# CHAPTER V

## HISTOCHEMICAL LOCALIZATION OF LIPIDS AND LIPASE IN THE TESTES OF CERTAIN VERTEBRATES

The localization of lipids, and seasonal changes in the pattern of distribution in the testes of various vertebrates, have been studied by earlier investigators (Oslund, 1928; Long and Engle, 1952; Montagna, 1952; Melampy and Cavazos, 1954; Marshall and Lofts, 1956; Lofts and Marshall, 1957; Marshall and Woolf, 1957; Lofts and Boswell, 1960; Lofts and Boswell, 1961; van Oordt and Lofts, 1963). In a recent study Lofts (1964) has described cyclical changes in lipid in the Leydig and Sertoli cells of Rana esculenta, and has correlated the appearance of the secretory lipid globules in the Leydig cells with the development of the thumb-pads. According to this author the post-nuptial accumulation of lipid globules and cholesterol in the interstitial cells, was due to the cessation of male hormone production and the appearance of cholesterol-positive lipid droplets in the Sertoli cells was found to coincide with the process of spermiation. In the late post-nuptial period, however, he observed the lipid laden Sertoli cells to be getting detached and passing down the tubular lumen. This was found to coincide with decreased interstitial cells stimulating hormone (ICSH) out-put and initiation of follicle stimulating hormone (FSH) secretion from the anterior pituitary.

Miller (1959) reviewed the literature on lipids and their distribution pattern in Leydig cells of reptiles and the changes occurring in the seasonal breeders. The significance of the refractory period as a preparatory phase in bird migration was discussed by Marshall (1951) on the basis of the cyclical changes in the lipids occurring in the Leydig cells and seminiferous tubules. Among mammals, studies on the variations in the distribution of lipids in the Leydig cells and the seminiferous tubules have been carried out in a single species of a seasonal mammal - Talpa europea (Lofts, 1960).

In the light of the observations mentioned above and those discussed in chapter I, it is evident that lipids do have an important role in the metabolism of the testis. It was, therefore, thought desirable to extend the earlier investigations to a study of the localization and distribution of lipase, an enzyme of primary importance in lipid metabolism, in the testes of certain vertebrates. In conjunction with this, a reinvestigation of the distribution of lipids was also undertaken in order to obtain an integrated picture of the localization of the substrate as well as the enzyme.

## MATERIAL AND METHODS

In the present investigation the following vertebrates were used. Indian bull frog - Rana tigrina, the common garden lizard - Calotes versicolour, a migratory starling (Rosy pastor) - Sturnus roseus, the house sparrow - Passer

domesticus, the blue rock pigeon - Columba livia, the rabbit Oryctolagus ~~Lepus~~ cuniculus, the cat - Felis domesticus, the dog - Canis familiaris, the sheep - Ovis sp., and the rhesus monkey - Macacus rhesus. Of these animals the frog and the garden lizard were procured during their breeding as well as non-breeding periods. The rosy pastors arrive in Baroda (India) during August/September from their breeding grounds abroad (probably Middle East and South Europe and U.S.S.R.) and leave for breeding in April. These birds were captured in September (post migratory phase) and in April (pre migratory phase). In the house sparrow and pigeon the testes showed active spermatogenesis throughout the year, except during winter (December). In these birds the testis was studied only during the active period. The testes of the rabbit, dog and sheep were obtained during the breeding season. In the case of the cat the testis was studied at the time when the breeding season was approaching. The monkey testis was examined during the period of sexual inactivity.

Frogs and lizards were freshly collected, killed and the testes were fixed. The birds were shot with an air rifle and brought to the laboratory within 20 minutes, after which the testes were carefully removed. The testes of the rabbit, cat, dog and monkey were removed under anaesthesia.

In all cases the glands were first frozen to -10°C immediately after removal. The methods employed for the demonstration of lipids and lipase activity were the same as those described in chapter I.

