CHAPTER III

IN VIVO EFFECT OF TESTOSTERONE PROPIONATE AND GONADOTROPIN ON THE LIPID CONTENT AND THE LIPASE AND SUCCINIC DEHYDROGENASE ACTIVITIES IN THE RAT TESTIS AND EPIDIDYMIS

It is well known that the male sex hormone governs the growth and maintenance of the male reproductive organs and that the administration of testosterone propionate has a direct stimulatory effect on spermatogenesis (Albert, 1961; Greep, 1961). Chorionic gonadotropin has been observed to stimulate androgen production by the Leydig cells, which in turn stimulates the growth of the entire reproductive system. Leuteotropic factor of the chorionic gonadotropin is believed to be responsible for such an effect (Burrows, 1949; Albert, 1961; Greep, 1961).

Apart from this, various other effects of these hormones on the male reproductive system, under different conditions, have been studied. The administration of testosterone propionate (TP) has been shown to prevent the damaging effects of castration. It has been observed that castration leads to atrophic changes, nuclear pycnosis, and loss of lipids in the Leydig cells, and these changes are reversed to normalcy by the administration of human chorionic gonadotropin (HCG) (Albert, 1961; Greep, 1961). Kincl *et al.*(1964), and Maqueo and Kincl (1964) demonstrated

that the administration of oestradiol benzoate to young male rats results in strong atrophic changes in the testis which are reversed by the administration of TP and the pregnant mare serum preparation.

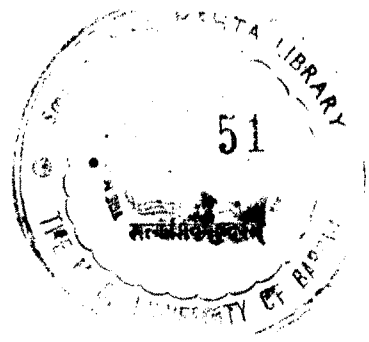
Besides the literature cited above, a large body of information on the effects of various steroid hormones on different enzyme systems in many tissues is also available. One of the most prominent effects of steroid hormones is found to be the inhibition of the respiratory enzyme systems (Gilbert et al., 1951; Kochakian, 1951; Endahl and Kochakian, 1961; Strittmatter, 1962). Yielding et al. (1960) suggested that the locus of the steroid inhibition of pyridine nucleotide requiring dehydrogenation reaction, such as that of reduced diphosphopyridine nuleotide-cytochrome c -reductase system, was between the flavoprotein and either coenzyme-Q or cytochrome b. Stoppani et al. (1962 a & b) also studied the reduced diphosphopyridine nucleotide oxidation system and found that though the steroid hormones interfere with electron transfer reactions between reduced diphosphopyridine nucleotide and cytochrome c, it would be premature to pinpoint the inhibitory effect at cytochrome b or coenzyme-Q levels. McKerns (1962, 1963), who studied the reduced triphosphopyridine nucleotide specific dehydrogenases of the anterior pituitary, demonstrated that steroid hormones inhibited the oxidative system and they also suggested that such a mechanism may play an important role in the regulation of the pituitary secretion of trophic hormones and the state

of the endocrine tissues in vivo (McKerns, 1963).

Another aspect of the study of the action of the sex hormones is concerned with the metabolic changes in the accessory sex organs. Damaging effects of gonadectomy on the accessory sex glands and their reversal by the administration of the appropriate sex hormones are discussed by Price and Williams-Ashman (1961). There is ample evidence that androgens stimulate protein biosynthesis in the prostate gland (Hancock et al., 1962; Liao and Williams-Ashman, 1962; Williams-Ashman et al., 1964; Kochakian, 1964). Williams-Ashman et al. (1964) go further and suggest that the steroid hormones might directly modify the action of genetic repressors, like histones, to facilitate the activation of specific messenger RNA (Ribonucleic acid) synthesis to provide genetically coded information for the biosynthesis of specific proteins in the prostate gland.

It was suggested earlier (Chapters I & II) that lipids have an important role in the metabolism of the testis and the epididymis. Considering this suggestion and the literature cited above, which indicates subtle changes in the growth and maintenance of the entire male reproductive system, it was thought worth-while to study the effect of TP and HCG on the lipid content and, the two key enzymes of lipid metabolism - lipase and succinic

dehydrogenase in the rat testis and epididymis.



