In Vitro Synthesis of Cholesterol and Testosterone from Acetate by Rat Epididymis and Vas Deferens[1] (34546)

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Baillie *et al.* (1) first demonstrated steroid metabolizing enzymes histochemically in hamster epididymis. Subsequently, a number of workers (2–4) verified biochemically that steroid enzymes are present in epididymal tissue in a number of species by measuring *in vitro* conversion of steroids such as pregnenolone and progesterone to compounds further along in the steroid pathway. To our knowledge, Hamilton *et al.* (5) were the first to show, however, that the epididymis and vas deferens of the mouse can incorporate acetate into the steroid cholesterol *in vitro*. The present experiments were initiated to study more fully steroid biosynthesis by the mammalian epididymis and vas deferens and to correlate this function with the morphology of the organ. This report is to present experimental evidence that both cholesterol and testosterone are synthesized by rat epididymis and vas deferens.

Materials and Methods. Rats, weighing from 250 to 500 g, were killed by cervical dislocation. The epididymis and vas deferens from each side were dissected free of the testis and epididymal fat pad, put into cold (4°) bicarbonate buffered Krebs–Ringer's (KRB) and all vestiges of adipose tissue were removed with fine irridectomy scissors under a dissecting microscope. After division into head, body, and tail portions, the epididymis and the vas deferens were blotted, weighed, sliced with a razor blade, and then incubated at 37° for 2 hr in 5 ml of gassed (95% O_2, 5% CO_2) KRB containing 1 mg/ml of glucose and 1 μCi/ml of [1-^{14}C]-acetate (sp act: < 2 mCi/mm). Control flasks contained the complete incubation mixture without tissue. Reaction was stopped by pouring the incubation mixture into chloroform: methanol (2:1). Subsequent extraction of the total lipid fraction followed the procedure of Folch *et al.* (6).

Total lipid fractions were separated by thin layer chromatography after additions of tracers of [7-^{3}H]-cholesterol and [7-^{3}H]-testosterone of known purity, and of carriers of pure testosterone and cholesterol. The solvent systems used have been reported elsewhere (5).

After spraying the chromatograms with Rhodamine 6G, the UV fluorescent cholesterol spot and the UV absorbent testosterone spot were scraped, eluted in chloroform, and aliquots of the eluates were taken for counting on a Nuclear-Chicago model 722-723 liquid scintillation spectrometer. Subsequent purification of the cholesterol eluate followed the protocol given by Hamilton *et al.* (5), except that the cholesterol cleaved from the dibromide was not counted until it had been recrystallized from aquaeous acetone, methanol, and ethanol. Aliquots from each recyrstallization were counted. The remaining eluate from the testosterone spot was acetylated at room temperature (20°) overnight in a mixture of pyridine and acetic anhydride (2:1). The acetate derivative formed was subsequently extracted in a mixture of chloroform:methanol (2:1), 33% acetic acid, and *n*-heptane (4:3:5). The top phase was taken, dried under nitrogen (37°) and the extract

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TABLE I. Examples of ^{14}C:^{3}H Ratios Derived from Recrystallizations.

Solvent	Cholesterol				Testosterone acetate			
	Head	Body	Tail	Vas	Head	Body	Tail	Vas
Eluate of spot	0.75	0.92	0.43	0.10	0.10	0.02	0.010	0.01
Aqueous acetone	0.047	0.47	0.23	0.06	0.02	0.01	0.006	0.01
methanol	0.045	0.39	0.18	0.04	0.01	0.01	0.008	0.01
ethanol	—	0.36	0.17	0.04	0.01	0.01	0.010	—

was chromatographed on a thin layer of silica gel, using benzene–ethyl acetate (2:1) as solvents, against a standard of pure testosterone acetate. Three spots resulted which corresponded to 17α-hydroxyprogesterone and the acetates of testosterone and 20α-dihydroprogesterone, respectively, in order of increasing R_f values. The testosterone acetate spot was eluted in chloroform, the eluate was dried under nitrogen (37°), and (after addition of 50 mg of pure testosterone acetate) the product was recrystallized once from each of aqueous ethanol, methanol, and acetone. Aliquots from each recrystallization were taken for counting. Examples of ^{14}C:^{3}H ratios derived from the recrystallizations are given in Table I.