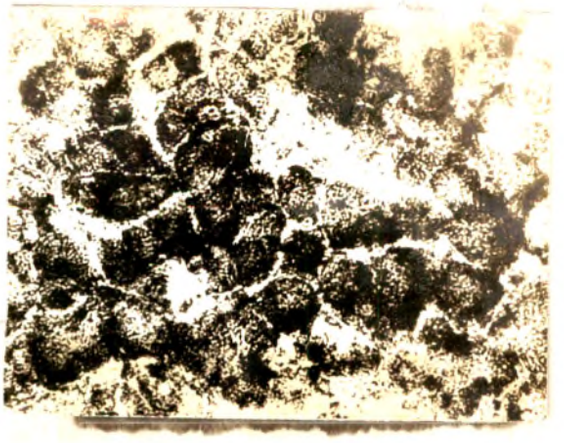
## RESULTS

Frog testis

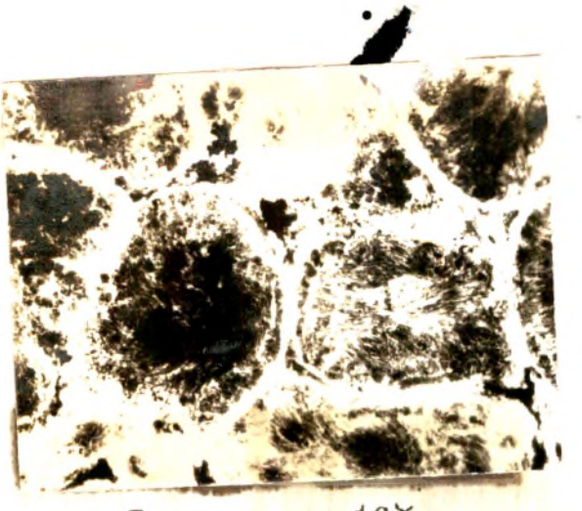
(a) Non-breeding season : The testes were found to be small cylindrical bodies containing brownish-green pigment cells. The diameter of the seminiferous tubules was about 102 μ. The Leydig cells had a considerable number of lipid globules whereas the seminiferous tubules were almost devoid of them. Most of the tubules contained cell nests having spermatogonia and one or two generations of spermatocytes. A few tubules had some spermatozoa adhering to the Sertoli cells.

The Leydig cells had a number of sudanophilic globules. A few such globules were also present in the Sertoli cells and the spermatogonia. The rest of the germinal elements were devoid of lipid globules. Where the spermatozoa occurred attached to the Sertoli cells, fine lipid globules were present near and around the spermatozoa. Lipase activity was negligible in the seminiferous tubules, excepting the Sertoli cells. The Leydig cells showed high lipase activity.

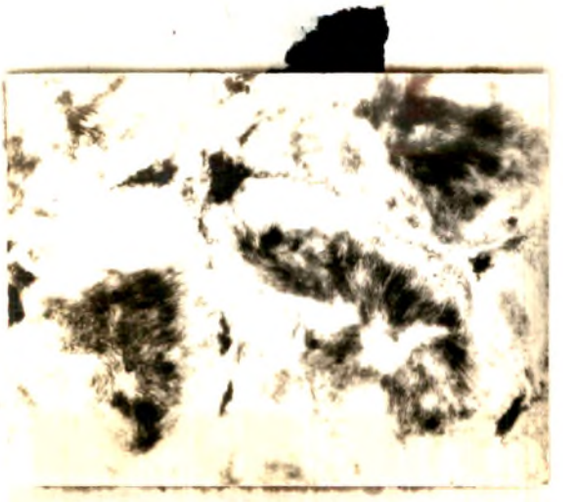
(b) Breeding season : The testes were much enlarged and the average tubule diameter was 150 μ. All the seminiferous tubules had many groups of spermatozoa attached to the Sertoli cells. The chromatophores were much dispersed and the testes were pale yellow in colour. In the central part of the testis many chromatophores were present in the interstitial tissue. Spermiation had, however, not occurred.



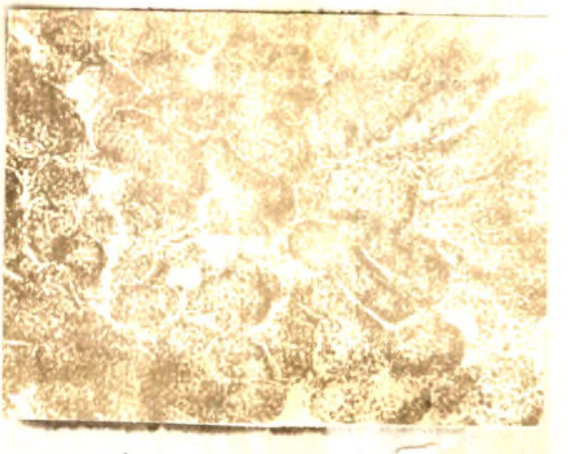
1 35×



3 10×



2 35

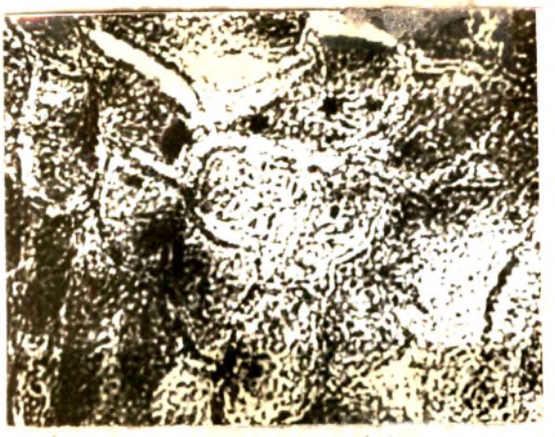


4

Photomicrographs of the sections of the frog testes.

