CHAPTER I

HISTOCHEMICAL OBSERVATIONS ON THE RAT TESTIS

Studies on the structure and physiology of the vertebrate testis have been largely confined to the process of spermatogenesis, and the role of testis as an endocrine gland. The various stages of spermatogenesis in the rat testis have been described with great precision on the basis of the definitive cellular associations in the seminiferous tubule (Leblond and Clermont, 1952). Roosen-Runge (1952) discussed the kinetics of spermatogenesis in mammals. In a recent review of the new advances concerning the process of spermatogenesis in mammals the same author (1962) called attention to the fact that there is scanty information on the role of lipids in spermatogenesis.

The presence of lipids in the interstitial tissue as well as the seminiferous tubules in the vertebrate testis is well known (Long and Engle, 1952; Montagna, 1952; Cavazos and Melampy, 1954). As early as 1928, Oslund reviewed the literature on the periodic accumulation and discharge of lipids in the interstitial tissue of the testes of fishes, amphibians, birds, and seasonal mammals. Several others have studied the seasonal changes in the lipid content of the testes with respect to spermatogenesis in various vertebrates and also with reference to the behavioural pattern of

these animals (Lofts and Marshall, 1957; Marshall and Woolf, 1957; Lofts, 1960; Lofts and Boswell, 1961). Schieb (1959) has traced the developmental changes in the pattern of distribution and nature of lipids in the gonads of the developing chick embryo. Lacy (1960), using light and electron microscopes, demonstrated the distribution of lipids in the rat testis during different stages of spermatogenesis. He also pointed out that as spermatogenesis progresses there is a regular and gradual increase in the intra-tubular lipids and a shift of lipids towards the center of the lumen of the tubule. It is known that in senescence and cryptorchidism, the low production of spermatozoa is always accompanied by lipid accumulation in the testicular tissue.

Previously the interest in the lipid material in the testicular tissue was mainly centered on its role in the nutritional requirement of the gland at the cellular level. Recently, however, the possibility of this lipid material being of a steroidal nature has been suggested. The Leydig and Sertoli cells, therefore, have been studied extensively on the basis of the assumption that the interstitial Leydig cells are the probable sites where androgenic compounds are elaborated (Baillie, 1961; Christensen and Fawcett, 1961; Leeson, 1963). With the advances in histochemical techniques, it became possible to demonstrate the activity of one of the key enzymes of the system synthesizing steroid hormones viz. - steroid-3β-ol dehydrogenase in the interstitial

Leydig cells of the rat testis (Niemi and Ikonen, 1961). Lynn and Brown (1958) demonstrated the capacity of the testicular tissue, under *in vitro* conditions, to convert progesterone to androgens. They showed that there are four enzymes involved in this process, all of them being associated with the microsomal fraction. These enzymes have also been shown to require reduced triphosphopyridine nucleotide (TPNH) as an essential cofactor, two of them also needing the presence of molecular oxygen. It is well known that the activity of hexose monophosphate shunt produces TPNH and the latter is necessary for fatty acid synthesis (Langdon, 1957; Levy, 1961) as well as for the biosynthesis of steroids (Lynn and Brown, 1958). The glucose-6-phosphate dehydrogenase (G-6-PD) activity has been histochemically demonstrated in the cells of Leydig in the fetal as well as adult rat testes (Niemi and Ikonen, 1962) and in the fetal and prepubertal Leydig elements in the human testes (Wolfe and Cohen, 1964). These investigators could not detect the enzyme activity in the seminiferous tubules either in the prenatal or postnatal testes of the species investigated by them.

Scott and Lynch (1952) have brought forth some evidence to indicate the possibility of Sertoli cells being the site of steroidogenesis.

Apart from the above information a large volume

of literature on the metabolic pathways of semen and isolated spermatozoa has accumulated. Several aspects of the glycolytic metabolism in the semen (Mann, 1957) and spermatozoa of various species of vertebrates are now fairly well understood. Rothschild (1951) has reviewed the advances in the morphology and physiology of the sea-urchin spermatozoa. Certain aspects concerning the motility of spermatozoa have recently been reviewed by Bishop (1962). It is now well established that the fructose, present in the seminal plasma of vertebrates, can support, under anaerobic as well as aerobic conditions, the life and motility of spermatozoa after ejaculation.

Lardy and Phillips (1941 a & b) showed that washed bull spermatozoa can survive only under aerobic conditions, possibly by utilizing the endogenous phospholipids. Bomstein and Steberl (1957) also showed that phospholipids can be utilized by bovine spermatozoa. Lardy, Phillips, and Hansen (1945) found that phospholipids are utilized by the bovine epididymal spermatozoa and suggested that this may be the source of energy for the sperm in the epididymis where no glycolysable sugar is present. Rothschild and Cleland (1952) concluded that - "...the oxidative breakdown of phospholipids, located in the middle piece, is the principal energy source....." for the echinoderm sperm for which no seminal sugar is available. Terner (1962), and, Terner and Korsh

(1962) have not only confirmed the capacity of the bull spermatozoa to utilize lipids as a source of energy but have, in addition, shown that these sperms can also synthesize lipids. Occurrence of fatty acids in mammalian semen (Scott et al., 1961) and their oxidation by spermatozoa (Hartree and Mann, 1959 & 1960; Scott et al., 1962 a & b) have also been demonstrated.

Very little, however, is known about the utilization of lipids in the testicular tissue for its general metabolism and, in particular, for the continuous activity of sperm production in the rat testis. Roosen-Runge (1962) citing the work of Von Ebner says that the latter observed that during the transformation of spermatids; Sertoli cells seem to supply lipid material, which he had described as a nutritional current - Ernahrungsstrom. It would be pertinent to mention here that a similar observation was made by Montagna (1952) regarding the glycogen transfer in the human Sertoli cells and the maturing spermatids.

As a prerequisite to dealing with the problem on the role of lipids in the rat testis it was thought desirable to make a closer study than has hitherto been made of the enzyme lipase which could hydrolyze fat into fatty acids and glycerol. It was also thought necessary to study histochemically the distribution of lipids in the rat testis.

