Activity and Androgenic Control of Enzymes associated with the Tricarboxylic Acid Cycle, Lipid Oxidation and Mitochondrial Shuttles in the Epididymis and Epididymal Spermatozoa of the Rat

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1. Enzyme activities (units/g wet wt.) were determined in the caput and cauda epididymidis and in epididymal spermatozoa of the rat. 2. The activity of most enzymes in the cauda was between 50 and 100% of that in the caput, except that ATP citrate lyase was barely detectable in the cauda. 3. Spermatozoa, unlike epididymal tissue, contained sorbitol dehydrogenase but lacked ATP citrate lyase. NADP⁺-malate dehydrogenase, mitochondrial glycerol 3-phosphate dehydrogenase, succinate dehydrogenase, carnitine acetyltransferase and citrate synthase were 5 to 400 times as active in spermatozoa as in epididymal tissue. 4. 2-Oxoglutarate dehydrogenase was the least active member of the tricarboxylic acid cycle in all tissues and most closely matched the measured flux through the cycle. 5. The concentrations of hydroxyacyl-CoA dehydrogenase and carnitine palmitoyltransferase were equivalent to the more active enzymes of the tricarboxylic acid cycle, indicating the capacity for extensive lipid oxidation, and the presence of 3-hydroxybutyrate dehydrogenase suggests that these tissues can also oxidize ketone bodies. 6. Transfer of reducing equivalents from cytoplasm to mitochondrion is unlikely to occur by means of the glycerol phosphate cycle because mitochondrial glycerol 3-phosphate dehydrogenase is relatively inactive in epididymal tissue, whereas the cytoplasmic enzyme has little activity in spermatozoa, but transfer may be accomplished by the malate-aspartate shuttle. 7. Transfer of acetyl units from mitochondrion to cytoplasm could be effected by the pyruvate-malate cycle in the caput of androgen-maintained rats, but not in the other tissues because of the low activity of ATP citrate lyase. Acetyl unit transfer could take place via acetylcarnitine, mediated by carnitine acetyltransferase. 8. Castration resulted in a decrease in the concentration of nearly all enzymes, although subsequent administration of testosterone restored concentrations to values similar to those in animals maintained by endogenous androgen. The extent to which enzyme concentration was changed by an alteration in androgen status was highly variable, but was most marked in the case of pyruvate carboxylase.

A number of experimental approaches may be followed to identify enzymes that play a specific role in controlling the flux of metabolites through metabolic pathways (Newsholme & Gevers, 1967). One of the aids in the identification of enzymes that catalyse non-equilibrium reactions *in vivo* is the determination of maximum enzyme activities *in vitro*. Regulatory enzymes tend to form a low-activity group, whereas non-regulatory enzymes form a high-activity group. The flux of metabolites through metabolic pathways can also be altered in the longer term by the formation of new steady-state enzyme concentration as a result of dietary or hormonal

manipulation of the animal. Comparison of enzyme activities in tissue from animals under different hormonal regimes can then provide additional indicators of points of regulation.

The male accessory sex organs are highly dependent on androgenic hormones to maintain their normal structure and functions. The prostate gland has usually been taken as the type organ for studying the mechanisms of action of androgenic hormones (Mainwaring, 1977). However, the epididymis is another component of the male reproductive tract that is highly androgen-dependent and plays a vital role in male fertility. It is only during passage through the epididymis that immature spermatozoa from the testis become capable of fertilizing eggs, although the mechanisms by which the epididymis provides a

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

unique intraluminal environment to effect this maturation are poorly understood.

Androgenic hormones reach the epididymis via the bloodstream and also in the fluid that accompanies the spermatozoa from the testis (Vreeburg, 1975). By ligating the efferent ducts that lead from the testis to the epididymis, spermatozoa are prevented from entering the epididymis, and those spermatozoa already in the epididymis are slowly decreased in number by loss into the urine or ejaculate. This procedure depletes the spermatozoal content of the epididymis, but hormonal support is still maintained by androgens carried from the testis to the epididymis via the blood. In contrast, when the testis is removed by castration, the source of androgens is removed and the epididymis regresses. These two operations (efferent-duct ligation compared with castration) can therefore be used to compare the epididymis with and without androgenic stimulation.

In previous studies of the epididymis, it has been shown that only certain enzymes of the glycolytic sequence were decreased in specific activity after androgen deprivation (Brooks, 1976*a,b*). Further, these enzymes were identical with those considered to play a regulatory role in the glycolytic sequence in other tissues. The examination of the androgenic control of enzyme activity in the epididymis has been extended in the present study to enzymes involved in the tricarboxylic acid cycle, lipid oxidation and mitochondrial shuttles. As in the previous work, the androgen-dependence of the enzymes has been assessed both by measuring the change in specific activity after castration and by determining the extent of restoration of specific activity after the administration of testosterone propionate to regressed castrated animals. The activities of these enzymes have also been measured in epididymal spermatozoa to assess the metabolic capabilities of these cells and to compare with activities in epididymal tissue.

Materials and Methods

Coenzymes, crystalline enzymes and substrates were from C. F. Boehringer und Soehne G.m.b.H., Mannheim, West Germany, and from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals were of analytical-reagent grade where available and solvents were redistilled before use. $\text{Na}_2^{14}\text{CO}_3$ (60 mCi/mmol), sodium [$1\text{-}^{14}\text{C}$]pyruvate (14.4 mCi/mmol) and $n\text{-}[1\text{-}^{14}\text{C}]\text{hexadecane}$ (0.509 $\mu\text{Ci/g}$) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Cytochrome *c* was reduced by adding 166 μl of 1.2M- $\text{Na}_2\text{S}_2\text{O}_4$ to 1 ml of cytochrome *c* (10 mg/ml) in 0.1M-potassium phosphate, pH 7.0, and shaking in air to destroy excess dithionite. Acetoacetyl-CoA and acetyl-CoA were prepared by treating CoA with diketene (Ritchie *et al.*, 1971) and acetic anhydride (Stadtman, 1957) respectively, and the former was

standardized enzymically with purified hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (Decker, 1974). L-Carnitine and L-acetylcarnitine were kindly given by Dr. A. M. Snoswell, Dept. of Agricultural Biochemistry, University of Adelaide, South Australia, and L-palmitoylcarnitine was prepared by the procedure of Bremer (1968).

A heat-detoxified, dialysed skim-milk preparation was prepared by adaptation of procedures given by Harrison (1976). Fresh milk was centrifuged at 6000g for 45 min at 2°C and the infranatant was collected, heated for 10 min at 90–95°C and rapidly cooled. After centrifugation at 100000g for 60 min at 2°C the supernatant was dialysed at 2°C for 12 h periods against four changes of 40 vol. of water. Additions were then made to give the following concentrations: 20 mM-Hepes, 10 mM-glucose, 2 mM- Na_2HPO_4 , 4 mM- MgCl_2 , 136 mM- NaCl , 2.5 mM-KOH, 0.56 mg of penicillin G and 0.94 mg of streptomycin sulphate/ml. The final pH was adjusted to 7.5 with 1M-NaOH.

