CHAPTER 6

HISTOCHEMICAL LOCALIZATION OF LIPASE AND ESTERASE IN THE GONADS

There exists an extensive literature dealing with the lipase and esterase activities in the gonads of various vertebrates but comparatively little is known about these enzymes in the gonads of invertebrates, especially of crustaceans. It is well known that lipids do have an important role in the metabolism of the gonads, in the production of eggs and sperms. Ambadkar (1965) studied the distribution of lipids and the localization and distribution of lipase, an enzyme of primary importance in lipid metabolism, in the testis of certain vertebrates belonging to the various classes of Vertebrata. In the rat testis, George and Ambadkar (1963), observed that both the distribution of the enzyme as well as the substrate follow a definite parallel pattern, and the physiological significance of the enzyme lipase in the lipid metabolism of the testis was thus revealed. Since the available literature on this subject in crustaceans is very scanty, it was thought desirable to make a study of the fat splitting enzymes, lipases and esterases. Such an attempt should also help to obtain a better understanding of the utilization of fat by the gonads during the different stages of the gonadal development, based on cyclic changes in the fat distribution presented earlier (Chapter 5).

MATERIAL AND METHODS

Common freshwater crabs, Paratelphusa jacquemontii, collected from local rivers were used. The animals were killed and the gonads were quickly dissected out and fixed in 6% buffered formalin at 4°C, for a period of 16 hours. Gelatine blocks were prepared and 10 μ thick sections were cut on a freezing microtome. The sections were washed in distilled water and treated for the histochemical demonstration of esterases and lipases, employing the Tween method of Gomori (1953) - "Tween 85" which was shown to be a specific substrate for "true lipase" in rat testis (George and Ambadkar 1963), was used as the substrate to demonstrate the lipase activity. Non-specific esterases were demonstrated with the help of lower Tweens, having shorter chain length as the substrates viz., Tween 20, 40, 60 and 80.

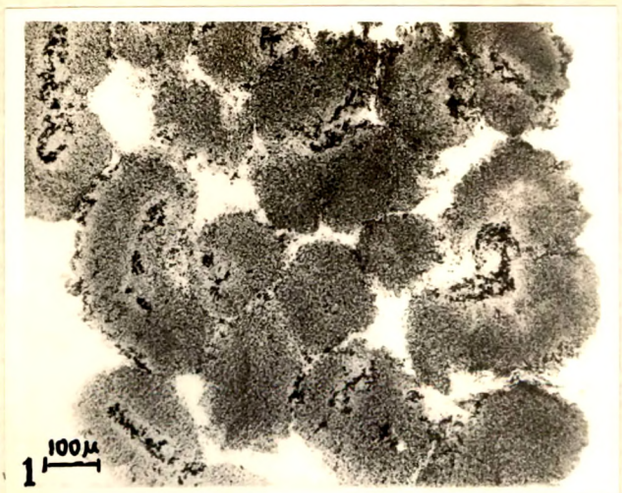
OBSERVATIONS

In the present study the enzyme activity obtained with "Tween 85" is considered as that of a true lipase and the enzyme activity obtained with the lower 'Tweens' as that of non-specific esterases. The enzyme activity is demonstrated as brownish black precipitate at the sites where the enzyme was localized.

In June, soon after the aestivation, the sections of testis treated with "Tween 85" showed moderate uniform

activity in the tubules and comparatively less activity in the interstitial connective tissue (Fig. 1). With the lower "Tweens" the interstitium did not show any activity and the seminiferous tubules possessed lower enzyme activity except with "Tween 80", which showed a combined esterase and lipase activity (Fig. 2). However, an increase in the lipase as well as esterase activities was noted towards the beginning of spermatogenesis. During the early stages of spermatogenesis,the tubules showed fairly high concentrations of both the enzymes. Among the various Tweens used the activity was more intense with "Tween 85" in the interstitium (Fig. 3). Enzyme activity with "Tween 80" and other lower "Tweens" was comparatively more in the tubules than in the interstitium (Fig. 4). Towards the late spermatogenesis the spermatozoa in the centre of the tubules as well as the peripheral germinal epithelial cells of the tubules showed high lipase activity (Fig. 5). But esterase activity was seen only in the peripheral cells of the tubule and slightly in the interstitium. The spermatozoa showed no noticeable esterase activity (Fig. 6). After the sperm formation lipase activity was much reduced in the tubules and in the interstitium (Fig. 7). But increased esterase activity was noted in the interstitium and tubules during that period (Fig. 8).

