CHAPTER IV

HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON ASCORBIC ACID LEVELS IN NORMAL AND X-IRRADIATED TISSUES

INTRODUCTION

Ascorbic acid biosynthesis is known to occur in mammals (Burns, 1960; Rey Chaudhary and Chatterjee, 1969). Homig and coworkers (1972a, 1972b) clearly indicated that after dosage of either compound of $^{14}$C dehydroascorbic acid or $^{14}$C ascorbic acid, the radioactivity was widely distributed in tissues of guinea pig and rat. The metabolic role of AA in mammalian tissues has been discussed by Chinoy (1972) and Homig et al (1972a) and a correlation was established between the highest levels of AA and a greater turnover by the tissues. It seems likely that AA functions in mammalian cells as a cofactor of certain redox and hydroxylation reactions (Sebrell and Harris, 1967).

It is known that administration of ascorbic acid, cystein and cyanide prior to radiation affords some protection against radiation damage (Warren and Brues, 1950; Shapiro et al, 1967). Although number of enzymes and metabolites have been investigated in irradiated tissues, yet, not much work seems to have been carried out on the ascorbic acid concentrations, its utilization as well as binding with macromolecules by tissues subjected to low dose of X-irradiation. Hence the present study was undertaken to investigate histochemically on AA and biochemically on the
concentrations of free AA, its bound form or ascorbigen (ASG), the enzymic utilization of ascorbic acid (AAU) and its complexing capacity with other macromolecules (AA-MM complex) in the normal and X-irradiated guinea pig, rat and mouse tissues.

**MATERIALS AND METHODS**

**Animals:** Normal guinea pig (*Cavia porcellus* L.), weighing 350-400 gm; albino rat (*Rattus norvegicus* L.), weighing 170-200 gm and albino mouse (*Mus musculus* L.), weighing 25-30 gm were used in the present experiments. They were bred and maintained in the temperature conditioned laboratory on standard feeds (Hindustan Lever Ltd.) and water ad libitum.

**Irradiation procedure:** The animals under light ether anaesthesia were X-irradiated between 11.00 a.m. to 1.00 p.m. in the thoracic region (ventral side facing the source) by Copper K alpha lines (Philips model). The skin target distance was 20 cm. The diameter of the circular portion of thorax exposed to X-irradiation was 1.78 cm and the total area exposed was 5.6 square cm, at a voltage of 40 kV and 20 mA. The dosage employed was 24 r/second. The dose rate was measured with a Victorean dosimeter.

**Experimental procedure:** The animals were exposed to single doses of 72 r, 120 r and 240 r X-irradiation and compared with those of non irradiated control animals. They were sacrificed at 1 h, 24 h, 48 h and 72 hours after X-irradiation. Histochemical and biochemical studies were carried out on liver, kidney, pectoralis major muscle, adrenal, spleen, cerebellum (brain), ovary, uter
caput and cauda epididymides and testis of locally X-irradiated guinea pig, rat and mouse.

Histochemical localization of AA was carried out by the modified method of Chinoy (1969b) and Dave et al (1969) using alcoholic acidic silver nitrate reagent. The normal and experimental animals were beheaded and bled. The small pieces of the required tissue were dropped in chilled alcoholic, acidic silver nitrate reagent after blotting free of blood, for the localization of AA.

Quantitative estimations of free AA, bound ascorbic acid or ascorbigen (ASG), its utilization (AAU) and macromolecular complexing (AA-MM complex) of various tissues of the normal and X-irradiated (72 r, 120 r and 240 r) guinea pig, rat and mouse, were carried out. The method employed was that of Chinoy et al (1969) using the dye 2,6-dichlorophenol indophenol. The optical density was read at 520 mp in the Spectronic 20 (Bausch & Lomb) with 0.005 mg to 0.1 mg ascorbic acid (BDH) as standard. The concentration of AA was expressed as mg of AA or ASG or AAU or AA-MM complex/100 gm wet weight of the tissue.

Histochmical localization of ascrobic acid:

Liver:

Guinea pig: A uniform deposition of silver granules was observed in the nuclei, while the cytoplasm was comparatively free of the vitamin (Fig. 1). X-irradiation with 72 r showed more or less normal distribution at 72 h post-irradiation (Fig. 2). On the other hand, after 120 r and 240 r irradiation, the concentration of AA decreased and did not show normal localization three days
after X-irradiation (Figs. 3 & 4).

Rat: The hepatic cells show heavy deposits of silver. The nuclei of these cells are comparatively darker than the cytoplasm. Liver also possess large granules of AA (Fig. 5). X-irradiation with 72 r showed significant reduction in the AA localization (Figs. 6 & 7). After 120 r and 240 r irradiation normal distribution pattern was not observed but liver showed large deposits of AA (Fig. 8). This deposition did not observed throughout the liver, but seen only in the peripheral part of the liver.

Mouse: Mouse liver contains very less concentration of AA as compared with guinea pig and rat liver (Fig. 9). With 72 r and 120 r X-irradiation (Fig. 10) the AA deposition showed increased in liver AA, whereas 240 r X-irradiation resulted in significant decrease in AA as compared with normal liver (Figs. 11 & 12).

Kidney: -

Guinea pig: The intensity of staining was more in the cortex as compared to the medulla. The unifromous tubular cells contained brownish black deposits of silver in their nuclei and intertubular areas (Fig. 13). With 72 r X-irradiation normal localization of AA was noticed at 72 h (Fig. 14). X-irradiation with 120 r and 240 r showed large black deposits of AA in intertubular areas but the nuclei did not show AA staining at 72 h after treatment (Figs. 15 & 16).

Rat: Intertubular areas were rich in AA of normal rat kidney (Fig.
X-irradiation with 72 r resulted in an increased AA deposition in the tubular lumen, while its concentration was decreased after 120 r irradiation as compared with normal kidney. Following 240 r X-irradiation AA localization showed noticeable accumulation of AA in the cortical region at 48 h after irradiation (Fig. 20).

Mouse: The concentration of the vitamin is less as compared with guinea pig and rat kidney. The concentration was significantly reduced by 72 r, 120 r and 240 r X-irradiation and did not show any recovery at 72 h after treatment.

Muscle:

Guinea pig: A denser deposition of the vitamin was found in the interfibral regions as well as in the muscle fibres (Fig. 21). AA deposition increased by 72 r and 120 r X-irradiation (Figs. 22 & 23), while its concentration reduced by 240 r irradiation (Fig. 24).

Rat: The denser deposition of AA was seen in the interfibral regions (Fig. 25). After 72 r irradiation the fine granular as well as large deposits of the vitamin are seen in interfibral and the myofibres (Fig. 26). After 120 r X-irradiation the concentration of AA was seen only in the muscle fibres as compared with interfibral areas (Fig. 27). Same holds good after 240 r X-irradiation (Fig. 28).

Mice: The AA deposition in the muscle was localized in the
interfiberal spaces as well as myofibres. X-irradiation with 72 r, 120 r and 240 r resulted in decreased AA concentration. The reduction was maximum after 240 r irradiation.

Adrenal:

Guinea pig: The concentration of AA was more in the cortex as compared with medulla. The nuclei stained darkly than the cytoplasm and large black deposition of the silver were also noticed in the adrenal cortex (Fig. 29). X-irradiation with 72 r and 120 r resulted in decreased AA deposition (Figs. 30 & 31). After 240 r irradiation the AA showed decreased concentration at 48 h after treatment (Fig. 32). At 72 h normal concentration of AA was noticed.

Rat and mouse: AA deposition was more in the cortex as compared with medulla. X-irradiation with 72 r, 120 r and 240 r resulted in increased AA deposition (Figs. 32 to 40).

Spleen:

Guinea pig, rat and mouse: The concentration of AA was more in the splenic nodules than the other region of the spleen in all the three animals investigated. The concentration of AA increased by 72 r, 120 r, and 240 r X-irradiation in guinea pig, rat and mouse (Figs. 41 to 44).

