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Activity and Androgenic Control of Glycolytic Enzymes in the Epididymis and Epididymal Spermatozoa of the Rat

By DAVID E. BROOKS
Department of Animal Physiology, University of Adelaide, Waite Agricultural Research Institute, Glen Osmond, South Australia 5064, *Australia*

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1. Procedures were developed for the extraction and assay of glycolytic enzymes from the epididymis and epididymal spermatozoa of the rat. 2. The epididymis was separated into four segments for analysis. When rendered free of spermatozoa by efferent duct ligation, regional differences in enzyme activity were apparent. Phosphofructokinase, glycerol phosphate dehydrogenase and glucose 6-phosphate dehydrogenase were more active in the proximal regions of the epididymis, whereas hexokinase, lactate dehydrogenase and phosphorylase were more active in the distal segment. These enzymes were less active in the epididymis of castrated animals and less difference was apparent between the proximal and distal segments. However, the corpus epididymidis from castrated rats had lower activities of almost all enzymes compared with other epididymal segments. 3. Spermatozoa required sonication to obtain satisfactory enzyme release. Glycolytic enzymes were more active in spermatozoa than in epididymal tissue, being more than 10 times as active in the case of hexokinase, phosphoglycerate kinase and phosphoglycerate mutase. 4. The specific activities of a number of enzymes in the epididymis were dependent on the androgen status of the animal. These included hexokinase, phosphofructokinase, aldolase, glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase, glycerol phosphate dehydrogenase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphorylase. 5. The caput and cauda epididymidis differed in the extent to which enzyme activities changed in reponse to an altered androgen status. The most notable examples were hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase, 6-phosphogluconate dehydrogenase and phosphorylase.

The mode of action of steroid hormones is generally considered to be mediated via an action on the genome to bring about specific protein synthesis. For instance the sequence of events involved in the action of oestrogen on the uterus has been well established (Jensen & DeSombre, 1972). A similar system appears to operate in the male. Testosterone is converted into dihydrotestosterone (Bruchovsky & Wilson, 1968) followed by binding to specific receptor molecules (Anderson & Liao, 1968; Mainwaring, 1969*a*,*b*) before interaction with the genome to induce specific mRNA synthesis (Mainwaring *et al.*, 1974). Although cyclic AMP has been proposed as a mediator of androgen action (Singhal & Sutherland, 1975) the direct involvement of this cyclic nucleotide has been questioned (Mangan *et al.*, 1973).

The prostate gland has usually been chosen as the model to study the mechanism of action of androgens and in this tissue the activities of a number of enzymes are androgen-dependent. By contrast, the epididymis has received scant attention as an androgen target tissue. This highly convoluted organ leads spermatozoa from their site of production in the testis to the ductus deferens from where they pass into the ejaculate. It takes approx. 2 weeks for spermatozoa to pass through the epididymal duct, during which time a number of changes take place, culminating in their attainment of the ability to fertilize an egg. Processes such as the accumulation by the epididymis of glycerylphosphorylcholine (Dawson & Rowlands, 1959; Brooks *et al.*, 1974), carnitine (Marquis & Fritz, 1965; Brooks *et al.*, 1974) and sialic acid (Rajalakshmi & Prasad, 1968) have been shown to be androgen-dependent. Only a few epididymal enzymes have been examined for their androgen-dependency, and those whose activity is controlled include acid and alkaline phosphatases and adenosine triphosphatase (Allen & Slater, 1957, 1958, 1959), glycosidases (Conchie & Findlay, 1959), ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase (Majumder *et al.*, 1974) and there is an indication that glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are also androgen-dependent (Sholl & Leathem, 1973).

The object of the present work was to establish conditions for the extraction and measurement of glycolytic and related enzymes in the epididymis and epididymal spermatozoa and to determine whether the specific activity of any of the epididymal enzymes is under androgenic control.

Table 1. *Composition of assay media for glycolytic enzymes and stability of enzymes in tissue homogenate*

All assays were carried out in a total volume of 1 ml at 25°C. The stability of enzymes in the 600*g* supernatant is expressed as a percentage of the activity measured approx. 45 min after homogenization. The actual times of assay during the 4–6 h period are indicated by the values in parentheses. The results represent the means ± S.E.M. of extracts of four caput epididymides and four cauda epididymides.