As for the ovary in the immature stage, the small developing ova showed uniformly distributed precipitate and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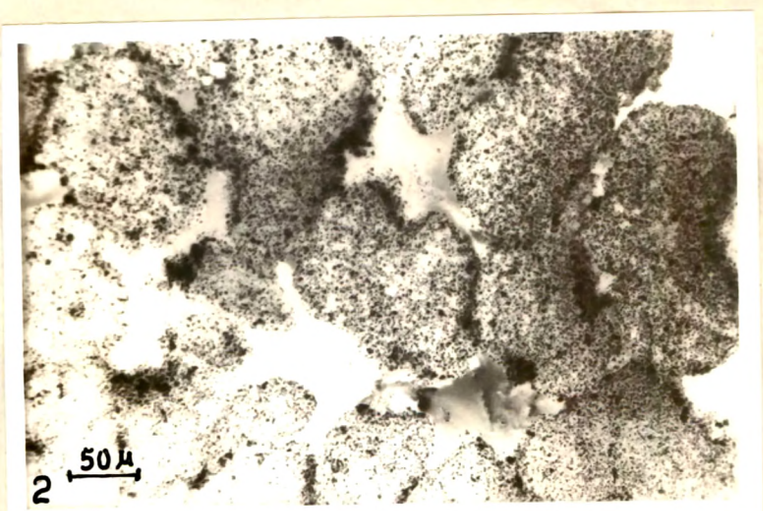
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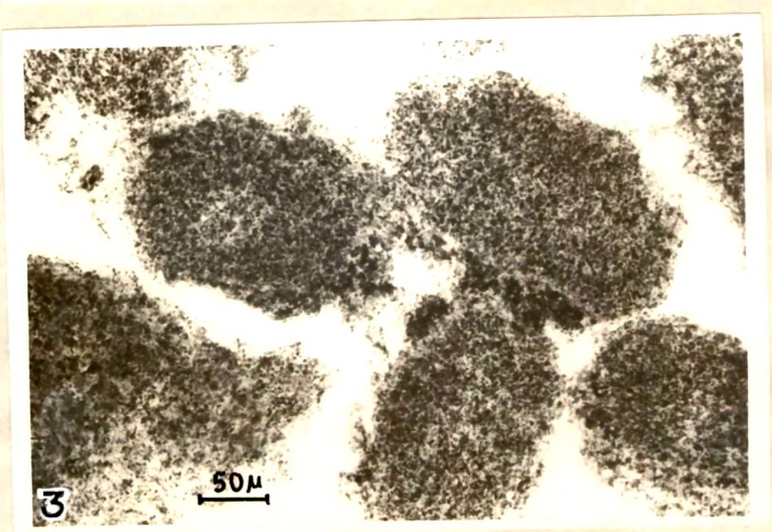
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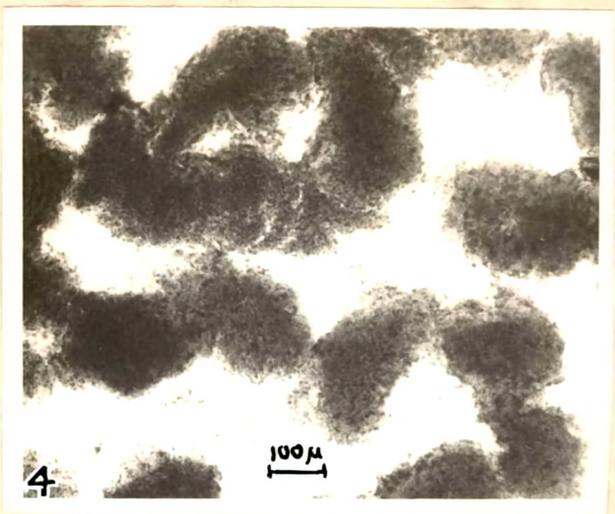
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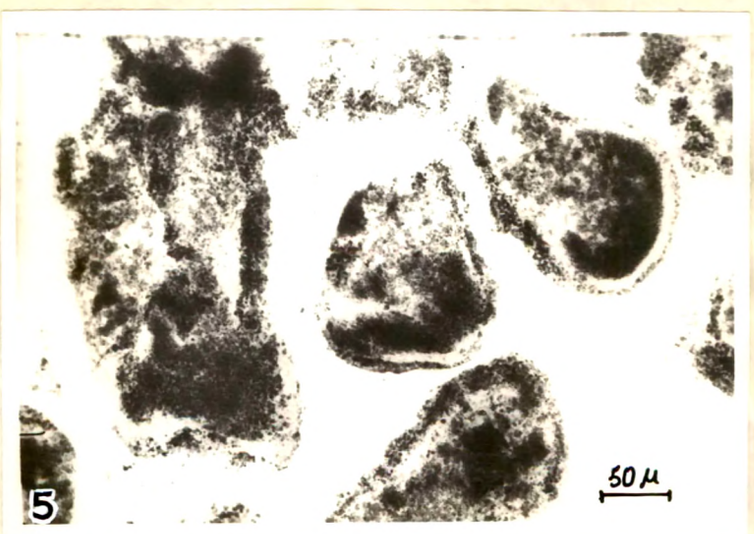
