EFFECT OF OESTRADIOL-3,17β DIPROPIONATE ON SPERMATOGENESIS AND LYSOSOMAL ENZYMES IN THE RAT TESTIS

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Abstract

Daily intramuscular injections (20 μg/day) of oestradiol-3,17β dipropionate in adult male rats depressed body growth and reduced the weight of the testes and seminal vesicles between 4 and 8 days of treatment.

The proportion of soluble acid phosphatase (E.C. 3.1.3.2), acid proteinase (E.C. 3.4.4.23), and β-glucuronidase (E.C. 3.2.1.31), increased during oestradiol treatment. There was also an increase in the total amount of acid proteinase and β-glucuronidase but no significant change in total acid phosphatase activity with oestrogen treatment. These changes in hydrolase activity coincided with a decrease in the yield of late primary spermatocytes from early primary spermatocytes, and long spermatids from round spermatids. Histochemical evidence supported the biochemical and histochemical findings, with increasing tubular staining of acid phosphatase and lipid in the testis during treatment.

It is concluded that lysosomal enzymes are important in testicular degeneration and probably in normal spermatogenesis.

I. INTRODUCTION

Oestradiol causes regression of the testes and accessory reproductive organs of various mammals [see Emmens and Parkes (1947) and Burrows (1949) for extensive reviews]. Oestrogenic extract of amniotic fluid reduced the gonadotropic activity of the pituitary glands of castrated male and female rats (Meyer *et al.* 1932), and Moore and Price (1930) showed that oestrogen caused a depression of pituitary function in the male, due to a depression of gonadotropin output by the pituitary (Greep and Jones 1950). It has been shown that oestrogen concentrates in the hypothalamus and pituitary gland of the rat (Stumph 1968; Attramadal 1969; Kato *et al.* 1969), which suggests that oestrogen may inhibit the secretion of gonadotropins and their releasing factors from the pituitary and hypothalamus respectively.

Oestrogens are powerful inhibitors of spermatogenesis, the descent of the testes in immature animals (Gardner 1949), Leydig cell function (Selye and Friedman 1941), and follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion (Nelson and Patanelli 1960). In man, De La Blaze *et al.* (1962) showed that oestrogens cause atrophy of the seminiferous tubular epithelium to spermatogonia, spermatocytes, and Sertoli cells, with an apparent increase in lipid and glycogen in the tubules. They also demonstrated the disappearance of Leydig cells and appearance of fibroblast-like cells, which may be the morphological products of the Leydig cells. Lacy and Lofts (1965) suggest that the effect of oestrogen on the rat testis is indirect due to reduced secretion of FSH and LH from the pituitary. Inhibition of LH secretion is thought to decrease the testosterone production by the Leydig cells and depress testis and seminal vesicle weight, while depression of FSH secretion may decrease testosterone production by Sertoli cells (Lacy 1967) resulting in a cessation of spermatogenesis.

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Observations on the effect of oestrogen on enzymes in the testis have been concerned mainly with dehydrogenases involved in steroid and glucose metabolism. Oestrogen inhibits 3-β-hydroxysteroid dehydrogenase in the testes of some animals (Collenot 1964; Bongiovanni *et al.* 1967; Botte and Delrio 1967) and causes a marked decrease in 17α-hydroxylase, 17,20-lyase, and 17-β-hydroxysteroid dehydrogenase (Samuels, Uchikawa, and Huseby 1967). Oestrogen also decreases the histochemical reaction of lactate dehydrogenase (LDH-X) (Zinkham, Blanco, and Clowry 1964), and glucose-6-phosphate dehydrogenase in the rat testis (Elkington and Blackshaw 1970). According to Baust, Goslar, and Tonutti (1967), the activity of acid phosphatase was only slightly affected by oestrogen treatment, although hyaluronidase was decreased by both oestrogen treatment and hypophysectomy (Steinberger and Nelson 1955).

