CHAPTER IV

POST-NATAL LEVELS OF LIPIDS, LIPASE AND SUCCINIC DEHYDROGENASE IN RAT TESTIS

From the vast array of information available from developmental studies, it is being increasingly realised that the gross morphogenetic changes are the manifestations of the underlying adaptive mechanisms at the biochemical level. The role of the enzymes and the enzyme systems in effecting these changes is also being well established. Changes in enzyme concentrations have been noted in conjuction with functional changes in several organ systems during embryonic as well as post embryonic development.

Rogers (1960) and Rogers et al. (1960), have shown progressive increase in alkaline phosphatase and cholinesterase activities in the chick brain during ex-ovo development which could be correlated with differentiation preparatory to the attainment of the functional status. It has also been shown (Rogers, 1963 a & b), with respect to alkaline phosphatase activity, that the attainment of function in the developing brain of an altricial bird is a delayed process when compared to that in the precocius chick.

In the rat brain cholinesterase activity was shown to rise to a maximal level between 20 and 22 days of post-natal development (Metzler and Humm, 1951). Crevier (1958) observed that the PAS-reactive material and cholinesterase

increased progressively in the brain of the rat during post-natal development. Cohen and Lin (1962) found that the maturation of the rabbit brain during post-natal development was accompanied by a progressive increase in the concentrations of phosphocreatin and adenosine triphosphate, in order to meet the increasing energy demands. However, ascorbic acid and phosphoethanolamine were reduced to minimal levels during the first 20 days after birth (Cohen and Lin, 1962). Progressive increase in phosphorylase (Shimizu and Okada, 1957), and 5'-nucleotidase (Naidoo, 1962). activities in the brain tissue has also been demonstrated.

The relation of the alkaline phosphatase activity to the process of differentiation of the small intestine, and the developmental enzymic adaptations have been discussed by Moog (1962). McAlpine (1951 and 1954) found that alkaline glycerophosphatase activity in the developing endocrine pancreas, thyroid and thymus of the rat attained a high level before these organs were properly differentiated for their respective functions.

Studies on the biochemical changes occurring prior to, during, and after metamorhposis in various animals have also brought forth valuable information regarding the role of enzymes in development (Bennett and Frieden, 1962).

In the light of the knowledge obtained from the studies mentioned above, significant biochemical changes

in the testis prior to and/or during the process of attainment of sexual maturity should be expected. It has already been shown that lipids are utilized by the rat testis in the process of spermatogenesis (chapter I). It should therefore be worth-while to study lipid metabolism in the rat testis during post-natal development. In the present investigation, therefore, a study of the lipid content, and the levels of lipase, a lipid splitting and esterifying enzyme, and succinic dehydrogenase, the key oxidative enzyme of the Krebs cycle, was undertaken.

MATERIAL AND METHODS

Developing young albino rats were killed by decapitation at weekly intervals from the fifth to the sixteenth week. The testes were removed and quickly frozen to -10°C. One of the testes from each animal was homogenized in a chilled mortar with ice-cold distilled water, and the other gland was weighed on a Mettler balance and kept for drying in an air oven at 90° C. The homogenate prepared in distilled water was used to estimate lipase and succinic dehydrogenase activities. Lipase activity was assessed by a manometric method using tributyrin as the substrate as described in chapter III. The enzyme activity is expressed in terms of µl of CO_2 per mg protein per hour. A modified colorimetric method was adopted to estimate succinic dehydrogenase (SDH) activity which is expressed as µg diformazan produced per mg protein for 15 minutes at 37° C (cf. chapter III). The amount

of protein in the homogenate was estimated colorimetrically using the biuret reaction as described in chapter III. The tissue dried to constant weight was extracted for lipids in the Soxhlet apparatus employing 1:1 mixture of ethanol and ethyl ether as solvent. Lipid content is expressed as per cent mg on the basis of the dry weight of the tissue.

RESULTS

The level of the lipase activity of the testis of the 5 week old rat was found to be the highest (Table 1 and Figs. 1 to 4) whereas, the lipid content was the lowest. Succinic dehydrogenase (SDH) also was observed to be very low. Thereafter, lipase activity showed a progressive decrease upto the age of 9 weeks when it reached the lowest level. During this period the lipid content steadily increased upto the age of 8 weeks and was followed by a significant decrease in the ninth week. The SDH activity gradually decreased upto the seventh week, and showed a slight increase in the eighth week. Then there was a decrease touching the minimal level at the age of ninth week.