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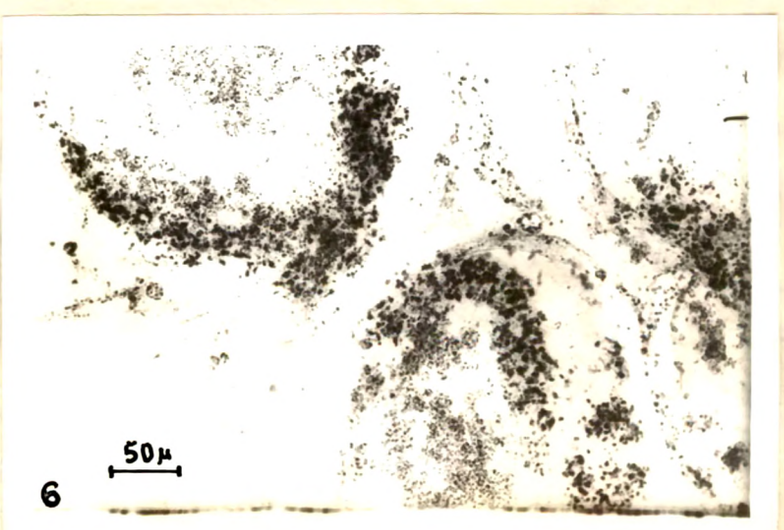
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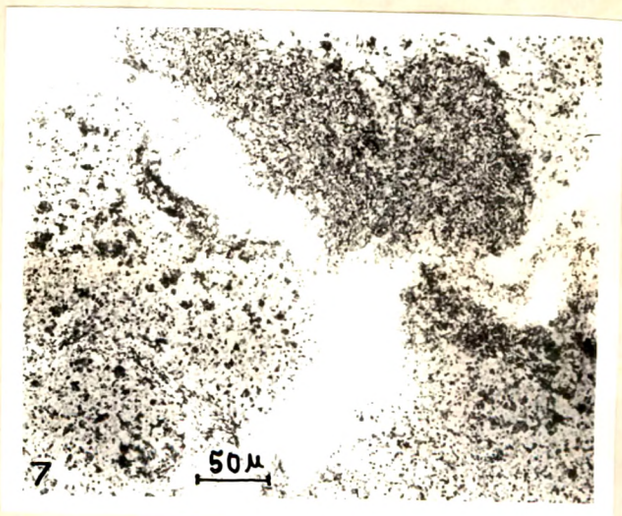
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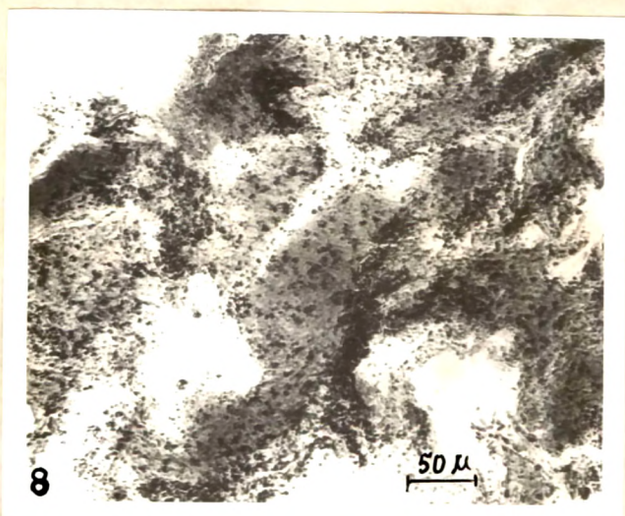
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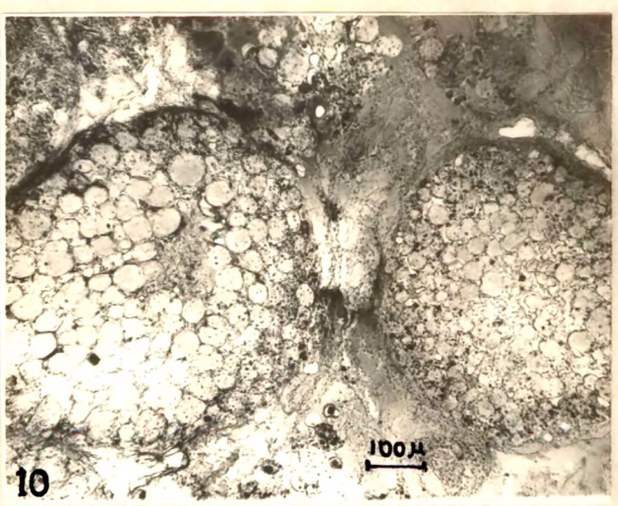
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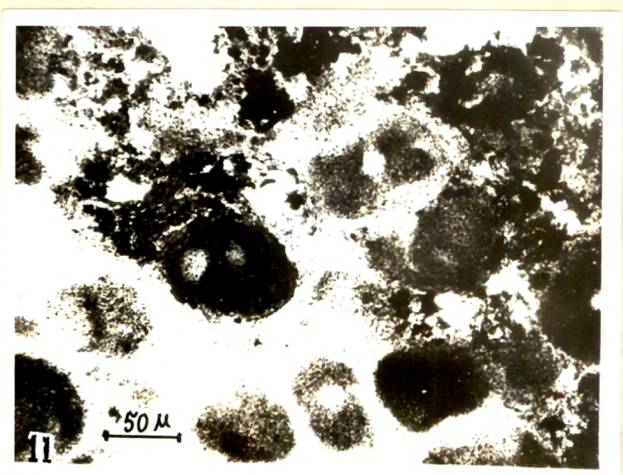
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EXPLANATION TO THE FIGURES

