CHAPTER VIII

INCORPORATION OF ACETATE-1-C^{14} INTO LIPIDS OF THE RAT TESTIS

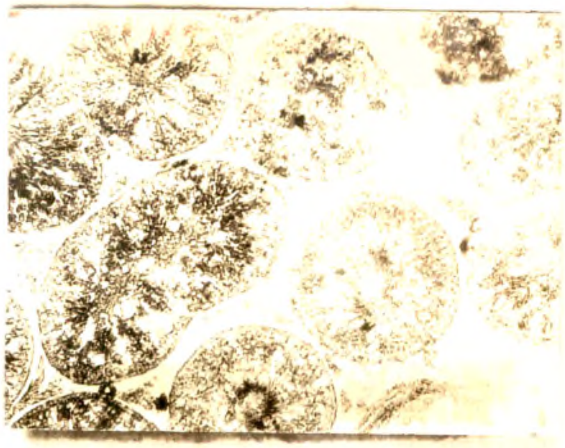
That blood vessels are found only in the interstitium of the testis and that they do not enter the seminiferous tubules, is known (Albert, 1961). It is also known that Leydig cells are often found in close relation with the blood vessels of the interstitium (Leeson, 1963). Therefore, the supply of the nutrient and other materials from the blood should pass through the basement membrane to the Sertoli cells (and perhaps spermatogonia too) and finally to the germinal cells. With this assumption it was conjectured that the cells intervening between the blood and the germinal cells might pass on to the latter some specific materials such as lipids and proteins. The assumption is that the Sertoli cells might sequester some specific lipid components which are transported to the germinal cells. It is known that the testicular lipids show a specific chemical constitution, which is not altered to any significant extent even if the dietary ingestion of lipids is changed (Bieri and Prival, 1965). Such specificity of testicular lipids is obviously of great significance since it would help to minimize fluctuations in the biochemical environment at a site where precise and accurate genetic information is to be passed down through the spermatozoa. An attempt was, therefore, made employing the autoradiographic technique, to investigate the possibility of demonstrating the transport of lipids through the above mentioned route, to the germinal cells in the rat testis.

MATERIAL AND METHODS

Labelled sodium acetate(1-C^{14}) was administered by an intraperitonial injection to a mature male albino rat weighing about 90 gm. A total dose of 60 µc in 0.3 ml solution was given. The animal was sacrificed after 30 minutes. Pieces of the testis were fixed in Elftman's fixative for 48 hours. The tissue was embedded in paraffin-wax and 7 µ thick sections were cut on a sling microtome. Adjacent sections were mounted on separate slides for parallel control and experimental purposes. The sections were mounted on slides coated with 0.5% gelatin containing 0.05% chrome alum. Glass-distilled water was used to float the sections. The sections were deparaffinized with xylene and brought down to distilled water through alcohol grades. The sections were washed three times in 0.5% non-labelled sodium acetate solution to remove excess of carrier acetate, if any. The control sections were extracted for lipids with a chloroform : methanol mixture at 60°C for one hour. All the sections were then coated with Ilford's G.5 nuclear research emulsion and all the slides were kept in a light-proof container for exposure. The sections were exposed to the emulsion for a period of 12 weeks, developed in Fieg's developer, stained for lipids with Sudan black B in propylene glycol (Pearse, 1960), and finally mounted in glycerine jelly. The photographs were taken on a phase contrast microscope using a dark green filter.

RESULTS

After 30 minutes of labelling with acetate-1-C^{14}, all the cell types of the seminiferous tubules as well as the Leydig cells were found to be radio-labelled (Figs. 1 & 2). The Leydig and Sertoli cells showed comparatively higher granular density than the rest of the cells. Granular density was found to decrease progressively from the periphery to the centre of the seminiferous tubules when there were no fully formed spermatozoa. This roughly corresponded to stages I to VI according to the classification method of Leblond and Clermont (1952). In the sections which represented stages IX to XI, maximum radio-activity was found to be present in the spermatogonia, primary spermatocytes and also the Sertoli cells. At such stages were found many labelled granules sticking to the outer side of the tubule wall. In certain sections the activity was observed to be present in the Sertoli cells, primary spermatocytes and the developing spermatids, which represented stages XII to XIV. In those sections which showed stages VII and VIII, considerable amount of labelling was present in the Sertoli cells and in the receding kinoplasmic droplets. Where the mature spermatozoa had already been shed the label was seen in the residual bodies very prominently (Fig. 3). At this stage,



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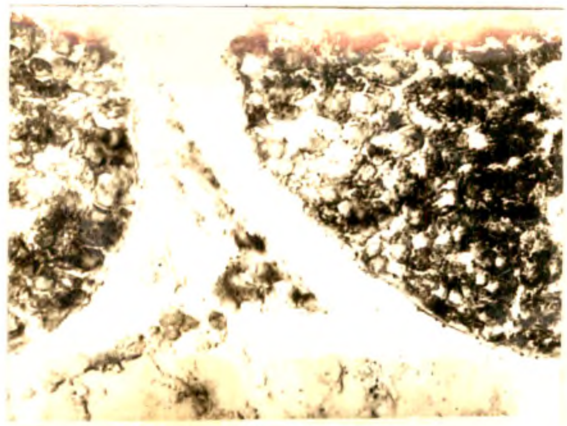

