THE EFFECT OF HYPOPHYSECTOMY ON TESTICULAR HYDROLASES, LACTATE DEHYDROGENASE, AND SPERMATOGENESIS IN THE RAT

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Abstract

Hypophysectomy of the adult male rat depressed testis and seminal vesicle weight between 4 and 8 days after hypophysectomy.

The activity of soluble acid proteinase (E.C. 3.4.4.23) and β-glucuronidase (E.C. 3.2.1.31) increased between 8 and 16 days after the operation, while that for acid phosphatase (E.C. 3.1.3.2) increased until 16 days after hypophysectomy and then decreased to the end of the experiment.

There was an increase in the total acid proteinase and β-glucuronidase activity per gram testis between 8 and 16 days after hypophysectomy; there was no change in the total activity of acid phosphatase per gram testis until 16–32 days after treatment, when there was a decrease. After hypophysectomy there was a decrease in acid phosphatase activity per testis after 16 days, and acid proteinase activity per testis after 8 days, while there was an increase in both free and total β-glucuronidase activity per testis after 16 days. A decrease in lactate dehydrogenase isoenzyme (LDH–X) activity in the testis occurred between 8 and 16 days after the operation.

Histochemical evidence showed an increase in acid phosphatase activity in the seminiferous tubules 4–8 days after hypophysectomy, which coincided with a decrease in the yield of round spermatids from late primary spermatocytes and of long spermatids from round spermatids.

It is concluded that lysosomal enzymes of Sertoli cell origin are important in testicular degeneration and, probably, in normal spermatogenesis. Acid phosphatase and acid proteinase are found mainly in spermatocytes and spermatids, while β-glucuronidase is found mainly in the Sertoli cells and spermatogonia. LDH–X occurs in the seminiferous tubular epithelium associated with late primary spermatocytes and spermatids.

I. INTRODUCTION

It has been clearly demonstrated that the pituitary gland is necessary for the maintenance of spermatogenesis (Smith 1927; Greep and Fevold 1937) and the degeneration of spermatogenesis which follows hypophysectomy has been attributed to the removal of the pituitary gonadotropins (see review by Albert 1961). At the electron-microscopic level Vilar (1968) has demonstrated degeneration after hypophysectomy of the Leydig cells, which are not replaced by fibrocyte-like cells, as suggested by De La Blaze *et al.* (1962). There was a progressive reduction in the number of spermatocytes and degenerative changes in long spermatids, but young spermatids progressed into the early stages of spermiogenesis (Vilar 1968). In the Sertoli cells the changes involved both cytoplasm and nucleus.

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Studies on the effect of hypophysectomy on enzymes in the testis has concerned mainly the dehydrogenases (see Blackshaw 1970 for references) and lipid metabolism (see Johnson 1970 for references). The testicular hydrolases have received little study in mammals (Elkington and Blackshaw 1971; Vanha-Perttula 1971*a*, 1971*b*; Vanha-Perttula and Nikkanen 1971) but in the testis of the reptile *Leptodactylus chaquensis* acid phosphatase was located in degenerating cells after hypophysectomy (Bertini *et al.* 1969). Hypophysectomy has been shown (Males and Turkington 1971) to elevate the total amount of β-glucuronidase and depress the total amount of acid phosphatase in the immature rat testis.

The normal adult testis of man (Blanco and Zinkham 1963; Goldberg 1963), rat, rabbit, mouse, dog, guinea-pig, bull, and pigeon (Zinkham *et al.* 1964), contain unusual isoenzymes of lactate dehydrogenase, designated LDH–X and composed of C subunits (Zinkham *et al.* 1964) distinct from the A and B subunits of other LDH isoenzymes. The testis-specific isoenzymes of LDH are a convenient metabolic marker for the spermatogenic activity of the testis, as they are associated with the spermatogenic cycle from the stage of the pachytene primary spermatocytes (Blackshaw and Elkington 1970*a*).

This paper describes experiments using hypophysectomy to study acid phosphatase, acid proteinase, β-glucuronidase, and lactate dehydrogenase isoenzymes in the rat testis in order to correlate changes in enzyme activity and spermatogenesis with lysosomal function in the testis.