Fig. 1. T.S. of testis (July) showing uniform lipase activity (T. 85) in the tubules and in the interstitial connective tissue.

Fig. 2. T.S. of testis (July) showing esterase activity (With lower Tweens) in the tubules. The interstitium is negative to the enzyme reaction.

Fig. 3. T.S. of testis showing high lipase activity towards the beginning of spermatogenesis.

Fig. 4. T.S. of testis showing high esterase activity (with lower Tweens) towards the beginning of spermatogenesis. Note the interstitium is completely negative.

Fig. 5. T.S. of testis showing high lipase activity towards the late spermatogenesis. Note the activity is seen only in the spermatozoa in the centre of the tubules and at the periphery of the tubules. No activity in the interstitium.

Fig. 6. T.S. of testis showing slight esterase activity (with lower Tweens) only at the periphery of the tubule and in the interstitium. Note the absence of enzyme activity in the spermatozoa.

Fig. 7. T.S. of testis showing less lipase activity after the completion of the spermatogenesis.

Fig. 8. T.S. of testis showing comparatively high esterase activity after the completion of the spermatogenesis.

Fig. 9. T.S. of ovary showing uniform lipase activity in the developing ova and in the ovarian connective tissue.

Fig.10. T.S. of ovary showing high lipase activity in the stroma of the ovary before the completion of the vacuolization in the egg cytoplasm.

Fig.11. T.S. of ovary showing high esterase activity (with lower Tweens) in the ovarian stroma before the vacuolization in the egg cytoplasm.

the ovarian connective tissue also showed moderate uniform activity (Fig. 9). The egg nucleus was completely devoid of any enzyme activity. In the gradually developing ova the peripheral fat globules at the cortical region showed high lipase activity whereas the medullary portion showed lower uniformly distributed granules. In the fully yolk laden ova only slight activity was noted at the cortex, and the germinal epithelium showed decreased activity. When the vacuolization started in the egg cytoplasm in September, the stroma showed high lipase activity (Fig. 10). The degenerating cells present in the immature ovary contained high esterase activity (Fig. 11), while lipase activity was not so intense in those cells.