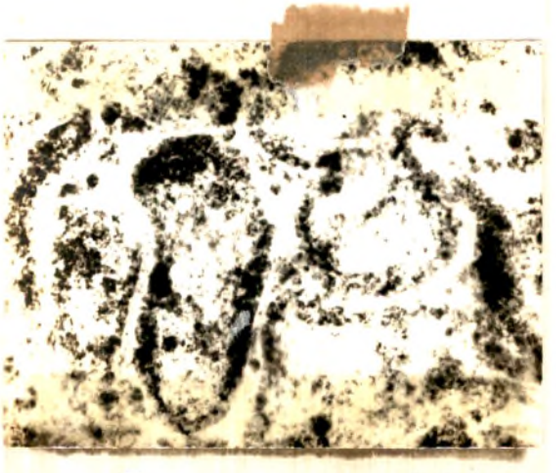
Figs. 1 & 3. Showing the distribution of lipids in the breeding and the non-breeding seasons, respectively.

Figs. 2 & 4. Showing the pattern of lipase activity in the breeding and the non-breeding seasons, respectively.

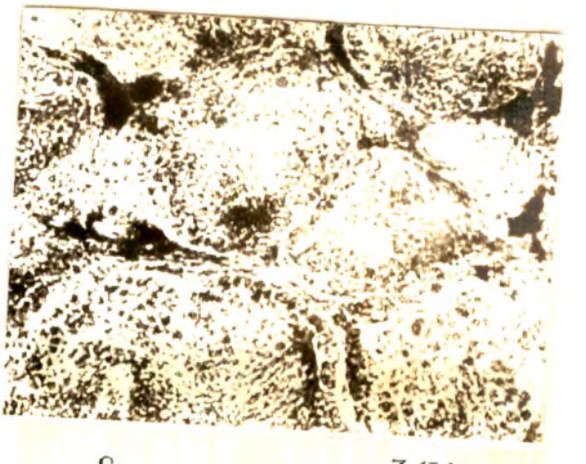


5 10×

6 10×



7 35×



8 35×

Photomicrographs of the sections of the calotes testes.

Figs. **5** & **7**. Showing the distribution of the lipids in the breeding and the non-breeding seasons, respectively.

Figs. **6** & **8**. Showing the pattern of lipase activity in the breeding and the non-breeding seasons, respectively.

The interstitial Leydig cells possessed fine sudanophilic globules in the cytoplasm but the number and size of such globules was much more reduced than that found in the non-breeding season. The cells of the nests and the Sertoli cells showed a few lipid globules in the cytoplasm while the rest of the germinal elements in the tubules showed negligible sudanophilia. Leydig cells possessed a considerably high lipase activity. In the seminiferous tubules only the Sertoli cells and the spermatogonial cells were observed to have lipase activity. The mid-pieces of the spermatozoa showed low enzyme activity.

Calotes testis

(a) Non-breeding season : The testes were very small and bright golden yellow in colour. The pigment was found to be dissolved in lipid globules dispersed in the interstitial tissue. The average tubule diameter was about 45 to 50 μ and the tubules were found to possess thick walls measuring 20 μ.

The cytoplasm of the Leydig cells was found to be loaded with sudanophilic globules. The seminiferous tubules possessed only the Sertoli cells and a single layer of spermatogonia both of which showed the presence of fine sudanophilic globules in the basal portions of the cells. In addition to this there were some brown spherical bodies in the apical portions of these cells but such bodies were non-sudanophilic. High lipase activity was present in the interstitial cells but the cells in the seminiferous tubules had low enzyme activity.