The experiments described in this paper show some of the effects of oestradiol on the hydrolytic enzymes, acid phosphatase (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.2), β-glucuronidase (β-D-glucuronide glucuronohydrolase, E.C.3.2.1.31), and acid proteinase (E.C.3.4.4.23) in the rat testis; this is correlated with effects on spermatogenesis, to show the possible relationship between some testicular hydrolases and testicular degeneration.

II. Materials and Methods

Young adult male Wistar rats (60–80 days), maintained on commercial pellets and water, were selected randomly and treated with 20 μg/day of oestradiol-3,17β dipropionate (Ovocyclin, CIBA), or maize oil, for 1, 2, 4, 8, 16, or 32 days by intramuscular injection. The rats were weighed at the beginning and end of the experiment.

The seminal vesicles (empty of secretion) and testes were weighed, and half of one testis was frozen to a stainless steel microtome chuck, in a dry ice–acetone mixture, for histochemical studies. The remaining half of the testis was fixed in Susa (Humason 1967) for histological examination. The second testis was homogenized (10% w/v) in ice-cold 0·25M sucrose with a motor-driven glass–Teflon homogenizer. The homogenate was immediately contrifuged at 2500 r.p.m. in a Christ UJ15 centrifuge for 5 min at 4°C.

Aliquots (4·5 ml) of the crude supernatant were diluted with 0·5 ml of 0·25M sucrose or 0·25M sucrose containing 1% Triton X-100, for the determination of free and total enzyme activities respectively. The Triton X-100 disrupts lysosomes and releases bound enzymes into the medium (Wattiaux and de Duve 1956). After standing for 10 min at 4°C, the preparations were centrifuged for 30 min at 20,000 *g* and the supernatants used for the enzyme determinations. A supernatant was diluted 25 times for acid phosphatase (Torriani 1960), 10 times for β-glucuronidase (Plaice 1961), and used undiluted for acid proteinase measurements (Barrett 1967). The substrates for these reactions were 0·05M *p*-nitrophenyl phosphate (acid phosphatase), 0·025M phenolphthalein glucuronide (β-glucuronidase), and 4% denatured sheep haemoglobin (acid proteinase). All these methods gave a linear response over the times of incubation and the substrate levels were optimal for the reaction. The incubation times (37°C) for acid phosphatase and β-glucuronidase were 30 min and 18 hr respectively, while acid proteinase was incubated at 45°C for 1 hr. Enzyme activity was expressed as the change in optical density per gram tissue at the indicated wavelengths (acid phosphatase, 404 nm; acid proteinase, 280 nm; and β-glucuronidase, 550 nm).

Thin sections (10 μm) of the testis were made with a Lipshaw Cryotome at −25°C and placed on slides for staining of acid phosphatase (Pearse 1960) or with oil red O for lipid. Sections were mounted in Kaiser's glycerol jelly. The fixed testis was dehydrated and embedded in paraffin wax by standard procedures. Sections (7 μm) were made on a Minot microtome 1212 (Leitz) and stained by the Feulgen reaction, the counterstain being alcian blue (Humason 1967).

The volumetric proportion of particular elements in the testis was examined using a procedure described by Chalkley (1943) and applied to spermatogenesis by Kennelly and Foote (1964). The theoretical basis of this procedure has been discussed by Weibel and Elias (1967). The method used a counting graticule (Maxta) divided into four quadrants and with 25 randomly placed spots in the field, to give an unbiased estimate of the volumetric proportions of testicular elements. Only testicular elements (nuclei or other structures) touching the spots were counted in 10 fields of a paraffin section (7 μm) from each rat testis; the magnification for counting was 500 times. The elements of the testicular sections were classified as spermatogonia, early (leptotene and zygotene) and late (pachytene and diplotene) primary spermatocytes, round and long spermatids, and Sertoli cells, together with all extratubular and non-nuclear intratubular elements.

All results are expressed as the mean response for each treatment, and have been assessed by standard analyses of variance or covariance (Steel and Torrie 1960). Percentage data (soluble enzymes and volumetric proportions) were transformed to angles (arcsin $x^{-\frac{1}{2}}$, where x = percentage) for analysis. Mean squares for individual treatment effects and their interactions were isolated and tested for significance using the residual variance as error. The analyses are presented in summary form, giving degrees of freedom and variance ratios for each source of variation, with the error variance at the base of each variance ratio column.