To isolate rat epididymal spermatozoa, rats were anaesthetized by an intraperitoneal injection of sodium pentobarbital (80 mg/kg body wt.) and a retrograde aortic perfusion (Vitale *et al.*, 1973) was performed with Krebs–Ringer phosphate buffer (DeLuca, 1972) to clear the epididymis of blood. The epididymis was then removed, trimmed of excess fat and cut into small fragments (1 mm³) which were suspended in 10 ml of milk diluent and shaken at 100 strokes/min at 30°C for 20 min. The suspension was strained through a Terylene mesh to remove large tissue fragments and centrifuged at 15g for 10 min. The supernatant was transferred to another tube and centrifuged at 150g for 15 min; the supernatant from this centrifugation was discarded and fresh milk diluent added to make a total volume of 10.5 ml, and the spermatozoa were resuspended by gentle repeated inversion of the tube. Then 10 ml of the spermatozoal suspension was centrifuged at 250g for 10 min to give a pellet, which was frozen for subsequent enzyme assay. The remaining spermatozoal suspension (0.5 ml) was used to check for spermatozoal motility by microscopic observation and, after dilution in 0.9% NaCl containing 0.2% (w/v) formaldehyde, to determine cell numbers by haemocytometer counts. The respiration rate of spermatozoa prepared in milk diluent was determined in Warburg manometers at 25°C with 100% O_2 as the gas phase, in flasks of 6 ml capacity and with 0.1 ml of 10% (w/v) KOH in the central well to absorb CO_2 .

Details of the animals, their care and the techniques for castration and efferent-duct ligation as well as the protocol for hormone replacement therapy have been given (Brooks, 1976*a*). To obtain tissue for enzyme assay, animals were killed by decapitation and the caput and cauda epididymidis were isolated and stored frozen at –20°C (Brooks, 1976*a*).

The tissue was minced with scissors, and homogen-

ates were prepared in 10 vol. of homogenizing medium by using a glass Potter-Elvehjem homogenizer fitted with a motor-driven Teflon pestle. The composition of the homogenization media is given in Table 1. For the extraction of certain enzymes, the homogenate was further disrupted by sonication as indicated in Table 1. Since spermatozoa are particularly difficult to disrupt (Harrison, 1971; Brooks, 1976a), all homogenates of spermatozoa were sonicated for a total of 1 min. With the homogenate kept in an ice bath, sonication was carried out in 15 s bursts with 45 s cooling intervals by using a 20 kHz sonicator (Labsonic 1510, from B. Braun Melsungen A. G., Melsungen, West Germany) fitted with a needle probe and operated at 75 W. Enzymes were assayed in the supernatants obtained by centrifugation of homogenates at 600g for 10 min at 2°C, except for sorbitol dehydrogenase (EC 1.1.1.14), NADP⁺-isocitrate dehydrogenase (EC 1.1.1.42) and ATP citrate lyase (EC 4.1.3.8), where the supernatant from centrifugation at 100000g for 60 min was used.

Enzymes were assayed at 25°C and blanks were run concurrently. For direct spectrophotometric assays, the final assay volume was 1 ml and changes in absorption were followed in an SP.1800 recording spectrophotometer (Pye Unicam, Cambridge, U.K.). The final assay volume for other assays was: 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) (1.2 ml), glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) (375 µl), pyruvate dehydrogenase (EC 1.2.4.1) (500 µl), carnitine palmitoyltransferase (EC 2.3.1.21) (100 µl) and pyruvate carboxylase (EC 6.4.1.1) (125 µl). All assays were tested for proportionality with the amount of tissue homogenate and for linearity with time. For some assays (e.g. glycerol 3-phosphate dehydrogenase and carnitine palmitoyltransferase) it was necessary to incubate for up to 4 h to obtain sufficient chromagen formation, and for glycerol 3-phosphate dehydrogenase the activity measured was multiplied by a factor of 2.5 to correlate with the rate obtained in the presence of phenazine methosulphate (Crabtree & Newsholme, 1972a).

The procedures for the enzyme assays were based on the following publications: sorbitol dehydrogenase (EC 1.1.1.14) (Mills & Means, 1972); 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) (Walker, 1954; Lehninger *et al.*, 1960); hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (Marriott & Northcote, 1975); NAD⁺-malate dehydrogenase (EC 1.1.1.37) (Bergmeyer & Bernt, 1974a); NADP⁺-malate dehydrogenase (EC 1.1.1.40) and ATP citrate lyase (EC 4.1.3.8) (Ballard & Hanson, 1967); NAD⁺-isocitrate dehydrogenase (EC 1.1.1.41) and NADP⁺-isocitrate dehydrogenase (EC 1.1.1.42) (Alp *et al.*, 1976); glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) (Zammit & Newsholme, 1976); pyruvate dehydrogenase (EC 1.2.4.1) (Taylor *et al.*, 1973;

Read *et al.*, 1977); 2-oxoglutarate dehydrogenase (EC 1.2.4.2) (Read *et al.*, 1977); succinate dehydrogenase (EC 1.3.99.1) (Rabinowitz & DeBernard, 1957); cytochrome oxidase (EC 1.9.3.1) (Smith, 1955); carnitine acetyltransferase (EC 2.3.1.7) (Fritz *et al.*, 1963); carnitine palmitoyltransferase (EC 2.3.1.21) (Crabtree & Newsholme, 1972b); glutamate-oxaloacetate transaminase (EC 2.6.1.1) (Bergmeyer & Bernt, 1974b); citrate synthase (EC 4.1.3.7) (Srere, 1969); fumarase (EC 4.2.1.2) and aconitase (EC 4.2.1.3) (Racker, 1950); pyruvate carboxylase (EC 6.4.1.1) (Saggerson, 1974). The final concentrations of components of the reaction media are shown in Table 1.

The activity of enzymes in homogenates of fresh tissue was compared with that in homogenates prepared from tissue stored at -20°C for 2 weeks. In most cases there was no loss of enzyme activity resulting from freezing (Table 1), except for succinate dehydrogenase and cytochrome oxidase, which lost considerable activity as a result of freezing, and assays for these enzymes were therefore carried out on homogenates prepared from fresh tissue. In addition, homogenates were tested for the stability of enzyme activity with time and for the proportion of enzyme activity present in a soluble form (Table 1). On the basis of these results, the assays were carried out in increasing order of enzyme stability.