Such an attempt should help to obtain a better understanding of the capacity of the testis to mobilize fat and make it available for the general metabolic activity of the testis. It should also throw some new light on the metabolism of lipids with special reference to the different stages of spermatogenesis. If fat is mobilized, then its utilization logically demands the presence of a well organized oxidative system side by side with the lipid mobilizing one. However, direct studies on the oxidative enzymes of the testis have been few -- (Steinberger and Wagner, 1961; Annison et al., 1962; Niemi and Ikonen, 1962; Blackshaw, 1963; Schor et al., 1963) -- and the knowledge regarding the localization of such enzymes is far from complete.

In the light of the above observations it was thought desirable to locate histochemically and assess the nature of lipids in the testis and also to study the localization and distribution of two enzyme systems, a lipolytic (lipase) and an oxidative (succinic dehydrogenase) one, which are possibly involved in the process of lipid utilization. In order to obtain a wider picture of the metabolic processes, certain other enzymes viz. - β-hydroxybutyric dehydrogenase, lactic dehydrogenase, malic dehydrogenase, glucose-6-phosphate dehydrogenase, acid- and alkaline-phosphatases were also studied histochemically.

MATERIAL AND METHODS

In the present investigation adult male laboratory bred albino rats (Haffkine Institute strain) were used. The animals were killed by decapitation and exsanguination. The testes were quickly dissected out and frozen at -20°C. Thin slices of the tissue were cut out and processed as described below:-

1. Lipids - Tissue slices were fixed in cold calcium-formol for 24 hours. After required washing the tissue was embedded in gelatin, cut on a freezing microtome and stained with Sudan black B and Sudan red 7 B (Fettrot 7 B) as described by Pearse (1960).

2. Lipase - 6% buffered formalin at 4°C was used as the fixative, period of fixation being 16 hours. Gelatin blocks were prepared and sections were cut on a freezing microtome. Lipase activity was demonstrated by an improved method of Gomori as modified by George and Iype (1960). Recently some doubts have been raised regarding the substrate specificity of "Tween 80" for lipase, therefore, in order to distinguish between non-specific esterase and true lipase activity the following procedure was adopted. "Tweens" 20, 40, 80, and 85 were used separately as substrates. In each case some sections were preincubated in 0.1 M sodium taurocholate for 1/2 hour and 1 hour, respectively. Preincubation was preferred to the use of sodium taurocholate as an ingradient of the incubation mixture to avoid changes in the

incubation medium such as pH.

3. Acid- and alkaline- phosphatases:- The tissue slices were fixed for 24 hours in cold 10% neutral formalin, washed, embedded in gelatin and sections were cut on the freezing microtome. The enzyme activities were demonstrated by Gomori's lead-phosphate method and calcium-cobalt method respectively (Pearse, 1960).

4. SDH, BDH, LDH, and MDH* - Thin slices of testis frozen at -20 °C were cut and washed for 5 minutes in chilled phosphate buffer (pH 7). This period of washing is sufficient to remove the endogenous substrates. It should, however, be admitted that in addition some of the cofactors might also be lost. This loss could be made good by either having comparatively larger volume of the incubation mixture than the one employed for thin sections or by addition of extra amounts of the cofactors, substrate, respiratory inhibitor, and neotetrazolium blue (NT) in the subsequent treatment. After washing the thin slices of tissue in the chilled phosphate buffer, they were incubated at 37°C for 1 hour in the different media containing the respective specific substrates for the demonstration of the various enzyme activities.

SDH activity was demonstrated according to the modified method adopted by George and Talesara (1961). Other three enzymes were demonstrated by the method described by Pearse (1960). In all

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* SDH - Succinic dehydrogenase, BDH - B-hydroxybutyric dehydrogenase, LDH - Lactic dehydrogenase, MDH - Malic dehydrogenase.

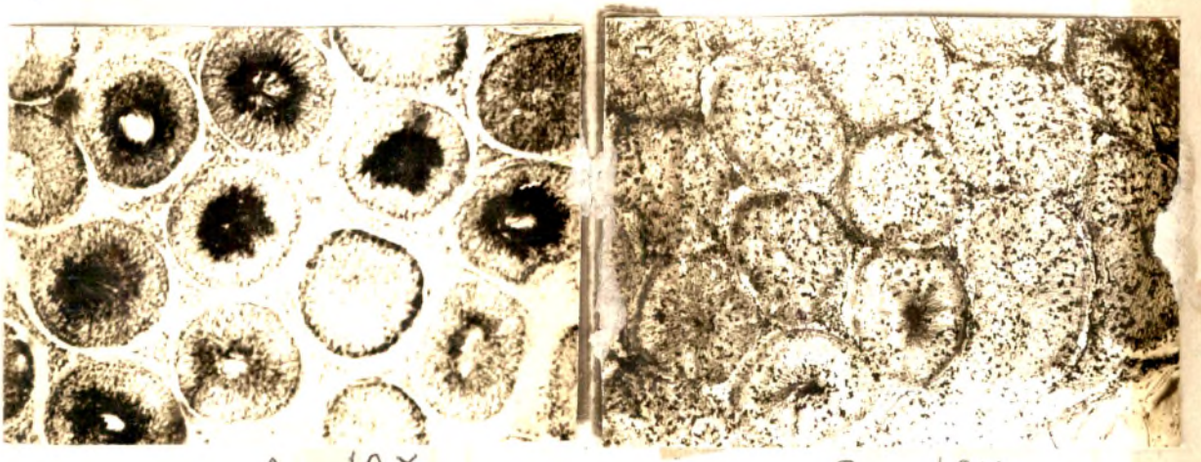
cases the final electron acceptor used was Neotetrazolium blue (NT). After the required period of incubation the tissue slices were fixed in 20% neutral formalin for one hour, washed in distilled water, and embedded in gelatin. Sections were cut on a freezing microtome at a uniform thickness of 20 μ, fixed on gelatinized slides and mounted in glycerine jelly.

5. Glucose-6-phosphate dehydrogenase(G-6-PD) - Slices of testis frozen at -20 °C were cut and quickly mounted on the microtome chuck and transferred to the cryostat chamber maintained at -20°C. 8 μ thick sections were cut on the cryostat. The sections were mounted on clean and dry coversslips, finger-thawed and dried in a current of air. No adhesive was used to mount the sections on the cover slips. The sections were incubated at 37°C for 10 to 15 minutes in the incubation mixture described by Pearse (1960). Some of the sections were incubated in an incubation mixture containing 0.5 ml of 0.1% solution of phenazine methosulphate in addition to the usual constituents.