Cerebellum:

Guinea pig: The cerebral cortex show heavy concentration of AA (Fig. 45). The concentration decreased by 72 r and did not show
normal localization of AA, while 120 r and 240 r X-irradiation showed increased concentration of AA in cerebellum (Figs. 46 to 48).

**Rat and mouse:** These animals also showed increased AA deposition after all the three doses of irradiation as compared with normal (Figs. 49 to 52).

**Ovary:**

**Guinea pig, rat and mouse:** Fine granular deposits of silver are seen in ovary of guinea pig. In rat and mouse the peripheral part of the ovary showed intense deposits of argentophilic granules. The concentration of AA increased by all the radiation experiments in all the three animals studied (Figs. 53 to 56).

**Uterus:**

**Guinea pig:** The concentration of AA was more in the uterine stroma (Fig. 59). After 72 r, 120 r and 240 r X-irradiation the concentration of AA greatly increased in the uterine epithelium and the uterine glandular tissue (Fig. 60).

**Rat:** In control animals the concentration of AA was more in the myometrium as compared with endometrium (Fig. 61), while after irradiation AA deposition increased and localized in the uterine stroma (Fig. 62).

**Mouse:** In normal animals the concentration was very less in uterine stroma, while AA showed increased deposition in the myometrium after irradiation (Figs. 57 & 58).
Epideridymes and testis:

Guinea pig, rat and mouse: The testicular and epididymal tissue was deeply stained. The AA localization was more in the intertubular region and tubular lumen of epididymides and testis. After all the three doses of X-irradiation, AA did not show significant variation as compared with control ones.

B - Biochemical:

The results are presented in figures 63 to 78. A minimum of six sets were carried out for each tissue and treatment. The data was statistically analyzed. The standard error of each mean value has been indicated by vertical bars in the histograms.

Control group:

The concentration of free AA was higher in kidney, spleen, caput epididymis and testis of guinea pig; rat liver and muscle and mouse adrenal, ovary, uterus and cauda epididymis were rich in AA content amongst the three animals.

The concentration of ASG was more in liver, spleen, brain, caput epididymis and testis of guinea pig; muscle and adrenal of rat and kidney, ovary, uterus and cauda epididymis of mouse.

The AAU was more in liver, kidney, muscle, spleen, brain and caput epididymis of guinea pig and remaining tissues of mouse.

The AA-MM complexing was high in liver, brain, ovary, caput epididymis of guinea pig; muscle of rat and in other tissues of mouse.
X-irradiated group:

Liver: The concentration of AA in guinea pig showed significant increase by all the three doses of irradiation. In rat liver, 72 r, 120 r and 240 r irradiation showed noticeable reduction at 72 h. In mouse liver, AA concentration was found increased by 72 r and 120 r and the levels were decreased by 240 r irradiation (Fig. 63).

The ASG content in guinea pig liver were seen increased by 72 r, did not show marked variation by 120 r and were decreased with 240 r irradiation. In rat liver, 72 r showed significant increase upto three days, marked reduction was obtained by 120 r and 240 r irradiation. In mouse liver, 72 r radiation resulted in decreased AA content, whereas 120 r and 240 r radiation showed remarkable increase upto three days post-irradiation (Fig. 64).

The AA in guinea pig, rat and mouse liver after 72 r, 120 r and 240 r X-irradiation resulted in marked increase at all post-irradiation intervals (Fig. 65).

The AA-MM complex in guinea pig liver showed increased concentration following 72 r, 120 r and 240 r X-irradiation. In rat liver, 72 r and 240 r irradiation caused decreased complexing, while 120 r radiation showed normal levels at 72 h post-irradiation. On the other hand, 72 r and 240 r radiation resulted in increased AA-MM complex, whereas 120 r irradiation showed normal levels at 72 h post-irradiation (Fig. 66).

Kidney: In guinea pig kidney, AA showed increased concentration by 72 r and 120 r, being manifold at 72 h after 120 r radiation
Fig. 63 to 66. The concentration of AA or ASG or AAU or AA-MM complex (mg/100 gm fresh weight) in guinea pig, rat and mouse tissues of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r and 240 r X-irradiation. The values are mean ± S.E.M.
FIG. 63

CONCENTRATION OF FREE ASCORBIC ACID (AA) mg/100 gm WET TISSUE

HOURS POST X-IRRADIATION
FIG. 64
FIG. 65
FIG. 66
and control levels were recorded at third day after 240 r irradiation. Rat kidney also showed same pattern as that of guinea pig but 70% decrease was obtained at 72 h after 240 r irradiation. On the contrary, mouse kidney showed significant increase by all the three doses of X-irradiation (Fig. 63).

The ASG concentration in guinea pig kidney after 72 r irradiation showed noticeable increase up to three days, whereas ASG was undetectable at 48 h and 72 h after 120 r irradiation but 80% increase was recorded at 72 h following 240 r irradiation. In rat kidney, ASG was undetectable at 72 h after 72 r and the concentration was decreased by 120 r and 240 r irradiation. In mouse kidney, ASG showed 70% inhibition up to 24 h, 100% and 80% decrease at 48 h and 72 h respectively after 72 r irradiation. X-irradiation with 120 r caused significant reduction up to 24 h but threefold increase was recorded at 72 h after irradiation. The concentration of ASG in mouse kidney after 240 r radiation showed 50% and 100% reduction at 1 h and 24 h, showed 50% increase at 48 h and ASG levels were not detectable at 72 h post-irradiation (Fig. 64).

The AAU in guinea pig kidney showed 35% and 85% increase at 72 h after 72 r and 120 r irradiation respectively, whereas the utilization was found decreased at 72 h following 240 r irradiation. Rat kidney also showed increased utilization after all the three doses of irradiation. The AAU in mouse kidney was found significantly enhanced up to 72 h by all the three doses of X-irradiation (Fig. 65).
The AA-MM complexing in guinea pig kidney was found gradually increased following 72 r, 120 r and 240 r irradiation. In rat kidney 72 r irradiation showed normal levels at 72 h, whereas 120 r irradiation resulted in slight increase at 72 h but after 240 significant increase was obtained at 72 h post-irradiation. On the other hand, in mouse kidney, AA-MM complexing decreased by 72 r, 120 r and 240 r irradiation (Fig. 66).

Muscle: The AA concentration in guinea pig, rat and mouse muscle significantly increased by all the three doses of irradiation (Fig. 67).

The ASG concentration in guinea pig muscle increased at 72 h after 72 r irradiation, whereas marked decrease was observed after 120 r and 240 r irradiation. On the other hand, 72 r, 120 r and 240 r irradiation resulted in marked reduction in rat muscle. In mouse pectoralis major, 72 r and 240 r resulted in increased ASG, whereas 120 r radiation did not show control levels at 72 h post-irradiation (Fig. 64).

The AAU concentration in guinea pig muscle was found significantly increased up to 72 h after 72 r, 120 r and 240 r irradiation. X-irradiation with 72 r showed two-fold increase at 72 h, whereas 120 r and 240 r irradiation showed 45% increase after irradiation. In mouse, AAU by 72 r X-irradiation showed 85% increase at 1 h and 60% increase was recorded up to 72 h post-irradiation, while 120 r radiation showed two-fold increase at 1 h and 80% increase was observed up to three days after irradiation. On the other hand, 240 r irradiation showed 80% increase was recorded at
Fig. 67 to 70. The concentration of AA or ASG or AAU or AA-MM complex (mg/100 gm fresh weight) in guinea pig, rat and mouse tissues of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r and 240 r X-irradiation. The values are mean ± S.E.M.
FIG. 67
FIG. 68
CONCENTRATION OF ASCORBIC ACID UTILIZATION (AAU) mg/100 gm WET TISSUE

GUINEA PIG SPLEEN  RAT SPLEEN  
5000  4000  3000  2000  1000  0

GUINEA PIG ADRENAL  
4000  5000  3000  2000  1000  0

RAT ADRENAL  
4000  5000  3000  2000  1000  0

MOUSE ADRENAL  
4000  5000  3000  2000  1000  0

FIG. 69
FIG. 70

CONCENTRATION OF AA-MM COMPLEX mg/100 g WET TISSUE

HOURS POST X-IRRADIATION

CONTROL
PP h
PD h
240 h
1 h and 24 h. However, remarkable increase was obtained at 48 h and showed twofold increase at 72 h after exposure to radiation (Fig. 65).