In the 10 weeks old rats, the testicular lipase activity was found to be raised to a higher level and the lipid content also showed a slight increase. The most stricking change was observed in the case of SDH activity which abruptly shot up to the highest level in the tenth and eleventh weeks.

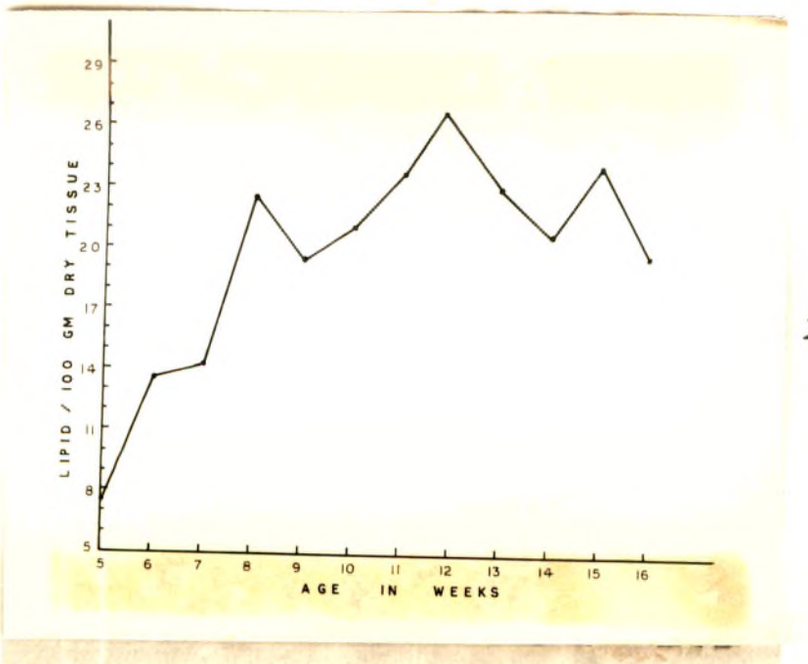
TABLE 1

Showing the lipid content and the levels of lipase and SDH activities during post-natal development of rat testis

Age in weeks	Lipids	Lipase	SDH
5	7.5 (3.5-14.8)	35.2 (26-80)	172.8 (80-250)
6	13.65 (11.8-15)	25.0 (21-28)	132.7 (80-150)
7	14.28 (13.7 -17.5)	22.1 (19-27)	117.1 (100-200)
8	22.73 (18.5 -27.9)	21.6 (12-22)	156.5 (100-200)
9	19.5 (16.6 -23.4)	13.87 (10-20)	87.5 (70 -110)
10	21.13 (19.5 -23.4)	19.78 (14-24)	234.6 (200-300)
11	23.88 (21.1 -25.4)	16.4 (14-19)	465 (400-550)
12	26.86 (23.0 -28.2)	16.2 (14-18)	435 (400-500)
13	23.1 (20.1 -25.0)	21.88 (19-25)	239 (200-300)
14	20.81 (18.5 -24.4)	19.26 (17-22)	215 (190-250)
15	24.4 (22.5 -27.1)	23.75 (22-25)	277.5 (200-350)
16	19.91 (14.5 -23.0)	21.8 (16-22)	280 (200-300)

Figures in paranthesis represent the range.

The values given are the average of not less than 5 experiments.



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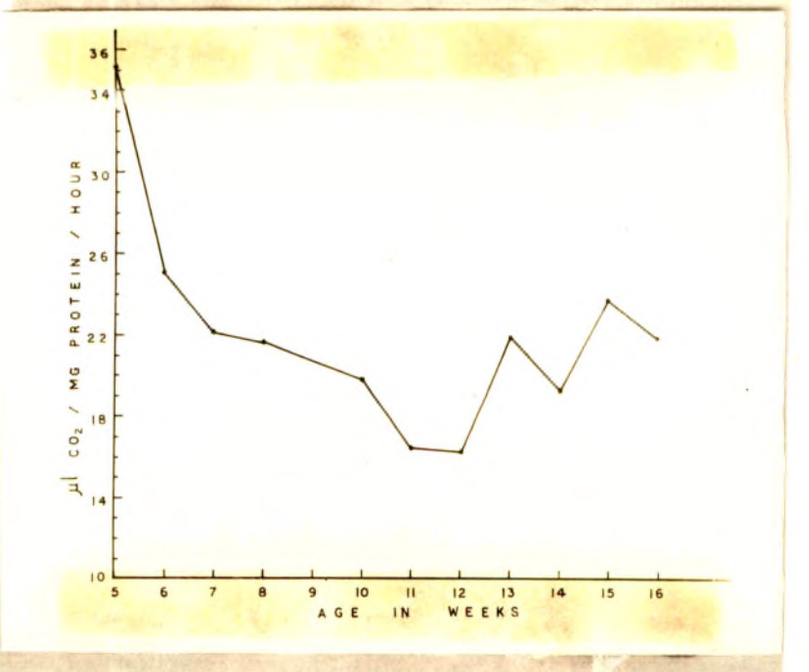

