CHAPTER V

SUMMARY

PART I

a) Ascorbic acid localization

The specificity of alcoholic, acidic silver nitrate staining method for the histochemical localization of ascorbic acid was reappraised. It was found that the method is by and large, better suited for the localization of ascorbic acid in animal tissues due to its greater specificity which is ensured by employing the reagents made in carbon dioxide saturated glass distilled water as well as by carrying out the reaction at low temperature (0-4°C) and at a pH of 2 to 2.5. An intense localization of ascorbic acid was found in the germinal epithelium, the spermatozoa and interstitial tissue of the testis as well as in the nuclei of the epithelial cells, stereocilia, spermatozoa and intertubular regions of the epididymides.

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b) Ascorbic acid metabolism

The metabolic turnover pattern of ascorbic acid was studied in the spermatozoa, luminal plasma and tissue components of rat testis and epididymides. The results revealed that the epididymal spermatozoa possessed a higher turnover of ascorbic acid than the tissue component and luminal plasma, probably due to the high energy requirement of spermatozoa for their motility and metabolism. The data confirms the postulation that ascorbic acid plays an important role in sperm motility and metabolism of spermatozoa as well as reproductive tissues via the formation of its free radical, monodehydroascorbic acid (MDHA) which functions as source of electron energy in addition to the energy obtained by the conventional breakdown of ATP.

PART II

STUDIES ON MALE CONTRACEPTIVE TECHNIQUES AND THEIR EFFECTS ON MALE REPRODUCTIVE FUNCTIONS OF ALBINO RATS

1. VASECTOMY

The short term effects of vasectomy and vasectomy + ascorbic acid feeding were studied with reference to the
metabolism of tissue components and fluids of rat testis and epididymides respectively. The androgenic parameters were found to decrease after treatment but recovery was obtained by 20 days of post vasectomy or else by ascorbic acid feeding. The enhanced turnover of ascorbic acid in the tissue is probably due to the stress imposed by vasectomy. However, decreased ascorbic acid metabolism in the luminal plasma suggests a greater uptake, storage and utilization by the tissues or else less secretion into the luminal plasma. The data corroborate with the earlier studies from this laboratory that ascorbic acid has a beneficial, prophylactic effect for maintaining tissue metabolism during and following vasectomy without interference with its contraceptive effects. Vasectomy as such, therefore, has no adverse effects on the metabolism of testis and epididymis.

2. STUDIES ON THE EFFECTS OF CYPROTERONE ACETATE ON RAT SPERMATOZOA

The effects of cyproterone acetate (CA) were investigated on the morphology, density, motility and fertilizability of rat epididymal spermatozoa. The
loss of motility and fertilizability have been correlated with the head and tail abnormalities and decapitation of spermatozoa. It is suggested that the occurrence of abnormal spermatozoa after CA treatment might be due to the alterations in the epididymal milieu or else a direct effect of CA on sperm metabolism affecting their maturation. The withdrawal of CA treatment for 30 days after administration for 7 days, was not adequate to restore the normal morphology and 100% fertility in the rats. Similar results were obtained by ascorbic acid feeding along with CA treatment. The results have been discussed in the light of recent work on cyproterone acetate in male animals.

3. STUDIES ON CADMIUM CHLORIDE

The effects of treatment of adult male albino rats with a single injection of cadmium chloride and its repercussions on testicular and epididymal spermatozoa were investigated and compared with animals which were treated with cadmium chloride as well as fed ascorbic acid extraneously. The results revealed that cadmium chloride injection has adverse effects on ascorbate metabolism especially of the testicular spermatozoa.
probably due to the marked changes in testis and the impairment of the pituitary gonadal axis. Cadmium chloride revealed antifertility effects which have been correlated with the decapitation, damage to the middle piece and unwinding of the tail end piece of the spermatozoa of treated rats. The results also suggest that ascorbic acid feeding along with cadmium chloride treatment has beneficial influence in maintaining the redox milieu in the testis and epididymal spermatozoa.