Enzyme	Final concentration of components in the assay system	Activity measured in tissue homogenate after various times of storage at 0°C: 4–6 h	24 h
Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12)	100 mM-Triethanolamine/HCl, pH 7.6, 100 mM-semicarbazide hydrochloride,* 1 mM-mercaptoethanol, 5 mM-$MgCl_2$, 1 mM-EDTA,* 3.75 mM-ATP,* 0.12 mM-NADH, 2.5 μM-rotenone, 12 mM-3-phosphoglycerate, 0.45 unit of phosphoglycerate kinase	82 ± 5 (5)	0.01
Phosphofructokinase (EC 2.7.1.11)	50 mM-Triethanolamine/HCl, pH 8.2, 1 mM-mercaptoethanol, 0.01% bovine serum albumin, 5 mM-$MgCl_2$, 1 mM-ATP,* 2 mM-ADP, 0.12 mM-NADH, 2.5 μM-rotenone, 1.5 mM-fructose 6-phosphate, 0.23 unit of aldolase, 10 units of triose phosphate isomerase, 0.16 unit of glycerol phosphate dehydrogenase	86 ± 5 (5)	33 ± 5
Phosphoglycerate kinase (EC 2.7.2.3)	100 mM-Triethanolamine/HCl, pH 7.6, 100 mM-semicarbazide hydrochloride,* 1 mM-mercaptoethanol, 10 mM-$MgCl_2$, 1 mM-EDTA,* 3.75 mM-ATP,* 0.12 mM-NADH, 2.5 μM-rotenone, 2 mM-3-phosphoglycerate, 0.8 unit of glyceraldehyde phosphate dehydrogenase	91 ± 3 (6)	40 ± 2
6-Phosphogluconate dehydrogenase (EC 1.1.1.44)	50 mM-Glycylglycine/NaOH, pH 7.6, 20 mM-$MgCl_2$, 0.4 mM-NADP, 2 mM-6-phosphogluconate	99 (4)	98 ± 2
Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)	As for 6-phosphogluconate dehydrogenase + 2 mM-glucose 6-phosphate	90 ± 1 (4)	40 ± 2
Glycerol phosphate dehydrogenase (EC 1.1.1.8)	200 mM-Triethanolamine/HCl, pH 7.6, 5 mM-EDTA,* 0.12 mM-NADH, 2.5 μM-rotenone, 0.3 mM-dihydroxyacetone phosphate	94 ± 1 (4)	71 ± 2
Pyruvate kinase (EC 2.7.1.40)	160 mM-Triethanolamine/HCl, pH 7.6, 67 mM-KCl, 10 mM-$MgCl_2$, 1 mM-EDTA,* 5 mM-ADP, 0.1 mM-fructose 1,6-bisphosphate, 0.12 mM-NADH, 2.5 μM-rotenone, 2 mM-phosphoenolpyruvate, 4 units of lactate dehydrogenase	97 ± 4 (5)	65 ± 2
Enolase (EC 4.2.1.11)	50 mM-Triethanolamine/HCl, pH 6.8, 5 mM-$MgCl_2$, 0.5 mM-ADP, 0.12 mM-NADH, 2.5 μM-rotenone, 0.5 mM-2-phosphoglycerate, 0.4 unit of pyruvate kinase 1 unit of lactate dehydrogenase	100 ± 1 (4)	99 ± 1
Phosphoglycerate mutase (EC 2.7.5.3)	50 mM-Triethanolamine/HCl, pH 6.8, 2 mM-$MgCl_2$, 1 mM-EDTA,* 1 mM-ADP, 0.12 mM-NADH, 2.5 μM-rotenone, 3 mM-3-phosphoglycerate, 0.08 unit of enolase, 0.4 unit of pyruvate kinase, 1 unit of lactate dehydrogenase	104 ± 2 (4)	101 ± 2
Triose phosphate isomerase (EC 5.3.1.1)	50 mM-Triethanolamine/HCl, pH 7.6, 1 mM-EDTA,* 0.12 mM-NADH, 2.5 μM-rotenone, 1 mM-glyceraldehyde 3-phosphate, 0.4 unit of glycerol phosphate dehydrogenase†	103 ± 1 (4)	97 ± 1
Hexokinase (EC 2.7.1.1)	100 mM-Triethanolamine/HCl, pH 7.6, 1 mM-mercaptoethanol, 10 mM-$MgCl_2$, 1 mM-EDTA,* 2.5 mM-ATP,* 0.4 mM-NADP, 1 mM-glucose, 0.35 unit of glucose 6-phosphate dehydrogenase	100 ± 2 (4)	100 ± 3

Aldolase (EC 4.1.2.13)	50mM-Triethanolamine/HCl, pH7.6, 1mM-mercaptoethanol, 0.12mM-NADH, 2.5μM-rotenone, 2mM-fructose 1,6-bisphosphate, 5 units of triose phosphate isomerase, 0.08 unit of glycerol phosphate dehydrogenase	101±3 (6)		96±3
Phosphoglucose isomerase (EC 5.3.1.9)	100mM-Tris/HCl, pH8.0, 0.4mM-NADP, 1.5mM-fructose 6-phosphate, 0.35 unit of glucose 6-phosphate dehydrogenase	101±1 (6)		102±1
Lactate dehydrogenase (EC 1.1.1.27)	100mM-Triethanolamine/HCl, pH7.2, 0.12mM-NADH, 2.5μM-rotenone, 1mM-pyruvate	98 (6)		105±4

* Adjusted to pH7.5 with NaOH.
† Dialysed against 1mM-EDTA as suggested by Saggerson & Greenbaum (1969).

Materials and Methods

Coenzymes, crystalline enzymes and substrates were from C. F. Boehringer und Soehne G.m.b.H., Mannheim, West Germany. Dihydroxyacetone phosphate and glyceraldehyde phosphate were prepared from their dimethylketal and diethylacetal forms respectively, following the manufacturers' instructions. Testosterone propionate was from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals were of analytical-reagent grade where available.

Male albino rats were housed at 25°C with alternate periods of 12h light and darkness. The animals were allowed free access to food (Special Mouse Cubes from Charlicks Feeds, Adelaide, South Australia) and water. Orchidectomy was performed as described previously (Brooks *et al.*, 1974) and ligation of the ductuli efferentes was carried out in an analogous manner with the ligature placed solely around the efferent ducts. Hormone replacement therapy was carried out by daily subcutaneous injections of hormone in maize oil (1mg/ml) at a dose of 1mg/kg body wt.