TABLE 1

EFFECT OF OESTRADIOL TREATMENT OF ADULT MALE RATS ON TESTIS AND SEMINAL VESICLE WEIGHT

Values are means of 10 replications

Time (days)	Testis Weight (g)		Seminal Vesicle Weight (mg)	
	Control	Oestrogen	Control	Oestrogen
1	2·58	2·42	344	306
2	2·47	2·38	315	251
4	2·51	2·48	317	224
8	2·58	2·15	320	217
16	2·24	1·61	308	123
32	2·61	0·80	376	103

Summary Analysis of Covariance

Source of Variation	D.F.	Adjusted Variance Ratios	
		Testis Weight	Seminal Vesicle Weight
Treatment	1	1244·00**	11·89**
Time	5	351·00**	199·99**
Time × treatment interaction	5	358·00**	15·95**
Replication	9	73·90**	7·75**
Error variance	98	*0·01*	*23·73*

** $P < 0{\cdot}01$.

III. RESULTS

Because body weight showed significant changes during oestrogen treatment, covariance analysis was applied to both testis and seminal vesicle weights. These were significantly depressed by oestrogen treatment (Table 1). The weight of the seminal vesicles showed an immediate and continued fall throughout treatment,

TABLE 2

EFFECT OF OESTRADIOL TREATMENT OF ADULT MALE RATS ON THE TOTAL ACTIVITY OF ACID PHOSPHATASE, ACID PROTEINASE, AND β-GLUCURONIDASE IN THE TESTIS

Acid phosphatase, acid proteinase, and β-glucuronidase determined as change in optical density per gram of tissue. The number of animals is shown in parenthesis

Time (days)	Acid Phosphatase (10)		Acid Proteinase (10)		β-Glucuronidase (5)	
	Control	Oestrogen	Control	Oestrogen	Control	Oestrogen
1	532	554	7·50	7·63	68·5	75·5
2	559	556	7·11	6·78	58·0	53·5
4	470	468	6·93	6·38	75·0	77·0
8	490	534	7·38	7·07	62·0	65·0
16	452	367	7·94	8·75	54·0	71·0
32	442	349	7·35	17·65	58·5	153·0

Summary Analysis of Variance

Source of Variation	D.F.	Variance Ratios		D.F.	Variance Ratio
		Acid Phosphatase	Acid Proteinase		β-Glucuronidase
Treatment	1	1·25	24·81**	1	9·75**
Time	5	9·90**	28·79**	5	5·29**
Time × treatment interaction	5	1·68	26·95**	5	5·84**
Error variance	108	*0·01*	*0·002*	48	*0·95*

** $P < 0·01$.

TABLE 3

EFFECT OF OESTROGEN TREATMENT OF ADULT MALE RATS ON THE FREELY SOLUBLE ENZYME ACTIVITY IN THE TESTIS

Activity expressed as the percentage of the total activity given in Table 2. The number of animals is shown in parenthesis