MATERIALS AND METHODS

Healthy, laboratory bred, male albino rats (Haffkine Institute strain); 15-20 weeks old and weighing 110-180 gms, were used. The hormones used in the present study were testosterone propionate (TP) and human chorionic gonadotropin (HCG). The testosterone propionate (Sigma Chemicals) was dissolved in pure tributyrin (British Drug House) to give a concentration of 4 mg / ml and this solution was used for intramuscular injections. Human chorionic gonadotropin (Sigma Chemicals) preparation, which contained a phosphate buffering system and urea, was dissolved in sterile distilled water diluted to a concentration of 100 i.u. / ml. Both the hormones were injected into the thigh muscle.

The animals were divided into four groups. The first group received 2 mg of TP (0.5 ml of the solution) per day per head. The second group received 0.5 ml tributyrin per day per head simultaneously with the first group to serve as control. Individuals of the third were administered 100 i.u. of HCG (1 ml of preparation) per day per head. Rats of the fourth group were injected with 1 ml of phosphate buffer (pH 7) to serve as control for the experimental animals of the third group. All the rats were given regular balanced diet and water ad libitum. Rats, treated in these different ways, were housed in separate cages along with healthy female rats.

The animals were sacrificed at the following total dosage levels along with appropriate control animals :

a) TP - 4 mg, 8 mg & 10 mg

b) HCG - 200 i.u., 400 i.u. & 500 i.u.

Soon after sacrificing the animals by decapitation the testes and epididymides were dissected out clear of adipose tissue and were quickly frozen to -10°C. The epididymides were divided into caput and cauda regions and were treated separately.

Pieces of testis, caput- and cauda-epididymis were homogenized with ice-cold distilled water in pre-chilled mortars. The homogenates of the respective tissues were assayed separately for lipase and succinic dehydrogenase(SDH) activities. Lipase activity was estimated by the manometric method of Martin and Peers (1953) as adopted by George and Scaria (1959). Lipase was allowed to act upon tributyrin (6% v/v suspended in 0.0148 M sodium bicarbonate solution and emulsified with a drop of "Tween 80"). The enzyme activity was assayed in a CO_2/bicarbonate buffer system. The fatty acids liberated by the lipolytic action reacted with bicarbonate giving out carbon dioxide which was measured from the pressure changes of the manometric reading. Lipase activity is expressed as µl of CO_2 per hour per mg protein.

SDH activity was assayed by a colorimetric method of Nachlas, Margulies and Seligman (1960) adopted with modi-

fication. Nachlas, Margulies and Seligman (1960), in their method, have recommended the use of 2-(-p-indophenyl)-3-p-nitrophenyl tetrazolium chloride (INT) with phenazine methosulphate (PMS) with the incubation mixture containing in addition to substrate and phosphate buffer (pH 7.7), a solution of gelatin (0.1%). In the present investigation 0.2 M substrate solution, 0.1 M phosphate buffer (pH 7.7) and 0.2% INT solution (1:1 distilled water: dimethyl formamide mixture) were used. Addition of PMS or gelatin was found to be of no advantage hence, these were omitted and the incubation mixture for assay was of the following composition:

0.2 M Sodium succinate	0.5 ml
0.1 M Phosphate buffer(pH 7.7)	0.5 ml
0.2% INT	1.0 ml
Homogenate	1.0 ml

After a 15 minute incubation period the reaction was stopped with 7 ml of acetone. The mixture was centrifuged for 5 min. at 3000 r.p.m. and the colour intensity was measured against a 540 mμ filter in a Klett-Summerson photoelectric colorimeter. The colorimeter was adjusted to zero with a control containing all the reagents and the homogenate except the substrate and in its place distilled water was added to correct the volume of the incubation mixture. The readings were calculated with reference to a

standard graph plotted for known quantities of reduced INT against colorimeter readings.

The lipid content of the testis, caput- and cauda-epididymis was estimated by a gravimetric method. Pieces of fresh tissue were weighed on a Mettler balance and dried in an air oven to constant weight. Lipids were extracted in 1:1 mixture of ethanol:ethyl ether using the Soxhlet apparatus. The percentage of extractable lipids is expressed as mg on dry weight basis.

The protein content of the homogenate was estimated by a colorimetric method based on the biuret reaction. The values for protein content were calculated with reference to the standard graph plotted on the basis of the colorimetric readings for known quantities of crystalline albumen.

The results are discussed on a comparative basis taking into account the readings obtained in the two sets of experiments conducted.