II. Materials and Methods

Young adult male Wistar rats (60–80 days old) were selected at random and divided into two groups; one group was hypophysectomized by the parapharyngeal route and the second group was used as controls. After hypophysectomy, the animals were treated with 1 mg of cortisol and maintained at 80°F on commercial pellets and an aqueous suspension of 5% whole milk powder and 5% glucose.

The animals were killed by cervical dislocation and the seminal vesicles (empty of secretion) and testes were weighed. Half of one testis was rapidly frozen to a stainless steel microtome chuck in a dry ice–hexane mixture for histochemical studies. The remaining portion of the testis was fixed in Susa and processed to paraffin wax for histological examination (Humason 1967). 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 centrifuged at 2500 r.p.m. in a Christ UJ15 centrifuge for 5 min (4°C).

Aliquots (4·5 ml) of the crude supernatant were diluted with 0·5 ml of either 0·25M sucrose or 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 enzyme determinations. A supernatant was diluted 25 times for acid phosphatase (Torriani 1960), 10 times for β-glucuronidase (Plaice 1961), and left 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 were found to give a linear response over the times of incubation and the substrate levels were optimal for the reaction. The incubation times at 37°C for acid phosphatase and β-glucuronidase were 30 min and 18 hr respectively, while acid proteinase was incubated at 45° for 1 hr. The enzyme activity was expressed as the change in absorbance *A* per gram of tissue at the indicated wavelengths (acid phosphatase 404 nm; acid proteinase 280 nm; β-glucuronidase 550 nm).

The fraction free from lysosomal components was used for total lactate dehydrogenase assay (Wilkinson 1962) with sodium pyruvate as substrate. A unit of LDH activity changed the absorbance of NADH at 340 nm by 0·001 in 1 min in a 3-ml assay mixture. Forty units of LDH activity were applied to polyacrylamide gels for separation of the LDH isoenzymes by disc electrophoresis (Ornstein and Davis 1962; Blackshaw and Samisoni 1966). Tetrazolium reductase, with nitroblue tetrazolium (NBT), was demonstrated as purple bands of formazan in the gel (Markart and Ursprung 1962) the substrate sodium lactate being used to demonstrate LDH and sodium DL-α-hydroxyvalerate for LDH–X isoenzymes (Allen 1961). The intensity of formazan deposition in each isoenzyme band was estimated using an integrating microdensitometer (Photovolt Densicord 542) and the activity of LDH–X in lactate-stained gels was calculated as LDH–X activity per milligram protein. The protein estimates were made by the Folion–Ciocalteau method (Lowry *et al.* 1951).

Sections (10 μm) of the frozen testis were cut with a Lipshaw "cryotome" at −25°C, placed on slides for staining of acid phosphatase (Pearse 1960), and mounted in Kaiser's glycerol jelly. Sections (7 μm) of paraffin-embedded testes were cut on a Minot microtome 1212 (Leitz) and stained with haematoxylin and eosin or the Feulgen reaction with alcian blue as counterstain (Humason 1967).

The volumetric proportion of particular elements in the testis was examined by 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). In this method a counting graticule (Maxta) divided into four quadrants and with 25 randomly placed spots in the field is used. This gives an unbiased estimate of the volumetric proportions of testicular elements. Only the 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. 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 were expressed as the mean response for each treatment, and have been assessed by standard analyses of variance or covariance (Steele and Torrie 1960). Percentage data for volumetric proportions were transformed to angles (arcsin$\sqrt{\text{percentage}}$) for analysis. Mean squares for individual treatment effects and their interactions were isolated and tested for significance using residual variance as error. The analyses are presented in summary form, giving degree of freedom and variance ratios for each source of variation, with the error variance at the base of each variance ratio column.

III. Results

Body weight showed significant changes following hypophysectomy, and covariance analysis was applied to both testis and seminal vesicle weights, which were significantly depressed after 8 days (Table 1).