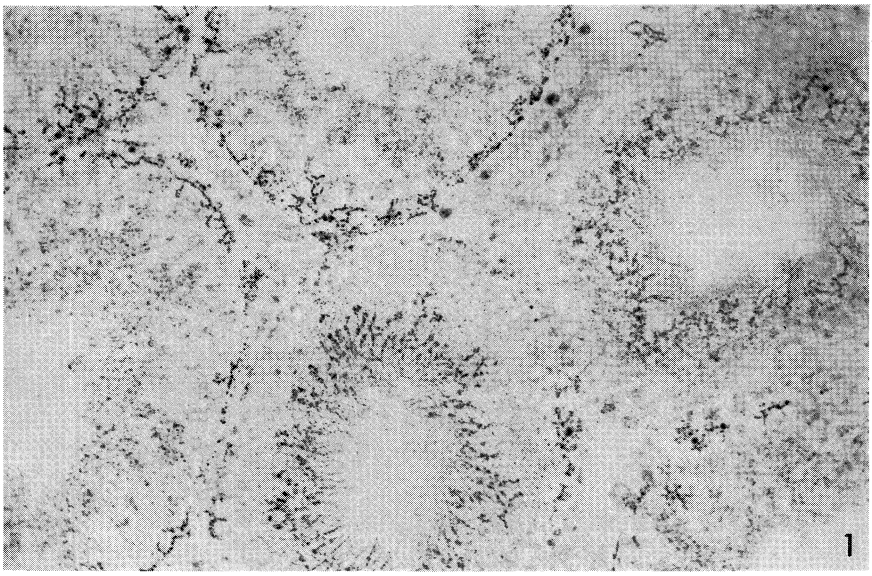
Time (days)	Acid Phosphatase (10)		Acid Proteinase (10)		β-Glucuronidase (5)	
	Control	Oestrogen	Control	Oestrogen	Control	Oestrogen
1	53·0	51·4	21·1	20·9	10·2	7·7
2	54·8	51·4	20·8	21·3	8·5	15·1
4	52·2	53·4	15·3	19·0	10·2	11·6
8	49·6	49·4	15·5	17·0	7·9	11·9
16	50·9	55·3	13·5	26·3	8·8	13·8
32	58·3	67·9	15·6	24·6	8·6	23·2

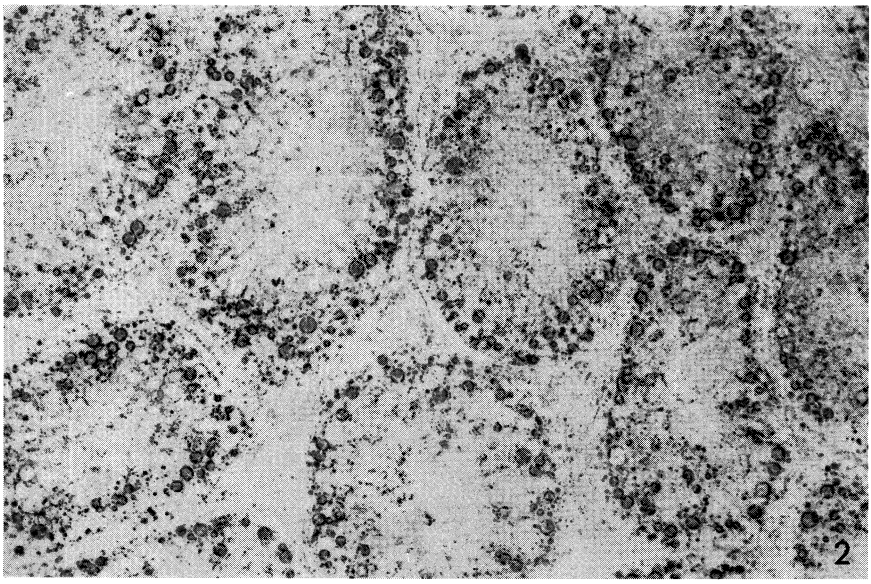
Summary Analyses of Variance

Source of Variation	D.F.	Variance Ratios		D.F.	Variance Ratio:
		Acid Phosphatase	Acid Proteinase		β-Glucuronidase
Treatment	1	2·26	6·02*	1	17·85**
Time	5	2·30*	0·96	5	2·97*
Time × treatment interaction	5	2·48*	1·31	5	3·12*
Error variance	108	*23·10*	*68·82*	48	*12·80*

* $P < 0·05$. ** $P < 0·01$.

but testicular weight did not change until between 4 and 8 days, when a steady decline occurred.





Figs. 1 and 2.—Acid phosphatase in the normal mature rat testis (Fig. 1) and in the testis of a rat treated for 32 days with 20 μg/day of oestradiol-3,17β dipropionate (Fig. 2). Both figures $\times$ 150.