Results and Discussion. The results given in Table II show that a significant amount of labeled acetate is incorporated into both cholesterol and testosterone along the whole length of the rat epididymis and in the vas deferens, although there is some variation from segment to segment, especially with respect to cholesterol. No label was detectable in cholesterol or testosterone in the flasks incubated without tissue.

This demonstration that epididymal tissue can synthesize an androgenic steroid suggests that steroids present in epididymal seminal fluid (7) are derived, at least in part, from the epididymis itself. It is known (7) that testicular seminal fluid collected from the rete testis contains a significant amount of testosterone, and that ejaculated semen also contains some testosterone (7, 8), although in lower concentration than testicular semen. Mounib (9), Baker *et al.* (10), and Gassner and Hopwood (11) report that free testosterone depresses oxygen uptake by spermatozoa, although Scott *et al.* (12) vigorously deny this and present experimental evidence to the contrary. It is difficult to resolve this inconsistency in experimental results, although it is possibly related to differing experimental conditions and to the fact that seminal steroids are almost entirely conjugated and not free compounds (8, 13–16). It is clearly of importance to determine whether the epididymis contains the enzymes necessary for conjugating steroids to sulfur, glucuronic acid, or *n*-acetylglucosamine, since only then are the steroids soluble in the water medium of the semen. Preliminary evidence indicates that the epididymis is indeed capable of forming dehydroepiandrosterone sulfate from an unconjugated cholesterol precursor. Since the semen and sperm contain significant amounts of enzymes for breaking down steroid conjugates (19), it seems probable that steroids are metabolized by the sperm as they move down the excurrent duct. The contribution by the epididymis and vas deferens then would act to renew the steroid pool that would be depleted by metabolism by the sperm.

The significance of the variation in cholesterol synthesis between segments is not clear. The relative lack of variation in incorpora-

TABLE II. Incorporation of [1-^{14}C]-Acetate into Cholesterol and Testosterone in Rat Epididymis and Vas Deferens.[a]

Segment	Cholesterol	Testosterone
Head	9438 ± 2000[b]	1498 ± 292
Body	85,888 ± 10,750	1967 ± 151
Tail	41,559 ± 3250	1272 ± 315
Vas	18,124 ± 1000	2776 ± 133

[a] Two hr incubation at 37°C.

[b] All numbers represent mean ^{14}C dpm/g (blotted wet wt of tissue) ± SE of the mean from four experiments.

tion of label into testosterone suggests that not all of the cholesterol present is related to androgen synthesis. This is not unexpected in view of the fact that cholesterol is known to be a structural component of membranes. Some of the cholesterol synthesis detected is probably related to membrane turnover.

Synthesis of testosterone in the rat epididymis using acetate as a precursor is surprising in view of the reports by Brady (17), and Sandler and Hall (18) that slices of rat testis cannot utilize acetate as a testosterone precursor, even though the organ can incorporate significant amounts of label into cholesterol from acetate, and into testosterone from exogenous cholesterol. The reasons for this are obscure but it may be that in the testis newly made cholesterol is shunted to a storage pool before it is utilized by the interstitial cells for androgen production, whereas in the epididymis it is possible that no such pool exists and that newly synthesized cholesterol is immediately utilized by the cells. Indirect evidence indicating that there is little storage of cholesterol in the rat epididymis is the fact that no label is detectable in cholesterol esters, and only a few lightly osmiophilic lipid droplets are seen in electron micrographs of rat epididymal principal cells.

Summary. The epididymis and vas deferens from the rat are capable of synthesizing cholesterol and testosterone from acetate. The significance of androgen synthesis by the epididymis is discussed.

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