Hypophysectomy decreased the specific activity of LDH–X 16 days after the operation, but the total testicular activity (units per testis × 10^{-3}) decreased after 8 days, falling to very low levels by 32 days (Table 2).

The total and free activities (activity per gram testis) of β-glucuronidase and acid proteinase increased 16 days after hypophysectomy [Tables 3 and 4; Figs. 1(*b*), 1(*c*), 2(*b*), and 2(*c*)] but when examined on an organ basis (activity per testis) there was a fall after 8 days of total acid proteinase [Table 4 and Fig. 3(*b*)] and little change in the free enzyme activity [Table 4 and Fig. 4(*b*)]. β-Glucuronidase activity on an organ basis followed that of specific activity, increasing after 16 days [Table 3; Figs. 3(*c*) and 4(*c*)]. Acid phosphatase activity per gram testis fell abruptly 32 days after hypophysectomy [Table 3; Figs. 1(*a*) and 2(*a*)] but on an organ basis the decrease began after 16 days [Table 3; Figs. 3(*a*) and 4(*a*)]. Significant changes in control levels of acid phosphatase also occurred during the experimental period, possibly reflecting changes in assay and homogenization technique.

TABLE 1

EFFECT OF HYPOPHYSECTOMY OF ADULT MALE RATS ON TESTIS AND SEMINAL VESICLE WEIGHT

Values given are adjusted mean weights

Days after hypophysectomy	No. of animals	Testis weight (g)		Seminal vesicle weight (mg)	
		Control	Experimental	Control	Experimental
1	7	2·08	1·88	170	108
2	6	2·19	2·00	185	144
4	4	2·12	2·05	243	113
8	5	2·38	1·62	232	58
16	5	2·35	0·78	195	61
32	4	2·83	0·43	237	61

Summary analysis of covariance

Source of variation	D.F.	Variance ratios	
		Testis weight	Seminal vesicle weight
Treatment	1	81·16**	8·73**
Time	5	24·70**	2·71*
Time × treatment interaction	5	61·56**	4·40**
Error variance	49	*0·03*	*1604·00*

* $P < 0·05$. ** $P < 0·01$.

TABLE 2

EFFECT OF HYPOPHYSECTOMY OF ADULT MALE RATS ON LDH–X ACTIVITY IN THE TESTIS

Values given are mean activities

Days after hypophysectomy	No. of animals	LDH–X activity (units/mg protein)		$10^{-3}\times$ LDH–X activity (units/g testis)		$10^{-3}\times$ LDH activity (units/testis)	
		Control	Exptl.	Control	Exptl.	Control	Exptl.
1	7	244	247	20·1	22·1	40·0	44·1
2	6	149	161	15·3	17·1	33·5	35·3
4	4	205	208	17·2	18·9	38·0	38·4
8	5	142	127	13·4	14·3	30·6	23·3
16	5	230	167	19·3	16·5	45·3	11·2
32	4	273	61	20·8	3·1	56·4	1·2

Summary of analyses of variance

Source of variation	D.F.	Variance ratios for LDH–X activity		
		Units/mg protein	Units/g testis	Units/testis
Treatment	1	3·1	1·7	23·4**
Time	5	2·9*	5·0**	4·0**
Time × treatment interaction	5	2·5*	4·6**	13·1**
Error variance	50	*6529·3*	*22·9*	*103·4*

* $P < 0·05$. ** $P < 0·01$.

TABLE 3

SUMMARY ANALYSES OF VARIANCE OF DATA IN FIGURES 1–4

Source of variation	D.F.	Variance ratios: Acid phosphatase		Acid proteinase		β-Glucuronidase	
		Per gram testis	Per testis	Per gram testis	Per testis	Per gram testis	Per testis
		(*a*) Data from Figures 1 and 3					
Treatment	1	0·4	60·7**	14·7**	17·9**	56·3**	4·4*
Time	5	8·5**	8·1**	15·6**	5·1**	25·4**	10·1**
Time × treatment interaction	5	7·8**	33·1**	10·7**	3·7**	25·1**	4·9**
Error variance	50	*3832·0*	*20056·0*	*19·0*	*17·6*	*4032·6*	*1022·8*
		(*b*) Data from Figures 2 and 4					
Treatment	1	12·5**	23·7**	10·4**	5·0*	33·4**	29·4**
Time	5	12·7**	11·4**	14·3**	3·3*	13·7**	10·2**
Time × treatment interaction	5	1·3	15·3**	6·3*	1·9	13·9**	9·5**
Error variance	50	*1421·0*	*7731·8*	*2·0*	*3·8*	*686·1*	*168·3*

* $P < 0·05$. ** $P < 0·01$.