Enzyme specific activities were calculated as units/g wet wt. of tissue, where a unit is defined as 1 µmol of substrate converted/min at 25°C. Results were evaluated by an analysis of variance. Individual means were tested for significant differences by using the effective standard error calculated from the error mean square and the appropriate value of *t*.

Results

Enzyme assays

Details of the procedures for extraction and assay of the enzymes are shown in Table 1. In several cases more enzyme activity was released from frozen than from fresh tissue. Tissue homogenized in some media was as a routine sonicated, although the effectiveness of this treatment for releasing additional activity was not tested, except with 3-hydroxybutyrate dehydrogenase. However, for those enzymes extracted in medium (a) or (b) (Table 1), sonication did not release additional activity (tested for hydroxyacyl-CoA dehydrogenase, NADP⁺-malate dehydrogenase, NADP⁺-isocitrate dehydrogenase, succinate dehydrogenase, cytochrome oxidase, citrate synthase and pyruvate carboxylase). To validate the assay systems further, enzyme activity was measured in other tissues (usually liver or heart) for comparison with values reported in the literature.

Although NAD⁺-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase are reported to be very

Table 1. *Composition of the media for homogenization and enzyme assay, and stability and solubility of enzymes in homogenates of rat epididymal tissue*

Enzyme	Homogenizing medium and total sonication time (s)	Final concentration of components in the assay system	Percentage of activity recovered in frozen tissue	Percentage of activity recovered in soluble fraction	Activity measured in tissue homogenate after various times of storage at 0°C	
					3-5 h	24 h
Sorbitol dehydrogenase (EC 1.1.1.14)	(c)	33 mM-Tris/HCl, 0.33 mM-NAD ⁺ , 50 mM-sorbitol, pH 8.6				100
3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30)	(d) (60)	40 mM-Tris/HCl, 100 mM-nicotinamide, 10 mM-2-mercaptoethanol, 2 mM-NAD ⁺ , 1 mM-KCN, 20 mM-DL-3-hydroxybutyrate, pH 8.5	106 ± 5	3		96 ± 5
Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)	(a)	50 mM-Potassium phosphate, 2 mM-EDTA, 1 mM-KCN, 0.24 mM-NADH, 0.1% bovine serum albumin, 0.1 mM-acetoacetyl-CoA, pH 7.0	126 ± 6	93 ± 2	85 ± 2 (5)	91 ± 2
Malate dehydrogenase (EC 1.1.1.37)	(a)	100 mM-Potassium phosphate, 40 mM-aspartate, 0.3 mM-2-oxoglutarate, 0.48 mM-NADH, 0.8 unit of glutamate-oxaloacetate transaminase/ml, pH 7.6	107 ± 1	100 ± 2		92 ± 2
Malate dehydrogenase (EC 1.1.1.40)	(b)	30 mM-Tris/HCl, 1.5 mM-MnCl ₂ , 0.3 mM-NADP ⁺ , 1 mM-L-malate, pH 7.6	116 ± 3	64 ± 3	103 ± 3 (5)	85 ± 4
Isocitrate dehydrogenase (EC 1.1.1.41)	(e) (30)	70 mM-Hepes, 2 mM-NAD ⁺ , 2 mM-ADP, 1 mM-MnCl ₂ , 8 mM-MgCl ₂ , 2.5 μM-rotenone, 20 mM-citrate, 3 mM-DL-isocitrate, pH 7.1	95 ± 4	97 ± 3	99 ± 3 (4)	62 ± 5
Isocitrate dehydrogenase (EC 1.1.1.42)	(b)	70 mM-Tris/HCl, 8 mM-MgCl ₂ , 1 mM-MnCl ₂ , 0.5 mM-NADP ⁺ , 3 mM-DL-isocitrate, 20 mM-citrate, pH 7.6	136 ± 8	97 ± 4	99 ± 1 (4)	79 ± 8
Glycerol 3-phosphate dehydrogenase (EC 1.1.1.99.5)	(a)	50 mM-Potassium phosphate, 1 mM-KCN, 1.7 mM-acetaldehyde, 20 mM-DL-glycerol 3-phosphate, 0.03-0.1% 2- <i>p</i> -iodophenyl-3- <i>p</i> -nitrophenyl-5-phenyltetrazolium chloride, 26.6 units of alcohol dehydrogenase/ml, pH 7.5	102 ± 9	2 ± 1		65 ± 3