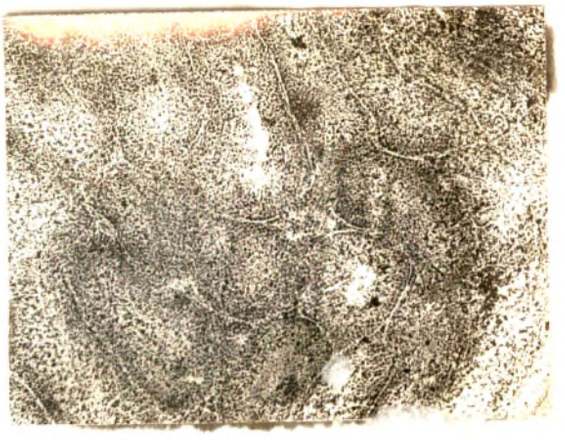
(b) Breeding season : The glands were much enlarged and the diameter of the seminiferous tubules varied between 220 to 250 μ. The basement membrane of the seminiferous tubules, which was about 20 μ thick in the non-breeding season, was hardly discernible in this period. The testes had very little of pigment and appeared pale yellow in colour. The seminiferous tubules showed all the spermatogenetic stages as well as mature spermatozoa. The Leydig cells were compressed in the corners of the widened tubules and were seen as small clumps or rows of few cells.

At this stage also the Leydig cells showed the presence of considerable number of lipid globules. Sertoli cells and spermatogonia also were richly sudanophilic. There were few sudanophilic globules in the center of the lumen of the seminiferous tubules. Lipase activity was present in the Leydig and Sertoli cells and in the spermatogonia. Other spermatogenetic cells showed low enzyme activity.

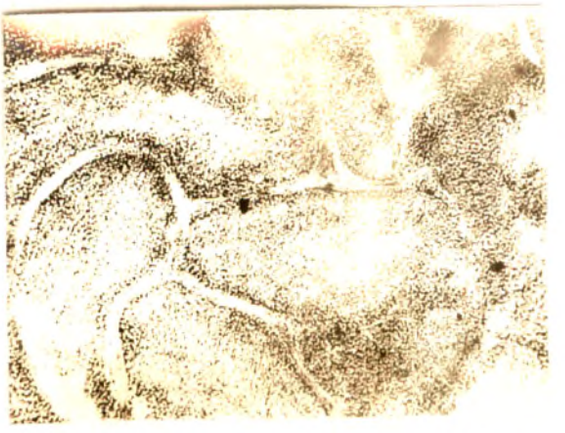
Rosy pastor testis

(a) Non-breeding season (post migratory phase) : Small recrudescent testes of these birds were found to contain numerous dark brown chromatophores in the interstitium. The seminiferous tubules were about 40 μ wide and contained only the Sertoli cells and a single layer of spermatogonia. Leydig cells were found in groups inbetween the chromatophores.

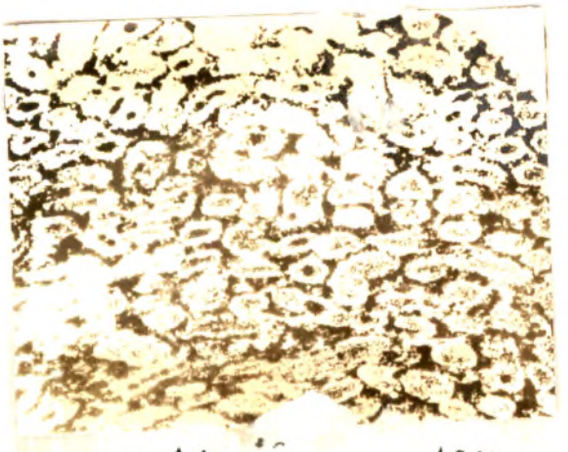
The Leydig cells contained many lipid globules.



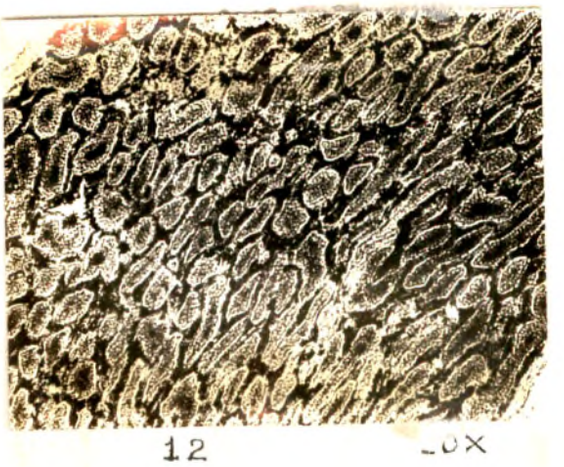
9 ×



10 10×



11 10×



12 10×

Photomicrographs of the sections of the Rosy Pastor testes.

