Effects of Postnatal Maturation and Castration on Rat Epididymal Carbohydrate Metabolism[1] (37083)

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An increase in fertilizing capacity of spermatozoa occurs during passage through the epididymis (1) suggesting that this organ provides a major source of substrates to support the maturation process. In this regard, an increase in carbohydrate metabolism relative to epididymal function has indicated that glucose is a major metabolic substrate and that a high pentose cycle activity exists in the organ (2–4). However, the activity of the pentose-shunt enzymes has not been studied during maturation.

Glycogen, which may serve as a source of energy, has been estimated in the epididymis histochemically (5) and found to decrease following castration (6) but glycogen content has not been examined. The present studies relate epididymal glycogen content and pentose-shunt dehydrogenase enzyme activities to maturation in the rat.

Materials and Methods. Long–Evans rats from the Bureau of Biological Research Colony were used. After weaning at 22 days of age, the animals were fed a semi-purified diet containing 20% casein and 25% fat (7).

The influence of endogenous testicular hormone levels on the epididymis was determined in intact rats at 22, 35, and 60 days of age. In addition, rats were castrated on day 22 and sacrificed at 35 and 60 days of age. All rats were killed by decapitation within 15 sec after removal from their cages. The epididymides were dissected free of adipose tissue, and the combined weight of both epididymides was recorded. In the analysis of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, the spectrophotometric procedure of Glock and McLean (8) as modified by Huggins and Fung (9) was used. Isocitrate dehydrogenase activity was estimated by the method of Ochoa (10). For all enzyme determinations the epididymis was homogenized in cold 0.154 *N* KCl to a final concentration of 2.5% (w/v), and an aliquot of the homogenate was analyzed for protein (11). After centrifugation at 1000*g* for 45 min, 0.1 ml aliquots of the supernatant were incubated in quartz cuvettes at 25° in the presence of appropriate substrates and phosphate buffer (pH 7.4). The production of NADPH per minute was used as an estimate of enzyme activity and was determined at a wave length of 340 nm in a Beckman-DU spectrophotometer.

Additional animals were used to assess changes in glycogen content as estimated by Seifter *et al.* (12). Results were statistically evaluated by analysis of variance and the Student's *t* test. A p value of 0.05 was considered as significant.

Results. Maturation from 22 to 35 days of age was accompanied by an increase in epididymal weight from 25.8 mg to 56.2 mg. Then to day 60, the organ increased to 297.3 mg. Activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate increased significantly ($p = 0.01$) between 22 and 35 days of age. No further increase was noted at 60 days despite the sharp increase in organ mass. In contrast, isocitric dehydrogenase activity declined between 22 and 60 days of age ($p = 0.05$). Castration prevented the maturational changes in weight and enzyme activity in the epididymis (Table I).

Glycogen concentration in the epididymis was 0.14% in rats at 22 days of age and declined significantly to 0.05% at 35 days of age (Table II). No changes were noted between 35 and 60 days in percent glycogen,

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TABLE I. Effect of Maturation and Castration on Pentose Shunt Dehydrogenase Enzyme Activity in the Rat Epididymis.

No. of rats	Age (days)	Body wt (g)	Organ wt (g)	Glucose-6-phosphate d. μmoles NADPH/min 100 mg	Glucose-6-phosphate d. μmoles NADPH/min mg protein	6-phosphogluconate d. μmoles NADPH/min 100 mg	6-phosphogluconate d. μmoles NADPH/min mg protein	Isocitrate d. μmoles NADPH/min 100 mg	Isocitrate d. μmoles NADPH/min mg protein
				Normal rats					
7	22	47	25.8 ±0.9[a]	0.214 ±0.02	0.016 ±0.001	0.201 ±0.01	0.015 ±0.001	0.64 ±0.01	0.048 ±0.002
9	35	90	56.2 ±7.3	0.451 ±0.03	0.039 ±0.004	0.281 ±0.02	0.024 ±0.002	0.56 ±0.05	0.048 ±0.004
9	60	192	297.3 ±26.4	0.430 ±0.02	0.039 ±0.003	0.313 ±0.02	0.028 ±0.003	0.42 ±0.03	0.038 ±0.004
				Castrated rats[b]					
8	35	98	20.6 ±1.0	0.194 ±0.01	0.016 ±0.001	0.176 ±0.008	0.015 ±0.001	0.56 ±0.02	0.048 ±0.002
9	60	196	19.8 ±0.7	0.174 ±0.01	0.016 ±0.001	0.158 ±0.006	0.014 ±0.001	0.58 ±0.02	0.053 ±0.003

[a] Values are means ± standard error of the mean.
[b] Castrated at 22 days of age.

but total organ glycogen increased with organ growth. Castration did not prevent the decrease in glycogen concentration associated with increasing age with a resultant decline in total glycogen.

Discussion. Several enzymes involved in carbohydrate metabolism have been examined in the rat epididymis (2). Glucose-6-phosphate dehydrogenase activity in this organ was six times greater than in the testis. A preferential use of glucose-1-phosphate over glucose-6-phosphate has been found in epididymal tissue *in vitro* from the rabbit, rat and mouse (3, 4) suggesting a prominent pentose shunt activity. Maturational changes in pentose-shunt enzyme have been observed in the present study. The increase in glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity occurred between 22 and 35 days of age, a time when androgen titers are known to be increasing (13). Indeed castration at 22 days of age prevented the increase in enzyme activity. The pentose shunt enzymes may be important in supplying NADPH for steroid and phospholipid synthesis in the epididymis (14–17) or in providing C-5 sugars for ATP, RNA and DNA.

TABLE II. Effects of Maturation and Castration on Glycogen of the Rat Epididymis.

No. of rats	Age (days)	Body wt (g)	Organ wt (mg)	Glycogen %	Glycogen Total (mg)
			Normal rats		
7	22	48	25.1 ± 0.5[a]	0.14 ± 0.01	3.5 ± 0.2
11	35	100	83.3 ± 4.2	0.05 ± 0.01	4.2 ± 0.2
13	60	200	247.3 ± 17.8	0.06 ± 0.01	14.8 ± 0.7
			Castrated rats[b]		
10	35	109	24.9 ± 0.5	0.08 ± 0.02	2.0 ± 0.2
6	60	190	30.7 ± 1.3	0.07 ± 0.02	2.1 ± 0.2

[a] Values are means ± standard error of the mean.
[b] Castrated at 22 days of age.

With maturation glycogen concentration in the epididymis declined. The glycogen may provide glucose-6-phosphate for the pentose shunt in the ovary (18), and a similar relationship may exist in the epididymis.

Isocitrate dehydrogenase activity was not androgen sensitive in rat prostate (19) but did exhibit a slow decline in the epididymis following castration.

Summary. An increase in epididymal weight and in pentose-shunt enzyme activity of glucose-6-phosphate and 6-phosphogluconate dehydrogenase occurred between 22 and 35 days of age in the rat. Both were prevented by castration. Further epididymal growth to day 60 in normal rats was associated with a decline in isocitrate dehydrogenase activity but no change occurred with the other enzymes. Glycogen concentrations in the epididymis declined in normal and castrated rats with increasing age.

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