Oestrogen treatment had no significant effect on total acid phosphatase activity per gram of testis, but there was a significant increase in total activity of acid proteinase and β-glucuronidase per gram of testis between 16 and 32 days of oestrogen treatment (Table 2).

The proportion of free enzyme, expressed as a percentage of total activity, and analyses of variance are given in Table 3. The proportion of free acid phosphatase showed a small increase in control testes during treatment, but oestrogen caused a major rise in this activity. There were no significant time trends in the proportion of free acid proteinase but, overall, oestrogen raised the percentage of free enzyme. The proportion of free β-glucuronidase was markedly increased by oestrogen, most of the rise occurring during the last half of the treatment period.

The histochemical reaction of the rat testis was tested by staining for acid phosphatase and lipid in frozen sections. Depending on the stage of spermatogenesis there was a discrete and regular staining of acid phosphatase either in the peripheral or central area of the seminiferous tubule of control animals (Fig. 1). After 4 days of oestrogen treatment the activity of acid phosphatase began to increase in the seminiferous tubules. This was first seen in the periphery of the tubules as enlarged granules in the Sertoli cells; these granules gradually increased in size until at 32 days there was generalized activity of acid phosphatase throughout the tubule (Fig. 2).

Oil red O was used to stain lipid in frozen sections of the rat testis, with haematoxylin for nuclear staining. The control rats showed negligible lipid staining while the lipid reaction in the testis of treated rats, also beginning at 4 days, showed a similar distribution to the acid phosphatase reaction and was generalized by 32 days (Fig. 3).

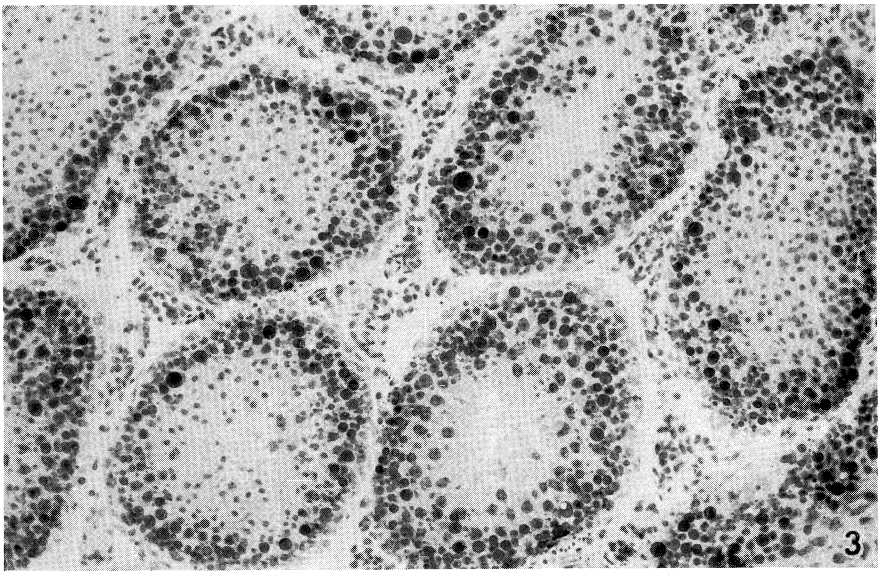


Fig. 3.—Lipid in the testis of a rat treated for 32 days with 20 μg/day of oestradiol-3,17β dipropionate and counterstained with haematoxylin. $\times$150.

Oestrogen caused a significant increase in the percentage nuclear volumetric proportions of spermatogonia and early and late primary spermatocytes (Table 4), although there was no significant change in the proportion of round spermatids and Sertoli cells (Table 4). In contrast, the nuclear proportions of the long spermatids

decreased significantly during oestrogen treatment (Table 4). The volumetric proportions of extratubular tissue and non-nuclear intratubular tissue (Table 4) did not

TABLE 4

EFFECT OF OESTRADIOL TREATMENT OF ADULT MALE RATS ON THE PERCENTAGE VOLUMETRIC PROPORTION OF TESTICULAR ELEMENTS IN THE SEMINIFEROUS TUBULES OF THE TESTIS