The AA-MM complex in guinea pig muscle showed significant increase by all the three doses of X-irradiation, being maximum at 48 h (fivefold) after 72 r, 2.5 times and 3.5 times after 120 r and 240 r X-irradiation respectively. Rat muscle did not show marked variation after 72 r irradiation. On the other hand, 120 r and 240 r irradiations showed slight decrease in the AA-MM complex after three days. In mouse, 72 r irradiation showed significant increase at 1 h but again its concentration increased upto three days. With 120 r irradiation the complexing increased at 1 h but showed significant decrease upto 72 h after exposure to radiation. After 240 r radiation AA-MM complexing increased at 1 h and normal values were recorded upto 72 h post-irradiation (Fig. 66).

**Adrenal:** In guinea pig and rat adrenal 72 r, 120 r and 240 r irradiation showed increased free AA at all post-irradiation intervals. In mouse adrenal AA was significantly decreased at all post-irradiation by all the three doses of radiations. The reduction was 30-40% at 72 h after all the three doses of irradiation in mouse adrenal (Fig. 67).

The AEG content in guinea pig, rat and mouse adrenal decreased by 72 r, 120 r and 240 r, X-irradiation (Fig. 68).

The AAD in guinea pig adrenal showed gradual and significant increase upto three days after 72 r and 120 r X-irradiation.
Irradiation with 240 r showed marked increase up to 48 h but normal levels were obtained at 72 h after irradiation. In rat adrenal, 72 r irradiation caused reduction in the AAU at 1 h, showed increased utilization at 24 h and control levels were recorded at 48 h and 72 h after irradiation, while with 120 r irradiation the utilization of AA did not show marked variation up to 24 h but showed increased utilization at 48 h and 72 h after irradiation. On the other hand, 240 r irradiation showed decreased utilization at 1 h, the concentration was normal upto 48 h and an increase was recorded at 72 h after irradiation. In mouse adrenal, AAU was significantly reduced by all the three doses of radiations (Fig. 69).

The AA-MM complexing in guinea pig adrenal increased after 72 r, 120 r and significantly decreased at 72 h after 240 r irradiation. The AA-MM complex in rat adrenal decreased after 72 r and 120 r and remarkable increase was obtained with 240 r X-irradiation. The AA-MM complex in mouse adrenal was significantly reduced up to 24 h, showed 30% increase at 48 h and normal levels were recorded at 72 h post-irradiation, whereas control levels were recorded at 72 h after 120 r irradiation. After 240 r the AA-MM complexing decreased up to 72 h (Fig. 70).

Spleen: In guinea pig, AA did not show significant change up to three days after 72 r irradiation. With 120 r irradiation marked increase was recorded at 1 h, the concentration was normal at 24 h, while threefold increase was recorded at 48 h and the concentration was gradually reduced at 72 h, as compared with
preceding phase. In rat, AA concentration was seen increased after 72 r and 120 r irradiation. On the other hand, 240 r showed noticeable reduction at 72 h (70%) post-irradiation. In mouse spleen, AA concentration showed significant increase at all recovery phases by 72 r, 120 r and 240 r X-irradiation (Fig. 67).

The concentration of ASG in guinea pig showed manifold increase at 72 h after 72 r irradiation. On the contrary, 120 r and 240 r radiation marked reduction was obtained at 72 h post-irradiation. In mouse spleen the ASG was not detectable up to 24 h but its levels were gradually increased at 48 h and threefold increase was recorded at 72 h after 72 r irradiation, whereas 120 r irradiation did not show any variation up to 72 h after 120 r irradiation. On the other hand, 240 r irradiation showed fivefold increase at 1 h, ASG was significantly reduced at 48 h and normal levels were obtained at 72 h post-irradiation (Fig. 68).

The AAU in guinea pig did not show remarkable variation up to 72 h after 72 r and 240 r irradiation. X-irradiation with 120 r did not cause marked variation up to 24 h but 55% increase was recorded at 48 h and 72 h after irradiation. The AAU in rat showed subsequent increase up to three days after 72 r and 120 r, while 240 r irradiation also caused significant decrease up to 48 h but normal levels were recorded at 72 h post-irradiation. In mouse, AAU showed significant increase up to three days after 72 r, 120 r and 240 r X-irradiation (Fig. 69).

The AA-MM complexing in guinea pig showed increase up to three days after 72 r and 240 r X-irradiation. On the other hand,
Fig. 71 to 74. The concentration of AA or ASG or AAU or AA-MM complex (mg/100 gm fresh weight) in guinea pig, rat and mouse tissues of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r and 240 r X-irradiation. The values are mean ± S.E.M.
CONCENTRATION OF FREE ASCORBIC ACID (mg/100 g wet tissue)

**GUINEA PIG UTERUS**

**RAT UTERUS**

**MOUSE UTERUS**

**GUINEA PIG OVARY**

**RAT OVARY**

**MOUSE OVARY**

**GUINEA PIG BRAIN**

**RAT BRAIN**

**MOUSE BRAIN**

**HOURS POST X-IRRADIATION**

**FIG. 71**
FIG. 72
CONCENTRATION OF ASCORBIC ACID UTILIZATION (AAU) mg/100 g WET TISSUE

■ CONTROL
□ 72 h
■ 120 h
□ 240 h

RAT UTERUS
GUINEA PIG UTERUS
GUINEA PIG OVARY
RAT OVARY
MOUSE OVARY

FIG. 73
CONCENTRATION OF AA-MN COMPLEX (mg/100 g W T TISSUE)

HOURS POST X-IRRADIATION

FIG. 74
120 r irradiation caused 50\% decrease at 1 h, showed control levels at 24 h and significant increase was obtained at 48 h and 72 h after radiation. The AA-MM complexing in rat showed 50-60\% increase up to 48 h and the concentration decreased at 72 h after 72 r radiation. Irradiation with 120 r and 240 r did not show any variation at 1 h, while the concentration was gradually increased up to 72 h. In mouse, AA-MM complexing was significantly increased at 1 h after 72 r, while normal levels were obtained at 24 h and the complexing showed 50\% increase at 72 h after exposure to radiation. After 120 r irradiation control levels were recorded at 48 h and 72 h. On the other hand, 240 r irradiation showed remarkable increase at 1 h, showed gradual decline towards control values at 48 h but the complexing was found noticeably reduced at 72 h post-irradiation (Fig. 70).

**Brain:** In guinea pig cerebellum, 72 r, 120 r and 240 r irradiation showed marked increase up to 72 h. The levels of free AA in rat resulted in 30\% decrease after 72 r, normal levels were obtained following 240 r irradiation and 120 r radiation resulted in two-fold increase at 72 h post-irradiation. In mouse the AA concentration was three-fold at 1 h after 72 r, showed gradual decrease towards normal up to 48 h but again significant increase was noticed at 72 h after irradiation. X-irradiation with 120 r showed significant variation at 24 h but subsequently noticeable increase was observed at 72 h post-irradiation. On the other hand, with 240 r irradiation significant increase was recorded up to 72 h (Fig. 71).
The ASG in guinea pig cerebellum after 72 r irradiation showed threefold increase at 1 h, while marked variation was not obtained from 24 h to 72 h post-irradiation. On the other hand, 240 r irradiation showed complete inhibition up to 24 h but recovered the radiation damage at 48 h and again showed 50% decrease at 72 h post-irradiation. The concentration of ASG in rat was more or less same at 1 h after 72 r irradiation, showed twofold and threefold increase at 24 h and 48 h respectively and gradually decreased at 72 h. After 120 r radiation normal levels were recorded at 72 h post-irradiation. With 240 r irradiation 40% reduction was noticed up to 72 h. In mouse, 72 r irradiation showed twofold increase at 1 h, the concentration was not detectable at 24 h and gradually increased up to 72 h (four fold) after irradiation. X-irradiation with 120 r caused significant increase up to 48 h but ASG was undetectable at 72 h after irradiation while 240 r irradiation showed significant increase up to 24 h and 48 h and 72 h ASG was completely undetectable after irradiation (Fig. 68).