TABLE 4

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

C, control; E, experimental rats

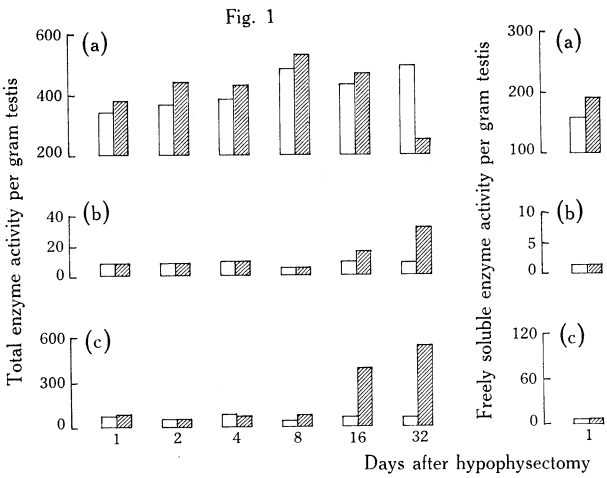
Days after hypophysectomy	No. of animals	10^{-3} × Nuclear index for: Spermatogonia		Early primary spermatocytes†		Late primary spermatocytes‡		Round spermatids		Long spermatids	
		C	E	C	E	C	E	C	E	C	E
1	7	27·9	37·3	47·1	8·8	394·6	107·4	182·4	204·4	94·2	105·6
2	6	51·6	30·1	49·5	48·9	102·2	104·5	199·7	219·1	157·6	130·0
4	4	35·3	34·4	46·0	53·9	94·2	114·6	195·0	217·5	135·9	160·8
8	4	23·3	41·0	39·2	27·6	101·2	62·8	254·9	152·6	145·2	61·6
16	5	52·9	25·8	50·3	31·8	84·4	43·3	281·7	51·5	155·7	4·4
32	4	66·3	21·6	71·5	12·2	123·8	8·8	248·5	0·0	162·9	1·6

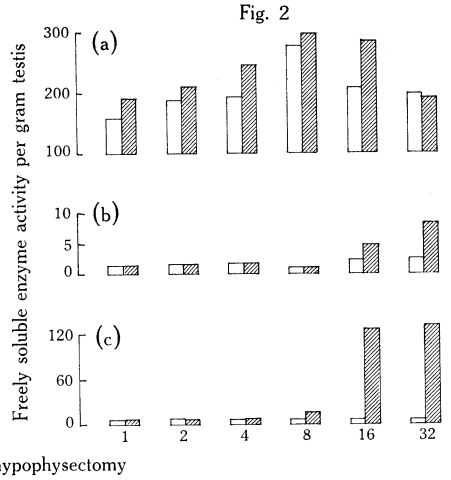
Analysis of variance using the nuclear indices for Sertoli cells as the covariate

Source of variance	D.F.	Variance ratios: Spermatogonia	Early primary spermatocytes†	Late primary spermatocytes‡	Round spermatids	Long spermatids
Treatment	1	10·92**	9·21**	9·67**	33·02**	35·43**
Time	5	0·85	1·60	4·72**	3·61**	6·19**
Time × treatment interaction	5	5·17**	5·00**	5·93**	15·15**	11·43**
Error variance	47	*2·05*	*3·00*	*9·62*	*24·35*	*13·95*

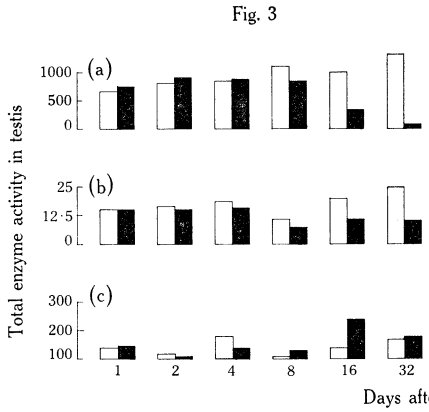
** $P < 0·01$ † Leptotene + zygotene. ‡ Pachytene + diplotene.

