SUMMARY

It is being increasingly realized that lipids are an important source of energy in all active systems hitherto studied. The testis seems to be no exception. Considerable amounts of lipids were found to be present in the interstitial cells as well as in all the cells of the seminiferous tubules of the rat testis. The localization of lipids and of the lipolytic enzyme, lipase, presented a closely parallel pattern of distribution, in four distinct phases, giving a clear indication of the spermatogenetic activity along the seminiferous tubules. Over a decade ago, the concept of the spermatogenetic wave in the seminiferous epithelium was developed by Perry et al. (1961) on the basis of their histological studies on the rat testis. The particular pattern of localization of the substrate as well as of the enzyme concerned, observed during the course of the present studies, shows a well organized shift in the loci of enzyme (lipase) and its substrate (lipids) from the periphery to the centre of the seminiferous tubule as the spermatogenesis progresses. This colateral shift in the activity of the enzyme and its substrate is strongly suggestive of the metabolic adaptation at the sub-cellular level. If the lipids are to be utilized after degradation by lipase, it is logical to expect a parallel oxidative system also for the complete oxidation of the fatty acids thus liberated through enzymatic action. It was indeed found that the β-hydroxybutyric dehydrogenase, and succinic dehydrogenase, two important and essential enzymes involved

in the oxidation of the fatty acids, also showed a similar pattern of localization, thereby, giving additional support to the hypothesis of lipid utilization during spermatogenesis. In addition, two other oxidative enzymes - Lactic dehydrogenase and Malic dehydrogenase - were also found to present same distribution. If lipid metabolism is thus intimately associated with the process of spermatogenesis, it should be expected that lipids as well as the various enzymes would present a configuration of gradients along the radial axis closely paralleling the stages in spermatogenesis. This has also been confirmed.

Considerably high activity of the glucose-6-phosphate dehydrogenase (G-6-PD), the key enzyme leading to the hexose- monophosphate shunt (HMP shunt), was also observed to be present in the interstitial cells as well as in the seminiferous tubules. It is known that this alternative pathway of glucose metabolism is of considerable significance in the generation of reduced triphosphopyridine nucleotide (TPNH). The latter has been shown to be a necessary cofactor for the enzyme system of fatty acid biosynthesis (Langdon, 1957; Levy, 1961) as well as steroidogenesis (Lynn and Brown, 1958). It may, however, be mentioned here that the G-6-PD activity was not found to show phase-wise localization, which was clearly observable in the case of all other enzymes studied.

On the basis of the present observations, it is suggested that the rat testicular tissue is capable of

synthesizing lipids, and that some specific pre-formed lipid moieties that might be synthesized in the Sertoli cells are passed on as such to the germinal cells of the seminiferous tubules. It is well known that the Sertoli cells are in intimate contact with most germinal cells, forming the so called "symbiotic units" (Roosen-Runge, 1962) and they have been ascribed with the function of supplying certain metabolites to the developing spermatozoa (Von Ebner, 1888; Montagna, 1952). Vilar (1962) described them as "bridge cells" between the basement membrane and the germinal cells. Baillie (1962) studied the development of the basement membrane of the seminiferous tubules in the mouse testis, and from the observations reported by him, it appears that the basement membrane is structurally and functionally suitable for the transport of metabolites. In the light of this it is reasonable to assume that simple metabolites pass from the blood vessels through the basement membrane to the Sertoli cells. Sertoli cells then may synthesize specific lipid moieties which are subsequently passed on to the germinal cells. In order to test this hypothesis an attempt was made by injecting labelled acetate ($1\text{-}C^{14}$) and following its course in the rat testis employing the autoradiographic technique. It was observed that within 30 minutes after injection, the label appeared in the lipids of all cells of the seminiferous tubules as well as in the interstitium. This indicates that there is continuous movement of metabolites from the blood to the

germinal cells. It should, however, be mentioned that the present study does not give conclusive information regarding the formation and the transport of specific lipid moieties by the Sertoli cells, nevertheless, it does give support to the above contention.

Recently, Bieri and Prival (1965) have shown that rat testicular lipids maintain a characteristic fatty acid pattern, particularly the 22-carbon-polyunsaturated fatty acids, which is not altered to any significant extent even with a wide variation in the ingested unsaturated fatty acids. Such a "lipid specificity" must have important bearings on spermatogenesis. This may be considered as a mechanism to minimize fluctuations in the biochemical environment at a site where precisely and accurately coded genetic information is to be passed down to the future generations, through the spermatozoa. The particular assembly of certain selected fatty acids in the lipid material in the Sertoli cells and its handing down to the germinal elements may be, however, regarded as an effective mechanism behind the maintenance of the specific lipid composition in the testis.