Spermatozoa were isolated by chopping the epididymis into small pieces of approx. 1 mm^3 and agitating the tissue pieces in 10ml of Krebs–Ringer phosphate buffer (DeLuca, 1972) for 15min at 30°C. The suspension was strained through a fine silk mesh to remove large fragments and centrifuged at 15*g* for 10min to remove smaller debris and epithelial cells. The supernatant was removed and centrifuged at 500*g* for 10min and the sedimented spermatozoa were resuspended to 10.5ml in fresh Ringer solution. A portion (0.5ml) of the spermatozoal suspension was added to 2.5ml of 0.15M-NaCl containing 0.2% formaldehyde for spermatozoal counting by absorption at 650nm using a calibration line derived from haemocytometer counts (Bishop *et al.*, 1954). The remaining 10ml of spermatozoal suspension was centrifuged at 800*g* for 10min, the supernatant removed and the sample frozen at −20°C until assay. Isolated epithelial cells from the epididymis were prepared as described by Brooks (1975).

To prepare tissue for enzyme assay, animals were anaesthetized with an intraperitoneal injection of sodium pentobarbital at a dose of 80mg/kg body wt. The epididymis was dissected free of fat and separated into four portions based on segments of the epididymis as illustrated by Brooks *et al.* (1973). Segment 2 formed the initial segment, and segments 3–6, 7–10 and 11–14 were taken as the caput, corpus and cauda epididymidis respectively. The tissue was wrapped in Nescofilm (Brando Chemical Ind., Kobe, Japan) and aluminium foil to prevent change in water content, and stored at −20°C until assay.

Tissue segments were weighed on a torsion balance and extracted in a glass Potter–Elvehjem homogenizer fitted with a motor-driven Teflon pestle in 10vol. of

50mM-triethanolamine containing 1mM-EDTA, 2mM-$MgCl_2$ and 30mM-2-mercaptoethanol adjusted to pH7.6 at 2°C with 1M-HCl. The homogenate was centrifuged at 600*g* for 10min at 2°C and the supernatant used for the determination of enzyme activity. In some instances, homogenates were further disrupted by sonication before centrifugation. Sonication was carried out for two 30s periods in an ice-cooled container with a 60W, 20kHz sonicator (MSE, London S.W.1, U.K.).

All enzyme assays were performed at 25°C against blanks in which the substrate was omitted in cells of 1cm light-path in an SP.1800 recording spectrophotometer (Pye Unicam Ltd., Cambridge, U.K.) by following the oxidation or reduction of nicotinamide coenzymes at 340nm. Assay conditions were chosen to maximize enzyme activity and to ensure zero-order kinetics. Reaction rates were linear over the 5min recording period and proportional to the amount of tissue homogenate. The enzyme assays were adapted from those of Glock & McLean (1953), Noltmann (1966), Shonk & Boxer (1964), Opie & Newsholme (1967), Saggerson & Greenbaum (1969), Crabtree & Newsholme (1972) and Vaughan *et al.* (1973). The final concentration of components in the 1ml assay volume is shown in Table 1 and the enzymes were assayed in order of increasing stability as listed in Table 1. In all cases where the enzyme was not stable for 24h, the assays were completed within 4h of homogenization. Although rotenone was included in the assay systems, NADH and NADPH oxidase activity in the homogenates were found to be negligible. Results were calculated as units of activity/g wet wt. of tissue, where a unit is defined as 1 μmol of substrate converted/min at 25°C.

For the determination of phosphorylase (EC 2.4.1.1) activity the tissue was homogenized in a different buffer, namely 100mM-maleic acid, 40mM-mercaptoethanol and 20mM-NaF adjusted to pH6.5 with 1M-NaOH, and assayed at 25°C as described by Crabtree & Newsholme (1972).

The changes in enzyme activity, which were recorded as a result of the various animal treatments, were shown to be true differences in the amount of enzyme as the activities of samples were additive when assayed in the same cuvette.

Results were analysed by an analysis of variance after logarithmic transformation. Individual means were tested for significant differences by using the error mean square in a *t* test.

Results

Characteristics of enzyme extraction and assay

A comparison of enzyme activities in the caput and cauda epididymidis between freshly excised tissue and tissue stored at −20°C for 2 weeks revealed no differences except in the case of hexokinase and phosphorylase. The mean activity ± S.E.M. from four samples each of caput and cauda epididymidis in extracts of frozen tissue as a percentage of the activity in extracts of fresh tissue was 155±11 for hexokinase and 81±10 for phosphorylase. All assays were therefore carried out with frozen tissue, except for phosphorylase, which was assayed by using homogenates of fresh tissue. Loss of phosphorylase activity was still apparent in the homogenate of fresh tissue, the activity being 83±8% at 5h and 18±12% at 24h after preparation of the homogenate. In spermatozoa, equivalent enzyme activities were measured in sonicated extracts of freshly prepared cells (four samples) and those which had been stored for several days at −20°C (four samples).

Sonication for 1min of homogenates of spermatozoa-free epididymal tissue prepared in the Potter–Elvehjem homogenizer did not release additional enzyme activity except in the case of hexokinase, glycerol phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. In four caput and cauda epididymidis samples, the percentage activity in unsonicated homogenates compared with sonicated homogenates was 77±2 for hexokinase, 88±3 for glycerol phosphate dehydrogenase and 81±5 for 6-phosphogluconate dehydrogenase. In contrast with epididymal tissue, disruption of spermatozoa with the Potter–Elvehjem homogenizer resulted in poor release of enzymes. A comparison of the two extraction methods indicated that the proportion of enzyme extracted in the Potter–Elvehjem system was by no means constant, but varied from 13% in the case of pyruvate kinase to 106% for glucose 6-phosphate dehydrogenase. The refractoriness of spermatozoa to the usual methods of homogenization has been demonstrated by Harrison (1971).