The A\&U in guinea pig brain did not show significant variation by 72 r and 240 r X-irradiations, but at 24 h after 240 r irradiation, the levels showed 50% increase in A\&U. In rat, A\&U was significantly increased by all the three doses and did not show control levels even after three days of irradiation. But the increase was maximum by 72 r irradiation. On the other hand, A\&U in mouse was found significantly increased by all the three doses, being maximum by 240 r irradiation (Fig. 73).
The concentration of AA-MM complex in guinea pig brain showed normal levels up to 24 h but 50% decrease was obtained at 72 h after irradiation. With 120 r irradiation significant decrease was recorded at 1 h, was normal at 24 h but 30% increase was observed at 48 h and again decreased at 72 h after irradiation. X-irradiation with 240 r showed marked increase up to 24 h but normal levels were recorded by three days after radiation. In rat, AA-MM complexing was remarkably increased up to 48 h but control levels were observed at 72 h after 72 r irradiation. On the other hand, two-fold increase was observed up to 24 h, was slightly decreased at 48 h as compared with preceding phase and again remarkably increased at 72 h after 120 r irradiation. On the contrary, three-fold increase was observed up to 24 h, followed by a gradual decrease up to 72 h but did not show normal concentration after three days. In mouse, 72 r and 120 r irradiations showed control values up to 48 h and 30% increase was recorded at 72 h after irradiation. X-irradiation with 240 r showed slight increase at 1 h but normal levels were observed at 48 h and the complexing was slightly reduced at 72 h post-irradiation (Fig. 70).

Ovary: X-irradiation with 72 r and 120 r in guinea pig showed increased AA at 72 h, while 240 r radiation showed normal levels at third day post-irradiation. In rat AA did not show much variation by 72 r but after 120 r and 240 r the concentration was found increased at 72 h. In mouse 72 r and 120 r irradiation showed marked increase, whereas with 240 r normal levels were
recorded at 72 h post-irradiation (Fig. 71).

The concentration of ASG in guinea pig did not show any change at 1 h after 72 r but significant decrease was observed at 24 h, while 40% increase was recorded at 48 h and 72 h after radiation. On the other hand, the ASG concentration was not detectable upto 24 h, whereas at 48 h and 72 h normalcy was not restored after 120 r irradiation. Irradiation with 240 r caused marked reduction upto 72 h post-irradiation. In rat, ASG was remarkably increased at 1 h after 72 r irradiation but normal levels were attained upto 72 h. X-irradiation with 120 r also showed 65% reduction from 24 h to 72 h post-irradiation, while 240 r irradiation showed slight increase at 1 h, gradually decreased upto 48 h and again increased at 72 h after irradiation. In mouse ASG concentration showed 40% increase at 72 h after 72 r irradiation. After 120 r normal concentration was recorded at 1 h, showed marked reduction upto 48 h and increased at 72 h post-irradiation. On the contrary, 240 r irradiation caused 50% decrease at 72 h after irradiation (Fig. 72).

In guinea pig A& showed decreased concentration by 72 r and 240 r and normal levels were recorded at 72 h post-irradiation. The A& in rat ovary did not show significant variation upto 72 h after all the three doses of irradiation. The A& in mouse ovary showed decreased utilization after 72 r, marked increase after 120 r and normal values were obtained after 240 r irradiation (Fig. 73).

The AA-MM complexing in guinea pig was found significantly reduced by all the three doses of radiations investigated. In rat
ovary, noticeable increase was obtained after 72 r, showed normal levels at 72 h after 120 r and 240 r irradiation. In mouse ovary, 72 r and 120 r irradiation showed increased complexing at 72 h, whereas 240 r radiation resulted in decreased AA-MM complexing at 72 h post-irradiation (Fig. 74).

Uterus: In guinea pig, 72 r, 120 r and 240 r irradiation resulted in increased AA up to 72 h post-irradiation. The AA in rat uterus showed increased concentration by 72 r and 240 r, whereas 120 r radiation showed control levels at 72 h after irradiation. In mouse AA was found increased at 1 h but the concentration was decreased up to three days after 72 r irradiation, whereas by 120 r irradiation an increase was observed up to three days. On the other hand, 1 h after 240 irradiation the concentration did not show marked variation but slight decrease was recorded at 24 h and subsequently 80% increase was obtained at 72 h after irradiation (Fig. 71).

In guinea pig, significant change was not observed in ASG at 1 h after 72 r irradiation but showed fourfold, twofold and manifold increase at 24 h, 48 h and 72 h respectively. After 120 r irradiation ascorbigen was not detectable at 1 h as well as at 72 h after irradiation. With 240 r irradiation the concentration was undetectable at 1 h, showed normal amount at 24 h and again ASG was undetectable at 48 h and 72 h after irradiation. In rat, ASG did not show significant variation by 72 r and 120 r X-irradiation. On the other hand, 240 r irradiation showed normal levels up to 48 h and ASG values were decreased at 72 h after
irradiation. In mouse, ASG concentration did not show significant variation at 1 h after 72 r and 120 r irradiation but ASG was undetectable up to three days after irradiation. On the other hand, 240 r irradiation did not show significant difference at 1 h, was remarkably decreased up to 48 h but normal levels were recorded at 72 h after irradiation (Fig. 72).

The A4U in guinea pig uterus showed 20% increase at 1 h, the values were more or less similar to normal levels up to 48 h and again 35% increase was observed at 72 h after 72 r irradiation. A4U did not show significant variation up to 72 h after 120 r as well as 240 r x-irradiation. In rat no change was observed in A4U at 1 h after 72 r irradiation but showed significant increase at 1 h after 72 r irradiation but showed significant increase at 24 h (70%) and 50% increase was recorded at 72 h after irradiation whereas 120 r irradiation did not show any change at 1 h, but was subsequently increased up to 48 h and control values were recorded at 72 h after irradiation. On the other hand, up to 24 h significant variation was not obtained after 240 r irradiation but utilization was subsequently increased up to 72 h (50%) after irradiation. In mouse, A4U showed slight increase at 1 h and the utilization decreased up to three days after 72 r irradiation, whereas 60% increase was observed at 1 h after 120 r irradiation, but showed normal values up to 72 h after irradiation, while 240 r irradiation did not show significant variation up to 48 h but 70% increase was recorded at 72 h after irradiation (Fig. 73).
The AA-MM complexing in guinea pig showed noticeable decrease at 1 h, which was slightly increased at 24 h, again decreased at 48 h while 50% increase was observed at 72 h after 72 r irradiation. No significant variation was recorded up to three days after 120 r irradiation while 240 r irradiation caused nearly twofold increase up to 24 h but control levels were recorded at 48 h and 72 h after irradiation. In rat AA-MM complexing showed 50% increase at 1 h after 72 r irradiation, but the concentration was found decreased at 24 h and again enhanced up to three days post-irradiation. On the other hand, 120 r irradiation showed significant decrease at all the recovery phases. Irradiation with 240 r caused significant decrease at 1 h, showed 40% increase at 24 h and gradually decreased up to 72 h after irradiation.

The AA-MM complexing in mouse uterus decreased by 72 r and 120 r irradiation but showed slight increase at 1 h after 240 r irradiation and again reduced up to 72 h, as compared with control levels (Fig. 74).