Pyruvate dehydrogenase (EC 1.2.4.1)	(f) (15)	21 mM-Potassium phosphate, 1.1 mM-EDTA, 0.1 mM-EGTA, 2.8 mM-MgCl ₂ , 0.1 mM-CaCl ₂ , 1.6% bovine serum albumin, 1.2 mM- dithiothreitol, 1.6 mM-NAD ⁺ , 0.08 mM-thiamin pyrophosphate, 0.16 mM-CoA, 0.1 mM-dichloroacetate, 0.7 mM-pyruvate, 0.12 μ Ci of [1- ¹⁴ C]pyruvate/ml, pH 7.5	166 $\pm$ 9	0	88 $\pm$ 5 (4)	68 $\pm$ 5
2-Oxoglutarate dehydrogenase (EC 1.2.4.2)	(g) (15)	100 mM-Hepes, 10 mM-2-mercaptoethanol, 1 mM-KCN, 2 mM-NAD ⁺ , 0.02 mM-NADH, 0.1 mM-thiamin pyrophosphate, 1 mM-2-oxoglutarate, 0.5 mM-CoA, pH 7.4	116 $\pm$ 7	5 $\pm$ 5	96 $\pm$ 7 (3)	118 $\pm$ 4
Succinate dehydrogenase (EC 1.3.99.1)	(a)	40 mM-Potassium phosphate, 1 mM-KCN, 20 mM-succinate, 0.2 mg of oxidized cytochrome c/ml, pH 7.6	36 $\pm$ 2	2	88 $\pm$ 1 (3)	48 $\pm$ 2
Cytochrome oxidase (EC 1.9.3.1)	(a)	50 mM-Potassium phosphate, 0.8 mg of reduced cytochrome c/ml, pH 7.0	36 $\pm$ 3	1 $\pm$ 1	105 $\pm$ 4 (4)	96 $\pm$ 11
Carnitine acetyltransferase (EC 2.3.1.7)	(a)	100 mM-Tris/HCl, 1 mM-KCN, 2.5 mM-NAD ⁺ , 5 mM-L-malate, 0.12 mM-CoA, 0.06 mM-NADH, 15 mM-L-acetyl carnitine, 5.5 units of malate dehydrogenase/ml, 1.1 units of citrate synthase/ml, pH 8.0	121 $\pm$ 7	102 $\pm$ 1		92 $\pm$ 2
Carnitine palmitoyltransferase (EC 2.3.1.21)	(a)	500 mM-Hydroxylamine, 8 mM-CoA, 12 mM-L-palmitoylcarnitine, pH 7.0	95 $\pm$ 2	70 $\pm$ 2		105 $\pm$ 4
Glutamate-oxaloacetate transaminase (EC 2.6.1.1)	(a)	80 mM-Potassium phosphate, 100 mM-aspartate, 0.24 mM-NADH, 12 mM-2-oxoglutarate, 1.2 units of malate dehydrogenase/ml (in glycerol), 1.2 units of lactate dehydrogenase/ml (in glycerol), pH 7.6	113 $\pm$ 5	90 $\pm$ 5		99 $\pm$ 2
Citrate synthase (EC 4.1.3.7)	(a)	100 mM-Tris/HCl, 0.1 mM-5,5'-dithiobis- (2-nitrobenzoic acid), 0.3 mM-acetyl-CoA, 0.5 mM-oxaloacetate, pH 8.1	129 $\pm$ 3	102 $\pm$ 2		94 $\pm$ 2
ATP citrate lyase (EC 4.1.3.8)	(b)	15 mM-Tris/HCl, 10 mM-MgCl ₂ , 20 mM-citrate, 10 mM-2-mercaptoethanol, 0.24 mM-NADH, 5 mM-ATP, 0.5 mM-CoA, 1 mM-KCN, 5.5 units of malate dehydrogenase/ml, pH 7.6	111 $\pm$ 11	122 $\pm$ 5	90 $\pm$ 5 (4)	33 $\pm$ 13
Fumarase (EC 4.2.1.2)	(a)	50 mM-Potassium phosphate, 50 mM-L-malate, pH 7.6	89 $\pm$ 2	104 $\pm$ 2		88 $\pm$ 2
Aconitase (EC 4.2.1.3)	(a)	50 mM-Potassium phosphate, 30 mM-citrate, pH 7.6	90 $\pm$ 5	94 $\pm$ 2	37 $\pm$ 1 (4)	10 $\pm$ 1
Pyruvate carboxylase (EC 6.4.1.1)	(a)	100 mM-Hepes, 5 mM-MgCl ₂ , 2.5 mM-ATP, 0.4 mM-CoA, 2 mM-acetyl phosphate, 25 mM-NaHCO ₃ , 5 μ Ci of Na ₂ ¹⁴ CO ₃ /ml, 0.5 unit of phosphotransacetylase/ml, 1.1 units of citrate synthase/ml, 7 mM-pyruvate, pH 7.8	478 $\pm$ 85	59 $\pm$ 8	50 $\pm$ 1 (5)	7

unstable in homogenates of other tissue (Alp *et al.*, 1976; Read *et al.*, 1977), this was not found to be the case in homogenates of epididymal tissue. Most enzymes, except for aconitase and pyruvate carboxylase, lost little activity within 4 h of homogenization.

Inhibition of fumarase activity by deoxycholate, as has been reported with liver homogenates (Szkudlarek & Turski, 1975), was not observed in the present

study. This could possibly be because a lower concentration of deoxycholate was used and bovine serum albumin was added. The addition of the latter was necessary to prevent release of DNA from epididymal tissue of castrated animals.

By using the assay for carnitine acetyltransferase of Fritz *et al.* (1963), it was found that the reaction rate increased as NADH was formed. The reasons for the

Fig. 1. Characteristics of the assay for carnitine palmitoyltransferase in homogenates of epididymal tissue. Carnitine palmitoyltransferase was determined by measuring the A_{520} of the hydroxamate complex formed from the reaction product. Details of the assay procedure are given by Crabtree & Newsholme (1972b). The enzymic reaction was carried out in a volume of 100 μ l with the usual concentration of components as shown in Table 1. Effect of altering (a) palmitoylcarnitine concentration and (b) CoA concentration on the formation of reaction product after 2 h of incubation using 30 μ l of homogenized caput from an animal with ligated efferent ducts. (c) Linearity of reaction with time using 10 μ l of homogenized caput from an animal with ligated efferent ducts. (d) Formation of reaction product resulting from the incubation of various volumes of homogenate from either the caput of an animal with ligated efferent ducts ($\bullet$, 2h incubation) or the cauda of a castrated animal ($\blacktriangle$, 4h incubation). (e) Influence of L-carnitine on the formation of reaction product after 2 h of incubation using either 30 μ l ($\bullet$) or 10 μ l ($\blacktriangle$) of homogenized caput from an animal with ligated efferent ducts.

non-stoichiometry of conversion have been described by Pearson (1965), who indicated that addition of NADH to the medium would result in stoichiometric conversion of acetyl-CoA into NADH. In the assay system used (Table 1) it was found that the inclusion of 0.06 mM NADH satisfied this condition.

The characteristics of the assay for carnitine palmitoyltransferase from epididymal tissue are shown in Fig. 1. The concentrations of palmitoyl-carnitine and CoA required to saturate the enzyme were greater than those used by Crabtree & News-holme (1972b). The reaction rate remained linear for incubation times up to at least 4 h (Fig. 1c). However, when tested for proportionality to the amount of tissue homogenate, the response was not linear with homogenates prepared from rats with ligated efferent ducts, although a linear response was found with tissue homogenates from castrated rats (Fig. 1d). The epididymis normally accumulates large amounts of carnitine (Brooks *et al.*, 1974) and it was possible that carnitine present in the homogenate might cause some inhibition in the reaction when larger volumes of homogenate were used in the assay. Calculations based on the data of Brooks *et al.* (1974) indicated that with 30 μ l of homogenate from efferent-duct-ligated rats, the final concentration of carnitine in the assay would be 0.06 and 0.4 mM for the caput and cauda epididymidis respectively. However, dialysis of the homogenate for 24 h at 2°C against homogenizing medium did not alter the non-linearity in subsequent assays, although addition of L-carnitine to the assay medium did cause some decrease in enzyme activity with undialysed homogenates (Fig. 1e). Owing to the non-proportionality of response to homogenate volume, no more than 5 or 10 μ l of homogenate from efferent-duct ligated animals was used in the assay.

Pyruvate dehydrogenase exists in two enzymically interconvertible forms (Randle *et al.*, 1974). The medium used for homogenizing the tissue was designed to promote the activation and minimize the inactivation of pyruvate dehydrogenase (see Read *et al.*, 1977). By using this extraction procedure and the assay system of Taylor *et al.* (1973), frozen rat heart contained 4.8 ± 0.1 units/g (mean $\pm$ S.E.M. of four replicates) of pyruvate dehydrogenase. This value is comparable with that measured by a different assay procedure (3.4 units/g; Read *et al.*, 1977) and similar to that found when homogenates were treated with pyruvate dehydrogenase phosphatase to obtain complete conversion into active pyruvate dehydrogenase (4.5 units/g; see calculations of Read *et al.*, 1977). Because epididymal homogenates contained a lower concentration of pyruvate dehydrogenase compared with heart, it was necessary to extend the incubation time from 1 min to 30 min, nevertheless the reaction rate remained linear with time during the incubation and was proportional to

the homogenate volume. The proportion of pyruvate dehydrogenase assayed in the active form in epididymal homogenates is not known, but on the basis of the results from rat heart it seems probable that the measured activity was similar to the maximum activity.