DISCUSSION

It has been shown by George and Ambadkar (1963), that in rat testis "Tween 85" is by and large a specific substrate for the histochemical demonstration of lipase activity, since the enzyme activity was not inhibited by sodiumtaurocholate, which is known to inactivate non-specific esterases and activate true lipase. Wallach, Ko and Marshall (1962) isolated and purified "Tween hydrolysing" enzymes from the rate adipose tissue and showed that this enzyme readily hydrolyses "Tween 20" but had no effect on "Tween 85". This also supports the view that "Tween 85" is acted upon only by a true lipase, and

not by non-specific esterases. There is a controversy regarding the substrate specificity of water soluble substrates in recent years. Even though the "Tweens" are water soluble substrates, quantitatively "Tween 85" (Polyoxyethylene sorbitan trioleate) showed a comparable enzyme activity to the activity obtained with triolein which is known to be hydrolysed only by the enzyme lipase being a long chain fatty acid ester (Chapter 4). Esterases are enzymes which hydrolyse the glycerides of short chain fatty acids, and are not appreciably active towards the true fat glycerides of long chain fatty acids, which are hydrolysed by lipases.

After the aestivation period when the animals started active feeding, considerable amounts of interstitial neutral fat was seen (Chapter 5). The presence of an enzyme capable of hydrolysing "Tween 85" at these sites indicate that the neutral fat was easily hydrolysable in the interstitium. The localization of enzyme activity in the cellular elements of the germinal epithelium of the seminiferous tubules was almost the same with both Tweens 85 and 80, but not with lower "Tweens". This difference in the interstitial activity on different Tweens is interesting. Due to the active feeding after aestivation an increase in the lipid content in the seminiferous tubules followed by an increase in both lipase and esterase activities was noted towards the early stages of

spermatogenesis. This increase in the substrate followed by the increase in the hydrolysing enzymes in the early stages of gonodal development indicates the possibility of lipids being utilized for the production of the spermatogenic elements. It should be mentioned that during this period lipid from other sources were also being utilized for the same purpose e.g., a reduction in the stored lipids of the hepatopancreas (Chapter 3) with a parallel increase in lipase activity (Chapter 4) was noted.

In later stages of spermatogenesis, the spermatophores move towards the centre of the seminiferous tubules. These spermatophores contained neutral fat and showed high lipase activity with "Tween 85". The germinal epithelial cells of the seminiferous tubules showed no neutral fat and this region was negative to the enzyme reaction. Only the peripheral portion showed slight activity. The spermatophores in the centre of the seminiferous tubule did not show any esterase activity. So the fat contained in the spermatophores, was hydrolysable only by "Tween 85" specific lipase present in the spermatophores. The few fat globules present in the interstitium were the sites for remarkably high esterase activity. It could be assumed from this observation that the fat which is present in the interstitium may be glycerides of short-chain fatty acids as indicated by the nature of enzyme active on lower "Tweens".

In the immature ovary, also lipids are being utilized during the early stages, for the production and development of ova, whereas in the later stages fat is stored as large globules in the mature ova. In the immature developing ova, only acidic fat was seen (Chapter 5) and a uniformly distributed brownish black precipitate denotes the enzyme activity in the developing ova and in ovarian connective tissue. The presence of acidic lipid together with hydrolysing enzyme indicates the possibility of lipids being synthesised and utilized for the development and growth of the ova. In the later stages, i.e., when the ova becomes gradually mature and the yolk deposition starts taking place, the lipase activity decreases considerably because at that time lipids are being stored up in the ova and not being utilized. In the fully yolk laden ova only at the periphery of the egg cortex slight enzyme activity was seen. Before the vacuolization of the fat starts in the ova, a great amount of acidic fat was seen in the connective tissue surrounding each ova (Chapter 5). An increased lipase and esterase activity was also noted at that time. There is the possibility that fat present in the connective tissue might have been hydrolysed by these enzymes and utilized for storing in the vacuoles formed in the egg cytoplasm. The presence of high esterase activity in the degenerating cells as well as the presence of cholesterol positive lipids in these cells

(Chapter 5) is worthy of consideration, since lipids are known to be utilized in the synthesis of hormones such as steroid hormones, and thereby giving support to the attributed function of ovary, as an endocrine organ.