In other tissues it has been reported that a lower activity of phosphofructokinase is obtained if the pH of the extraction buffer is below 7.8 (Saggerson & Greenbaum, 1969). Therefore 50mM-Tris containing 1mM-EDTA, 5mM-$MgSO_4$ and 10mM-mercaptoethanol adjusted with 1M-NaOH to pH8.2 at 2°C (Opie & Newsholme, 1967) was compared as an extracting buffer with the usual triethanolamine buffer at pH7.6. Equal activity was extracted with either buffer and therefore phosphofructokinase was subsequently extracted in the same buffer as the other enzymes.

The pyruvate kinase extracted from epididymal tissue was activated by fructose 1,6-bisphosphate as has been found for this enzyme in liver and yeast (Hess *et al.*, 1966; Bailey *et al.*, 1968). The spermatozoal enzyme did not, however, show this activation.

A considerable proportion of the lactate dehydrogenase in spermatozoa is attributable to a specific isoenzyme designated as lactate dehydrogenase-X

which has distinctive catalytic properties compared with other isoenzymes (Battellino & Blanco, 1970; Goldberg, 1972). In order to establish the most suitable pyruvate concentration to use, a comparison was made between whole epididymal tissue, isolated epididymal epithelial cells (which represent a large proportion of the total epididymal mass) and spermatozoa. The lactate dehydrogenase from epithelial cells exhibited near maximum activity at 1 mM-pyruvate, but this concentration was sub-optimal for the spermatozoal preparation (Fig. 1). Alterations in the epididymal content of spermatozoa would have little influence on the apparent optimum pyruvate concentration for the whole organ, as spermatozoa represent only a small proportion of the total epididymal mass and their lactate dehydrogenase activity did not differ greatly from that of epididymal tissue (Tables 2 and 3). However, spermatozoal lactate dehydrogenase (Table 2) is underestimated by about 20% as the determinations were made with 1 mM-pyruvate.

Enzyme activities in different segments of the epididymis

The activities of enzymes in different segments of the epididymis, which had been rendered free of spermatozoa by efferent duct ligation, are shown in Table 3. Androgen support to the epididymis is still maintained from the testis via the blood stream. In general, lower activity was found in the initial segment compared with the caput epididymidis and in the corpus compared with the caput and cauda epididymidis. Along the duct, some trends in enzyme activity were apparent. Phosphofructokinase, glycerol phosphate dehydrogenase and glucose 6-phosphate dehydrogenase decreased in activity along the duct, whereas hexokinase increased. Lactate dehydrogenase and phosphorylase were more active in the cauda epididymidis than in other segments.

In castrated animals, in addition to becoming devoid of spermatozoa, the androgen support to the epididymis is also removed. After this treatment regional differences and trends in enzyme activity were less marked than in animals in which the efferent ducts were ligated, except that activity in the corpus epididymidis was lower than in other segments (Table 4).

Androgen-dependence of enzymes in the epididymis

The results shown in Table 3 are not directly comparable with those in Table 4 as they were determined in two unrelated groups of animals. To

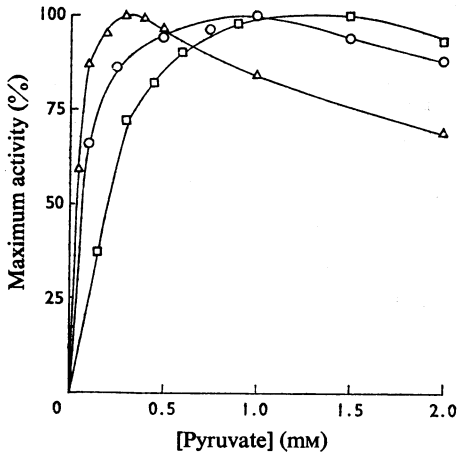


Fig. 1. *Influence of pyruvate concentration on the activity of lactate dehydrogenase from the rat epididymis and spermatozoa*

The components of the assay mixture are given in Table 1. ○, Caput epididymidis from an adult rat; △, epididymal spermatozoa; □, isolated epididymal epithelial cells. Spermatozoa were disrupted by sonication, whereas the other tissues were treated in a Potter–Elvehjem homogenizer. The 600*g* supernatant was used for assay in all cases.

Table 2. *Activities of glycolytic enzymes in rat epididymal spermatozoa*

Spermatozoa were prepared as described in the Materials and Methods section and were disrupted by sonication. The results represent the means±S.E.M. for eight preparations. Conversion of units/1 × 10^9 spermatozoa to units/g wet wt. is based on a spermatozoal volume of 0.2 plitre (Roosen-Runge, 1955) and assumes a sp.gr. of 1.

Enzyme	Activity in sonicated spermatozoa (units/10^9 spermatozoa)	(units/g wet wt.)
Hexokinase	5.57±0.18	27.9
Phosphoglucose isomerase	30.3±0.7	152
Phosphofructokinase	0.444±0.025	2.22
Aldolase	1.37±0.02	6.85
Triose phosphate isomerase	212±7	1060
Glyceraldehyde phosphate dehydrogenase	8.14±0.43	40.2
Phosphoglycerate kinase	61.5±5.2	308
Phosphoglycerate mutase	27.9±1.1	140
Enolase	13.0±0.3	65
Pyruvate kinase	13.0±0.3	65
Lactate dehydrogenase	16.1±0.7	81
Glycerol phosphate dehydrogenase	0.09±0.01	0.45
Glucose 6-phosphate dehydrogenase	0.07±0.02	0.35
6-Phosphogluconate dehydrogenase	0.12±0.004	0.60

Table 3. *Activities of enzymes in segments of the epididymis from adult rats 6 weeks after efferent duct ligation*

Enzyme activities are expressed as units/g wet wt. and the results represent the mean±S.E.M. of four animals weighing 270±20g. The activity of phosphorylase was determined in six adult rats that had not been subjected to efferent duct-ligation. Significant differences between adjacent segments are indicated by asterisks (*, $P<5\%$; **, $P<1\%$; ***, $P<0.1\%$).

