SUMMARY

CHAPTER I:

Certain follicles large and small, simple and sacculated, single and in groups with dense colloid inside were observed in the connective tissue of the various tissues studied except that of muscle of the freshwater crab, Paratelphusa jacquemontii. The diameter and the shape of these follicles varied widely even in the same animal and comparatively more follicles were seen in males than in females. An increase in the number and size of the follicles was noted prior to spermatogenesis and aestivation in males and only before aestivation in females. The colloid inside the follicles was mostly acidophilic in nature, was tyrosine rich, and showed strong affinity towards PAS reaction and Ferric ferricyanide test. Antithyroid compound like thiourea had a remarkable decreasing or destroying effect on these follicles and the colloid content, whereas eye-stalk removal was without any effect on them. The colloid showed a tendency to incorporate labelled iodine when subjected to autoradiographic experiments, thereby indicating that they were thyroidal in function. Considering all these various histological and histochemical staining reactions and properties these follicles are comparable to the thyroid follicles of higher animals.

CHAPTER 2:

Incorporation of labelled iodine into the follicles present in the various tissues of the crab Paratelphusa jacquemontii, was studied. The animals were given a tracer dose of 25 to 35 μc of carrier free I^{131} and the various tissues such as the hepatopancreas, gonads, brain, thoracic ganglion and muscle were utilized for the autoradiographic studies and radioactive countings. Radioautographic studies showed that the incorporation of I^{131} in the thyroid-like follicles. The radioactive assay gave remarkably high radioactive countings in almost all the tissues excepting muscle which was taken as the control tissue where no follicles were present. The radio activity was comparatively more in the tissues of the male crabs than in those of the females. The results obtained were based on the amount of protein present in the tissue which was estimated colorimetrically. The significance of the incorporation of I^{131} into the thyroid-like follicles of the various tissues of the crab are discussed.

CHAPTER 3:

Since it is known that the crab's hepatopancreas which is comparable to the vertebrate liver is the main storage organ of protein, fat and glycogen, seasonal variation in these metabolites in the hepatopancreas was studied. Histochemical observations on the cyclic changes in the fat content

of the hepatopancreas were also carried out. Blood glucose which is the transporting form of glycogen was estimated seasonally. It was seen that the chief energy reserve in the hepatopancreas was fat. Histochemical observations on fat showed an increase in the storage of triglycerides towards the aestivation period in summer with a substantial decrease in the water content, whereas after the aestivation in June/July only acidic fat was seen in the tubules and in the connective tissue, indicating the utilization of stored fat during aestivation. Comparatively high protein content was observed in July, soon after the aestivation. Blood glucose was also high in June/July which might help in the formation of new chitin prior to moulting during this period. Glycogen content of the hepatopancreas was comparatively high in September/October as a source of energy by supplying the necessary oxaloacetate for the metabolic requirements.

CHAPTER 4:

A quantitative and histochemical study of the enzyme lipase which catalyses the hydrolysis of fat into fatty acids and glycerol was carried out in the hepatopancreas. Histochemically the enzyme was localized with the help of "Tween 85" as the substrate. For the quantitative estimations, triolein, a long chain fatty acid ester and tributyrin, a short chain fatty acid ester were used as the substrates.

Since true lipases are known to hydrolyse the glycerides of long chain fatty acids, the lipase activity obtained with triolein was considered as that of a 'true lipase'. The result of the histochemical observations using "Tween 85" as the substrate was comparable with that of triolein. So the lipase activity obtained in the hepatopancreas was found to be that of a true lipase.

CHAPTER 5:

Cyclic changes in the fat content of the gonads were studied histochemically. After the breeding season a lesser amount of fat deposition was seen in the testis as well as in the ovary which was acidic in nature. Before the onset of spermatogenesis, in early August the seminiferous tubules showed acidic fat and the interstitium contained neutral fat which was strongly positive to the Schultz's cholesterol test. After the sperm formation the spermatophores inside the tubules showed the presence of neutral fat and the interstitial fat decreased considerably. The neutral fat in the tubules showed a reduction in amount towards the aestivation and the preceding breeding period as the neutral fat containing spermatophores were transferred from the tubules to the vas deferens.

In the immature ovary the developing oocytes contained only acidic fat while, the fully mature ova were found to

be filled with numerous fat globules of triglycerides. Cholesterol positive lipids were also seen in the degenerating cells of the immature ovary.