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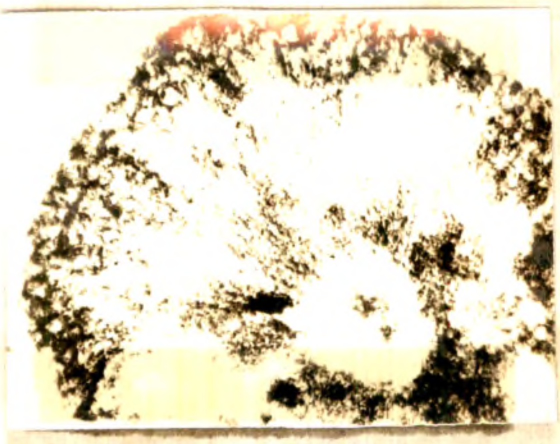

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Figs. 1 & 2. Photomicrographs of the sections of rat testis showing the distribution of the radio-labelled lipids.

Fig. 3. Section of the rat testis showing the strongly labelled residual bodies after spermiation has occurred.

Fig. 4. Section of the rat testis showing the distribution of label after extraction with chloroform-methanol.

Sertoli cells showed a considerable amount of labelling. The Leydig cells were also heavily labelled.

Hot chloroform-methanol mixture almost completely removed the labelled material and only negligible activity was found to have remained (Fig. 4). Very little activity, which resisted the extraction procedure, was found to be confined to the Leydig cells, Sertoli cells and the residual bodies in the lumen.

DISCUSSION

The results show that much of of the labelled acetate ($1-C^{14}$) was incorporated into lipids which were removed by hot chloroform-methanol extraction. There is enough evidence that the testicular tissue incorporates labelled acetate into long chain fatty acids (Hall, Nishizawa and Eik-Ness, 1963). It is also known that the Leydig cells are capable of synthesizing lipids (Leeson, 1961). The present study suggests that the lipids as a metabolite, quickly pass through various cellular elements in the seminiferous tubules. The finding that the Sertoli cells at all stages of spermatogenesis showed the presence of incorporated radioactivity may be taken to mean that these units quickly take up the labelled acetate through the basement membrane and supply it to the germinal cells. It is well known that a single Sertoli cell is in intimate contact with several germinal cells. Such an association of the Sertoli cell with others has been referred to as a "symbiotic unit" (Roosen-Runge, 1962). Vilar et al (1962) have described the Sertoli cells as the "bridge-cells" between the basal membrane and the germinal cells. Von Ebner (1888) demonstrated that fine lipid granules line up between the central region of the Sertoli cell and the attached developing spermatozoa, which he called "Ernahrungsstrom" (nutritional current). It was suggested by Montagna (1952) that the glycogen granules in the human Sertoli cell may be regarded as an energy source to the attached spermatozoa. From the present observations it is seen that lipids are also transported by the Sertoli cells to the various germinal elements which are in contact with them. The assumption stated earlier that specific lipid moieties are probably transported by the Sertoli cells to other cell types was, however, not clearly established since there was a rapid run of the labelled material through the seminiferous epithelium. Lacy (1960) has shown that rat Sertoli cells phagocytose the residual bodies, and that the RNA content of such bodies is reabsorbed into the Sertoli cytoplasm. The lipid moiety, however, was found to be maintained as such and subsequently converted to a hormone-like substance which, according to Lacy (1960), may regulate and synchronize spermatogenetic activity. He also noted that this lipid factor is passed on to the developing germinal elements by the Sertoli cells. Such a fate of the lipid material seems to be an attempt on the part of the testis for maintaining some specific conditions during spermatogenesis.

In the present study it was difficult to confirm the actual passage of incorporated acetate through the basement membrane to the Sertoli cells since the label appeared almost everywhere in the testis within a period of only 30 minutes after administration. It may be mentioned that the basement membrane serves as an ideal structure for the transport of various materials. Alkaline phosphatase activity has been found to be associated with transfer of metabolites across the cell membranes (Moog, 1946; Bradfield, 1950; Rosenberg and Wilbrandt, 1952; Prankerd, 1956). This enzyme activity was shown to be present in the basement membrane and Sertoli cells (chapter I). Baillie (1962) reported that alkaline phosphatase activity is slowly acquired by the basement membrane of the seminiferous tubules of the mouse during post-natal development and reaches the maximum level by the time spermatogenesis begins. This enzyme activity was shown to be present in the Sertoli cells of the rat, guinea pig, and human testes (Vilar et al., 1962). It, therefore, seems clear that the basement membrane and the Sertoli cells are functionally well adapted for the transport of metabolites and other materials of physiological significance in the metabolism of the germinal elements.