	Initial segment		Caput epididymidis		Corpus epididymidis		Cauda epididymidis
Tissue wt. (mg)	17.8±0.9	***	69.4±3.7	***	16.8±1.3	***	99±6
Hexokinase	0.57±0.05		0.59±0.08		0.74±0.21		1.07±0.17
Phosphoglucose isomerase	37.6±2.0	***	50.3±1.4	***	35.5±2.8	**	45.1±1.6
Phosphofructokinase	4.10±0.16	**	5.19±0.22	***	2.24±0.27		1.86±0.05
Aldolase	0.71±0.07	***	1.63±0.06	**	1.18±0.14	**	1.67±0.11
Triose phosphate isomerase	251±11	**	316±12		281±22		291±8
Glyceraldehyde phosphate dehydrogenase	29.9±1.4	**	38.1±1.6	**	29.0±1.5	*	34.5±1.7
Phosphoglycerate kinase	12.9±0.4	***	18.7±1.0		16.6±1.0		18.3±0.7
Phosphoglycerate mutase	12.3±0.6		14.4±0.7		11.6±0.9		12.3±0.3
Enolase	8.79±0.27	**	10.6±0.4	***	6.95±0.52	**	8.57±0.18
Pyruvate kinase	24.0±2.1	**	30.7±1.1	***	21.4±1.4	***	34.3±0.8
Lactate dehydrogenase	23.8±1.1		25.6±0.5		25.7±2.1	***	34.3±0.8
Glycerol phosphate dehydrogenase	3.21±0.26	**	1.11±0.09		0.97±0.12		0.80±0.09
Glucose 6-phosphate dehydrogenase	4.86±0.33	***	3.08±0.34	***	1.23±0.13		1.60±0.08
6-Phosphogluconate dehydrogenase	0.72±0.08	***	1.21±0.03	***	0.74±0.10		0.88±0.02
Phosphorylase	1.61±0.13		1.36±0.09		1.44±0.09	***	2.80±0.10

Table 4. *Activities of enzymes in segments of the epididymis from adult rats 5 weeks after orchidectomy*

Enzyme activities are expressed as units/g wet wt. and the results represent the mean±S.E.M. of four animals weighing 316±11 g. Significant differences between adjacent segments are indicated by asterisks (*, $P<5\%$; **, $P<1\%$; ***, $P<0.1\%$).

	Initial segment		Caput epididymidis		Corpus epididymidis		Cauda epididymidis
Tissue wt. (mg)	9.4±0.6	***	20.5±1.70	***	7.6±0.6	***	30.9±3.5
Hexokinase	0.41±0.02		0.47±0.04		0.29±0.05		0.41±0.08
Phosphoglucose isomerase	29.7±0.9		33.4±1.4	***	23.8±0.7	***	33.5±1.7
Phosphofructokinase	1.40±0.08	*	1.66±0.08	***	0.80±0.03	*	1.04±0.08
Aldolase	0.44±0.02		0.58±0.05	**	0.29±0.04	***	0.88±0.08
Triose phosphate isomerase	219±9		236±9	**	187±3	***	258±11
Glyceraldehyde phosphate dehydrogenase	22.7±1.1		23.7±1.4	**	16.6±0.5	**	23.2±1.5
Phosphoglycerate kinase	11.5±0.55		13.1±1.1	*	10.6±0.1	***	15.7±1.2
Phosphoglycerate mutase	8.49±0.26	*	9.21±0.12	***	6.34±0.1	***	8.51±0.22
Enolase	7.61±0.21	*	8.84±0.56	***	5.36±0.29	***	7.51±0.31
Pyruvate kinase	19.6±0.9		21.9±0.6	***	13.7±0.4	***	22.9±1.1
Lactate dehydrogenase	21.0±0.8		20.7±0.2	***	14.9±0.4	***	25.6±1.3
Glycerol phosphate dehydrogenase	1.00±0.07		0.82±0.09		0.77±0.12		0.55±0.11
Glucose 6-phosphate dehydrogenase	1.23±0.13		1.06±0.08	***	0.56±0.03		0.56±0.04
6-Phosphogluconate dehydrogenase	0.37±0.05		0.47±0.08		0.24±0.04		0.43±0.04

obtain a direct comparison of the effects of efferent duct ligation, castration and testosterone replacement therapy, mature male rats were divided into five treatment groups as indicated in Fig. 2.

Efferent-duct ligation resulted in a considerable reduction in weight of both the caput and cauda epididymidis, whereas castration resulted in an even larger loss of weight. Although the epididymides from castrated animals appeared to be completely devoid of spermatozoa, there still appeared to be some spermatozoa remaining in the cauda epididymidis of animals with ligated efferent ducts.

Efferent duct ligation resulted in altered activities of a number of enzymes in the caput and cauda epididymidis. Hexokinase, aldolase, phosphoglycerate kinase, phosphoglycerate mutase and glycerol phosphate dehydrogenase in particular were markedly decreased. A number of other enzymes showed smaller, but statistically significant, changes. However, as a consequence of the incomplete release of

spermatozoal enzymes in the Potter–Elvehjem homogenizer, the differences cannot be taken as absolute differences owing to the presence or the absence of spermatozoa.

