, the initial phase of increase of liver and serum ascorbic acid content and activity of drug metabolizing enzymes, followed by a second phase with the extension of fast, produced a marked decrease.

The increase in ascorbic acid during early period of fasting that was observed could be due to increased synthesis and/or decrease in catabolism of ascorbic acid. The increase in the serum ascorbic acid during the early period of starvation could indicate an increase in the release of ascorbic acid from storage tissue to serum. This observation is supported by the observation reported by Vorek (15) in the similar studies on piglets. Ascorbic acid was found to be depleted significantly from adrenals at the end of 12 hours'
fasting in the male rats indicating the stimulation of adrenals for steroidogenesis.

The increase in the activities of drug metabolizing enzymes observed during the early period of fasting could be due to a change in hormonal balance associated with stress. This observation is supported by the similar observation reported by Kato and others (3, 6, 14) in their studies on rats fasted for 72 hours. The observed increase in cytochrome P-450 during early period of starvation suggests that the activities of drug metabolizing enzymes in fasted rats were probably due to the increased level of cytochrome P-450 content. It is also possible that stability and/or integrity of the endoplasmic reticulum components or turnover time of these enzymes may be altered during starvation.

The decline observed in the activity of acetanilide hydroxylase and aminopyrine N-demethylase in 84 hours fasted rats could be due to the change in hormonal imbalance during this period of starvation.

Our results demonstrate a very interesting parallelism between the activation of microsomal enzymes and increase in the serum ascorbic acid level during early period of starvation. When serum ascorbic acid level was declined below normal, the drug metabolizing enzymes activities were also decreased. It is quite possible that the increased seems not the case for acetanilide hydroxylation vs. serum level of AsA.
serum ascorbic acid level could at least be partly responsible for the induction of hepatic mixed function oxidase system observed during the early period of starvation. The increased level of liver ascorbic acid content during early period of starvation could protect the cytochrome P-450 from inhibition as evidenced by Zannoni (16) in his studies in ascorbic acid deficient animals. However, it seems unlikely, because it would not explain the observation reported by Kato (14) that increase in drug metabolizing enzymes activities during starvation was due to increase in de novo synthesis of protein enzymes.

Mgbodile (17) observed that restricted feeding for varying periods resulted in significant increase in endogenous corticosteroids production and further concluded that such hormonal changes during starvation might be an important factor in increasing microsomal enzyme activities. It has been shown in the previous Chapter that ascorbic acid has no direct action on drug metabolism but it acts through mediator(s). Therefore, it seems possible that the rise in the activity of drug metabolizing enzymes during early period of starvation could be due to an increase in serum ascorbic acid level and also due to hormonal changes.
SUMMARY

The effect of starvation at varying periods on the activities of drug metabolizing enzymes, electron transport components and tissue ascorbic acid content was studied in male rats.

The present studies indicate that

(1) Liver and serum ascorbic acid content were increased slightly but significantly during the early period of starvation followed by decrease with further increase of starvation period;

(2) Initial period of starvation caused a decrease in adrenals ascorbic acid content and then increased after 36 hours of fasting;

(3) Starvation up to 84 hours have no significant effect on kidney ascorbic acid level;

(4) Aminopyrine N-demethylase and cytochrome c reductase activities were increased during 36 hours of fasting and then decreased; and

(5) Acetanilide hydroxylase activity and cytochrome P-450 content were increased with increase of starvation period up to 60 hours and then decreased at 84 hours of fasting.
REFERENCES