RESULTS

Lipids - Lipids stained with Sudan black B were seen to be present in the interstitial cells of the fibroblast type as well as the cells of Leydig. The walls of the capillaries were also stained with Sudan black B. The Leydig cells were found to be loaded with lipids. All the cells of the germinal epithelium were seen to be sudanophilic (Fig. 1).

The distribution pattern obtained with Sudan red 7B was different from that obtained with Sudan black B. The cells



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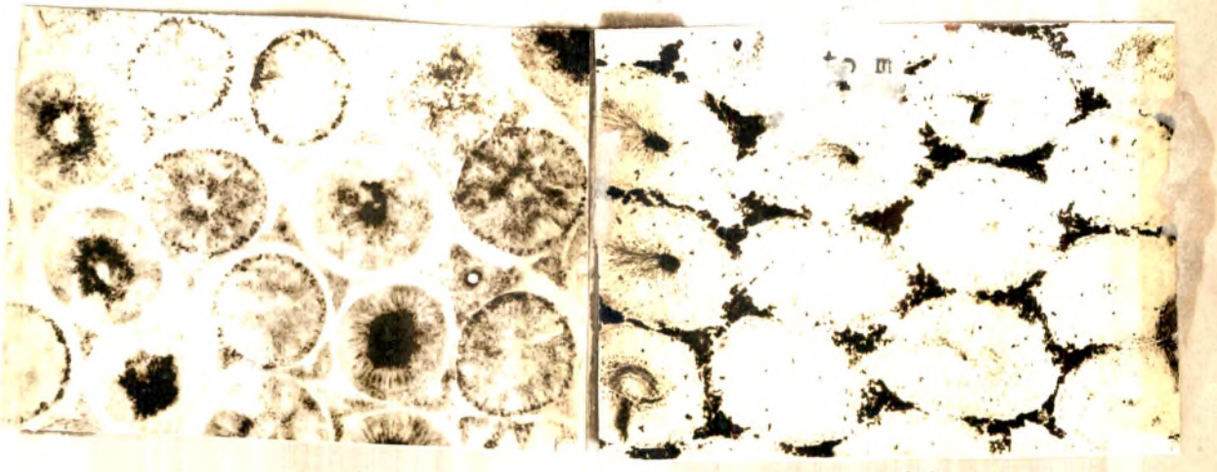

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D 10×

Photomicrographs of the sections of rat testis.

Figs. A, B, C and D showing the enzyme action on "Tweens" 20, 40, 80 and 85 respectively.

of the germinal epithelium, and the residual protoplasmic bodies, when present, were stained while the whole of the interstitial tissue remained unstained (Fig. 2).

Lipase - In order to distinguish between non-specific esterase and true lipase, as already said, "Tweens" 20, 40, 80, and 85 were used separately. In each case some sections were preincubated in 0.1 M sodium taurocholate for half hour and one hour, respectively. All sections preincubated half hour with sodium taurocholate showed little enzyme activity thereby denoting the inactivation of nonspecific esterase action (Figs. 3-6). On the other hand, the sections preincubated one hour showed high activity but with a different pattern of localization (Table I and Figs. 7-10). This reaction is to be considered as the result of true lipase activity which is in conformity with the findings of Gomori, in that sodium taurocholate inhibits all nonspecific esterase action but activates true lipase. With "Tween 80" the enzyme activity in the interstitium was higher (Fig. 8 and Table I) than that obtained with "Tween 85", whereas with "Tween 85" the distribution of lead sulphide precipitate was uniform in the tubule as well as the interstitium (Fig. 7). The intensity of the enzyme activity in the seminiferous elements was almost the same with both "Tweens" 80 and 85. This difference in the interstitial activity on two different "Tweens" is interesting. The lipase present in the interstitium appears to be active more specifically on the mono-oleate (Tween 80) than on tri-oleate (Tween 85). With the "Tweens" 20

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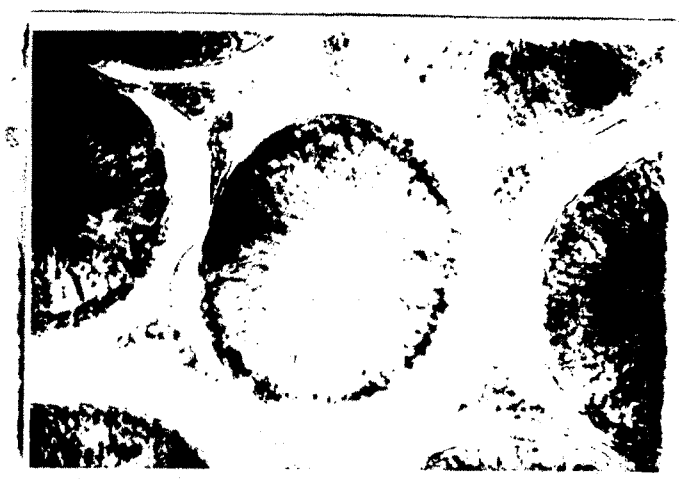

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Fig. 1. Section of the rat testis showing the distribution of lipids stained with Sudan black B.

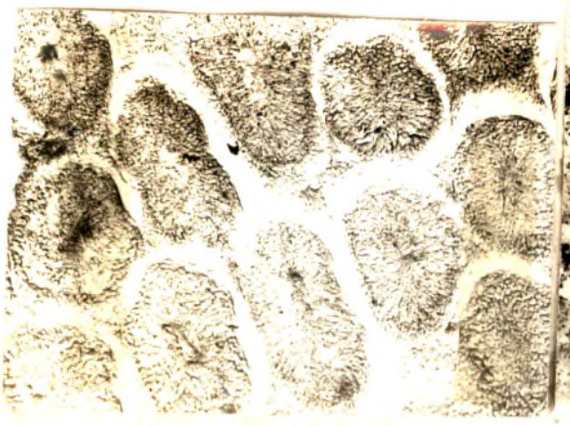
Fig. 2. Section of the rat testis showing the lipids stained with Fettrot 7B.