Castration resulted in a significant lowering of activity of all enzymes in the caput epididymidis except for an increase in phosphorylase and no change in triose phosphate isomerase and lactate dehydrogenase. In the cauda epididymidis a significant lowering of activity was observed for all enzymes except triose phosphate isomerase and enolase.

In the group of animals maintained on testosterone after castration, all enzymes were maintained at values similar to those in the group with ligated efferent ducts. In the testosterone regenerated group, testosterone increased the concentrations of all enzymes in the caput epididymidis except for lactate dehydrogenase and phosphorylase. In the cauda epididymidis, phosphoglucose isomerase, triose phosphate isomerase, phosphoglycerate kinase, phosphoglycerate mutase, enolase and lactate dehydrogenase activities were not increased.

There were several enzymes in the cauda epididymidis (phosphoglucose isomerase, triose phosphate isomerase, phosphoglycerate kinase, phosphoglycerate mutase and lactate dehydrogenase) that were maintained at higher concentrations than in castrated animals by immediate testosterone replacement, but that did not respond to delayed testosterone treatment. The reason for this difference between the testosterone-maintained and testosterone-regenerated groups probably lies in the fact that the cauda epididymidis in the former group still appeared to contain an appreciable number of spermatozoa in contrast with the latter.

Discussion

Enzyme activities in the epididymis and epididymal spermatozoa

The activities of glycolytic enzymes in the epididymis have not been studied before. Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are the only enzymes whose activity can be compared with those of previous reports (Lunaas *et al.*, 1968; Sholl & Leathem, 1973), and in this case the activities were similar to those in the present study. However, the activities of the complete sequence of glycolytic enzymes in segments of the epididymis (Table 3) can be compared with the activities of these enzymes in other rat tissues. In this regard, the data collected by Shonk & Boxer (1964) are useful. In comparison with an intensely glycolytic tissue such as skeletal muscle, the epididymis has a lower amount of all glycolytic enzymes, the concentrations being more comparable with those measured in liver and kidney.

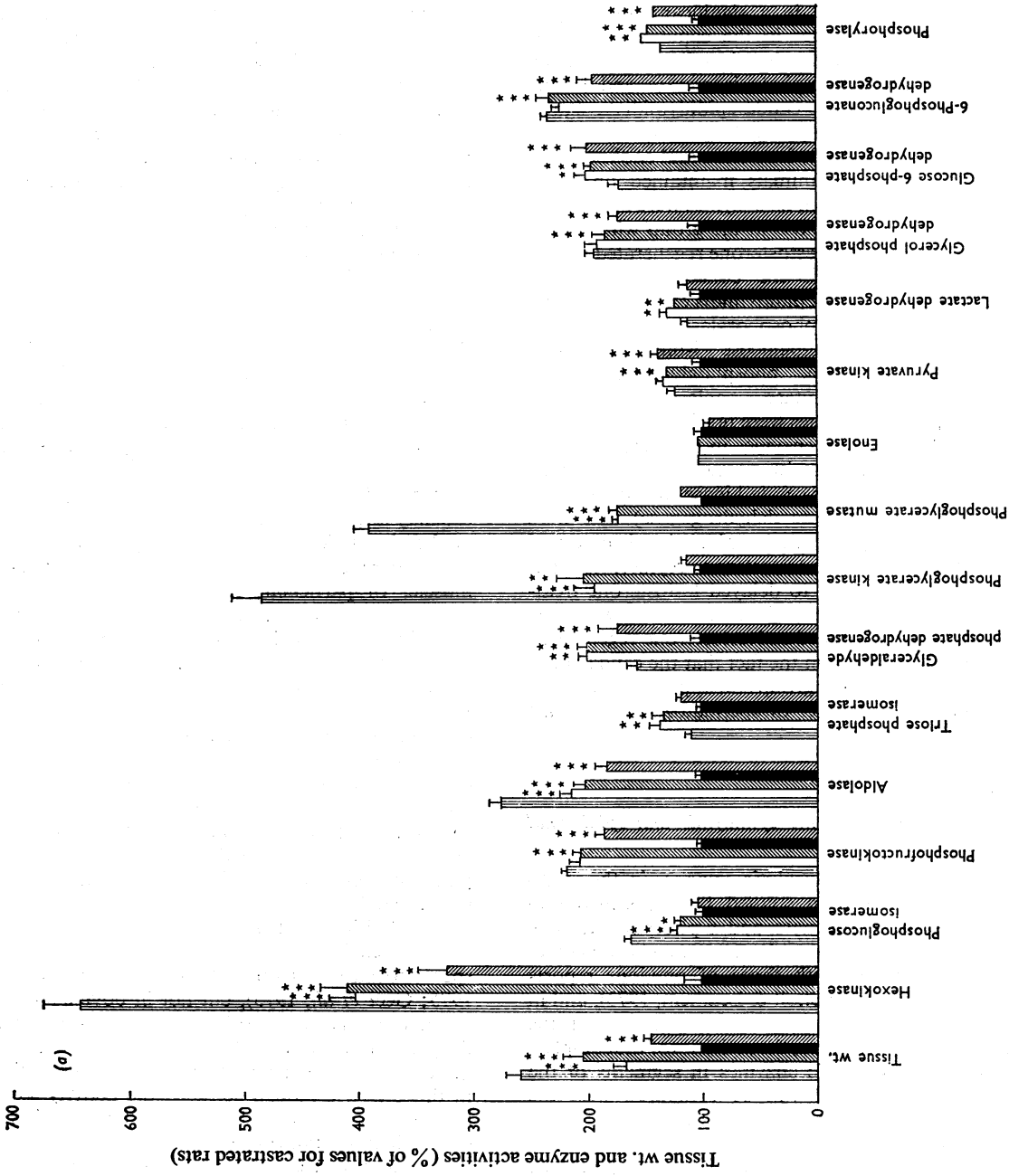
The first enzyme of the pentose phosphate cycle, glucose 6-phosphate dehydrogenase, is quite active in epididymal tissue, particularly in the initial segment. Johnson & Turner (1971) have provided evidence for the operation of an active pentose phosphate cycle in the rat epididymis. Both phosphofructokinase and glucose 6-phosphate dehydrogenase decrease from the initial segment to the cauda epididymidis, but their ratio remains relatively constant. This would suggest that although the potential flux through the glycolytic pathway and pentose phosphate cycle may decrease along the length of the epididymis, they may retain a constant relationship to each other. This interpretation is supported by the constant ratio of $^{14}CO_2$ formation from [1-^{14}C]-glucose and [6-^{14}C]glucose determined in the caput and cauda epididymidis by Kraft & Johnson (1972).

