Estrogen-Induced 16-Hydroxysteroid Dehydrogenase Activity in Rat Kidney

Abstract. *The activity of an enzyme system which readily converts estriol to 16-ketoestradiol is present only in the kidneys of mature female rats and is absent in the kidneys of male rats and immature rats of either sex. The full enzyme activity occurs in kidneys of rats of either sex at any stage of maturity after administration of estradiol for 2 weeks. The time course of the appearance and disappearance of the enzyme, the absence of detectable inhibitors and activators, and evaluation of cofactor requirements suggest that this may be an example of enzyme induction by the hormone. Furthermore, the enzyme activity itself represents a metabolic pathway for estriol metabolism whose potential quantitative significance has not heretofore been recognized.*

Interest in the mechanisms of hormone- and substrate-induced formation of enzymes has led to extensive study of differences in enzyme activities between the sexes and of differences at various levels of maturity (*1*). Examples of hormone-induced enzyme systems have been reported, as well as several examples of enzyme action, influenced by the sex of the individual, which result in the metabolism of steroid hormones (*2*).

In the course of studies on estriol metabolism a striking example of an estrogen-induced dehydrogenase activity was found. Estriol had long been considered a metabolic dead end of estrogen catabolism, but in 1958 Levitz *et al.* demonstrated the in vivo conversion of estriol to 16-ketoestradiol in low yield (*3*). Subsequently, King reported an enzyme in rat kidney which converted estriol to 16-ketoestradiol in 20-percent yield (*4*). This report confirms King's observations but, in addition, demonstrates that this 16α-hydroxy steroid dehydrogenase activity is dependent on estrogen and is inhibited by administration of testosterone.

A crude homogenate prepared from kidneys of the female rat converted 200 μg of estriol to 16-ketoestradiol in 85-percent yield. The enzyme activity did not occur in kidneys of male rats or immature rats of either sex, but it occurred in males and immature animals after administration of estradiol. The enzyme activity could be decreased in mature females by castration and administration of testosterone, or by administration of testosterone alone, but not by castration alone. Administration of estradiol to intact, mature males was less effective in stimulating enzyme activity than administration of the hormone to castrated animals (Table 1).

The enzyme could be found in immature females after they were 45 days old, but its occurrence was blocked by administration of testosterone. It occurred in castrated immature females, but to a much lower degree than in intact animals. When castrated females were given estradiol, the enzyme occurred earlier and in larger amounts. It occurred in intact or castrated immature males given estradiol but not in immature males given testosterone (Table 2).

The amount of enzyme was measured in terms of its activity in cell-free systems under conditions where the possibility that activators, inhibitors, or cofactors were functioning could be reasonably evaluated. The enzyme was prepared from kidneys of Sprague-Dawley rats, of established sex and maturity, by homogenizing the excised tissue in 3 volumes of a buffer consisting of 0.05*M* potassium phosphate, 0.25*M* sucrose, and 0.04*M* niacinamide at *p*H 7. The homogenate was fractionated by differential centrifugation, and suitable samples were incubated in 50-ml erlenmeyer flasks, in air, at 37°C for 1 hour in the presence of nicotinamide adenine dinucleotide (NAD) and chromatographically pure estriol added in 0.1 ml of either ethanol or dioxane. The incubation mixture was directly extracted three times with 6 volumes of chloroform, the chloroform was evaporated, and the residue was dissolved in ethanol. The 16-ketoestradiol formed was measured in suitable aliquots by the "blue

Table 1. 16α-Hydroxy steroid dehydrogenase activity in kidneys of mature rats (in micrograms of 16-ketoestradiol formed from estriol). The incubation systems had the following components in a total volume of 5 ml: supernatant fraction of 800*g* kidney homogenate (2 ml); estriol (200 μg); NAD (10 μmole in 0.05*M* potassium phosphate buffer, at *p*H 7, containing 0.25*M* sucrose and 0.04*M* nicotinamide).

Dose of hormone* (μg)	Activity			
	Females		Males	
	Intact	Rats 80 days old, castrated 2 wk earlier	Intact	Rats 69 days old, castrated 2 wk earlier
0	179	186	4	4
		Estradiol		
30			0	8
70–100		178	44	66
140			51	173
		Testosterone propionate		
7×10^3				14
14×10^3	162	29		2
28×10^3	69			

* Amounts represent total cumulative dose administered subcutaneously in oil, daily.

Table 2. 16α-Hydroxy steroid dehydrogenase activity in kidneys of immature rats (in micrograms of 16-ketoestradiol formed from estriol). The incubation system is described in Table 1.

Dose of hormone* (μg)	Age of rats (days)	Activity			
		Females		Males	
		Intact	Castrated at 32 days	Intact	Castrated at 37 days
0	30–45	6	20	4	
0	45–60	179	69	6	24
		Estradiol			
70	30–45		80		
140	45–60		162	187	197
170	45–60			190	194
		Testosterone propionate			
7×10^3	30–45	13	14		
14×10^3	45–60	7	26		7

* Amounts represent total cumulative dose administered subcutaneously in oil, daily.

tetrazolium" method (*5*). The estriol substrate did not react during measurement by this method. The 16-ketoestradiol was identified by chromatographic comparison with standard material in toluene-methanol (75 percent) and chloroform-formamide systems, as well as by thin-layer chromatography in a system of ethyl acetate, *n*-hexane, and ethanol (80:15:5) (*6*). The metabolite, separated as a phenolic substance (reduced blue tetrazolium), gave a characteristic Kagi-Miescher reaction (*6*) and, upon reduction with borohydride, was converted to 16-epiestriol (*6*).