TABLE 1

Effect of Sodium taurocholate on Lipase Activity in Rat Testis

Treatment	Reaction*							
	Tween 20		Tween 40		Tween 80		Tween 85	
	Intra-tubular	Inter-stitial	Intra-tubular	Inter-stitial	Intra-tubular	Inter-stitial	Intra-tubular	Inter-stitial
Control (not treated with sod.taurocholate)	+	2+		4+	2+	2+	4+	4+
With 1/2 hr pre-incubation in 0.1M sod. taurocholate	-	-	-	+	-	+	4+	4+
With 1 hr pre-incubation in 0.1M sod. taurocholate	2+	3+	2+	3+	3+	5+	4+	4+

* - Activity nil; + minimum activity; 5+ maximum activity.



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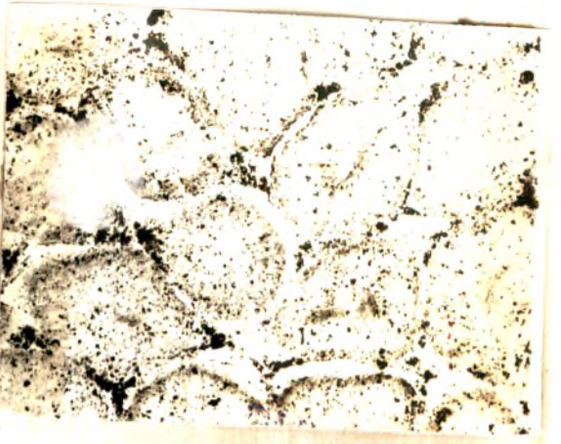

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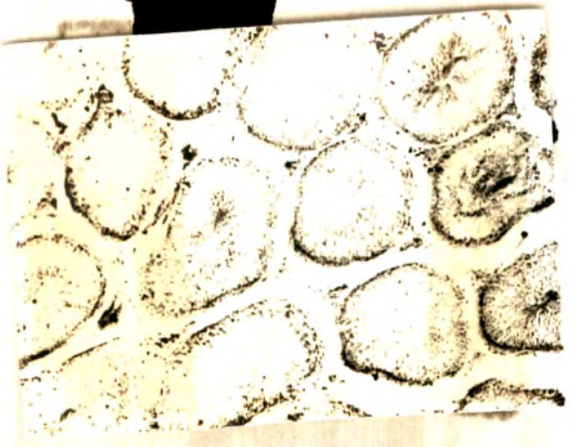

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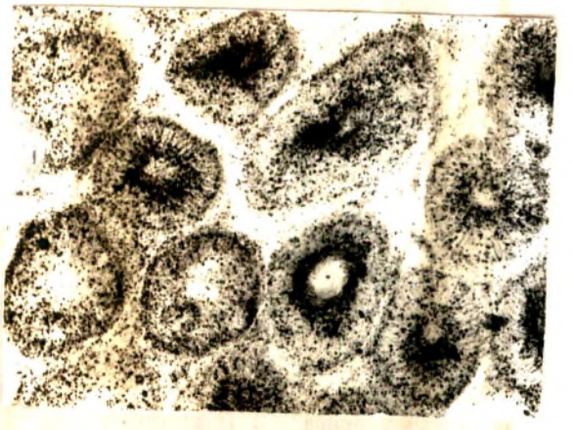

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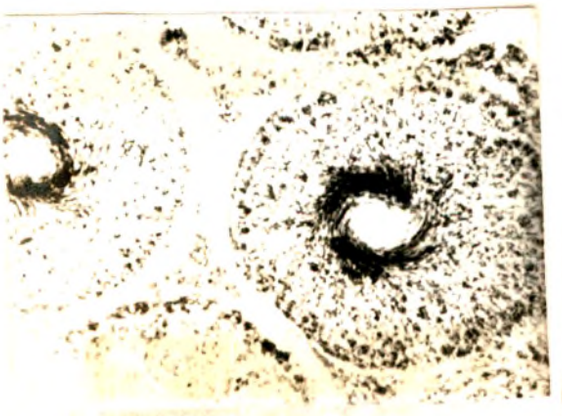
Figs. 3 to 6. Sections of the rat testis showing the action of the enzyme on the "Tweens" 20, 40, 80 and 85, respectively, after 1/2 hour of preincubation in 0.1 M sodium taurocholate.



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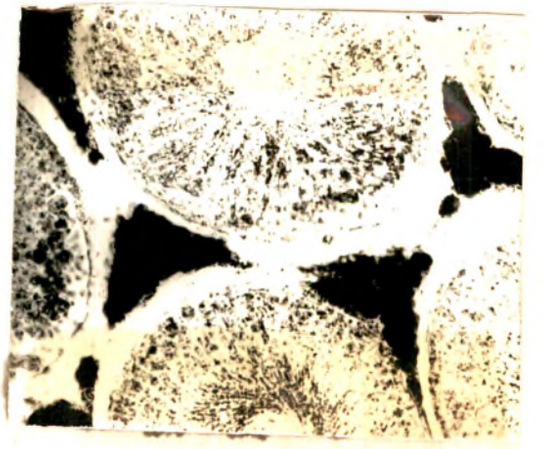

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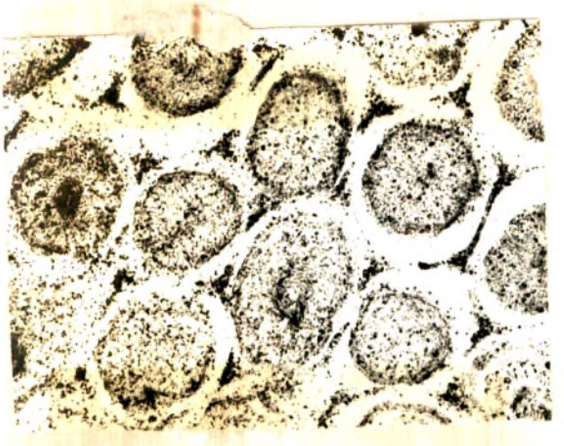

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Figs. 7 to 10. Sections of the rat testis showing the enzyme action on the "Tweens" 85, 80, 40 and 20, respectively, after 1 hour of preincubation in 0.1 M sodium taurocholate.

and 40, sections preincubated in sodium taurocholate for one hour showed greater activity in the interstitium thereby indicating that the enzyme of the interstitium is not the same as that of the tubules. On the other hand, sections preincubated with sodium taurocholate for half hour and treated with "Tween 85" did not show the distinct decrease in the activity of the enzyme as seen with other "Tweens" (Fig. 6). This further indicates that what is obtained with [illegible] 85" is true lipase.