Glycerol phosphate dehydrogenase is present in low amounts in the epididymis compared with other tissues. This enzyme is considerably more active in the more proximal regions of the epididymis, indicating that these regions are more active in lipid synthesis, which is consistent with the greater quantities of total lipid found in these segments (Brooks, 1976).

Mammalian spermatozoa have a high glycolytic capacity and this is reflected in the greater activities of the glycolytic enzymes (Table 2), these activities being more similar to those recorded in skeletal muscle. In particular, hexokinase is very active. Spermatozoa depend on hexose sugars almost exclusively, especially fructose (Mann, 1964), as an exogenous energy substrate. Mammalian spermatozoa contain no glycogen (Mann & Rottenberg, 1966) and in the present study glycogen phosphorylase could not be detected.

Although the operation of the pentose phosphate cycle has not been demonstrated in spermatozoa (Scott *et al.*, 1962; Voglmayr *et al.*, 1970), histochemical studies have indicated the presence of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in spermatozoa of several species (Balogh & Cohen, 1964; Blackshaw, 1964; Bolton & Linford, 1970). These enzymes have also been detected in extracts of mouse, human and rat spermatozoa (Peterson & Freund; 1970; Erickson, 1975; Geer *et al.*, 1975), but not in bovine spermatozoa (Hammerstedt, 1975). Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were detected in rat spermatozoal preparations in this study, although some of the activity would have been contributed by the small proportion of contaminating erythrocytes.

The low concentration of glycerol phosphate dehydrogenase in spermatozoa (Geer *et al.*, 1975; the present study) is also consistent with the limited synthetic activity of these cells. The mitochondrial form of the enzyme, however, is particularly active in rat spermatozoa (Shenkman *et al.*, 1965).


Tissue wt. and enzyme activities (% of values for castrated rats)
700
600
500
400
300
200
100
0
(a)
Tissue wt.
Hexokinase
Phosphoglucose isomerase
Phosphofructokinase
Aldolase
Triose phosphate isomerase
Glyceraldehyde phosphate dehydrogenase
Phosphoglycerate kinase
Phosphoglycerate mutase
Enolase
Pyruvate kinase
Lactate dehydrogenase
Glycerol phosphate dehydrogenase
Glucose 6-phosphate dehydrogenase
6-Phosphogluconate dehydrogenase
Phosphorylase