The enzyme activity occurred in the whole homogenate from kidneys of female rats and resulted in the conversion of 85 percent of 200 μg of estriol to 16-ketoestradiol in the presence of 10 μmole of NAD. From three to ten rats were used in each experiment. Upon further fractionation, the entire activity was found to reside in the supernatant fraction obtained by centrifugation at 105,000*g*. A system of, for example, lactic dehydrogenase and pyruvic acid was provided to oxidize any reduced NAD that might be formed, and thus to insure completion of the reaction when the supernatant fraction was used alone. Most of the studies (see Tables 1 and 2) were performed with the 800*g* supernatant fraction of the crude homogenate, for which no exogenous system for reoxidizing the reduced NAD was necessary.

When compensation had been made for dilution, mixing the extracts from kidneys of male and female rats gave activities which were simply additive—a finding which suggests that no inhibitors or activators were operative. The enzyme system metabolized 16-epiestriol only slightly, suggesting relative stereospecificity for the 16α-hydroxy configuration. Details of the specificity for other types of substrates are not available; however, the pattern for steroid dehydrogenases revealed so far has shown a high degree of substrate specificity. The enzyme reaction was barely demonstrable in liver extracts prepared and tested under conditions similar to those described for rat kidney.

The time course for the appearance of the enzyme activity suggests that this activity may well be another example of enzyme formation induced by steroid hormones, although until further data are available this cannot be proved conclusively. This enzyme provides a system for studying the mechanism of hormone action in metabolic adaptation. There is a clear-cut, almost absolute, sex difference, which can be affected by castration and hormone administration. Since slight activity occurs in castrated females, the enzyme may well be influenced by other factors, in addition to the hormones used in this study.

The conversion of estriol to 16-ketoestradiol may have a quantitative significance heretofore unrecognized. In addition, the data suggest that the extent of estriol metabolism may well be different in male and female rats (*7*).

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Biodegradation of Alkylbenzene Sulfonate in a Simulated Septic Tank and Drain Field

Abstract. *A straight-chain alkylbenzene sulfonate was 90 to 95 percent biologically degraded in a laboratory bench-scale septic tank and drain field. Under the same conditions, only about 35 percent of a highly branched alkylbenzene sulfonate was degraded.*

Straight-chain alkylbenzene sulfonates are more rapidly degraded than highly branched alkylbenzene sulfonates in sewage treatment and in surface waters (*1*). However, one of the principal causes for concern over the stability of detergents after disposal is the contamination of ground waters, and consequently wells, in suburban areas using septic tanks for waste disposal. The relative rates of biological degradation of various detergents under these less aerobic conditions are therefore of considerable interest.

A simulated septic tank and drain field system was used to study the biodegradation of two alkylbenzene sulfonates from (i) a mixture of secondary phenylalkanes with straight chains containing 10 to 13 carbons and (ii) a typical alkylate derived from propylene tetramer and pentamer. Each septic tank consisted of two cylindrical compartments, the first having a liquid capacity of 2 liters and the second 1 liter. Each drain field was a series of columns packed with soil to a level just below the inlet, then gravel past the inlet, topped off with more soil. These were connected so that any excess over the capacity of the first column would pass through the gravel into the second, and so forth. After the septic tank had been seeded with sludge from an operating full-scale septic tank, sterilized raw whole sewage containing 10 parts per million of alkylbenzene sulfonate, tagged with sulfur-35, was fed into the septic tank automatically for 4 minutes once each hour for 16 consecutive hours each day, with an average residence time of 5 days. Effluent samples from the septic tank and soil columns were acidified with HCl, extracted with ether, deposited on activated carbon, and counted by the technique of House and Fries (*2*).

The efficiency of the system for removing organics was followed by measuring chemical oxidation demand (*3*). During the period of the tests, oxidation demand removals were 60 to 65 percent in the septic tank and about 80 percent over-all. Weekly averages of the percentage of alkylbenzene sulfonate remaining in the septic tank effluent are plotted in Fig. 1 Removal in the septic tank alone averaged about 15 percent for the branched-chain sample and 30 to 40 percent for the straight-chain compound. Almost all of this branched-chain removal and about one-third of the straight-chain removal were by adsorption on new sludge formed throughout the run. Biodegradation accounted for the other two-thirds of the straight-chain compound removed. Figure 2 shows the analyses of the soil column effluents. The overall removal in the system was about 35 percent for the polypropylene alkylbenzene sulfonate and 90 to 95 percent for the straight-chain sulfonate.

A typical household effluent contains 10 to 20 parts of alkylbenzene sulfonate per million. A full-scale septic tank drain field system operating with the same efficiency as the laboratory system and receiving polypropylene benzene sulfonate might discharge an