Activities of enzymes in epididymal segments and in spermatozoa

The activity of enzymes in the androgen-maintained epididymis (from rats with ligated efferent ducts) is given in Table 2 together with the activity of these enzymes in the epididymis of castrated rats and in the epididymis of castrated rats after the administration of testosterone propionate.

For many enzymes, activities were similar in the caput and cauda epididymidis, whereas for others, activities differed by a factor of 2. However, in the epididymis of animals with ligated efferent ducts, ATP citrate lyase was about 7 times as active in the caput region, but this difference was not present in the castrated epididymis.

The pattern of enzyme activities in spermatozoa (Table 3) contrasted with that in epididymal tissue. Those enzymes that were considerably more active in spermatozoa included NADP⁺-malate dehydrogenase, glycerol 3-phosphate dehydrogenase, succinate dehydrogenase, carnitine acetyltransferase and citrate synthase, whereas ATP citrate lyase could not be detected. Mounib (1974) has reported the presence of two decarboxylating malate dehydrogenases in bovine and human spermatozoa. One of these is specific for NAD⁺, and the other is active with either NAD⁺ or NADP⁺. Sorbitol dehydrogenase could not be detected in epididymal tissue, but was present in spermatozoa. Previous studies have indicated that in the testicular excurrent duct system sorbitol dehydrogenase is an enzyme specific to spermatozoa (e.g. Mills & Means, 1972). However, sorbitol dehydrogenase is not a characteristic constituent of all spermatozoa, as it is present in ram but not boar spermatozoa (Brooks & Mann, 1972).

3-Hydroxybutyrate dehydrogenase was detected both in epididymal tissue and in epididymal spermatozoa. However, as with sorbitol dehydrogenase, 3-hydroxybutyrate dehydrogenase appears not to be present in all mammalian spermatozoa, as 3-hydroxybutyrate cannot be oxidized by boar spermatozoa, although it can be by bull and ram spermatozoa (Lardy & Phillips, 1945; Mohri *et al.*, 1965; Brooks & Mann, 1972). The occurrence of a highly active mitochondrial glycerol 3-phosphate dehydrogenase in rat spermatozoa has been noted by Schenkman *et al.* (1965), but the enzyme is considerably less active in spermatozoa of several other species. Carnitine acetyltransferase has also been demonstrated to be highly active in rat spermatozoa (Marquis & Fritz, 1965), but its activity in the spermatozoa of other

Table 2. Activity of enzymes in the epididymis of androgen-maintained rats compared with that of androgen-deprived rats, and the influence of subsequent administration of testosterone propionate to castrated animals

Either bilateral ligation of the efferent ducts or bilateral castration was carried out and animals were kept for at least 6 weeks before daily subcutaneous injections began and were continued for 3 weeks as follows: animals with ligated efferent ducts as well as some castrated animals received a daily injection of oil only, and other castrated animals were injected with 1 mg of testosterone propionate in oil/kg body wt. Owing to the limited amount of tissue available from one animal and the need to use a variety of extraction media, five unrelated groups of rats were used to obtain the results. The enzymes assayed and the body weights (mean $\pm$ S.E.M., in g) of the groups at the start of injections were as follows: NAD⁺-isocitrate dehydrogenase and pyruvate carboxylase (355 $\pm$ 8); pyruvate dehydrogenase (339 $\pm$ 8); 2-oxoglutarate dehydrogenase (301 $\pm$ 6); glycerol 3-phosphate dehydrogenase, succinate dehydrogenase and cytochrome oxidase (389 $\pm$ 12); all other enzymes (318 $\pm$ 7). 3-Hydroxybutyrate dehydrogenase was determined in animals that did not receive injections. Enzyme activity is expressed as units/g wet wt. and is shown as the mean $\pm$ S.E.M. of five replicates, and tissue wt. (mg) is the mean of 25 replicates. The values in parentheses represent the mean $\pm$ S.E.M., expressed as a percentage of that in the castrated group. The following statistical comparisons were made: caput versus cauda in animals with ligated efferent ducts and in castrated animals (evaluated by a paired *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$); efferent duct ligation versus castration (evaluated by analysis of variance; † $P < 0.05$; ‡ $P < 0.01$; †† $P < 0.001$); efferent duct ligation versus castration + testosterone propionate (evaluated by analysis of variance; ‡ $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$). Calculation of the flux through the tricarboxylic acid cycle is based on the respiration rate of tissue slices in the Warburg manometer at 30°C (Brooks, 1978). The respiration rate at 25°C was determined by experiment to be 67% of that at 30°C (corresponding to a doubling of rate for a 10°C rise in temperature; i.e. a Q_{10} of 2). It was then assumed that 1 μ mol of acetyl-CoA produced from either glucose or fatty acids and oxidized by the tricarboxylic acid cycle is equivalent to the uptake of 3 μ mol of O₂.