**Caput epididymis**: Ascorbic acid in guinea pig caput epididymis showed significant increase up to three days, being maximum at 24 h after 72 r irradiation while 120 r irradiation caused slight decrease at 1 h, which was subsequently increased up to three days (threefold) after irradiation. On the other hand, 240 r did not show significant variation up to three days. Rat caput epididymal AA showed same pattern as that of guinea pig after 72 r X-irradiation while 120 r and 240 r irradiation showed significant increase.
Fig. 75 to 78. The concentration of AA or ASG or AAU or AA-MM complex (mg/100 gm fresh weight) in guinea pig, rat and mouse tissues of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r, and 240 r X-irradiation. The values are mean ± S.E.M.
CONCENTRATION OF FREE ASCORBIC ACID (AA) mg/mg WET TISSUE

![Graph showing concentration of free ascorbic acid over time for different species.]

FIG. 75
HOURS POST X-IRRADIATION

FIG. 76
FIG. 77
upto 48 h and normal levels were observed at 72 h after 120 r.
The AA was significantly decreased at 72 h after 240 r irradiation.
The AA concentration in mouse caput epididymis showed twofold increase from 1 h to 72 h post-irradiation. On the contrary, remarkable increase was observed after 120 r as well as 240 r irradiation (Fig. 75).

The ASG in guinea pig caput epididymis did not show significant variation after 72 r irradiation, while 120 r irradiation showed 75% decrease at 1 h and the concentration was undetectable upto three days after irradiation, while 20% decrease was observed at 1 h, which further reduced upto 72 h after 240 r irradiation. In rat, ASG did not variation at 1 h, but gradually increased upto 72 h (70%) after 72 r irradiation, whereas 120 r irradiation showed no significant variation upto 48 h but twofold increase was obtained at 72 h after irradiation. Irradiation with 240 r caused slight decrease at 1 h and remarkably increased upto three days, being maximum at 48 h (fourfold) after irradiation.

In mouse, ASG was undetectable at 1 h after 72 r X-irradiation, showed sixfold increase at 24 h and gradually declined towards control levels at 72 h after irradiation, while 120 r irradiation showed slight increase at 1 h, was normal at 24 h and increased upto 72 h after irradiation. On the other hand, the concentration was not detectable upto 24 h, showed threefold increase at 48 h and significant reduction was noticed at 72 h after 240 r irradiation (Fig. 76).

The AAU in guinea pig showed no significant variation by
72 r irradiation, while 120 r as well as 240 r X-irradiations did not show much variation up to 48 h but two-fold increase was observed at 72 h after irradiation. Rat caput epididymal AMJ showed significant increase by all the three exposures, being maximum by 72 r X-irradiation. AMJ in mouse caput epididymis was found significantly increased by 72 r, 120 r and 240 r irradiation. But the increase was maximum by 240 r irradiation (Fig. 77).

The AA-MM complexing was reduced in guinea pig at 1 h after 72 r irradiation but normal levels were recorded upto 48 h and three-fold increase was obtained at 72 h after irradiation. On the other hand, 120 r irradiation showed gradual increase from 1 h to 72 h after irradiation, while 240 r irradiation showed remarkable increase at 1 h, the concentration was normal at 24 h and the values were increased at 48 h and the AA-MM complex was normal at 72 h after irradiation. In rat AA-MM complexing was significantly enhanced by 72 r irradiation. X-irradiation with 120 r showed normal levels up to 24 h but gradually increased up to three days. On the other hand, 240 r irradiation showed increased AA-MM complexing up to 48 h, being maximum at 24 h and significant decrease was recorded at 72 h after irradiation. In mouse, AA-MM complexing, slight increase at 72 h after 72 r irradiation. On the other hand, 120 r irradiation showed significant increase up to 72 h post-irradiation, but values were recorded at 72 h after 240 r irradiation (Fig. 78).

Cauda epididymis: In guinea pig, AA showed control levels at 1 h, two-fold increase was obtained at 24 h which was gradually
decreased at 48 h and again twofold increase was observed at 72 h after 72 r irradiation. On the other hand, 120 r irradiation showed normal levels at 1 h and significant increase was recorded up to three days, while 240 r irradiation caused remarkable increase up to 24 h but gradually decreased at 48 h and again showed slight increase at 72 h post-irradiation. Bat cauda epididymis showed gradual increase in AA up to three days after 72 r irradiation. However, 120 r irradiation showed slight increase at 1 h, which was strikingly increased at 24 h, but gradually decreased up to three days, as compared with 24 h values, while 240 r irradiation showed twofold increase at 1 h, threefold at 24 h but gradually decreased up to 72 h after irradiation. In mouse AA showed significant increase by 72 r, 120 r and 240 r irradiation. The maximum increase was observed by 120 r irradiation at 72 h (4.5 times) after irradiation (Fig. 75).

The ASG in the guinea pig was slightly decreased by 72 r irradiation at 1 h, but gradual increase was observed up to 48 h and showed twofold increase at 72 h after irradiation. On the other hand 120 r irradiation did not show any activity up to 72 h after 72 r and 120 r irradiation but 240 r irradiation showed twofold increase at 1 h, significant decrease was obtained at 24 h, at 48 h the concentration was more or less same as that of control but again slight decrease was obtained at 72 h after irradiation (Fig. 76).

The AAI in the guinea pig did not show significant difference up to 48 h but 40% increase was observed at 72 h after 72 r irradiation, while 120 r as well as 240 r irradiation showed
no significant difference in the utilization up to three days after irradiation. On the other hand, AAU in the rat cauda epididymis showed significant increase by all the three doses, being maximum by 120 r irradiation at 24 h (fourfold) post-irradiation. Mouse also showed the same pattern of increase as that of rat but the increase was maximum by 120 r irradiation at 72 h (2.5 times) after radiation exposure (Fig. 77).

The AA-MM complexing in guinea pig significantly enhanced at 72 h after 72 r irradiation. On the other hand, 120 r irradiation showed normal levels at 1 h but slight decrease was observed up to 72 h, while 240 r irradiation showed significant increase at 72 h after irradiation. The AA-MM complexing in rat cauda epididymis showed no significant variation up to 72 h after 72 r irradiation. On the other hand, the complexing was remarkably increased up to 24 h but showed increased levels up to 72 h after 120 r irradiation. A dose of 240 r irradiation caused significant increase at 1 h but it was gradually decreased and showed normal levels at 72 h after irradiation. In mouse, AA-MM complexing was significantly decreased by 72 r as well as 240 r irradiation and did not show normal levels even at 72 h after radiation. With 120 r irradiation no significant change was observed up to 24 h but was gradually increased up to 72 h after irradiation (Fig. 78).

Testis: In guinea pig the AA concentration was increased by all the three doses of irradiation. The increase was 90% at 72 h by all the three doses of irradiation. In rat, AA levels were significantly increased by 72 r, 120 r and 240 r irradiations but the increase
was remarkable by 72 r and 120 r irradiation. Mouse also showed noticeable increase by 72 r, 120 r and 240 r irradiation. The increase was maximum at 1 h after 72 r X-irradiation (Fig. 75).

The ASG concentration in guinea pig testis was gradually increased up to 72 h and showed fourfold increase at 72 h after 72 r irradiation. A dose of 120 r irradiation also showed increased ASG levels up to 24 h, was normal at 48 h and undetectable at 72 h after irradiation. With 240 r irradiation normal levels were obtained from 24 h to 72 h after radiation exposure. In rat, ASG showed slight increase at 1 h, was nearly normal at 24 h and undetectable up to 72 h after 72 r irradiation whereas 120 r irradiation showed threefold increase was obtained at 72 h post-irradiation. However, 240 r irradiation showed significant increase at 1 h, which was gradually decreased up to 48 h but again remarkably increased at 72 h after irradiation. The ASG concentrations in mouse showed fourfold increase at 1 h, was not detectable at 24 h and again strikingly increased up to 72 h after 72 r X-irradiation. Hundred and twenty r irradiation showed significant increase up to 72 h, being maximum at 24 h after irradiation. On the other hand, 240 r irradiation showed significant increase at 1 h but the concentration was not detectable up to three days after treatment (Fig. 76).