4. STUDIES ON COPPER

1) In vitro studies

Different concentrations of analar copper sulphate solutions decreased the motility of cauda epididymal spermatozoa in vitro. The decrease was more rapid with increasing concentrations of copper sulphate solutions. The addition of ascorbic acid to copper sulphate solution inhibited the motility of the spermatozoa much more rapidly than the copper sulphate solution alone, probably due to the strong acidic properties of ascorbic acid which would cause a rapid fall in pH.
2) **Intra-epididymal and intra-scrotal copper devices**

The effect of implantation of an intra-epididymal copper device bilaterally and intra-scrotal copper device placed medially on testicular and epididymal spermatozoa of rats and the copper content in various reproductive tissues were investigated. The data was compared with another group of animals bearing the copper device and fed ascorbic acid extraneously.

The results revealed that some of the androgenic parameters of the testicular and epididymal spermatozoa decreased in the implant bearing rats. The decrease was more in the epididymal than the testicular spermatozoa, particularly in the IECB implanted rats. The change in sperm metabolism may be brought about by significant decrease in glutathione and inhibition of essential sperm specific enzymes by copper ions. The count, motility and fertilizability of epididymal spermatozoa also decreased in copper device bearing rats which has been correlated with the alterations in their morphology. In copper device bearing animals, an accumulation of copper occurred in several reproductive
tissues but recovery to almost normal level was obtained by ascorbic acid feeding to copper implanted rats. The data supports the view that ascorbic acid has a beneficial effect in copper toxicity. Although the intra-epididymal copper implantation was found to be a more effective male contraceptive device than the intra-scrotal, further work on the latter device would be more practicable in terms of reversibility of the effects and easy retraction of the device.

5. STUDIES ON PROSTAGLANDINS

The studies on the effects of subcutaneous injections of PGF2α and E1 to adult male rats on their sperm morphology, motility, density and fertilizability were investigated. The results showed that both prostaglandins reduced the fertility, percent motility and density of epididymal spermatozoa. The loss of fertility and motility after PG treatment have been related to the decapitation, furrow formation, distortion of the acrosome and unwinding of the tail end piece of the epididymal spermatozoa. It has been suggested that the reduced motility may be due to the probable decline in adrenergic response of the epididymis itself.
6. STUDIES ON VAS OCCLUSION BY ETHANOL

A single injection of 95% ethanol directly into the vas deferens of fertile albino rats was found to result in adverse effects on the structure and metabolism of testicular and epididymal spermatozoa after 30 days post injection. The vas lumen was occluded due to sperm granuloma formation. The absence of fertility has been correlated with the occurrence of decapitated spermatozoa and others bearing severe alterations in their morphology. The decrease in some androgen dependent enzymes of spermatozoa might reflect on the possible reduced androgenicity of the testis, circulating androgen levels or its metabolism following alcohol injection. That the redox milieu of the sperms after treatment is reduced, is evident from the fact that recovery in the levels of total ascorbic acid occurs upon feeding vitamin C to ethanol injected animals.

PART III

1. Effects of protein deficiency in albino rats

The protein free diet caused decrease in sperm metabolism, lack of mating behaviour, loss of libido
and severe abnormalities particularly in the acrosome and middle piece regions of the epididymal spermatozoa leading to 100% infertility in the animals.

The feeding of ascorbic acid to the protein deficient rats brought about a marked recovery in the levels of some androgenic parameters. The results suggest that ascorbic acid has a beneficial influence for the maintenance of the metabolism of spermatozoa in acute protein deficiency although it does not interfere with the antifertility effects of the treatment as fertility was not restored by feeding vitamin C.

2. Effects of vitamin C deficiency in guinea pigs

Vitamin C deficiency resulted in decreased levels of total ascorbic acid and dehydroascorbic acid in both TSS and ESS. The concentration of glutathione increased in TSS but declined in the ESS which may be one of the causative factors for the decrease in the motility of the epididymal spermatozoa. The increase in reduced ascorbic acid concentration in both sperm suspensions is correlated with its probable transport from storage organs via blood to sites of active metabolism such as testis and epididymis.
The 50% reduction in fertility has been correlated with the more intense rouleau formation and changes in acrosomal structure in vitamin C deficient animals. The alteration in epididymal milieu and androgenicity of testis in scorbutic guinea pigs may also be responsible for the changes observed in the sperm morphology and decline in fertility.