Values are means of five replications

Testicular Element	Volumetric Proportion (%) after					
	1 day	2 days	4 days	8 days	16 days	32 days
Spermatogonia						
Control	4·0	3·2	3·4	2·6	3·6	3·4
Oestrogen	4·4	3·8	4·6	3·6	5·4	13·2
Primary spermatocytes						
Early (= leptotene+zygotene)						
Control	2·4	3·0	3·6	2·2	3·4	2·4
Oestrogen	4·0	4·2	4·0	4·0	6·0	9·4
Late (= pachytene+diplotene)						
Control	8·9	10·6	9·8	9·4	9·2	5·8
Oestrogen	13·6	11·4	11·0	12·2	12·2	10·4
Round spermatids						
Control	20·8	20·4	19·4	20·2	21·6	19·6
Oestrogen	20·4	19·6	19·4	20·6	16·2	14·2
Long spermatids						
Control	14·0	12·6	14·6	13·8	12·4	13·6
Oestrogen	12·6	12·8	11·0	13·6	9·8	2·4
Sertoli cells						
Control	1·2	3·4	1·6	1·8	1·8	1·6
Oestrogen	1·8	2·0	2·2	1·4	2·4	2·2
Intratubular						
Control	34·0	34·8	33·4	38·0	32·2	36·2
Oestrogen	33·8	36·0	33·2	38·2	31·8	31·8
Extratubular						
Control	14·0	12·0	16·2	11·6	12·0	14·4
Oestrogen	11·8	10·2	14·2	18·4	18·4	15·6

Summary Analyses of Variance of Germ Cells

Source of Variation	D.F.	Spermatogonia	Primary Spermatocytes		Spermatids	
			Early	Late	Round	Long
Treatment	1	10·47**	26·45**	12·41**	2·44	20·44**
Time	5	3·79**	2·10	0·77	1·00	5·71**
Time × treatment interaction	5	3·21*	2·66*	0·42	1·51	5·66**
Replications	4	0·89	0·33	1·26	1·76	1·45
Error variance	44	*10·55*	*7·37*	*5·28*	*10·88*	*17·26*

* $P < 0·05$. ** $P < 0·01$.

alter significantly during treatment and the analyses are not included in the analyses of variance.

An index (the "nuclear index") was derived from the product of the nuclear volumetric proportions and the testicular weight (Table 5). It is clear (Table 5) that,

TABLE 5

EFFECT OF OESTRADIOL TREATMENT OF ADULT MALE RATS ON THE NUCLEAR INDICES OF ELEMENTS IN THE SEMINIFEROUS TUBULES OF THE TESTIS

Values are means of five replications

Cell Type	$10^{-2}\times$ Nuclear Indices after:					
	1 day	2 days	4 days	8 days	16 days	32 days
Spermatogonia						
Control	54·2	39·8	43·0	32·0	46·2	44·1
Oestrogen	54·6	42·0	55·0	38·2	35·0	45·9
Primary spermatocytes						
Early (= leptotene+zygotene)						
Control	35·2	36·3	45·0	25·7	36·5	30·9
Oestrogen	51·5	47·2	48·1	42·1	43·4	36·3
Late (= pachytene+diplotene)						
Control	113·6	128·7	124·7	117·6	119·5	95·9
Oestrogen	142·9	107·9	132·0	126·4	94·7	43·3
Round spermatids						
Control	272·1	258·1	221·0	244·4	276·9	252·5
Oestrogen	251·3	210·1	232·5	217·6	133·5	66·4
Long spermatids						
Control	181·8	151·4	183·7	167·7	163·7	178·6
Oestrogen	153·7	126·9	129·8	142·2	78·0	17·0
Sertoli cells						
Control	20·1	40·1	20·4	22·1	24·8	20·7
Oestrogen	22·0	21·8	26·9	14·3	20·8	8·9