Figs. 9 & 11. Showing the distribution of lipids in the breeding and the non-breeding seasons respectively.

Figs. 10 & 12. Showing the distribution pattern of the lipase activity in the breeding (pre-migratory period) and the non-breedin (post-migratory period) seasons, respectively.

The Sertoli cells and spermatogonia had few lipid globules. Lipase activity was very low in the Sertoli cells and the spermatogonia, but the Leydig cells showed enzyme activity which was mostly that of a non-specific esterase.

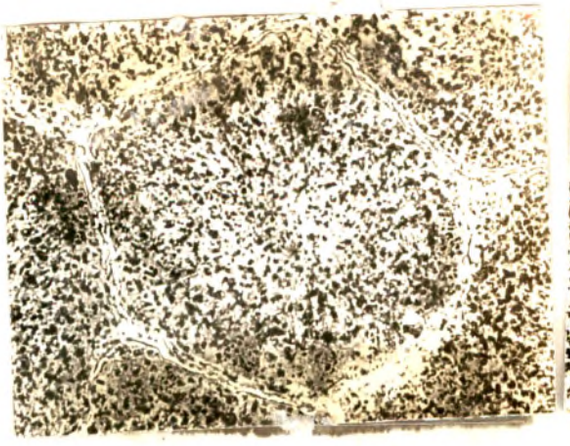
(b) Pre-breeding season (pre migratory phase) : The testes were very much enlarged and the chromatophores were widely dispersed to the extent that the glands were colourless. The average diameter of the seminiferous tubules was 175 μ. All spermatogenetic stages upto spermatids were observed, a few tubules showed the presence of spermatozoa also. The Leydig cells were compressed at the corners.

At this stage also the Leydig cells possessed lipid globules. Sudanophilia was observed in all the cells of the seminiferous tubules. Leydig cells at this time contained more of "true" lipase activity than that of the non-specific esterase. The pattern of lipase activity was similar to that of the lipids in the seminiferous tubules.

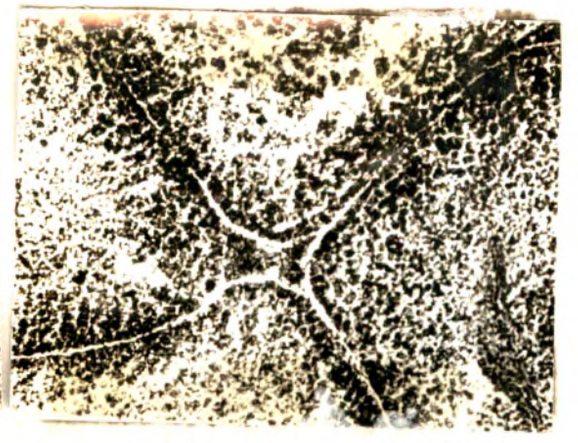
Pigeon testis

Sexually active period : The seminiferous tubules were enlarged and appeared polygonal in cross section and contained all the germinal cells including spermatozoa.

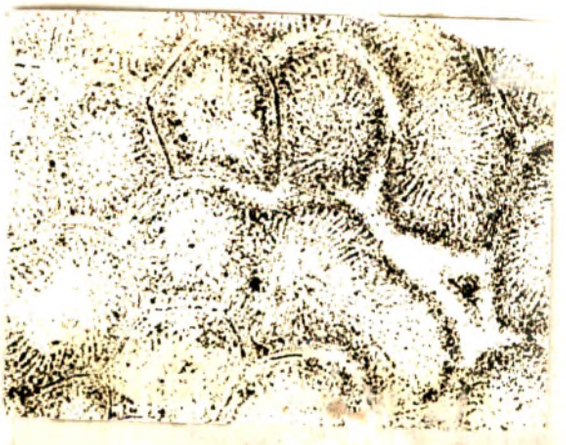
Leydig cells as well as the cells of the seminiferous tubules possessed sudanophilic cytoplasm. Lipase activity followed the distribution pattern of lipids in the interstitium and the seminiferous tubules.