RESULTS

The results obtained are presented in Table I showing the percentage increase or decrease in the values over those obtained in the control animals.

Effects of HCG

(A) Testis

(i) 200 i.u. - SDH activity and lipid content were reduced considerably but the lipase activity showed a variable response.

Table 1 A

Effects of HCG

Showing percentage increase or decrease in the lipid content and the levels of lipase and SDH activities in the rat testis and epididymis

		200 I.U.			400 I.U.			500 I.U.		
		T.	Cp.Epd	Cd.Epd	T.	Cp.Epd	Cd.Epd	T.	Cp.Epd	Cd.Epd
Lipase	Set I	-50	-15	-46	+67	+336	+375	+18	-40	-19
	Set II	+61	+46	-3	+65	+85	+91	-12	+85	+177
SDH	Set I	-61	-82	-80	+91	+100	+1370	-47	+39	+50
	Set II	-2	-18	-21	-15	+20	-48	+12	+32	+233
Lipids	Set I	-37.5	+14.5	+38.3	+42.8	+8.7	+40	-43.2	Nil	+25

+ = Increase; - = Decrease

Table 1 B

Effects of TP

Showing percentage increase or decrease in the lipid content and the levels of lipase and SDH activities in the rat testis and epididymis

		4 mg			8 mg			10 mg	
		T.	Cp.Epd	Cd.Epd	T.	Cp.Epd	Cd.Epd	T.	Cp.Epd	Cd.Epd
Lipase	Set I	+235	+264	+1760	+6	+5	-9	-5	+169	-17
	Set II	+16	+19	+31	+44	+48	+51	-7	+34	+87
SDH	Set I	+300	-50	-46	-13	+12	+1.2	-20	+82	+113
	Set II	-18	+1	-75	+7.3	+400*	+6600*	-4	+2300*	+1120*
Lipids	Set I	-25	-27.3	+18.2	+65.2	-27.7	+4.3	-6.3	-12.3	+64

\+ = Increase; - = Decrease

* The values obtained for the control animals were sub-normal

(ii) 400 i.u. - There was a considerable increase in lipase activity as well as lipid content while SDH activity showed a contradictory response in the two sets of the experiments.
(iii) 500 i.u. - There was a sharp decrease in the lipid concentration but the activities of both the enzymes responded variedly.

(B) Caput Epididymis

(i) 200 i.u. - There was a fall in the SDH activity and a considerable increase in the lipid level. For lipase activity a diverse response was obtained.
(ii) 400 i.u. - A considerable increase in the lipase and SDH activities and a decrease in the lipid content were noted.
(iii) 500 i.u. - There was a distinct increase in SDH activity whereas lipid content remained unaltered. Lipase activity showed a contradictory response.

(C) Cauda Epididymis

(i) 200 i.u. - There was a drop in the levels of lipase and SDH activities but the lipid content was increased.
(ii) 400 i.u. - Lipase activity was increased considerably and the lipid content also showed a slight increase. SDH activity showed a variable response.
(iii) 500 i.u. - The SDH activity was increased considerably and so also the lipid content. Lipase activity showed varied response.

From what has been noted above it is seen that with 200 i.u. of HCG, SDH activity in all the three tissues

viz. testis, caput- and cauda-epididymis was found to be decreased considerably. At the dosage level of 400 i.u. of HCG, lipase activity as well as lipid content in all the three tissues increased considerably. A dose of 500 i.u. induced a very variable response of lipase activity in all the three tissues. All the dosage levels of HCG continued to induce a definite increase in the lipid content of the cauda-epididymis.

Effects of TP

(A) Testis

(i) 4 mg - There was an increase in lipase activity and a decrease in the lipid level. Response of SDH activity was variable.

(ii) 8 mg - Here too an increase in the lipid content and lipase activity was recorded. SDH activity varied.

(iii) 10 mg - The decrease noted in the levels of lipase and SDH and lipid was not significant.

(B) Caput Epididymis

(i) 4 mg - The lipase activity was increased considerably and there was a significant decrease in the lipid level. SDH activity also tended to be low.

(ii) 8 mg - Lipase and SDH activities were enhanced but the lipid level continued to show a drop in the concentration.

(iii) 10 mg - The response of all the tissues in the case of the two enzymes as well as lipid content was similar to that obtained with an 8 mg dose.