From Figure 11 the distribution pattern of lipase activity can be seen distinctly in four different phases- A, B, C, and D. In phase A the enzyme activity is strongly localized only in the periphery of the seminiferous tubule. Phase B represents the distribution pattern of the enzyme activity in the shape of a broad band shifting toward the center of the tubule. In phase C, a uniform distribution of lead sulphide precipitation is obtained over all the tubule, and spermatids are clearly visible at this stage. In D the enzyme activity is seen in the form of a central band around and near the heads of the spermatozoa. It is clear that there is a regular sequence in the change of loci of the enzyme activity from the periphery of the seminiferous tubule to the center of the lumen of the tubule, as spermatogenesis proceeds.

This is the case not only with the enzyme [illegible]

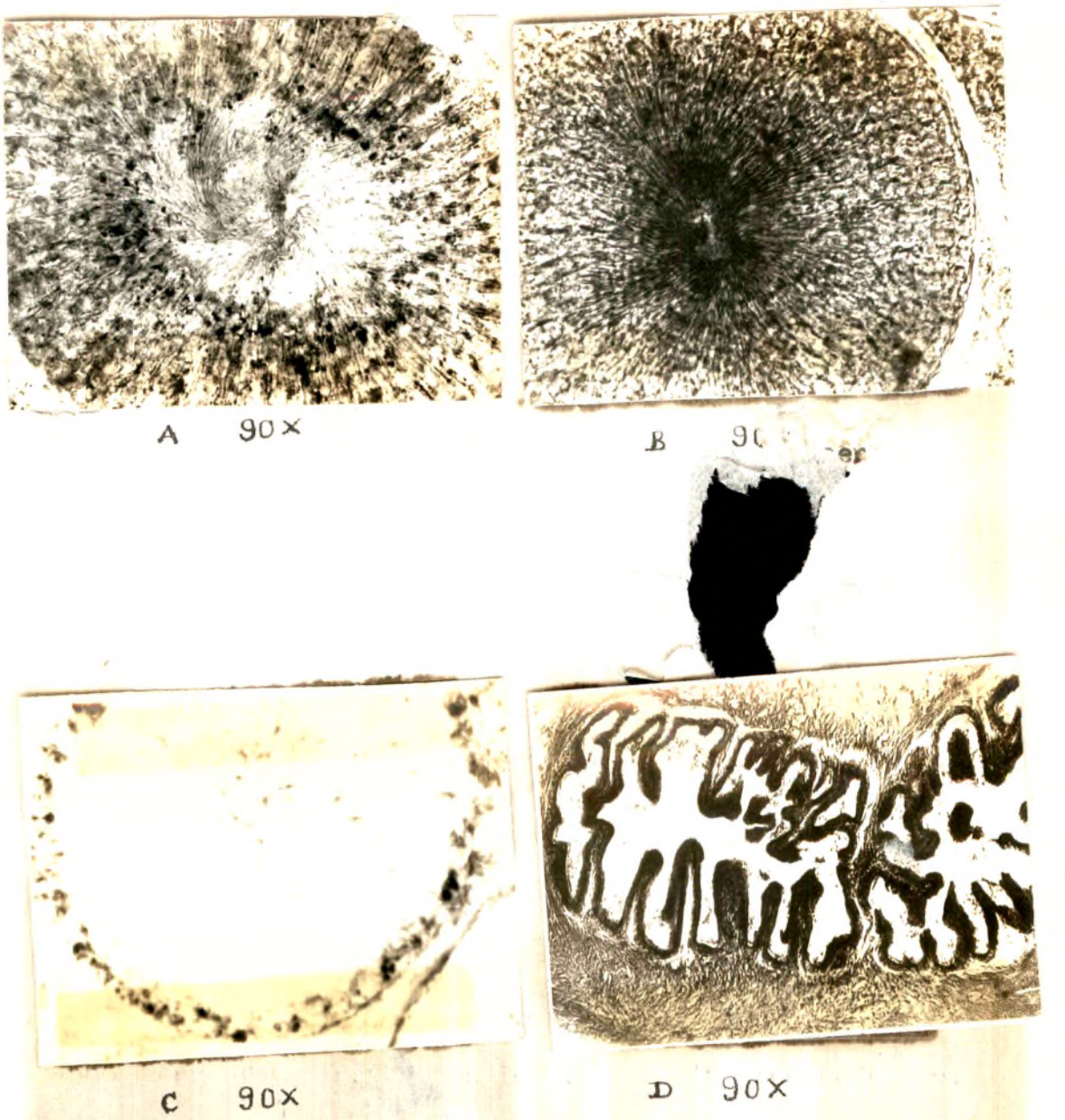

Fig. 11, A, B, C and D. Photomicrographs of cross sections of rat testis showing the four different phases of localization of the enzyme lipase in the seminiferous tubules.

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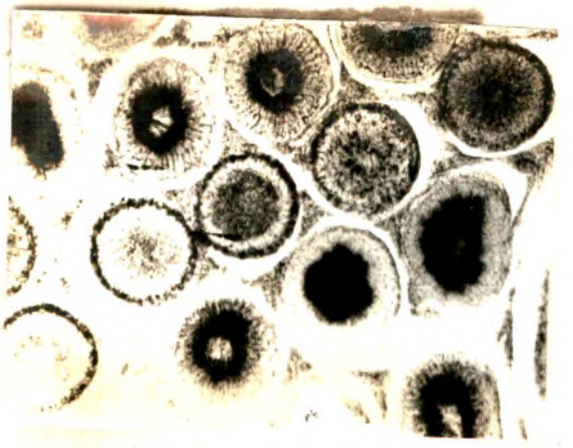