Summary Analyses of Variance

Source of Variation	D.F.	Variance Ratios: Spermatogonia	Early Primary Spermatocytes	Late Primary Spermatocytes	Round Spermatids	Long Spermatids	Sertoli Cells
Treatment	1	0·17	4·42*	0·88	20·54**	42·77**	2·19
Time	5	1·31	0·83	3·80*	3·45**	4·93**	1·41
Time × treatment interaction	5	0·41	0·24	1·66	4·36*	5·15**	0·96
Replication	4	0·25	0·74	0·29	1·79	2·95*	1·33
Error variance	44	*356·97*	*326·40*	*1312·52*	*3472·25*	*1394·94*	*214·33*

* $P < 0·05$. ** $P < 0·01$.

during oestrogen treatment, there were no changes in the values for spermatogonial and late primary spermatocyte nuclei but there was an increase in early primary

spermatocyte nuclear indices, while there were major falls in the indices of the other nuclear types after 8 days. The nuclear yield of a particular cell type from its immediate precursor may be obtained from the ratio of their nuclear indices [Figs. 4(*a*)–4(*d*)]. There were no changes in the yield of early primary spermatocytes from spermatogonia during oestrogen treatment [Fig. 4(*a*)], but after 8 days the yield of late primary spermatocytes declined [Fig. 4(*b*)]. Although the yield of round spermatids in the treated testes was consistently below that for the controls, the level was constant over the 32-day treatment period [Fig. 4(*c*)]. Conversion of round spermatids to long spermatids progressed normally until day 16 when a steep decline in the yield was apparent [Fig. 4(*d*)].

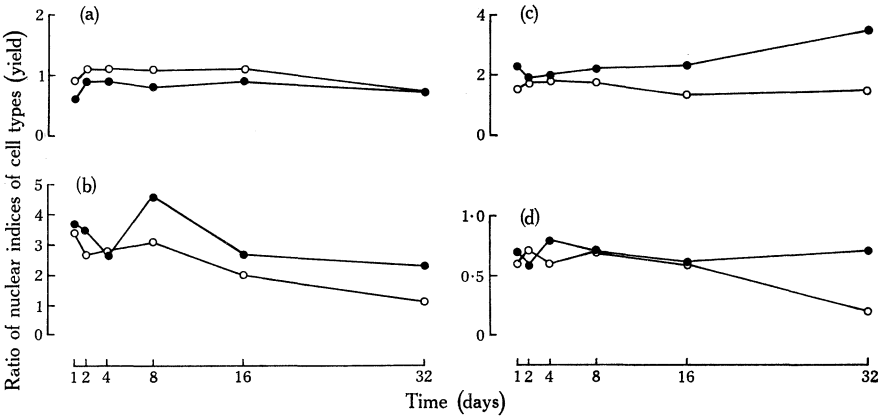


Fig. 4.—Effect of oestradiol (○) and oil (●) on the yield of cell types in the testis. Ratios of (*a*) early primary spermatocytes: spermatogonia; (*b*) late: early primary spermatocytes; (*c*) round spermatids: late primary spermatocytes; (*d*) long spermatids: round spermatids.

IV. Discussion

Oestradiol treatment depressed testis and seminal vesicle weight; the effect was probably due to the suppression of the output of gonadotropins from the pituitary gland (Moore and Price 1930).

In rats treated for 1 month with very large doses of oestrogen, Lacy and Lofts (1965) observed considerable variation in the extent of damage to different tubules in the same animal, and also in the response of the testes of different animals. The most advanced tubules contained Sertoli cells, spermatogonia, spermatocytes, and two layers of young spermatids. In the present experiments, the general histological picture at 32 days resembled that obtained by Lacy and Lofts (1965). Detailed examination of the cellular population has shown that the spermatogonia were unaffected by oestrogen treatment, the rise in volumetric proportions being balanced by the reduction in size of the testis.