The phospholipid content of both testis as well as ovary increased considerably towards the maturation of eggs and sperms. These cyclic changes in the types of lipids in the gonads are discussed in relation to the formation and development of the eggs and sperms.

CHAPTER 6:

The principla enzymes of lipid metabolism, lipases and esterases were studied histochemically in the male and female gonads using different "Tweens" as substrates. An increase in the lipase as well as the esterase activity was observed in the seminiferous tubules towards the onset of spermatogenesis in August. During this period the lipase activity with "Tween 85" was more intense in the interstitium when compared to the esterase activity with the lower "Tweens". The spermatophores inside the tubules showed only the lipase activity and no esterase activity. After the sperm formation a decreased lipase activity was observed all over the testis, whereas the interstitial connective tissue showed considerably high esterase activity. In the seminiferous tubule the esterase activity was moderately uniform.

In the immature ovary the cytoplasm of the small developing oocytes as well as the ovarian stroma showed uniform lipase activity. In the degenerating cells of the immature ovary only esterase activity was seen. In the developing ova the peripheral neutral fat globules were the sites of lipase activity, whereas the fully mature yolk laden ova showed a decreased activity and that too only at the egg cortex. The localization and distribution of these enzymes generally followed the pattern of fat indicating the utilization of fat by these tissues and are discussed in relation to the development of gonads.

CHAPTER 7:

Histochemical demonstration of acid and alkaline phosphatase activities in the gonads were carried out by the Naphthol AS phosphate method described by Burstone (1958a, 1961). For acid and alkaline phosphatases Naphthol AS-BI and Naphthol AS-MX were used as the substrates respectively. Fast blue B (BBN) was used as the coupling dye in both the cases. The spermatozoa showed a very high acid phosphatase activity, whereas the basal membrane and the spermatogenic cells were moderately active after the spermatogenesis. The interstitial connective tissue was completely negative to the acid phosphatase activity. But the spermatogenic cells and the interstitial connective tissue showed moderate enzyme activity in July, just after

the aestivation or before the starting of spermatogenesis.

No acid phosphatase activity was demonstrable in the various stages of formation of the ova. Very low activity was noted in certain fat globules of the fully yolk laden ova.

The lakaline phosphatase activity was comparatively less in the testis than the acid phosphatase activity. The spermatozoa showed less activity, whereas the interstitium and the germinal cells of the tubules showed uniform, moderate activity after the spermatogenesis. The thyroid-like follicles present in the interstitium showed high activity. Prior to aestivation, in April the seminiferous tubules and the spermatozoa showed high activity while the interstitium was less active. After the aestivation period less activity was seen in the tubules.

The ovarian stroma and the egg cytoplasm showed a faint activity in the early stages of development. As the ova attained maturity the peripheral cortical region of the egg cytoplasm showed increased activity. The germinal epithelium and the connective tissue showed moderate,uniform activity throughout the period of ova formation. The localization of this enzyme followed the pattern of fat and are discussed in relation to the fat metabolism during the various stages of gonadal development.

CHAPTER 8:

Histological features of the five different types of neurosecretory cells in the central nervous system were studied. The neurosecretory cells, particularly A-type cells showed thickly packed neurosecretory granules in the cytoplasm and large nucleus with conspicuous nucleolus soon after the aestivation or prior to the onset of gametogenesis, in early August. With the initiation of spermatogenesis and oogenesis numerous large neurosecretory releasing vacuoles started appearing at the periphery of the cells showing the neurosecretory discharging activity, and the cells showed a shrunken appearance. A considerable increase in the number of thyroid-like follicles was noted in the peripheral connective tissue of the brain, thoracic ganglion and the eye-stalk ganglion as well as in the other tissues studied viz: the hepatopancreas and gonads. The height of the neurosecretory activity was seen towards the later stage of spermatogenesis and oogenesis, and during this period the bulbous axon terminations of the sinus gland were filled with neurosecretory material. The resynthesis of the neurosecretory material was found to start in March/April i.e. prior to aestivation. Again an increase in the thyroid-like follicles was noted in the hepatopancreas and gonads during this period. The neurosecretory storage-release activity, the formation of the thyroid-like follicles and the gonadal development are correlated and discussed.