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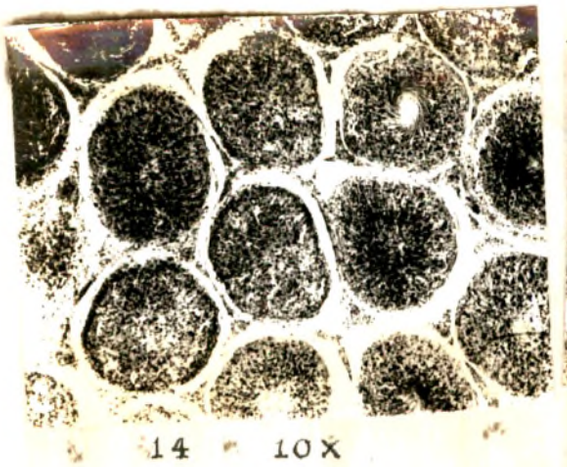

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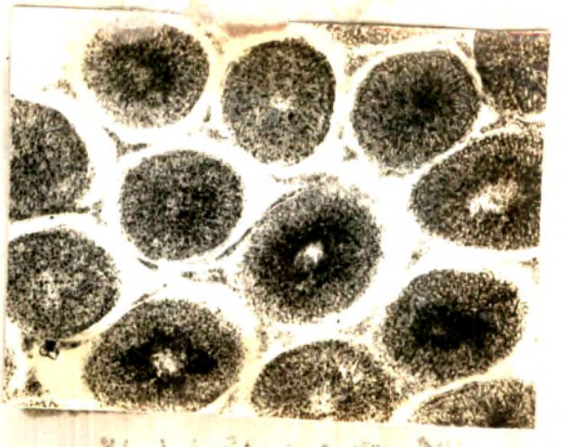

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Fig. 12. Section of the rat testis showing the lipids stained with Sudan black B.

Fig. 13. Section of the rat testis showing lipids stained with Fettrot 7B.

Figs. 14 and 15. Sections of the rat testis showing acid- and alkaline-phosphatse activities, respectively.

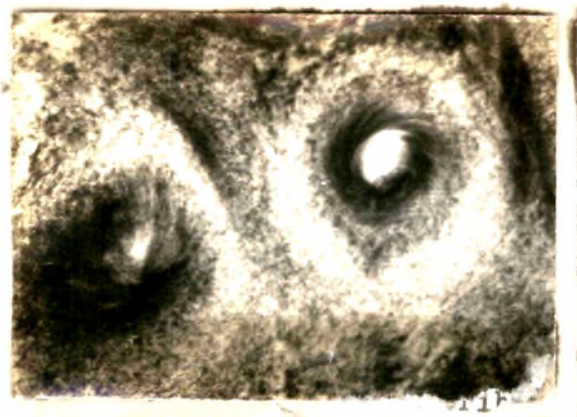
under investigation but also with its s lipids. Lipids demonstrated with Sudan red 7B are neutral lipids which are clearly seen to follow the same pattern of distribution as that of lipase activity. It is interesting that the substrate and the enzyme exist side by side and even move together, thereby indicating this as a metabolic adaptation at the subcellular level in the process of spermatogenesis.

In the distribution of lipids it is seen that the interstitium appears to contain the sudanophilic material (mostly phospholipids, Fig.12) and is comparatively free from Sudan red 7B - stainable material (neutral lipids, Fig. 13).

Acid- and alkaline-phosphatase activities - Both the enzymes were found to be located in the interstitium as well as in the seminiferous tubules (Fig. 14 & 15) and followed a similar pattern of distribution as obtained for lipase.

SDH, BDH, LDH, and MDH - All these enzymes were found to be present in the interstitium and the seminiferous tubules (Figs. 16-19). The intensity of the staining reaction for SDH, BDH, and LDH in the interstitial tissue was similar to that in the seminiferous tubules. In the seminiferous tubules the staining reaction was seen identical to the four phases as described for the lipase activity (Figs. 16-18). In the case of the MDH activity, however, though the pattern of enzyme localization in the tubule was similar to that obtained for the other three oxidative enzymes, it was conspicuously more intense in the interstitial tissue.

rate, viz.



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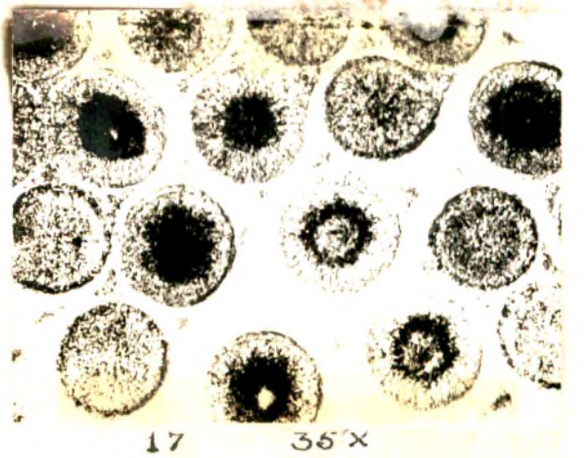

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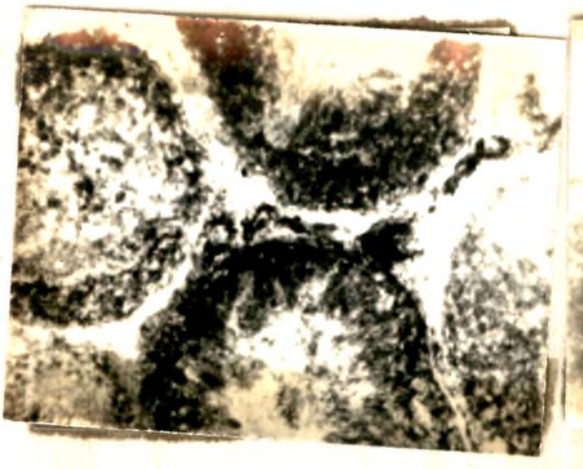