(C) Cauda Epididymis

(i) 4 mg - Lipase activity and the lipid content were increased but the SDH activity was low.

(ii) 8 mg - Lipase activity was influenced variably but SDH activity and lipid content were found to be increased.

(iii) 10 mg - The response was similar to that obtained with 8 mg dose.

The above observations may be summarised as follows :

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

(2) At all the three dosage levels 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.

(3) Cauda epididymis showed a continued increase in the lipid content with all the three doses of TP.

DISCUSSION

With different dosage levels of HCG, the response of the testis was quite variable but with 400 i.u. dose, there was increased lipase activity and also lipid content, which is indicative of an increased lipid biosynthetic activity in the testis.

With a 4 mg dose of TP the testis showed increased

lipase activity and a lower lipid level thereby indicating a trend towards acceleration of lipid utilization. The response with an 8 mg dose of TP was the reverse to that obtained with 4 mg, here an increased lipase activity was accompanied by an increased lipid concentration, thus suggesting increased lipid synthesis in the testis. The activities of lipase and SDH and the lipid content were all lowered by a 10 mg dose of TP. It is not possible to explain such a response.

The lipids in the rat testis were observed to be utilized at a faster rate under the influence of 4 & 10 mg of TP as well as 200 & 500 i.u. of HCG. It is known that TP has a fat mobilizing action in rats (Laron and Kowadlo, 1963 & 1964) and this is apparent at the different doses stated above. Under the influence of 4 mg of TP and 400 i.u. of HCG the testis showed increased levels of lipids, thus giving a reverse result to the one above. These observations lead to the conclusion that the effects vary with different doses of the hormones.

An intriguing response was shown by the caput epididymis under the influence of 400 i.u. of HCG. Here both the enzyme activities and lipid content were increased.

At 500 i.u. dose of HCG the caput epididymis showed increased SDH activity with no alteration in the lipid content, which means that either a balance is struck

between lipid synthesis and lipid utilization or lipid metabolism remained unaffected. The increased SDH activity is probably due to increased oxidative metabolism without the involvement of lipids.

The response of caput epididymis to all the three doses of TP was characteristic in that it showed a steady increase in lipase activity and a corresponding decrease in the lipid content. SDH activity too, was increased, at least with 8 and 10 mg doses.

Under the influence of 4 mg of TP, lipase activity and the percentage of lipids in the case of cauda epididymis were increased and SDH activity was decreased, thereby indicating that lipids are being synthesized in the cauda epididymis.

With 8 and 10 mg doses of TP, lipase activity showed a variable response but the lipid content and the SDH activity increased. According to Scott, Dawson and Rowlands (1963) the concentration of phospholipids gradually increases down the epididymal tract in the rat. Scott et al. (1963) have shown that there is an increase in the phospholipids, particularly glycerylphosphorylcholine, in the epididymal fluids of the ram and rabbit and that the caput- and cauda-epididymis of rabbit are capable of synthesizing glycerylphosphorylcholine. The extracting medium used in

the present work for lipids is known to extract phospholipids along with the simple lipids (Pearse, 1960). In this context it can be said that caput epididymis plays an important role in mobilizing the lipids and transporting them to the more distal parts where they are utilized for building up phospholipids. This possibility may explain the increase in lipids (extractable by the method employed in the present work) inspite of the rise in SDH activity in the cauda epididymis. The increased level of SDH activity under such experimental conditions is, in all probability, an indication of the higher rate of oxidative metabolism in the cauda epididymis. The results obtained are much in favour of such a conclusion since, at all doses of TP the caput epididymis showed continued increase in lipase activity and a corresponding decrease in lipid content whereas the lipid level increased in cauda epididymis.

In the light of the above observations it can be seen that lipid metabolism, in the tissues studied, is influenced by the circulating hormones depending on their specific concentrations. This is particularly so in the case of the epididymal portions. Evidence from histochemical studies is available to show that various metabolites and enzymes of the epididymis are affected by castration and hormone replacement therapy (Allen and Slater, 1957, 1958, 1959, 1960; Cavazos, 1958; Gohary, Cavazos and Manning, 1962). The present work also tends to show

a similar effect of hormones on the levels of lipids, lipase and SDH. It may also be suggested that the levels of circulating gonadotropin and androgen might influence the lipid metabolism of the epididymis so as to facilitate storage and further maturation of the spermatozoa. It should, however, be pointed out that the observations reported in the present work are insufficient to arrive at any definite conclusions.