The histochemical reaction of the rat testis for acid phosphatase was tested 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





Figs. 1. and 2.—Change in total (Fig. 1) and free (Fig. 2) hydrolytic enzyme activity per gram of testis in rat testis after hypophysectomy. (*a*) Acid phosphatase; (*b*) acid proteinase; (*c*) β-glucuronidase. Open bars, control rats; hatched bars, hypophysectomized rats.



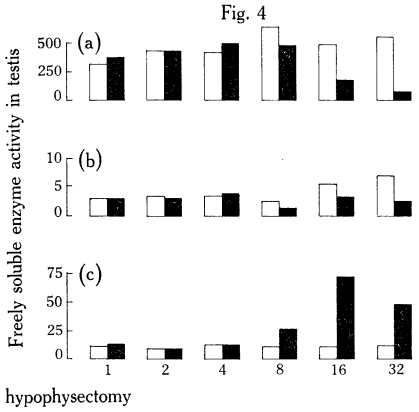


Fig. 3 and 4.—Change in total (Fig. 3) and free (Fig. 4) hydrolytic enzyme activity per testis in the rat testis after hypophysectomy. (*a*) Acid phosphatase; (*b*) acid proteinase; (*c*) β-glucuronidase. Open bars, control rats; solid bars, hypophysectomized rats.

the seminiferous tubule of control animals (Fig. 5). Between 2 and 4 days after hypophysectomy, there was an increase in the intensity of acid phosphatase activity in the tubules (Fig. 6) increasing further up to 8 days after hypophysectomy (Fig. 7). By 16 days and continuing up to 32 days after treatment, there was a general acid

phosphatase reaction throughout all the tubules (Figs. 8 and 9). At 4 and 8 days after hypophysectomy, the most intense acid phosphatase reaction was in tubules not containing spermatozoa (Figs. 6 and 7).

As a measure of the total contribution of the testicular elements to each testis, the nuclear index was introduced. This was derived from the product of the nuclear volumetric proportions and the testicular weight (Table 4). After hypophysectomy there was a decrease in the nuclear index of all germinal elements; the nuclear index for spermatogonia started to fall between 8 and 16 days, whereas that for spermatocytes and spermatids began to fall between 4 and 8 days post hypophysectomy.

TABLE 5

EFFECT OF HYPOPHYSECTOMY OF ADULT MALE RATS ON THE YIELD OF ELEMENTS OF THE SEMINIFEROUS TUBULAR EPITHELIUM

Values represent mean yields of cells from their immediate precursor cell. C, control; E, experimental rats

Days after hypophysectomy	No. of animals	Early† primary spermatocytes from spermatogonia		Late‡ from early primary spermatocytes		Round spermatids from late spermatocytes		Long from round spermatids	
		C	E	C	E	C	E	C	E
1	7	1·72	1·11	2·50	3·75	2·14	2·04	0·54	0·53
2	6	1·01	1·79	2·91	2·72	1·78	2·56	0·78	0·62
4	4	1·46	1·51	2·00	4·70	2·21	2·02	0·78	0·77
8	4	1·17	0·71	2·79	3·99	2·65	2·59	0·57	0·38
16	5	1·28	1·22	1·91	1·19	3·56	2·40	0·57	0·12
32	4	1·13	0·62	1·86	0·66	4·00	0·39	0·65	0·00

Summary analyses of variance

Source of variation	D.F.	Variance ratios			
		Early primary spermatocytes from spermatogonia	Late from early primary spermatocytes	Round spermatids from late primary spermatocytes	Long from round spermatids
Treatment	1	0·62	0·24	3·19	19·00**
Time	5	1·71	1·80	1·90	7·75**
Treatment × time interaction	5	2·23	0·64	2·89*	3·75
Error variance	*48*	*0·35*	*3·74*	*1·06*	*0·04*

* $P < 0·05$. ** $P < 0·01$. † Leptotene + zygotene. ‡ Pachytene + diplotene.