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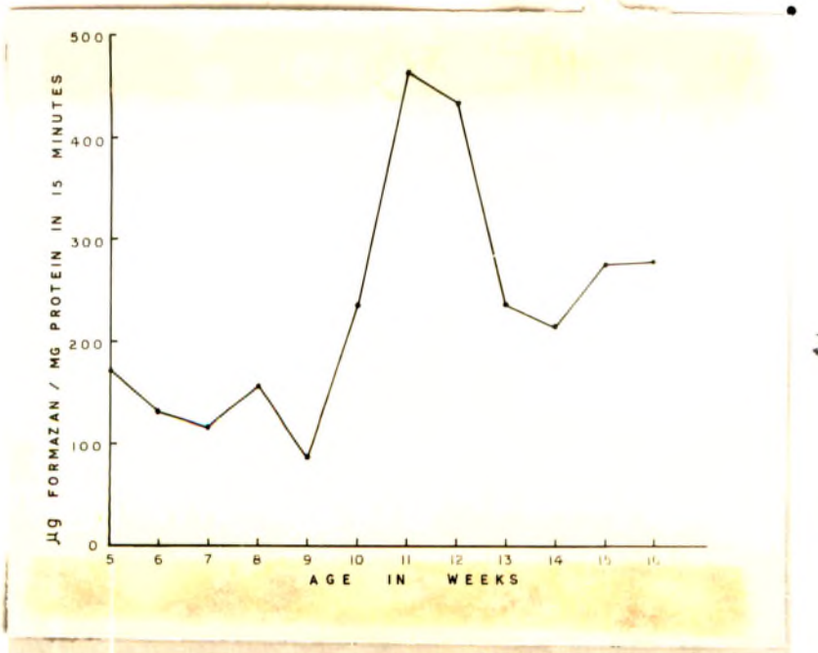
Fig. 1. Changes in the lipid content of the rat testes during post-natal development; from fifth to sixteenth week.

Fig. 2. Changes in the levels of testicular lipase activity in the rat during post-natal development.



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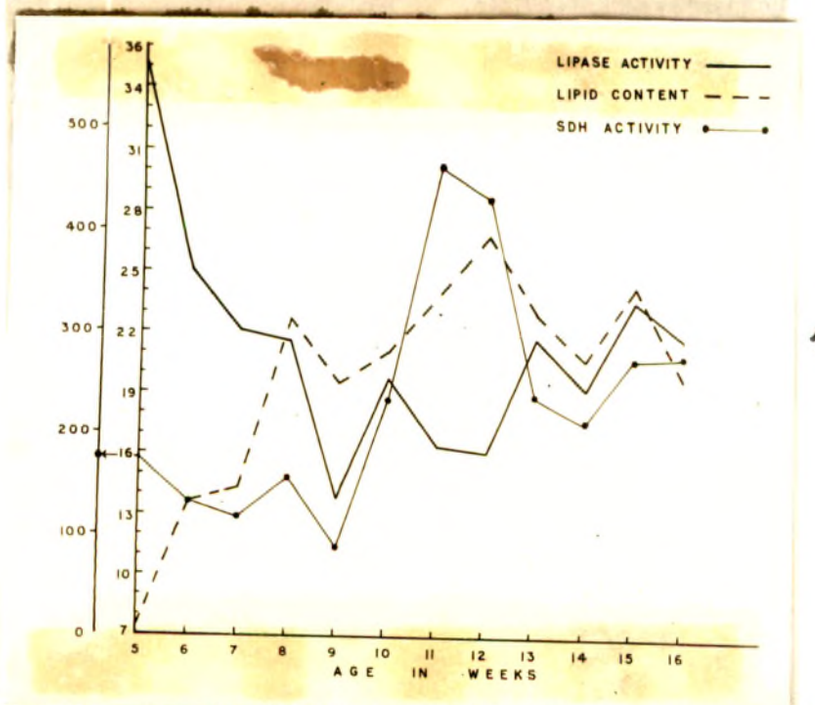

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Fig. 3. Changes in the levels of the succinic dehydrogenase activity of the rat testes during post-natal development.