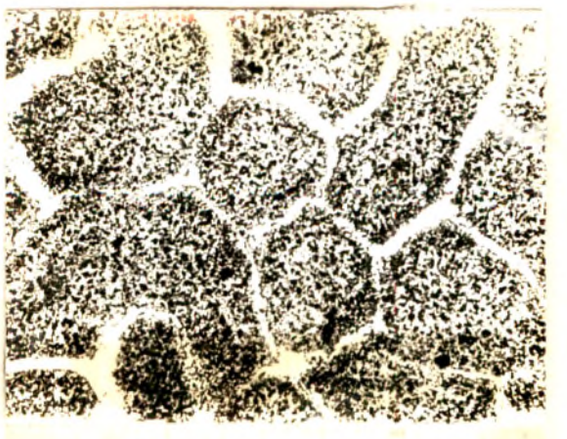
13 35×



14 35×



15



16 10×

Figs. 13 &14. Sections of the testes of pigeon showing the distribution patterns of lipids and lipase, respectively, during sexually active period.

Figs. 15 & 16. Sections of the testes of the house sparrow showing the distribution of the lipids and lipase respectively, during the sexually active period.

House sparrow testis

Sexually active period : The testes were spherical in shape and the seminiferous tubules appeared polygonal in cross section. All the germinal elements were present. The spermatozoa were seen in small bundles.

The Leydig cells possessed many lipid globules. All the cells of the seminiferous tubules were sudanophilic. Lipase activity was present in the Leydig cells as well as in all cells of the tubules.

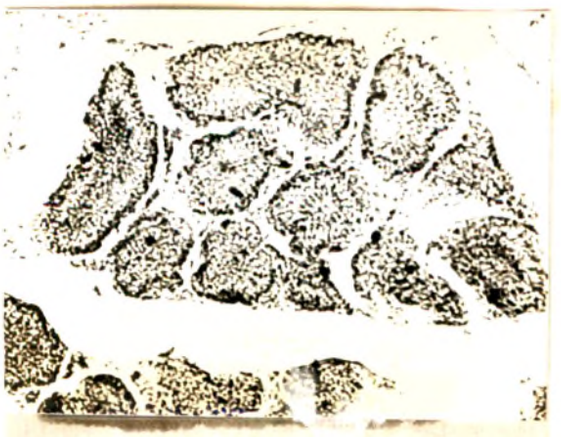
Rabbit testis

Breeding season : The average diameter of the seminiferous tubules was 190 μ and the tubules contained all the generations of spermatogenetic cells. Leydig cells were present as prominent groups in the interstitium.

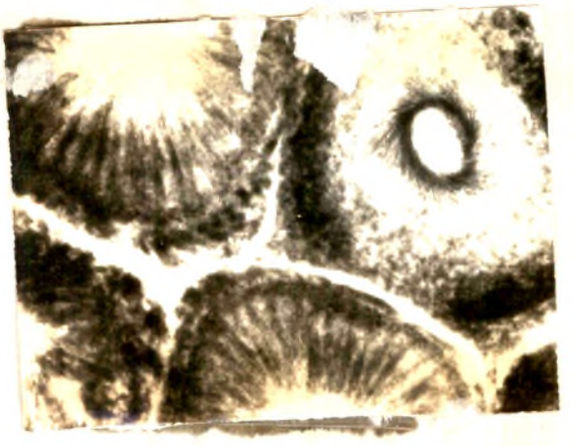
All the cells of the seminiferous tubules were sudanophilic. The Leydig cells contained more lipid material than any other type. Lipase activity was very high in the Leydig cells. The enzyme activity was also present in the cells of the tubules but at a lower level. Lipophanerosis was apparent and the residual bodies had lipids and only negligible lipase activity.

Dog testis

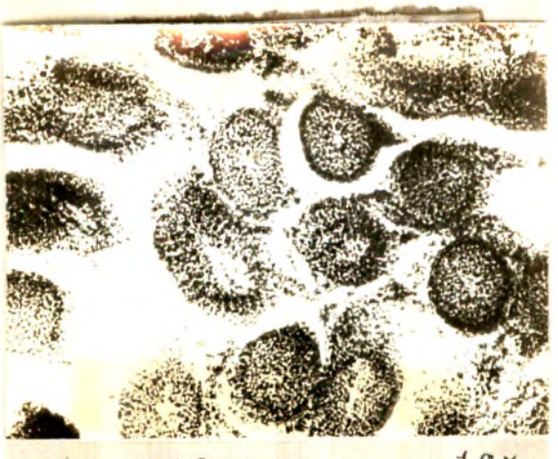
Breeding season : Average diameter of the seminiferous tubules was 190 μ. A high degree of sudanophilia was observed in the



17 10×



18 15×



19 10×

20 15×

Figs. 17 & 18. Showing the distribution of lipids and lipase of the rabbit testes, respectively, in the breeding season.

Figs. 19 & 20. Sections of the dog testes showing the lipids and lipase, respectively, in the breeding season.