	Caput epididymidis			Cauda epididymidis		
	Efferent-duct ligated + oil	Castrated + oil	Castrated + testosterone propionate	Efferent-duct ligated + oil	Castrated + oil	Castrated + testosterone propionate
Tissue weight (mg)	93.7 $\pm$ 2.7 (385 $\pm$ 11)†††	24.6 $\pm$ 1.3 (100 $\pm$ 5)	81.7 $\pm$ 2.4 (321 $\pm$ 9)†††	141 $\pm$ 6*** (329 $\pm$ 14)†††	42.8 $\pm$ 2.2*** (100 $\pm$ 5)	120 $\pm$ 5 (280 $\pm$ 12)††
3-Hydroxybutyrate dehydrogenase	0.250 $\pm$ 0.016 (1140 $\pm$ 70)†††	0.022 $\pm$ 0.005 (100 $\pm$ 22)		0.166 $\pm$ 0.005* (874 $\pm$ 26)†††	0.019 $\pm$ 0.001 (100 $\pm$ 5)	
Hydroxyacyl-CoA dehydrogenase	5.64 $\pm$ 0.26 (243 $\pm$ 11)†††	2.32 $\pm$ 0.12 (100 $\pm$ 5)	4.66 $\pm$ 0.11 (201 $\pm$ 5)††	3.70 $\pm$ 0.09*** (226 $\pm$ 5)†††	1.54 $\pm$ 0.06** (100 $\pm$ 4)	3.08 $\pm$ 0.24 (188 $\pm$ 15)†
NAD ⁺ -malate dehydrogenase	76.5 $\pm$ 5.7 (128 $\pm$ 10)	59.7 $\pm$ 5.5 (100 $\pm$ 9)	67.6 $\pm$ 5.4 (113 $\pm$ 9)	64.4 $\pm$ 3.9** (149 $\pm$ 9)†	43.3 $\pm$ 5.2*** (100 $\pm$ 12)	54.7 $\pm$ 4.7 (126 $\pm$ 11)
NADP ⁺ -malate dehydrogenase	0.370 $\pm$ 0.016 (264 $\pm$ 11)†††	0.140 $\pm$ 0.010 (100 $\pm$ 7)	0.281 $\pm$ 0.011 (201 $\pm$ 8)†††	0.170 $\pm$ 0.005*** (147 $\pm$ 4)††	0.116 $\pm$ 0.008** (100 $\pm$ 7)	0.136 $\pm$ 0.012 (134 $\pm$ 10)
NAD ⁺ -isocitrate dehydrogenase	0.273 $\pm$ 0.011 (151 $\pm$ 6)†††	0.181 $\pm$ 0.004 (100 $\pm$ 2)	0.283 $\pm$ 0.014 (156 $\pm$ 8)	0.370 $\pm$ 0.032 (228 $\pm$ 20)†††	0.162 $\pm$ 0.008* (100 $\pm$ 5)	0.349 $\pm$ 0.011 (215 $\pm$ 7)
NADP ⁺ -isocitrate dehydrogenase	2.42 $\pm$ 0.12 (96 $\pm$ 5)	2.52 $\pm$ 0.13 (100 $\pm$ 5)	2.02 $\pm$ 0.09 (80 $\pm$ 4)††	1.18 $\pm$ 0.06*** (100 $\pm$ 5)	1.18 $\pm$ 0.04*** (100 $\pm$ 3)	1.10 $\pm$ 0.07 (93 $\pm$ 6)
Glycerol 3-phosphate dehydrogenase (mitochondrial)	0.164 $\pm$ 0.014 (1170 $\pm$ 70)†††	0.013 $\pm$ 0.001 (100 $\pm$ 8)	0.102 $\pm$ 0.006 (785 $\pm$ 46)†††	0.071 $\pm$ 0.006** (507 $\pm$ 43)†††	0.014 $\pm$ 0.002 (100 $\pm$ 14)	0.072 $\pm$ 0.006 (514 $\pm$ 43)
Pyruvate dehydrogenase	0.137 $\pm$ 0.007 (264 $\pm$ 14)†††	0.052 $\pm$ 0.005 (100 $\pm$ 9)	0.134 $\pm$ 0.007 (258 $\pm$ 13)	0.134 $\pm$ 0.007 (328 $\pm$ 17)†††	0.041 $\pm$ 0.002 (100 $\pm$ 4)	0.144 $\pm$ 0.008 (351 $\pm$ 20)
2-Oxoglutarate dehydrogenase	0.123 $\pm$ 0.004 (143 $\pm$ 5)†††	0.086 $\pm$ 0.006 (100 $\pm$ 7)	0.127 $\pm$ 0.011 (148 $\pm$ 13)	0.118 $\pm$ 0.005 (128 $\pm$ 5)†††	0.092 $\pm$ 0.004 (100 $\pm$ 4)	0.155 $\pm$ 0.007 (168 $\pm$ 8)†
Succinate dehydrogenase	2.59 $\pm$ 0.21 (470 $\pm$ 38)†††	0.551 $\pm$ 0.033 (100 $\pm$ 6)	2.40 $\pm$ 0.17 (436 $\pm$ 31)	3.01 $\pm$ 0.06 (408 $\pm$ 8)†††	0.737 $\pm$ 0.042** (100 $\pm$ 6)	2.95 $\pm$ 0.20 (400 $\pm$ 27)
Cytochrome oxidase	19.5 $\pm$ 1.6 (322 $\pm$ 26)†††	6.06 $\pm$ 0.44 (100 $\pm$ 7)	19.7 $\pm$ 0.9 (325 $\pm$ 15)	21.9 $\pm$ 1.0 (447 $\pm$ 20)†††	4.90 $\pm$ 0.10 (100 $\pm$ 2)	22.3 $\pm$ 2.0 (455 $\pm$ 41)

Carnitine acetyltransferase	0.413 ± 0.047 (157 ± 18)††	0.263 ± 0.014 (100 ± 5)	0.391 ± 0.018 (149 ± 7)	0.518 ± 0.042 (229 ± 19)††	0.226 ± 0.011* (100 ± 5)	0.372 ± 0.020 (165 ± 9)†
Carnitine palmitoyltransferase	2.03 ± 0.10 (494 ± 24)†††	0.410 ± 0.040 (100 ± 10)	1.79 ± 0.08 (437 ± 20)	2.36 ± 0.13 (1050 ± 60)†††	0.224 ± 0.023*** (100 ± 10)	1.68 ± 0.15 (750 ± 67)††††
Glutamate-oxaloacetate transaminase	75.7 ± 3.3 (319 ± 14)†††	23.7 ± 1.0 (100 ± 4)	76.0 ± 2.2 (321 ± 9)	57.6 ± 1.4** (257 ± 6)†††	22.4 ± 0.9 (100 ± 4)	53.4 ± 2.0 (238 ± 9)
Citrate synthase	6.39 ± 0.37 (161 ± 9)†††	3.96 ± 0.18 (100 ± 5)	6.58 ± 0.71 (166 ± 18)	5.65 ± 0.20 (199 ± 7)†††	2.84 ± 0.18*** (100 ± 6)	5.26 ± 0.15 (185 ± 5)
ATP citrate lyase	0.163 ± 0.011 (362 ± 24)†††	0.045 ± 0.006 (100 ± 13)	0.134 ± 0.010 (298 ± 22)	0.021 ± 0.004*** (95 ± 18)	0.022 ± 0.006 (100 ± 27)	0.036 ± 0.010 (164 ± 45)
Fumarase	4.42 ± 0.55 (515 ± 64)†††	0.857 ± 0.233 (100 ± 27)	4.52 ± 0.39 (527 ± 46)	4.70 ± 0.55 (590 ± 69)†††	0.797 ± 0.141 (100 ± 18)	4.73 ± 0.52 (593 ± 66)
Aconitase	1.51 ± 0.12 (225 ± 18)†††	0.670 ± 0.070 (100 ± 10)	1.43 ± 0.09 (214 ± 13)	1.09 ± 0.06** (220 ± 11)†††	0.498 ± 0.038* (100 ± 8)	1.09 ± 0.07 (218 ± 13)
Pyruvate carboxylase	0.319 ± 0.012 (3190 ± 120)†††	0.010 ± 0.0004 (100 ± 4)	0.346 ± 0.008 (3460 ± 80)	0.184 ± 0.010*** (1150 ± 60)†††	0.016 ± 0.001** (100 ± 6)	0.259 ± 0.017 (1620 ± 110)††
Flux through tricarboxylic acid cycle at 25°C (μmol/min per g wet wt.)	0.094 ± 0.006	0.051 ± 0.004		0.082 ± 0.003	0.042 ± 0.004	

species has not been reported. One particularly striking factor in the present study was the difference in relative activities of the short- and long-chain carnitine acyltransferases between epididymal tissue and spermatozoa, the ratio being of the order of 0.25 in epididymal tissue and 8 in spermatozoa.