As it became known that lipids have an important role in the process of spermatogenesis in the rat testis, these studies were extended to certain other vertebrates. The testes of the following animals : frog, garden lizard, pigeon, house sparrow, a migratory starling - Rosy Pastor, cat, dog, sheep, rabbit and monkey, were examined histochemically for the localization of lipids and lipase activity.

It was observed that the testes in these animals also indicated active utilization of lipids during spermatogenesis.

Administration of testosterone propionate (TP) and human chorionic gonadotropin (HCG) to rats produced certain effects on the lipid levels, and lipase and SDH activities which varied with different doses of the hormones. Some of the important effects were :-

(1) With a dose of 200 i.u. of HCG, SDH activity in all the three tissues viz.- testis, caput and cauda epididymis was found to be decreased considerably.

(2) At the dosage level of 400 i.u. of HCG, lipase activity as well as lipid content in the three tissues increased considerably.

(3) A dose of 500 i.u. induced a variable response of lipase activity in the testis, caput and cauda epididymis.

(4) All the dosage levels of HCG continued to induce a definite increase in the lipid content of the cauda epididymis.

(5) At 4 mg dose level of TP, the lipase activity in the testis, and in the caput as well as cauda epididymis, was increased.

(6) At all the three doses of TP (4, 8, and 10 mg) the lipase activity of the caput epididymis was considerably more than that of the normal, but there was a corresponding decrease in the lipid content.

(7) The cauda epididymis showed a continuous increase in the lipid content with all the three doses of TP.

Three important phases of lipid metabolism were found

to occur during the post-natal development of the rat testis from the age of fifth week after birth upto the sixteenth week. In the first phase, it was observed that at the age of 5 weeks the lipolytic activity was the highest and the lipid content the lowest. The age of about 50 days marked the second phase, when the lipid concentration and levels of both lipase and SDH activities were seen to be lowered considerably. This is attributed to the onset of the gonadotropin secretion. A phenomenal and abrupt increase was noted in the case of SDH activity in the tenth and eleventh weeks. This was due to the influence of increased gonadotropin. The third phase started at the age of about 95 to 100 days, when the activities of both the enzymes and the lipid content decreased. Hereafter, the pattern characteristic of ~~of~~ the adult animals was found to be established.

The above observations suggest that the rat testis actively utilizes lipids by the age of five weeks but after this period the lipolytic activity is quickly lowered and the lipid content is increased in the testis, which may be due to the mobolization of lipids from other organs such as liver and adipose tissue. The increase in lipids during this period is probably due to the general growth of testis. The onset of gonadotropin secretion at the age of about 50 days after birth was found to have profound influence on lipid metabolism and the general oxidative capacity of the testis. The full maturity is attained by about 95 to 100 days of age and

obviously at this stage the adult pattern of testicular lipid metabolism is finally established.

Similar histochemical observations on the rat epididymis indicate that the glandular epithelial cells are capable of utilizing lipids to meet the continuous demands for energy during the secretory and absorptive activities.

It was found that there is a conspicuously different region in the cauda epididymis of the rabbit which is about 10 to 12 times wider than the ordinary epididymal duct. In this region the inner epithelial lining is produced into many branching and anastomosing projections. In order to obtain more information regarding the structure and function of this region a histological and histochemical investigation was carried out. The results obtained indicate that the foldings in the epithelium of this region are a structural modification for increasing the functional surface and that it has probably a secretory function. In discussing the homology of this region it has been homologized with the ductus aberrans, which might have secondarily established at both ends contacts with the epididymal duct.

During the course of the study on the mammalian testis, certain interesting features regarding the mediastinal rete testis of some seasonal mammals, like the rabbit, cat, dog, sheep and monkey ; were observed. This tissue was examined histologically and histochemically. The rete testis of the bull possesses a structural plan characteristic of a gland. The

epithelial lining of the rete testis of the bull is produced into many branching projections and it is supplied with numerous blood vessels. The rete testis of the rabbit also is similar to that of the bull. On the basis of the observations made, it is suggested that this tissue is probably an important glandular component of the excurrent duct system in the bull and the rabbit.