Leydig cells, Sertoli cells, and the spermatogonia, but the rest of the cells in the tubules were only faintly sudanophilic. Lipase activity followed a parallel pattern. Residual bodies contained many lipid globules and very little of lipase activity.

Cat testis

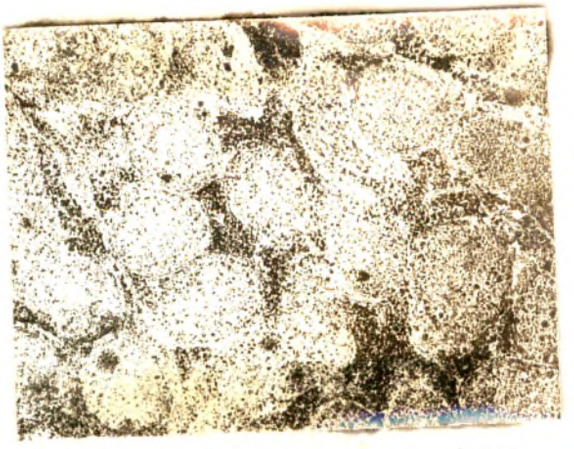
Near breeding season : The seminiferous tubules were about 175 μ wide and all stages in the germinal epithelium upto spermatids were observed. The Leydig cells were in groups as prominent patches in the interstitium which were observed to be loaded with lipids. All the germinal cells showed mi[illegible]udanophilia. Lipase activity followed an identical distribution pattern as of lipids.

Sheep testis

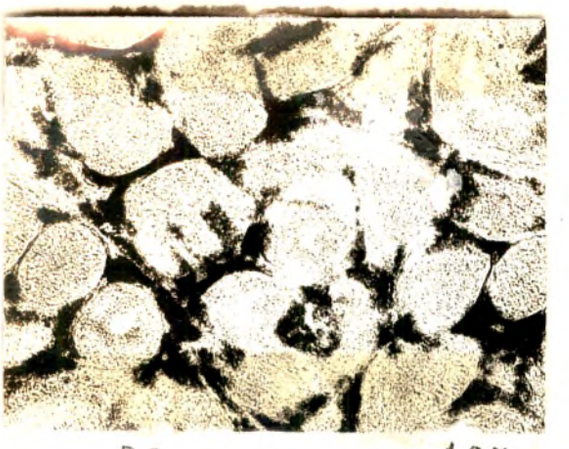
Breeding season : The seminiferous tubules contained all stages of spermatogenesis and were about 175 μ wide. Leydig cells possessed many lipid globules. Noncellular spherical bodies were found in the interstitium, some of which contained sudanophilic material. The Sertoli cells and the spermatogonia were observed to be more sudanophilic than the rest of the cells in the seminiferous tubules. The germinal elements as well as the Leydig cells showed a uniform distribution of lipase activity.

Monkey testis

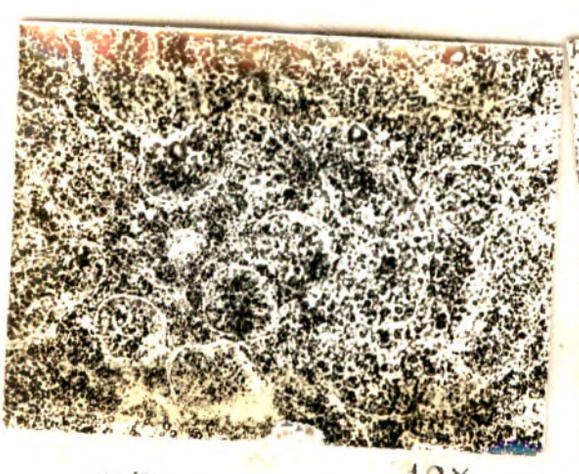
Nonbreeding season : The tubule diameter range was from 45 to 60 μ. Many of the tubules contained only a single layer of spermatogonia and a few Sertoli cells and only some tubules