Androgen-dependence of enzymes in the epididymis

The extent of androgenic control of enzymes was determined by comparing the specific activity of enzymes in the androgen-maintained epididymis (ligated efferent ducts) with the specific activity in the epididymis of androgen-deprived (castrated) animals. The androgen-dependence of enzymes was examined further by testing for the re-establishment of pre-castration activities by the administration of testosterone propionate to regressed castrated animals (androgen-regenerated). For greater ease of comparison between enzymes, the specific activities of enzymes in androgen-maintained and in androgen-regenerated animals have also been expressed as a percentage of that in androgen-deprived animals (Table 2). Although the time course of the alteration in activities was not determined for the enzymes in this study, a 3-week treatment period is sufficient to allow the establishment of a new steady-state concentration of the glycolytic enzymes (Brooks, 1976b).

In the caput epididymidis, manipulation of androgenic status caused an alteration in the specific activities of all enzymes except NAD⁺-malate dehydrogenase and NADP⁺-isocitrate dehydrogenase. NAD⁺-malate dehydrogenase was also not significantly influenced by androgen status in the cauda epididymidis, but in this segment ATP citrate lyase was also unaltered.

It is clear from the results in Table 2 that among those enzymes whose specific activities were altered by androgen status, the degree of alteration was variable. Those enzymes that showed the greatest change were glycerol 3-phosphate dehydrogenase and pyruvate carboxylase in the caput epididymidis, whereas in the cauda epididymidis carnitine palmitoyltransferase also showed extensive change. The relative activity of carnitine acetyltransferase under the two hormonal conditions was not as great as reported by Marquis & Fritz (1965). The difference is unlikely to be due to the assay conditions, because when expressed on the same basis (by using the dry-weight determinations of Brooks, 1977) the absolute activities in the androgen-deprived epididymis are similar in the two studies.

In most cases, the administration of testosterone propionate to regressed castrated animals resulted in restoration of enzyme specific activities to values equivalent to those in animals with ligated efferent ducts. There were four enzymes that were exceptions to this in the caput and five in the cauda epididymidis.

Table 3. *Activities of enzymes in epididymal spermatozoa of the rat*

Epididymal spermatozoa were prepared and extracted as described in the Materials and Methods section. The results represent the mean $\pm$ s.e.m. of four replicates. Conversion of units/10⁹ spermatozoa into units/g wet wt. is based on a spermatozoal volume of 80 fl (Brotherton, 1976) and assumes a sp.gr. of 1.1 [on the basis of values for epididymal bull spermatozoa from Lindahl & Thunqvist (1965) and Lavon *et al.* (1966)]. To determine the flux through the tricarboxylic acid cycle, the respiration rate of spermatozoa was measured at 25°C. It was then assumed, as in Table 2, that 1 μ mol of acetyl-CoA oxidized by the tricarboxylic acid cycle is equivalent to the uptake of 3 μ mol of O₂.

Enzyme	Enzyme activity	
	(units/10 ⁹ spermatozoa)	(units/g wet wt.)
Sorbitol dehydrogenase	0.134 $\pm$ 0.006	1.52 $\pm$ 0.07
3-Hydroxybutyrate dehydrogenase	0.014 $\pm$ 0.001	0.159 $\pm$ 0.011
Hydroxyacyl-CoA dehydrogenase	0.140 $\pm$ 0.018	1.59 $\pm$ 0.21
NAD ⁺ -malate dehydrogenase	8.16 $\pm$ 0.34	92.7 $\pm$ 3.9
NADP ⁺ -malate dehydrogenase	0.132 $\pm$ 0.004	1.50 $\pm$ 0.05
NAD ⁺ -isocitrate dehydrogenase	0.018 $\pm$ 0.001	0.205 $\pm$ 0.011
NADP ⁺ -isocitrate dehydrogenase	0.116 $\pm$ 0.010	1.32 $\pm$ 0.11
Glycerol 3-phosphate dehydrogenase (mitochondrial)	5.53 $\pm$ 0.63	62.8 $\pm$ 7.2
Pyruvate dehydrogenase	0.017 $\pm$ 0.001	0.193 $\pm$ 0.011
2-Oxoglutarate dehydrogenase	0.018 $\pm$ 0.003	0.205 $\pm$ 0.034
Succinate dehydrogenase	3.18 $\pm$ 0.28	36.1 $\pm$ 3.2
Cytochrome oxidase	2.87 $\pm$ 0.30	32.6 $\pm$ 3.4
Carnitine acetyltransferase	3.82 $\pm$ 0.20	43.4 $\pm$ 2.3
Carnitine palmitoyltransferase	0.472 $\pm$ 0.040	5.36 $\pm$ 0.46
Glutamate-oxaloacetate transaminase	2.08 $\pm$ 0.22	23.6 $\pm$ 2.5
Citrate synthase	3.20 $\pm$ 0.14	36.4 $\pm$ 1.6
ATP citrate lyase	Not detectable	
Fumarase	0.204 $\pm$ 0.013	2.32 $\pm$ 0.15
Aconitase	0.087 $\pm$ 0.012	0.989 $\pm$ 0.136
Pyruvate carboxylase	0.014 $\pm$ 0.002	0.159 $\pm$ 0.023
Flux through tricarboxylic acid cycle at 25°C (μ mol/min per 10 ⁹ spermatozoa or μ mol/min per g wet wt.)	0.017 $\pm$ 0.001	0.193 $\pm$ 0.011

In the latter tissue this difference was in two cases (2-oxoglutarate dehydrogenase and pyruvate carboxylase) due to the fact that testosterone treatment had restored activity to a value greater than that seen in animals with ligated efferent ducts.