The AAU in all the three animals was found significantly increased by 72 r, 120 r and 240 r irradiation. But the AAU was maximum in rat at 72 h after 72 r X-irradiation (Fig. 77).

The AA-MM complexing in guinea pig testis was slightly decreased at 1 h, showed normal levels at 24 h, again slightly
reduced at 48 h and remarkable increase was observed at 72 h after 72 r irradiation. On the other hand, 120 r irradiation did not show significant variation up to 24 h but 40% increase was obtained at 48 h after irradiation and showed gradual decrease towards normal levels at 72 h post-irradiation while 240 r irradiation did not show significant variation at 1 h but significantly increased at 24 h and gradually decreased at 72 h as in comparison with 24 h values. In rat testis AA-MM complexing showed decreased levels at 1 h but control levels were recorded from 24 h to 72 h after 72 r irradiation. On the other hand, after 120 r and 240 r irradiation significant reduction was observed at all post-irradiation. In mouse testis, AA-MM complexing did not show significant variation up to 24 h but was found significantly reduced at 48 h and 50% inhibition was observed at 72 h after 72 r irradiation. On the other hand, 120 r as well as 240 r irradiation caused significant reduction up to three days. The decrease was 60% (at 72 h) after 120 r and 240 r X-irradiation (Fig. 78).

DISCUSSION

Mammalian tissues such as liver and kidney are known to possess the enzymic makeup for the active synthesis of ascorbic acid (Hey Chaudhary and Chatterjee, 1969). Moreover, the tissues also possess large stores of bound ascorbic acid (Malakar, 1963; Szent Gyorgyi, 1957; Roe and Itscoitz, 1963). The histochemical localization pattern of AA in all tissues revealed a distinctly higher intensity in the nuclei as compared with cytoplasm. AA is
also associated with macromolecules, viz., nucleoproteins, mucoproteins and connective tissue-ground substances of the cells (Shah et al, 1973b) and higher AA concentration correlated with a corresponding high metabolic activity of tissues.

The absence of biosynthesis of AA by adult guinea pig liver is probably due to the lack of a key enzyme, gulunolactone oxidase into AA (De Fabro, 1968). De Fabro (1968) has demonstrated that the microsomal fraction of embryonic guinea pig liver possessed gulunolactone oxidase, an enzyme which converts gulunolactone to AA. This enzyme was found to be absent in adult guinea pigs. The absence of this key enzyme may account for the loss of capacity for biosynthesis of AA in adult guinea pig liver.

Functionally, AA has an important role to play in cellular metabolism (Sebrell and Harris, 1967). Thus, the activation of enzymes such as arginase, liver esterase and catalase is one of them. A correlation is, therefore, discernible between the high AA content of tissues and the high tempo of their metabolism. The participation of AA in cellular oxidoreduction reactions occur via the formation of its free radical. Staudinger et al (1961) have clearly demonstrated that AA helps in electron-transfer mechanism by donating one of its electrons forming an unstable free radical monodehydroascorbic acid (MDHA), which subsequently gets oxidized to dehydroascorbic acid (DHA). DHA can again be converted to AA. These reactions are enzyme mediated (Sebrell and Harris, 1967). Staudinger et al (1961) have also shown an AA dependent NADH oxidation occurs in rat liver.
Physiologically seen, an active energy-dependent transport of a compound offers the advantage of utilizing most of a substance as AA. DHA is more lipid soluble than AA is, therefore, suggested as the preferred form of the vitamin. Moreover, DHA was shown by in vitro experiments to be taken up by diffusion (Hornig et al., 1971a, 1971b, 1971c).

In normal guinea pig and rat liver, AA was localized in the nucleus (Figs. 1 & 5). Moreover, rat liver also possess large stores of AA (Fig. 5). On the other hand, mouse liver showed few large deposition of silver granules (Fig. 9). X-irradiation with 72 r caused significant alterations in AA localization in guinea pig liver. ASG content of guinea pig liver showed decrease at 24 h following 72 r irradiation, which might be due to increased release of bound AA, as free AA content increased at this post-irradiation phase. Histochemical localization of AA also showed decrease at 24 h after 72 r radiation (Fig. 2). AA localization demonstrated decreased AA concentration at 72 h after 120 r irradiation (Fig. 3), which may be due to marked increase in free AA and AAU after radiation. However, 240 r radiation resulted in significant decline in ASG at 72 h, which is probably due to reduced binding of AA with macromolecules. Histochemically also liver AA showed reduced AA at 72 h after 240 r radiation (Fig. 4).

With 72 r X-irradiation rat liver showed fine dense deposition throughout the liver at 1 h (Fig. 6), showing increased binding of AA with macromolecules. At 72 h (Fig. 7) large deposits of AA were observed after 72 r, which might be due to decreased
conversion to free AA. Thus, radiation seems to resulted in significant damage in AA biosynthesis or some changes in pituitary hormones, as they regulate the energy-dependent transport of AA (Hornig et al., 1972b). X-irradiation with 120 r resulted in significant decrease in AA, ASG, and AA-MM complex. This decreased AA content might be due to decreased synthesis of AA or inhibition of enzymes involved in AA biosynthesis. The decreased AA concentrations in the liver causes a decrease in the rate of the conversion of cholesterol to its main catabolic product, the bile acids, which involves several hydroxylation reactions (Guchhait et al., 1963). Ginter (1973) indicate that action of AA in the catabolism of cholesterol may be mediated by cytochrome P-450 in the liver microsomes. AA also stimulates the sulfation of cholesterol. X-irradiation with 240 r also showed decreased AA and ASG at 48 h and 72 h post-irradiation. Histochemical localization demonstrated large stores of AA in the liver. This deposition is not found throughout the liver but only at some places in the peripheral region of the liver (Fig. 8). This clearly suggests increased utilization of ascorbic acid following low dose X-irradiation.

Mouse liver, on the other hand, showed increased AA, ASG, AAU and AA-MM complex after all the three doses of radiation. AA localization showed large deposits after 72 r and 120 r radiation, while 240 r radiation showed fine granular deposition at 72 h post-irradiation (Figs. 10-12). In order to tide over the damage inflicted by radiation, there occurs increased synthesis of AA in
mouse liver. The increased AA contents might be due to increased AA metabolism, which might be brought about by an activation of enzymes involved in AA biosynthesis after radiation.

The uriniferous tubules of guinea pig contained brownish black deposits of silver, while cytoplasm had a fine deposition of silver granules (Fig. 13). X-irradiation with 72 r did not show marked variation in histochemical localization, but showed increased nuclear staining as well as large granules of AA in uriniferous tubules (Fig. 14). After 120 r and 240 r radiation ASG concentration decreased in guinea pig, which is due to increased release of bound form and its subsequent utilization after radiation. The large deposits of AA (Figs. 15 & 16) in the uriniferous tubules of guinea pig might be due to enhanced complexing with macromolecules after radiation.

Rat kidney, on the contrary, showed AA in the uriniferous tubules at 72 h after 72 r radiation, which might be due to either excretion or reabsorption of the vitamin by the kidneys. The ASG content decreased at 72 h (Fig. 19) after 120 r radiation but AA localization did not show significant variation, which might be due to increased complexing at this time interval. However, 240 r radiation caused increased AA in cortical region at 24 h (Fig. 20) post-irradiation, but sudden decrease in free AA and ASG at 48 h and 72 h might be due to excretion of the vitamin or decreased biosynthesis following irradiation. Mouse kidney also showed increased free AA and AAU which is due to increased release of bound AA.
The pectoral muscle of all the three animals showed marked alterations in the localization of AA as well as quantitative levels following all the three doses of radiations in guinea pig, rat and mouse (Figs. 21 - 23). In guinea pig 72 r irradiation caused marked increase in its localization (Fig. 22) and AA, ASG, AAU and AA-MM complex following irradiation. In rat and mouse, on the contrary, AA localization decreased following all the three doses of radiation (Figs. 24 to 26), which is due to its enhanced free form and utilization leading to depletion of AA in muscle.