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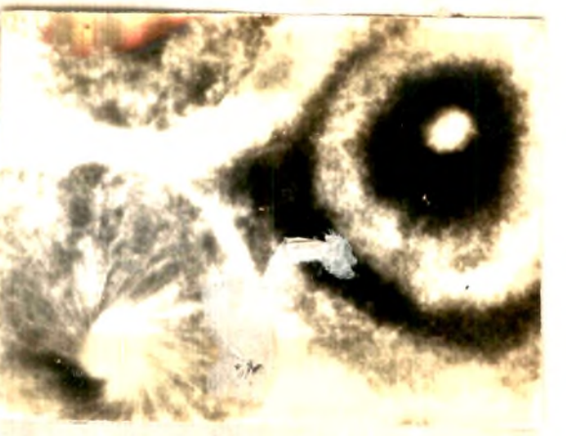
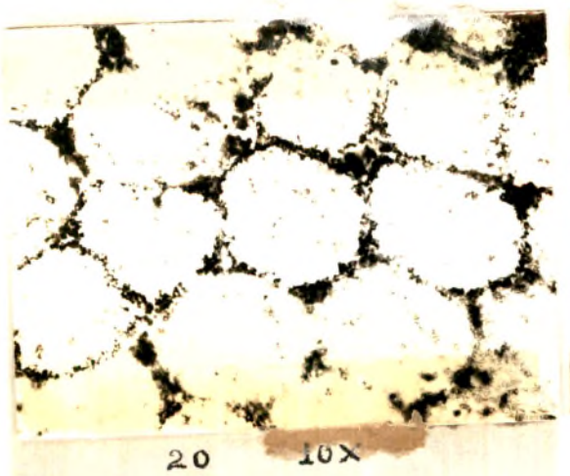


19 35×

Photomicrographs of the sections of the rat testis showing the localization of the respective enzyme activities.
Fig. 16. Succinic dehydrogenase. Fig. 17. β-hydroxybutyric dehydrogenase. Fig. 18. [illegible]ctic dehydrogenase. Fig. 19. Malic dehydrogenase.



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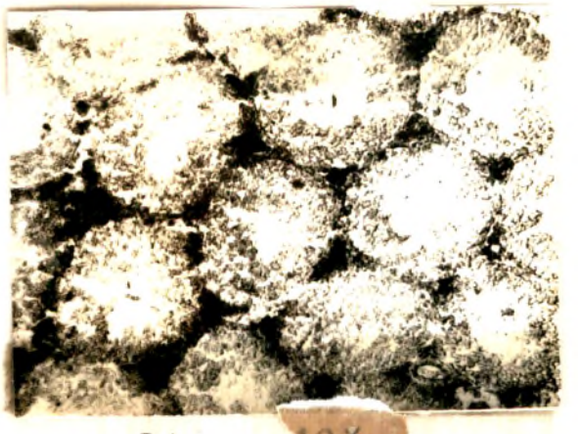

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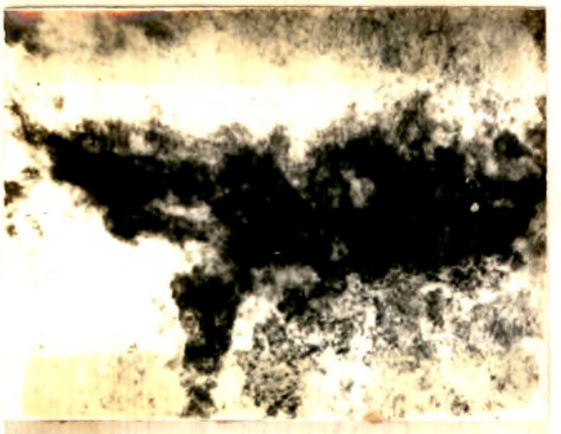

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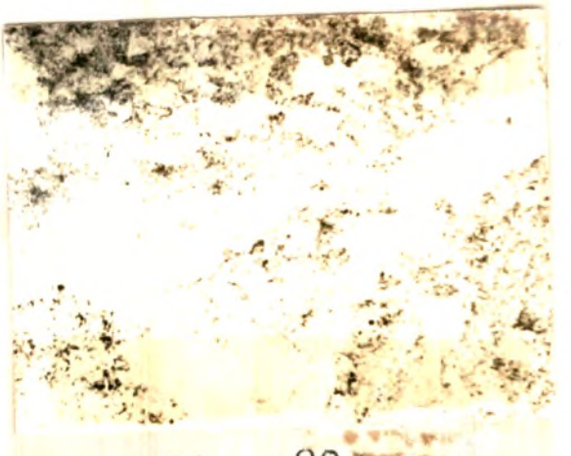

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Photomicrographs of the sections of rat testis.

Fig. 20. Showing the G-6-PD activity. Without PMS.
Fig. 21. Showing the G-6-PD activity. With PMS.

Fig. 22. G-6-PD activity in the interstitial tissue without PMS.
Fig. 23. G-6-PD activity in the interstitial tissue with PMS.

G-6-PD - This enzyme activity was present in the interstitium as well as in the seminiferous tubules. When phenazine-methosulphate (PMS) was not added to the incubation mixture the enzyme activity was considerably high in the interstitium and the basal membrane of the seminiferous tubules. These elements appeared deep blue with the reduced dye but in the rest of the seminiferous elements a fine granular deposition was noticed. On the addition of PMS to the incubation mixture, the deep blue colour developed in the interstitium and the basement membrane of the tubules was not observable but in the interstitial and germinal cells, a fine granular pattern was obtained. The spermatozoa in the tubule and the protoplasmic residual bodies were typically negative when PMS was present in the incubation medium. No phase-wise distribution of this enzyme in the seminiferous tubules was obtained as was the case with all other enzymes studied.

DISCUSSION

It is clear that "Tween 85" is by and large a specific substrate for the histochemical demonstration of lipase activity since the enzyme activity was not inhibited by sodium taurocholate which is known to inactivate non-specific esterase activity but to accelerate that of true lipase. Using the action of sodium taurocholate, a new histochemical method for lipase has been developed recently by Abe, Kramer and Seligman (1964), where naphthol-AS-nonanoate was used as the substrate. With this technique, the authors obtained a weak

lipase activity in the seminiferous tubules of the rat and the rabbit testes, which was mainly confined to Sertoli cells. Wallach, Ko and Marshall (1962) isolated and purified "Tween - hydrolyzing" enzyme from the rat adipose tissue and showed that this enzyme readily hydrolyzes "Tween 20" but had no action on "Tween 85". This also supports the contention that "Tween 85" is acted upon by true lipase and not by non-specific esterase, as was shown in the results obtained in the present work. Niemi et al.(1962) claimed that there is no true lipase in the rat testis but that there is only non-specific esterase activity. Their failure to obtain any activity with "Tween 80" was due to the procedure employed by them. Acetone is known to inhibit lipase completely (George and Scaria, 1958) and also calcium-formol (Eapen, 1960), the two fixatives used by Niemi et al.(1962). Furthermore, it should be noted that the enzyme could diffuse out of the sections as well as cells, especially so when loose sections are treated for the enzyme reaction.