21 10x



22 10x

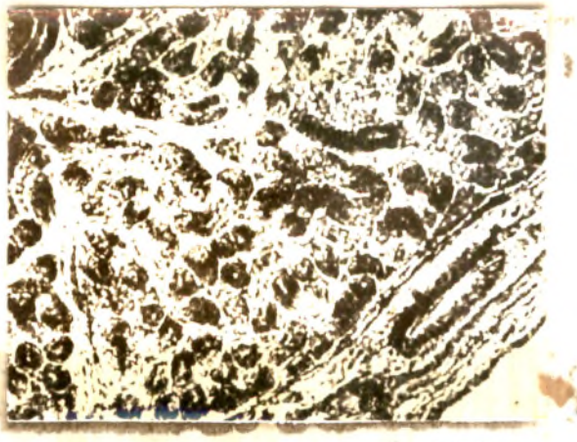


23 10x

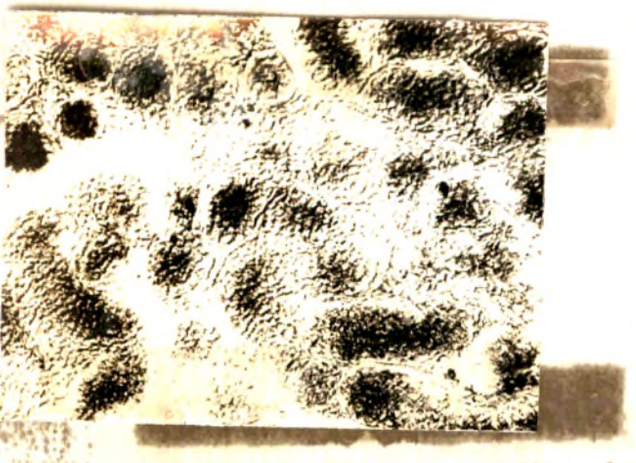
24 10x

Figs. 21 & 22. Sections of the cat testes showing the lipids and lipase, respectively, in the period nearing the breeding season.

Figs. 23 & 24. Sections of the sheep testes showing the lipids and lipase, respectively, in the breeding season.



25 10x



26 20x

Figs. 25 & 26. Sections of the testes of monkey showing the distribution pattern of the lipids and lipase, respectively, in the non-breeding season.

had a layer of spermatocytes. The interstitial Leydig cells were scattered about and were not prominent.

The germinal and Sertoli cells showed little sudanophilic material but the spermatogonia were comparatively rich in lipids. The localization of lipase activity was similar to that of lipids.

## DISCUSSION

In the animals studied in the present investigation the interstitial Leydig cells showed in the nonbreeding period, large quantities of sudanophilic material which were reduced considerably with the advent of the breeding season. Lipase activity in these cells also showed a corresponding decrease thereby indicating a lower enzyme gradient side by side with the decreasing substrate (lipid) levels. The lipid build up during the nonbreeding season follwed by its depletion in the breeding season shows that lipids are being metbolized by the Leydig cells. It was reported by Melampy and Cavazos (1954) that the interstitial lipids of the vertebrate testes studied by them, except those of chicken and fish, are positive to Schultz reaction for steroid compounds. It is now well established that the Leydig cells synthesize sex hormones and that lipids are intimately associated with steroid biosynthesis. In the light of this, it may be inferred that the lipids present in the Leydig cells are utilized for the formation of sex-steroids.

In all the other species studied in the present investigation, the Sertoli cells as well as spermatogonia invariably contained lipids and show lipase activity. From this it may be suggested that, at least, in the initial stages of spermatogenesis and in the final stages of spermatozoan development lipids are utilized. The Sertoli cells in the rat testis, have been found to phagocytose the lipid material from the residual bodies, which after some time give a positive Schultz reaction (Lacy, 1960). It was therefore, suggested that the regular ingestion of such lipid bodies by the Sertoli cells and the subsequent conversion to Schultz-positive substance(s) may be a regulatory mechanism in the initiation and synchronization of the spermatogenetic stages in the seminiferous tubules in relation to the process of spermiation (Lacy, 1960).

In the case of the pigeon and the house sparrow, which breed almost throughout the year, lipids and lipase follow a closely parallel pattern of distribution within the seminiferous tubules as well as in the interstitium. It may be said that, at least in these two species of birds, lipids are continuously utilized at all the stages of spermatogenesis. It should be mentioned here that in an animal such as rat, which breeds throughout the year, the concentrations of lipids and lipase presented a similar pattern. The fact that such an identical pattern in conjunction with constant spermatogenetic activity is seen in two different groups of vertebrates resulting in their ability to breed throughout the year, is

of considerable interest.

The Leydig cells in the testes of the rabbit, cat, dog and sheep were found to contain considerably more lipids than the germinal and Sertoli cells. The distribution pattern of lipase activity also presented an identical picture. Such a parallel distribution of the metabolite and the enzyme, has been explained as metabolic adaptation at the sub-cellular level (chapter I). The present observations lend further support to the fact that the lipids form an important metabolite in the process of spermatogenesis.