In situations where abrupt changes in germ cell numbers are not expected the yield of a cell type from its immediate precursor may provide useful information. The nuclear yield was obtained from the ratio of the nuclear volumetric proportions (Table 5). There was no significant change in yield of early primary spermatocytes from spermatogonia or of late from early primary spermatocytes. There was a significant decrease in the yield of round spermatids from late primary spermatocytes 16 days after hypophysectomy, while conversion of round spermatids to long spermatids was depressed 8 days after hypophysectomy (Table 5).

IV. Discussion

Clermont and Morgentaler (1955) and Clermont and Harvey (1967) showed that after hypophysectomy there was a moderate reduction in type A spermatogonia, but as the germ cells evolved the decrease in cell numbers became pronounced.

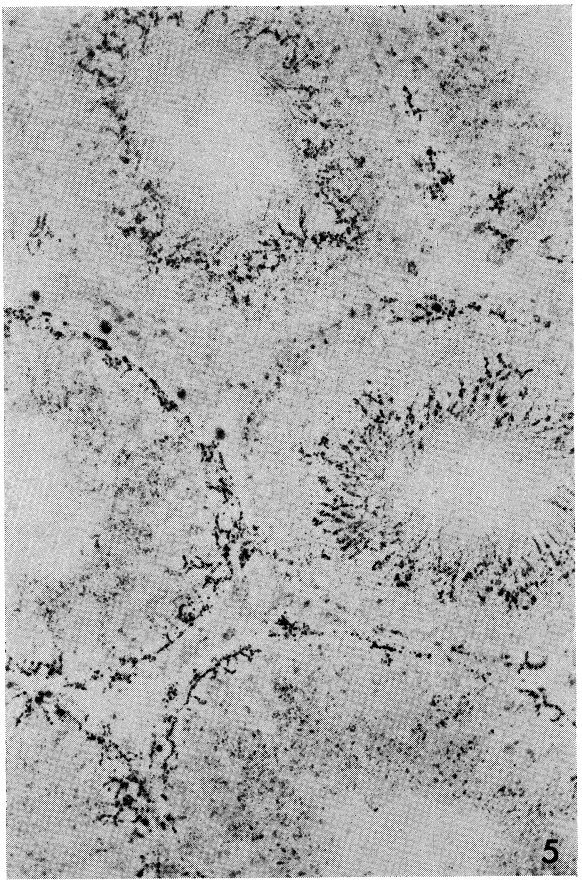
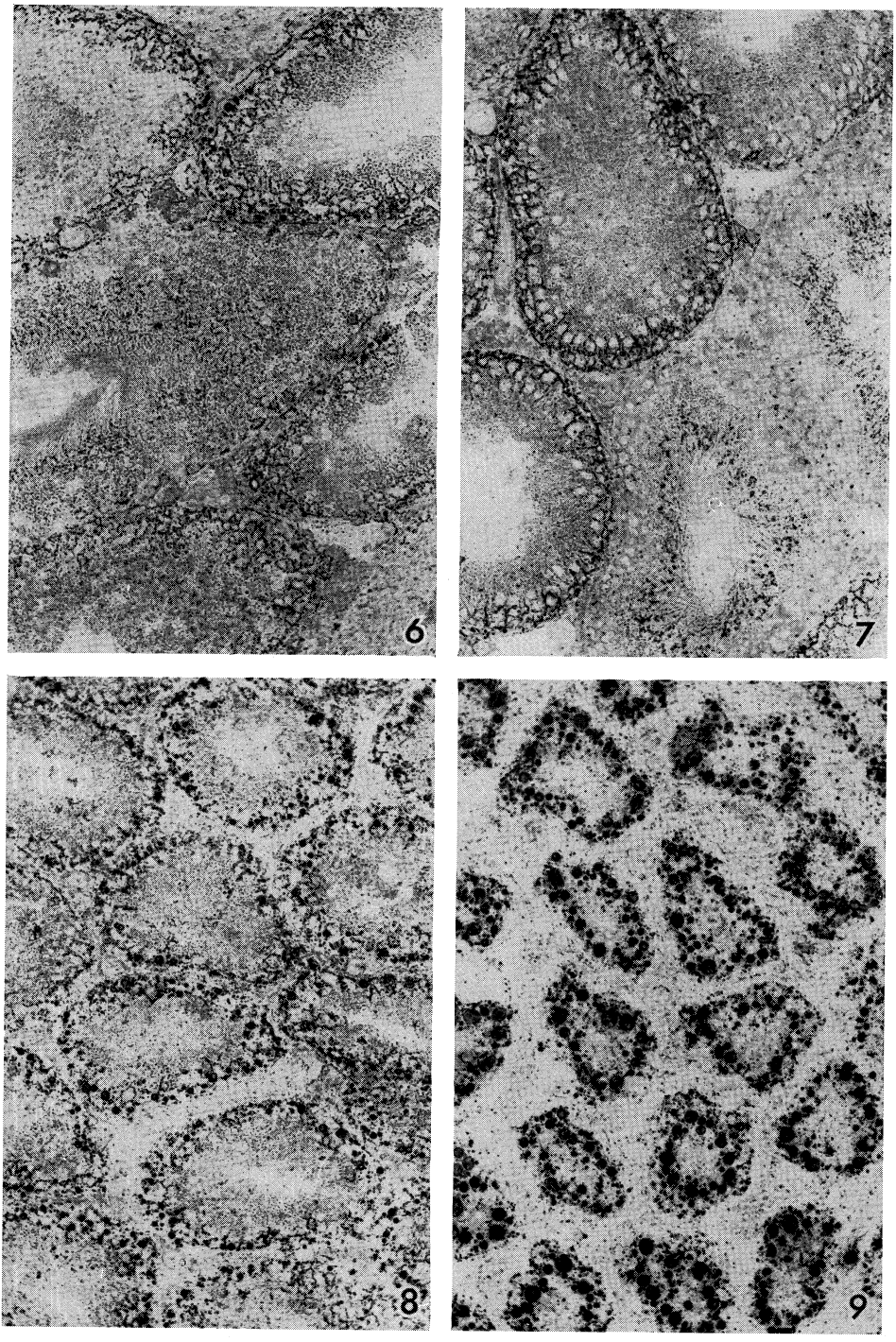