The results obtained after one hour preincubation in 0.1 M sodium taurocholate solution the enzyme action on "Tweens" 80 and 85 were interesting. With "Tween 80" the enzyme activity in the interstitium was considerably higher than in the seminiferous tubules, whereas with "Tween 85" the distribution was uniform in the tubule as well as the interstitium. The intensity of enzyme activity in the seminiferous tubules

was similar with "Tweens" 80 and 85. From this it is concluded that the lipase in the interstitium appeared to be active more specifically on mono-oleate (Tween 80) than on tri-oleate (Tween 85).

It is known that alkaline phosphatase can catalyze transphosphorylation reactions in addition to its hydrolytic action on organic orthophosphates (Pearse, 1960; Burstone, 1962), and that the former activity may regulate the level of d-glycerophosphate and consequently its availability for triglyceride synthesis (Wallach and Ko, 1964). Alkaline phosphatase is also known to hydrolyze the high-energy N-P bond of phosphocreatine (Morton, 1955) and in this way it may serve an important function in the general energy metabolism. That alkaline phosphatase activity is closely associated with the transport of the metabolites across the membrane-barrier is a well accepted fact. Tice and Barrnett, (1963) have studied the localization of varous phosphatases in the rat testis, and they have also suggested that phosphatase activity may be associated with the exchange of metabolites across the cell membrane. These observations indicate that alkaline phosphatase has a significant role in the metabolism of the rat testis.

With regard to the role of acid-phosphatase in tissues in general, however, it is not possible, in the present state

of our knowledge, to attribute any definite function. In the observations made in the present study also it is not possible to attribute any definite role to this enzyme in the metabolism of the testis. It is generally accepted that in a tissue having a high acid phosphatase activity, there is a low activity of alkaline phosphatase and vice versa (Moog, 1946). Results obtained here clearly show that activity of both the phosphatases is equally intensive . For an explanation of such a situation further investigations are necessary. However, it may be pertinent to mention the work of Rosenbaum and Lindeman (1958) who studied the phosphatase activity (pH 5.6) of the testis in growing rats. They found that the enzyme activity increases in an almost linear fashion from the age of 5 days to about 50 days. This indicates a progressive achievement of the functional status.

Walker and Seligman (1961) could not demonstrate SDH activity in the germinal elements of the seminiferous tubules of the rat testis except in the formed spermatozoa. Niemi and Ikonen (1962), in their study mainly concerning the cytochemistry of the Leydig cells, could not observe any oxidative enzyme activity in the seminiferous tubules of the rat testis except for a weak reaction for SDH. Turpienen et al. (1962) obtained intense SDH activity in the seminiferous tubules of normal rat testis. Posalaky et al.

(1961) could characterize histochemically the epithelial cycle in the rat testis on the basis of the SDH activity and certain other staining reactions.

In the present study, intense activity for all the oxidative enzymes investigated was obtained in the interstitial tissue as well as the seminiferous tubules. The presence of BDH and SDH clearly indicated the capacity of the testicular tissue to utilize the fatty acids liberated by the action of lipase which also has been localized in precisely similar locations. Annison, Scott and Wales (1962) showed that 1-C^{14}-acetate is oxidised *in vivo* by hamster testis. Apart from this no other investigation directly dealing with oxidation of fatty acids by the testicular tissue is known to be reported. Blackshaw (1963) has made only a quantitative assessment of the activities of SDH, LDH, and G-6-PD in the mouse testis.

In the present investigation intense MDH activity was obtained in the interstitial tissue, in sharp contrast to that of the other oxidative enzymes studied. In this connection it may be pointed out that Levy (1961) has observed that MDH activity, among other factors, is considerably higher in the lactating rat mammary glands actively synthesiszing fatty acids. Wise and Ball (1964) have postulated that malic enzyme has a role in lipogenesis, which involves an interplay

with MDH and pyruvate carboxylase. From the results obtained in the present studies it is difficult to ascribe any specific importance to the high MDH activity in the interstitium. It may be said, however, that this activity probably denotes a greater extent of interconversions of oxaloacetate and malate through the mediation of this enzyme, thereby providing a greater feed to the catalytic cycle involving succinate and fumarate.

The lipids as well as the oxidative enzymes viz.- SDH, BDH, MDH and LDH presented precisely the same pattern of distribution as that described for lipase activity, depicting the four phases. These findings strongly denote a gradual and well organized shift in the loci of the enzyme activities from the periphery of the seminiferous tubule to its center indicating a gradient in the spermatogenetic wave. Such a gradient also represents the metabolic adaptation at the subcellular level. These findings provide cytophysiological support to the concept of Perry et al. (1961) of the existence of a spermatogenetic wave along the lenght of the tubule.

In contrast to the phase-wise localization of all the enzymes mentioned above, action of the enzyme G-6-PD did not show such a clear pattern. Highly intense reaction for G-6-PD obtained in the interstitial tissue and in the basement membranes of the seminiferous tubules without PMS was not

observable as such when PMS was added to the incubation mixture indicating that there are certain non-enzymic reducing substances which gave a stronger reaction when PMS is not added. After the addition of PMS to the incubation mixture a fine granular distribution was evident throughout. Niemi and Ikonen (1962) in the rat testis and Wolfe and Cohen (1964) in the human could not obtain G-6-PD activity in the seminiferous tubules, whereas, this enzyme activity was clearly demonstrated in the present investigation. It is well known that G-6-PD is the key dehydrogenase to the hexose-monophosphate shunt, which yields reduced TPN as a byproduct. Reduced TPN has been shown to be a necessary cofactor for the biosynthesis of fatty acids (Langdon, 1957; Levy, 1961) and also for that of the steroid hormones (Lynn and Brown, 1958; Villee, 1961).