The adrenal gland contains AA in a high concentration in cortical region as compared with medulla (Figs. 29, 33 & 37) but the significance and functional importance of the vitamin in the cortex is not clear. It has been suggested that adrenal cortical functions be dependent on AA (Hornig et al., 1972a). Sharma et al. (1964) reported that \(^{14}C-\)AA in the guinea pig adrenal cortex is an energy-dependent process. In the present study, guinea pig and rat adrenal gland showed increased free AA, AAU and AA-MM complex, whereas mouse adrenal resulted in decreased AA, ASG, AAU and AA-MM complex at 72 h after 72 r, 120 r and 240 r irradiation. The localization pattern of AA in the adrenal gland was found to be almost identical with the biochemical alterations (Figs. 29 - 40). The decreased AA concentrations in mouse adrenal might be due to increased ACTH secretion. The corticosteroids produced in response to ACTH are the inhibitors of AA metabolism (De Nicola et al., 1968). Sharma et al. (1964) and Hornig et al. (1972a) showed
high coincident accumulation of radioactive AA in adrenal cortex and in the pituitary gland after intravenous injections of \textsuperscript{14}C-AA, which suggests a role of AA in the pituitary-adrenal axis. They further reported that pituitary gland also regulates transport of AA, its binding or catabolism in adrenal gland. The effect of X-irradiation on adrenal gland is, thus, assumed to be an indirect action through the pituitary adrenocortical mechanism (Hameed and Haley, 1964; Nakasone, 1972). Ungar \textit{et al.} (1955) demonstrated that gamma irradiation of the isolated, ACH-stimulated calf adrenal gland induced a significant decrease in steroidogenic activity. Guichhait \textit{et al.} (1963) reported decreased AA concentration resulted in accumulation of cholesterol, which is correlated with the decreased steroidogenesis following low dose X-irradiation.

The physiological role of AA in the brain is not known. Since AA is discussed as a cofactor of depamin B-hydroxylase (Hornig \textit{et al.}, 1972a), the vitamin might be involved in the regulation of catecholamine synthesis. Normal guinea pig cerebellum showed higher concentrations of AA, ASG, AAU and AA-MM complex. Histochemical localization also showed more concentration in guinea pig brain (Fig. 45), as in comparison with rat and mouse cerebellum (Figs. 49 & 51). Rat and mouse cerebellum showed significant increase in the AA localization at 48 h after 72 r and 240 r X-irradiation respectively (Figs. 50 & 52). Biochemical results also showed significant concentration of ASG at this time interval. The increased concentration of free AA as well as AAU
in all the three animals investigated might be a result of increased active transport process following irradiation (McIlwain et al., 1956). Since the complete blood-brain barrier has been postulated for AA in these animals (Martin and Mecca, 1961), the increased concentration of AA in cerebellum might be due to increased blood AA levels after X-irradiation. Addition of AA to the brain microsomal fraction greatly enhanced the oxidation of brain NADH₂ (Aghajanian, 1963). Thus, low dose radiations resulted in significant alterations in cerebellar AA metabolism.

The most remarkable feature in connection with AA in relation to mammalian ovary is its high content in both the interstitial tissue and the corpus luteum, though not in follicular fluid (Lutwak-Mann, 1958). Ovary of guinea pig, rat and mouse also showed marked concentration of AA (Fig. 53). At 1 h and 72 h after 72 r and 240 r radiation respectively interstitial tissue showed significant increase in the AA localization (Figs. 54 & 56). At this time interval ASG and AAU also showed increased contents following 72 r and 240 r irradiation. This increased utilization of AA is a well known index of active hormonogenesis by the ovary and since utilization was markedly increased at 72 h in rat (by 240 r) and in mouse (after 120 r & 240 r) ovary, it is likely that increased estrogen production is the outcome of low dose X-irradiation (Shah et al., 1974). An increase in the estrogen secretion has also been reported within several hours after transplanted sheep ovary (Ellis and Berliner, 1969), while decreased AA, ASG, AAU and AA-MM complex in mouse ovary by 72 r
and decreased utilization in guinea pig ovary (by 72 r and 240 r) might be resulted in decreased steroidogenesis, as AA helps in catabolism of cholesterol. The decreased steroidogenesis might be due to reduction of pituitary hormones, as they regulate the transport of AA, its binding or catabolism in the ovary (Dieter, 1969; Hornig et al., 1972b). Uterus also showed significant concentration of AA in the uterine stroma as well as in the myometrium in these animals (Figs. 57, 59, 61). After irradiation the AA concentration increased in uterine epithelium of guinea pig and rat at 72 h and 48 h after 72 r radiation respectively (Figs. 60 & 62). The increased localization in uterine epithelium might be due to increased secretory activity of the uterus following irradiation. In mouse the AA content decreased significantly at 72 h after 120 r radiation (Fig. 58). The alterations in the uterus might be due to variations in the ovarian hormones following low dose irradiation.

Ascorbic acid and particularly its free radical, monodehydro-ascorbic acid (MDHA), are known to be important sources of electron energy for several biosynthesis reactions in various tissues including reproductive. MDHA, by virtue of possessing unpaired electrons has a greater redox potential than AA itself. The involvement of AA, MDHA and DHA has also been implicated in steroidogenesis (Szent Gyorgyi, 1957; Kocen and Cavazos, 1958; Cavazos et al., 1961; Narendra Mohan Biswas and Deb, 1968). Thus, AA has an important role in oxidation of cholesterol as well as stimulation of hydroxylases leading to homonogenesis (Guchhait

AA and MDHA being reducing substances functions as important sources of energy for sperm metabolism. It has been suggested that AA and MDHA play an important role in restoring and maintaining the normal physiological milieu in the reproductive organs for their proper functioning (Cavazos et al, 1961). A recent study suggests the participation of AA in the maintainance of the NAD/NADH ratios in the reproductive tissues as well as in other tissues (Hornig et al, 1972a).

In testis of all the three animals there was an overall increase in free AA and its utilization following 72 r, 120 r and 240 r X-irradiation. This increase might be due to increased steroidogenesis by the testis, as AA helps in the breakdown of the side chain in addition to the hydroxylation of the sterol moiety (Guchhait et al, 1963). The reduction in ASG and AA-MM complex might be due to increased utilization of bound form of the vitamin after irradiation, whereas in epididymes decreased ASG and AA-MM complex would probably account for loss of motility of spermatozoa. Caput and cauda epididymes also showed similar pattern as that of testis of these animals after all the three doses of irradiation. The increase in free form of AA and AAU in epididymes occurs in order to protect the spermatozoa as well as to maintain the secretory activity of epididymis, especially steroid production, as epididymis is known to be involved in
steroidogenesis. Inano and Tamaoki (1968) have shown that X-irradiation activates some specific enzymes related to androgen biosynthesis in testis. Increased AA levels may have been produced by the increased functional activity of the irradiated testis or the over secretion of pituitary gonadotrophin, which occurred secondarily (Shimizu et al, 1972).