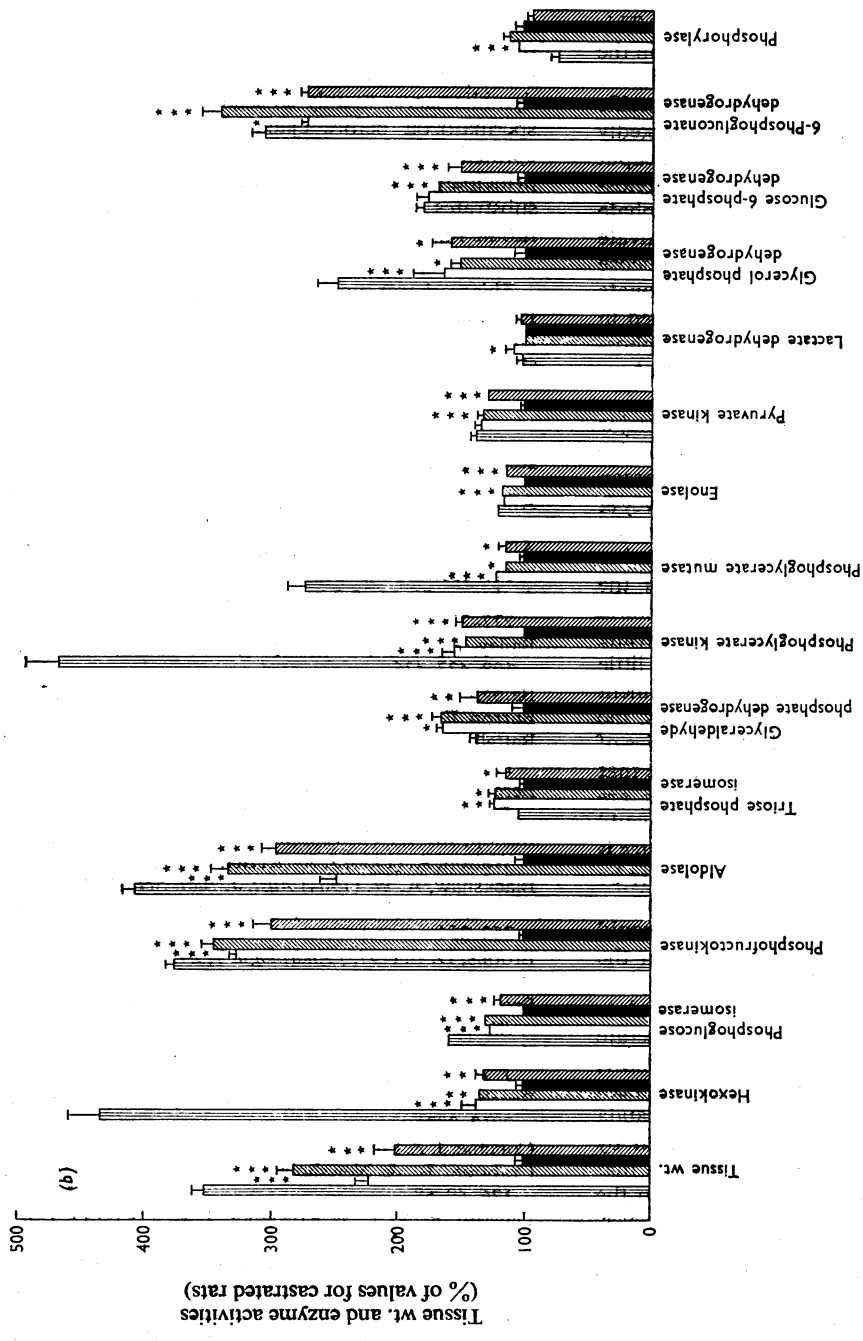


Fig. 2. *Influence of efferent duct ligation, orchidectomy and testosterone replacement therapy on the activity of enzymes in the rat epididymis*

(*a*) Cauda epididymidis; (*b*) caput epididymidis. Adult animals weighing 367 ± 5 g were treated as follows: ▨, untreated; □, bilateral efferent duct ligation for 2 weeks; ■, bilateral orchidectomy and daily injections of testosterone propionate at 1 mg/kg body wt. for 2 weeks (testosterone maintained); ■, bilateral orchidectomy with daily injections of maize oil for 2 weeks (castrated); ■, bilateral orchidectomy for 2 weeks followed by daily injections of testosterone propionate at 1 mg/kg body wt. for a further 2 weeks (testosterone regenerated). Enzyme activities, which were determined as units/g wet wt., are expressed as a percentage of the activity in the castrated group. The results represent the mean ± S.E.M. of six animals per group and significance levels as: *, $P<5\%$; **, $P<1\%$; ***, $P<0.1\%$. Asterisks above the efferent-duct-ligated column indicate a significant difference from the untreated group; asterisks above the testosterone-maintained and testosterone-regenerated group indicate a significant difference from the castrated group.

Influence of androgens on enzyme activities

The marked loss of epididymal weight after castration was offset by treating the animals with testosterone and could be reversed by administration of testosterone after regression had occurred. This anabolic response to testosterone was reflected in the absolute increase in the amounts of enzymes in the organ. However, when expressed in terms of specific activity, only selected enzymes were markedly stimulated (more than 25%) in activity. In the caput epididymidis, these enzymes were hexokinase, phosphofructokinase, aldolase, glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase, glycerol phosphate dehydrogenase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The cauda epididymidis behaved in a similar manner except that the activity of phosphorylase was also increased, whereas that of phosphoglycerate kinase was unchanged.

In the prostate and seminal vesicles, the major influence of androgen status is exerted on oxidative metabolism with little alteration in glycolysis (Barron & Huggins, 1944; Rudolph & Samuels, 1949; Levey & Szego, 1955), the effect on oxidative metabolism apparently being produced by a change in the number of mitochondria rather than an alteration of the specific activity of enzymes in the mitochondria (Edelman *et al.*, 1963). However, Elliot (1965), using homogenates of mouse epididymis, could find little effect of orchidectomy or testosterone replacement on either respiration or glycolysis. Despite this, the demonstration that in the epididymis the activities of a number of glycolytic enzymes, including those that are considered to be regulatory in other tissues, are dependent on the androgen concentration would indicate that androgenic control of glycolytic rates is likely to occur *in vivo*. There would also appear to be control at the metabolic branch points of the pentose phosphate cycle and glycerol formation. This would be consistent with greater synthetic activity in the androgen-maintained epididymis.

The reason for the difference in the absolute response of phosphorylase and phosphoglycerate kinase and the extent of response of hexokinase, phosphofructokinase, aldolase and 6-phosphogluconate dehydrogenase between the caput and cauda epididymidis is not clear. It has been assumed by analogy with the prostate and seminal vesicles that changes in enzyme activity reflect alterations in the epithelium rather than the connective tissue. However, the distal segments of the epididymis contain a greater proportion of smooth muscle than the proximal. It cannot be excluded that the muscular tissue responds to androgen in a similar way to the levator ani muscle in which the activities of several enzymes including hexokinase and phosphorylase increase after orchidectomy followed by testosterone administration (Bergamini *et al.*, 1969).

It is more likely, however, that the difference in response between the two segments reflects a difference in the function of the epithelium. The caput epididymidis is recognized as the region where spermatozoal maturation occurs, although the nature of the epithelial involvement in this process is entirely obscure. On the other hand, the cauda epididymidis serves mainly as a storage area for mature spermatozoa before ejaculation. The role of the epithelium in the latter segment may therefore be principally restricted to the provision of energy substrates to the spermatozoa contained in the tubule lumen.

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