Fig. 4. Super-imposed graphs showing the changes in the levels of lipid content and activities of lipase and SDH, to emphasize the changes occurring in the ninth and the fourteenth weeks. The scale on the left is for SDH activity (ug of formazan/mg protein in 15 minutes) and that on the right for lipase activity (ul CO_2/hr/mg protein) as well as for lipid content (per 100 gm).

During the subsequent development, the lipase activity decreased and remained at that level upto the twelfth week. By the thirteenth week it increased to a higher level to come down again in the next week. In the next week a further increase reaching the adult level (22 µl) was recorded. The lipid content increased progressively upto the twelfth week, decreased in the next two weeks, then slightly increased in the fifteenth week, and thereafter, was stabilized at the lower level (19 mg%) characteristic of the adult testis.

A progressive decrease was observed in the SDH activity from the eleventh week onwards upto fourteenth week. Then there was a slow progressive increase in the next two weeks so as to attain the adult level (275 µg diformazan).

However, there was a fairly significant decrease, though considerably lesser in magnitude, in the activities of both enzymes as well as lipid content at the age of 14 weeks, and thereafter, the fluctuations mainly led to the establishment of a pattern characteristic of the mature adult testis.

DISCUSSION

The results clearly show that in the fifth week the lipolytic activity is maximum, indicating that the testes utilize substantial amounts of lipids during this period. Thereafter, the lipolytic activity gradually decreases to

minimal levels through the succeeding three weeks and the testes accumulate lipids to a considerable extent (Fig. 1). This may be explained as due to the mobilization of lipids from other organs like adipose tissue and liver, during this period. This lipid accumulation may also be due to increase in the substance of the testes which is conspicuous during this period.

At the age of about 50 days, there is a considerable decrease in the lipid and enzyme levels occurring concomitantly. This seems to be a specific response of the testes coinciding with the increasing gonadotropin secretion. This suggestion finds strong support in the observation that the administration of gonadotropin to the adult rats results in a considerable decrease in the levels of lipids, lipase and SDH in the testes (chapter III). It is known that the albino rats attain maturity by about 60 days of age (Farris, 1954), and the present findings appear to be more or less in conformity with this. However, the author,maintains that this period only indicates the onset of gonadotropin secretion initiating rapid changes for maturity, and the testes get fully matured by about 95 to 100 days after birth. That this is the case is evident from the fact that rats can effect successful matings only after 90 days of age. This contention finds experimental support in the evidence that at about 95 to 98 days of age the lipid content and the levels of lipase and SDH activities again come down and gradually attain the characteristic adult levels.

The phenomenal rise in SDH activity soon after the onset of gonadotropin secretion needs explanation. From the literature cited in chapter I, it is evident that many steroid hormones affect various enzyme systems, particularly dehydrogenases. Recently some evidence has been brought forth to show that gonadotropins directly activate the dehydrogenases in the target glands (Schor, Cara and Perez, 1963). The dehydrogenases studied in these investigations were mainly those involved in steroid biosynthesis. It was observed that high doses of gonadotropin increase the testicular SDH activity (chapter III). On the basis of these observations it may be suggested that as the gonadotropin secretion reaches a certain level the SDH activity in the testes is greatly enhanced. It is, however, not possible to explain satisfactorily as to the cause of decrease in the enzyme activity during the subsequent few weeks of the post-natal development. The initial high level of gonadotropin prevailing at the age of about 50 days increases the SDH activity. When the gonadotropin thus stimulates the testicular biosynthesis of the male hormone, the latter in turn may be expected to inhibit the secretion of gonadotropins in the pituitary. Such a "feed-back" mechanism responsible for effecting a reduced gonadotropin level may bring about the decrease in the SDH activity.

Rosenbaum and Lindeman (1958) observed that the phophatase activity (pH 5.6) in the developing rat testes increases from fifth to fiftieth day after birth almost linearly,

and later maintains a steady level. Such a linear rise was not observed in the present investigation in any of the parameters and moreover, the changes were complicated by the gonadotropin secretion.

In the light of the above discussion, the following three phases in lipid metabolism may be recognized during the post-natal development of the rat testis: (1) Period of high lipolytic activity (fifth week) characterized by highest lipase activity, moderate SDH activity and lowest lipid content, (2) Period of high oxidative capacity (nineth to eleventh week) indicated by highest SDH activity. (3) Period of attainment of adult pattern of lipid metabolism (fourteenth week) characterized by fluctuations in the lipid and enzyme levels within narrow limits prior to attainment of the normal adult levels.