Fig. 5.—Acid phosphatase activity in normal rat testis. × 120.

Vilar (1968) found no change in the numbers or morphology of A and B spermatogonia at any period of a 149-day study, although the other cells of the germinal epithelium declined to very low levels. The losses of germinal elements in the present experiment began between 4 and 8 days after hypophysectomy, when there was a decrease in the yield of round spermatids. This indicates that both the reduction division of primary spermatocytes and spermiogenesis may be affected by the treatment. These results were similar to those of Nakai *et al.* (1967), but these authors consider that the main and immediate effect of hypophysectomy on the testis is on spermiogenesis.

Coinciding with the histological effects of hypophysectomy, between 4 and 8 days after treatment there was an increase in the histochemical reaction of acid phosphatase in the testis, which increased until 16–32 days, when there was a general



Figs. 6–9.—Acid phosphatase activity in the testis of the hypophysectomized rat at 4 (Fig. 6), 8 (Fig. 7), 16 (Fig. 8), and 32 days (Fig. 9) after the operation. × 120.

acid phosphatase reaction throughout the tubules. 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 of spermatogenesis (Roosen-Runge and Giesel 1950). Acid phosphatase-positive areas are found in the golgi apparatus of all cell types in the testis, as well as in the residual bodies and dense bodies of the Sertoli cells. Some acid phosphatase activity is found at the surface of most germinal cells, especially elongating spermatids (Tice and Barnett 1963).