Histochemical and biochemical studies obtained here clearly suggest that low dose local X-irradiation resulted in marked alteration in AA concentration in guinea pig, rat and mouse tissues. Amongst the three animals, normal guinea pig and rat tissues possess more concentration of AA as compared with mouse tissues. Mouse tissues possess less concentration of AA showing significant damage to cell metabolism, as alkaline and acid phosphatases were noticeably activated whereas DNA and protein content showed significant reduction following X-irradiation. Guinea pig and rat tissues, on the other hand, were rich in AA resulted in marked reduction in phosphatases and increased DNA, RNA and protein after 72 $r$, 120 $r$ and 240 $r$ X-irradiation. It has been proposed that AA might have showed protective action in decreasing the extent of all metabolic disturbances after irradiation. The adrenal, ovary and testis showed increased AA levels in all the three animals, as AA is involved in biosynthesis of steroidogenesis and resulted in increased rate of hormonogenesis to overcome the stress condition. The increased AA content in adrenal, ovary and testis might be due to stimulation of pituitary after low dose local X-irradiation.
SUMMARY

Ascorbic acid (Vitamin C) concentrations were studied both histochemically and biochemically in control and X-irradiated guinea pig, rat and mouse tissues. The levels of free ascorbic acid (AA), ascorbigen or bound form of the ascorbic acid (ASG), the enzymic utilization of ascorbic acid (AAU) and its complexing capacity with other macromolecules (AA-MM complex) were estimated.

Histochemical studies showed significant alterations in ascorbic acid localization upon X-irradiation. X-irradiation caused an overall decrease in ascorbic acid metabolism in mouse and rat tissues but an increase in those of guinea pig. The ascorbic acid metabolism in adrenal was geared up in all animals. It is suggested that increased levels of ascorbic acid in guinea pig tissues might be due to enhanced utilization of the bound form of the vitamin. The experimental animals differ in their synthesizing capacity of the vitamin. Guinea pig can not synthesize ascorbic acid in their organs, as one enzyme, gulono lactone oxidase was found to be absent in adult guinea pigs, whereas rats and mice are capable of synthesizing it. The significance of the histochemical and biochemical changes in ascorbic acid levels following low dose local X-irradiation have been discussed.

REFERENCES


Fig. 1. Normal guinea pig liver showing the localization of ascorbic acid. The nuclei were rich in the vitamin then the cytoplasm. x 400.

Fig. 2. Guinea pig liver stained for ascorbic acid at 72 h after 72 r X-irradiation. The liver has almost recovered the radiation damage. x 400.

Figs. 3 & 4. Guinea pig liver at 72 h and 48 h after 120 r and 240 r irradiation respectively. The concentration of ascorbic acid decreased after 120 r and 240 r irradiation. x 400.
Photomicrograph of the normal rat liver showing the localization of ascorbic acid. The nuclei of hepatic cells are comparatively darker than the cytoplasm. Liver also possess large deposits of ascorbic acid. x 400.

Photomicrograph of rat liver at 1 h and 48 h after 72 r irradiation respectively, showing significant reduction in ascorbic acid deposition. x 400.

Rat liver at 48 h after 240 r X-irradiation stained for ascorbic acid. The normal distribution pattern was completely lost by irradiation but large deposits of silver were observed only in the peripheral region of the liver. x 400.
Fig. 9. The photomicrograph shows the transverse section of normal mouse liver stained for ascorbic acid. x 400.

Fig. 10. Mouse liver at 1 h after 120 r irradiation. The AA deposition significantly increased following irradiation. x 400.

Figs. 11 & 12. Mouse liver at 1 h and 72 h after 240 r irradiation respectively, showing marked increase in AA localization at 1 h but at 72 h the AA localization showed fine granular deposition of silver. x 400.
Fig. 13. Localization of AA in control guinea pig kidney. The vitamin is localized in the intertubular areas and tubular cell nuclei. x 400.

Fig. 14. Guinea pig kidney at 72 h after 72 r X-irradiation. The localization of ascorbic acid is similar to that observed in the control guinea pig kidney. x 400.

Figs. 15 & 16. Guinea pig kidney at 48 h after 120 r and 240 r irradiation respectively. Large black deposits of silver are seen in intertubular areas, while the nuclei are unstained. x 400.
Fig. 21. Control guinea pig pectoralis major muscle showing localization of ascorbic acid. x 400.

Fig. 22. Guinea pig muscle at 48 h after 72 r irradiation. The AA deposition increased upon irradiation. x 400.

Figs. 23 & 24. Photomicrograph showing AA deposition in guinea pig muscle at 48 h after 120 r and 240 r irradiation respectively. Note the AA did not show normal localization pattern. x 400.
Fig. 25. Normal rat muscle showing the darker interfiberal and lesser intrafiberal localization of ascorbic acid. x 400.

Fig. 26. Photomicrograph of rat muscle at 72 h after 72 r irradiation. The fine granular as well as large deposits of the vitamin are seen in the muscle. x 400.

Figs. 27 & 28. Rat muscle at 72 h after 120 r and 240 r x-irradiation respectively. The AA deposits increased upon irradiation. x 400.
Fig. 29. Control adrenal cortex of guinea pig stained for AA. The nuclei and intercellular spaces were rich in AA. x 400.

Figs. 30 to 32. Guinea pig adrenal at 24 h after 72 r and 120 r and at 48 h after 240 r irradiation respectively, showing decreased AA localization. x 400.
Fig. 33. Normal rat adrenal demonstrating ascorbic acid localization. \( x \times 400. \)

Figs. 34 to 36. X-irradiation with 72 r, 120 r and 240 r resulted in increased AA deposition in rat adrenal cortex. The increase was maximum at \( 48 \) h after 240 r irradiation. \( x \times 400. \)
Fig. 37. Normal mouse adrenal stained for ascorbic acid. The cortical region is richer in vitamin, as compared with medulla. x 400.

Figs. 38 & 39. Mouse adrenal at 1 h and 24 h after 240 r irradiation, showing increased AA deposition. x 400.

Fig. 40. Photomicrograph of mouse adrenal at 72 h after 240 r irradiation, showing decreased AA localization. x 400.
Figs. 41 & 42. Normal and X-irradiated (at 72 h after 72 r irradiation) guinea pig spleen. The vitamin increases in the splenic nodules. x 400.

Figs. 43 & 44. Normal rat spleen and at 48 h after 72 r irradiation. The vitamin increases upon irradiation. x 400.
Fig. 45. Normal guinea pig cerebellum showing heavy deposition of AA. x 400.

Figs. 46 & 48. Cerebellum of guinea pig at 48 h and 1 h following 240 r irradiation respectively, showing increased AA deposition upon irradiation. x 400.

Fig. 47. Photomicrograph of guinea pig cerebellum at 48 h after irradiation. AA deposition increases upon irradiation. x 400.
Figs. 49 & 50. Normal and X-irradiated (at 48 h after 72 r) rat cerebellum. The ascorbic acid deposits significantly increased upon irradiation. x 400.

Figs. 51 & 52. Photomicrograph of normal and at 48 h after 240 r irradiated mouse brain. Note the increase of the vitamin following irradiation. x 400.
Fig. 53. Normal rat ovary stained for ascorbic acid. The peripheral part of ovary had an intense deposition of argentophilic granules. x 400.

Figs. 54 & 56. Photomicrograph of rat ovary at 1 h and 72 h following 72 r irradiation. At 1 h the vitamin showed significant increase in interstitial tissue of the ovary. At 72 h the peripheral part of ovum and the nucleus had an intense deposition of silver granules. x 400.

Fig. 55. Rat ovary at 72 h after 72 r irradiation. The deposition of AA increased upon irradiation. x 400.
Fig. 57. Normal mouse uterus stained for AA, showing very less concentration in the uterine stroma. x 400.

Fig. 58. Photomicrograph of mouse uterus at 72 h after 120 r irradiation. The myometrium is richer in AA. x 400.
Fig. 59. Control guinea pig uterus demonstrating AA in the uterine stroma. x 400.

Fig. 60. Photomicrograph of guinea pig uterus at 72 h after 72 r irradiation. The vitamin increased in uterine epithelium upon irradiation. x 400.

Fig. 61. Normal rat uterus showing more concentration of ascorbic acid in the myometrium. x 400.

Fig. 62. Localization of ascorbic acid in rat uterus at 48 h after 72 r X-irradiation. The AA deposits increased in uterine stroma upon irradiation. x 400.