The observations of Clermont and Morgentaler (1955) and Clermont and Harvey (1967) showed that after hypophysectomy there was a moderate reduction in A-type spermatogonia, but as the germ cells evolved the decrease in cell numbers became pronounced. However, Vilar (1968) found no change in the numbers or morphology of A- and B-type spermatogonia at any period of a 149-day study, although

the other cells of the germinal epithelium declined to very low levels. Steinberger and Nelson (1955), using very large doses of oestradiol (222 μg/day) or stilboestrol (1 mg/day), which would completely suppress pituitary gonadotropins (Steinberger and Duckett 1967), observed quantitative spermatogenic arrest similar to that observed after hypophysectomy.

Steinberger and Duckett (1967) have proposed that the qualitative progress of spermatogenesis is relatively independent of hormonal control up to the late pachytene stage of the primary spermatocytes, but that the reduction division requires testosterone rather than gonadotropic hormones as suggested by Cutuly and Cutuly (1940). Previous work (Elkington and Blackshaw 1970) has shown that testosterone and pregnant mare serum gonadotropin prevent the decline in enzymatic activity and qualitative histology of the oestrogen-treated rat testis.

In the present results the yield of late primary spermatocytes was reduced after 8 days of oestrogen treatment, indicating that hormonal support was needed. Although the yield of round spermatids from the pre-meiotic cells was unchanged during the treatment period, their maturation into long spermatids was prevented after 16 days of oestrogen treatment. In this context Steinberger and Duckett (1967) also proposed that the early maturation of the spermatids is independent of hormones, but that the completion of spermiogenesis requires FSH. Lacy and Lofts (1965) were able to completely reverse the effects of oestrogen on the rat testis by the administration of FSH, but LH was without effect.

Apart from the work of Clermont and Harvey (1967), valid quantitative estimates of the effects of hormones on spermatogenesis do not appear to have been made. Recalculation of their data to give yields of cell types from their immediate precursors suggest that both testosterone and FSH were slightly better than LH in restoring these values to normal. None of the hormones were successful in restoring the numbers of A-type spermatogonia to normal.

The role of the Sertoli cells in the maintenance of the germinal epithelium is not clear; Clermont and Harvey (1967) have suggested that hypophysectomy and the resulting absence of gonadotropins may influence the germ cells by a change in the nutritive or supporting function of the Sertoli cells; whereas Lacy and Lofts (1965) implicate the Sertoli cell as a source of intratubular testosterone necessary for spermatogenesis.

The function of lysosomes and their hydrolytic enzymes in degenerative changes of the testis is not clear. It has been shown by Blackshaw and Hamilton (1970) that lysosomal changes in pachytene primary spermatocytes are early and prominent features in acute heat-induced testicular degeneration but are probably not primary factors in damage. Under less drastic conditions such as hypoxia, cryptorchism (Blackshaw and Allan, unpublished data), and chronic oestrogen treatment, an increase in lysosomal acid phosphatase, presumably from the Sertoli cells, was the earliest signs of damage and this increased markedly as degeneration of the germinal cells took place.

There was an increase in the size of acid phosphatase-reactive granules [secondary lysosomes, Strauss (1967)] in the oestrogen-treated testis and increases in the proportion of "free" hydrolytic enzymes coinciding with the beginning of cellular alterations.

Increased fragility of lysosomes and the formation of autophagic and phagocytic vesicles is also seen in the degenerating corpus luteum (Dingle, Hay, and Moor 1968). Further evidence of cellular degeneration and phagocytosis is seen in the increase in lipid which Lacy and Lofts (1965) have shown to be due to phagocytosis of the damaged germ cells by the Sertoli cells. Increased phagocytic activity in the testis is also indicated by the rise in total activity of acid proteinase and β-glucuronidase after oestrogen treatment.

In the normal testis, acid phosphatase activity shows a cyclic pattern of activity (Posalaki *et al.* 1968), which is greatest in the Sertoli cells during stages 5–8 (Roosen-Runge and Giesel 1950) and lysosomal enzymes may be important in the normal differentiation of the spermatid. The regulation of the production and activation of these enzymes, by the Sertoli cells in particular, is presumably under the control of either testosterone or gonadotropins.

V. Acknowledgments

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