The dense bodies in Sertoli cells have been demonstrated to be increased in number after many forms of injury to the testis (Nabel 1959; Firlit and Davis 1965; Dietert 1966; Hugon and Borgers 1966) and are considered to be lysosomes, especially phagosomes.

The mechanism by which the germinal epithelium breaks down after hypophysectomy may concern the lysosomes of the Sertoli cells. Hugon and Borgers (1966) suggest that the breakdown of germinal elements in the mouse testis after whole-body X-irradiation is due to the dead spermatogonia inducing phagocytic activity by Sertoli cells. If there is generalized lysosomal breakdown in the testis, one might expect all cells of the germinal epithelium to be affected, which is not the case as some spermatogonia and early primary spermatocytes remain. Recent work at the ultrastructural level may explain this phenomenon, as it has been shown (Dym and Fawcett 1970) that spermatogonia and preleptotene spermatocytes are in a separate compartment of the germinal epithelium from the other germinal elements. Tight junctions between adjacent Sertoli cells isolate spermatogonia and preleptotene spermatocytes in a compartment near the basement membrane, with the other germinal elements in a compartment communicating only with the tubular lumen.

On an organ basis an increase in total hydrolase activity was seen only for β-glucuronidase at 16 days after hypophysectomy. This was not well sustained at 32 days, and in a severely degenerating organ this observation could be anomalous. It seems more likely that the enzyme content of the testis would remain fairly constant. Males and Turkington (1971) have correlated β-glucuronidase activity in the immature testis with the Sertoli cell and spermatogonial population; the present results also suggest that these cells, particularly the former, are associated with this enzyme. On the other hand, acid phosphatase and acid proteinase activity per testis decreased at a time when the nuclear indices for primary spermatocytes and spermatids were falling; this indicates that the enzymes may be located mainly in these cells.

Increase in activity of enzymes on a unit tissue basis appear to be related to reduced cytoplasmic volumes, as the number of Sertoli cells does not change but the size of the tubules is greatly reduced by hypophysectomy. The fragility of testicular lysosomes was altered by hypophysectomy. The net effect was to increase free enzyme activity, with acid phosphatase release preceding that of acid proteinase or β-glucuronidase.

The removal of gonadotropins by hypophysectomy may lead to a change in the environment provided by the Sertoli cell about the germinal cells in the luminal compartment (Dym and Fawcett 1970), with lysosomal activation. Castro *et al.* (1970) have shown that both follicle-stimulating hormone and luteinizing hormone are found in the Sertoli cell dense bodies, which Nakai *et al.* (1967) consider to be lysosomes, and these hormones may be directly or indirectly concerned with the regulation of lysosomal activity in the testis.

Lactate dehydrogenase has been used as a marker for active spermatogenesis (Blackshaw and Elkington 1970*a*, 1970*b*; Elkington and Blackshaw 1970). In this experiment the decrease in LDH–X activity between 8 and 16 days after hypophysectomy began somewhat later than the decrease in the nuclear index for late primary spermatocytes and spermatids, but this may be related to the relative insensitivity of the isoenzyme techniques and does not alter the view that this isoenzyme of LDH is found in the tubular epithelium of the rat testis. Dokov and Gerebtzoff (1970) consider that LDH–X is also present in the Leydig cells, but Blackshaw and Elkington (1970*a*) have shown that LDH–X appears in the immature rat testis at 20–30 days of age, a time when the Leydig cells are not producing a significant amount of testosterone (Inano *et al.* 1967; Steinberger and Fisher 1968). Also De Domenech *et al.* (1972) have shown that rat testicular LDH (LDH–X) is associated with specialized mitochondria, found only in primary spermatocytes, spermatids, and spermatozoa. It appears, therefore, that LDH–X is confined to the seminiferous epithelium and is absent from the Leydig cells. It is concluded that lysosomal enzymes of Sertoli cell origin are important in testicular degeneration and probably normal spermatogenesis. Acid phosphatase and acid proteinase are found mainly in spermatocytes and spermatids, while β-glucuronidase is found mainly in the Sertoli cells and spermatogonia.

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