Discussion

The flux through the tricarboxylic acid cycle can be estimated from the measured rate of O₂ uptake to be approx. 0.1 μ mol/min per g wet wt. at 25°C in the androgen-maintained epididymis (efferent-duct ligated rat) and about half this rate in the androgen-deprived epididymis (castrated rat) (Table 2). The activities of the cycle enzymes are sufficient to account for this flux. In particular, NAD⁺-isocitrate dehydrogenase is sufficiently active to account for this flux without requiring, in addition, NADP⁺-isocitrate dehydrogenase, as has been suggested in some vertebrate and invertebrate muscles (Alp *et al.*, 1976). Those enzymes involved with the cycle that are least active are pyruvate dehydrogenase, NAD⁺-

isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. However, if mitochondrial aconitase represents only 15% of the total tissue activity, as has been found in other tissues (e.g. Dickman & Speyer, 1954; Martin & Denton, 1970), then it would also fall into the group of low-activity enzymes. However, in other tissues such as liver it is considered that aconitase catalyses an equilibrium reaction (Gumaa *et al.*, 1971) and would therefore not fulfil a regulatory role.

As the procedure for the extraction of pyruvate dehydrogenase was designed to promote the activation and decrease the inactivation of this enzyme, the values recorded may not reflect the proportion of active enzyme in the tissue before extraction. The results do show, however, that sufficient pyruvate dehydrogenase is present to account for the entry of acetyl units from the glycolytic sequence into the tricarboxylic acid cycle. In the case of the androgen-maintained epididymis the concentration of pyruvate dehydrogenase is greater than the tricarboxylic acid cycle flux, indicating that additional acetyl units could be supplied for lipid synthesis (see Read *et al.*, 1977).

The enzyme whose activity correlates most closely with the measured flux through the tricarboxylic acid cycle in the epididymis is 2-oxoglutarate dehydrogenase. This result therefore supports the proposal made by Read *et al.* (1977) that 2-oxoglutarate dehydrogenase activity *in vitro* can be a useful index of the maximum rate of the tricarboxylic acid cycle *in vivo*.

In contrast with the androgen-deprived epididymis, the androgen-maintained epididymis derives a considerable proportion of its energy from the oxidation of lipids (Brooks, 1978). Two of the enzymes involved in fatty acid oxidation, carnitine palmitoyltransferase and hydroxyacyl-CoA dehydrogenase, are present in the epididymis with activities similar to the more active tricarboxylic acid-cycle enzymes (Table 2). It is probable that phosphatidylcholine may be a major source of fatty acids for oxidation in the epididymis with the consequent release of glycerophosphocholine, which accumulates in large quantities in the epididymis (Dawson *et al.*, 1957; Dawson & Rowlands, 1959; Scott *et al.*, 1963; Brooks *et al.*, 1974). In the epididymis of castrated animals, which does not appear to oxidize fatty acids, glycerophosphocholine does not accumulate. It is known that spermatozoa are unable to metabolize glycerophosphocholine (Dawson *et al.*, 1957), but its use by the epididymis has not been studied. However, the fact that glycerophosphocholine accumulates in the epididymis would militate against its use by this tissue. The presence of 3-hydroxybutyrate dehydrogenase in epididymal tissue and in spermatozoa suggests that ketone bodies could also act as a source of metabolic fuel for these tissues.

Reducing equivalents, in the form of NADH, are produced in the cytoplasm as a result of glycolysis. Under aerobic conditions these reducing equivalents can be re-oxidized within the mitochondrion, but as NADH is unable to cross the mitochondrial membrane, a shuttle system must operate to transfer the reducing equivalents across the membrane. In insect flight muscles this is achieved by the glycerol phosphate cycle (Estabrook & Sacktor, 1958), whereas in other tissues the transfer is thought to take place via the malate-aspartate shuttle involving NAD⁺-malate dehydrogenase and glutamate-oxaloacetate transaminase (Chappell, 1968). It is likely that the second rather than the first cycle operates in the epididymis and epididymal spermatozoa because NAD⁺-malate dehydrogenase and glutamate-oxaloacetate transaminase are both highly active, whereas the activity of mitochondrial glycerol 3-phosphate dehydrogenase is relatively low in the epididymis (Table 2), and cytoplasmic glycerol 3-phosphate dehydrogenase is relatively inactive in spermatozoa (Brooks, 1976a). Spermatozoa of the bull, like the rat, also have a highly active mitochondrial glycerol 3-phosphate dehydrogenase, but

lack the cytoplasmic enzyme to complete the cycle (Mohri *et al.*, 1965). Another possible mechanism for the transfer of reducing equivalents exists in spermatozoa. Owing to the unique intramitochondrial location of the spermatozoa-specific isoenzyme of lactate dehydrogenase (LDH-X) a pyruvate-lactate shuttle can operate (Storey & Kayne, 1977; Milkowski & Lardy, 1977), although it has not yet been resolved how the intra- and extra-mitochondrial compartments would be maintained at different redox states under this mechanism.

The synthesis of fatty acids requires that acetyl units be transported from the mitochondrion to the cytoplasm. Several mechanisms have been proposed for this transfer. In adipose tissue, the favoured mechanism involves citrate synthesis in the mitochondrion and subsequent cleavage in the cytoplasm. To complete the cycle, it is proposed that, through the activities of NAD⁺- and NADP⁺-malate dehydrogenases, oxaloacetate is converted into pyruvate, which re-enters the mitochondrion, where carboxylation results in the formation of oxaloacetate (Martin & Denton, 1970). In the caput epididymidis, the activities of the enzymes involved in this so-called pyruvate-malate cycle would appear sufficient for this cycle to operate (Table 2). In the cauda epididymidis, however, the activity of ATP citrate lyase is very low. It may be that lipid synthesis in this region of the epididymis is minimal or that transfer of acetyl groups occurs by way of acetylcarnitine mediated by carnitine acetyltransferase (Fritz & Yue, 1964). Glycerol phosphate, required for esterification, could be supplied by the activity of cytoplasmic glycerol 3-phosphate dehydrogenase (Brooks, 1976a).

The presence in spermatozoa of a relatively high concentration of carnitine acetyltransferase indicates that this enzyme may play a particularly important role in the metabolism of these cells. The acetylation state of carnitine in spermatozoa can be profoundly influenced by the presence of exogenous substrates (Casillas & Erickson, 1975; Milkowski *et al.*, 1976; Van Dop *et al.*, 1977) and one function of carnitine acetyltransferase may be to buffer the amounts of CoA and acetyl-CoA (Casillas & Erickson, 1975). Acetylcarnitine is present in rat epididymal plasma in substantial concentration (6mM, calculated from the data of Brooks *et al.*, 1974) and may act as an important energy substrate for spermatozoa within the epididymis. Acetylcarnitine is readily oxidized by spermatozoal mitochondria (Storey & Keyhani, 1974; Hutson *et al.*, 1977), although the rate of oxidation is depressed in the presence of carnitine, possibly because of competition with acetylcarnitine for mitochondrial transport (Calvin & Tubbs, 1976).

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