Steroid *N*-Acetylglucosaminyltransferase: Elevation of Activity in Rabbit Kidney by Oestrogen Treatment

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There have been a few reports on the effects of steroids on the ability of rat tissues to synthesize glucuronides *in vitro* (see Dutton, 1966), but there is little other evidence for endocrine effects on UDP transferases, in particular on the control of their synthesis in tissues. The work reported here was designed to determine whether oestrogens or other steroids administered to rabbits would affect the activities of the enzyme in tissues of this animal (Jirku & Layne, 1965; Collins, Jirku & Layne, 1968) that transfers *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the 17 α -hydroxyl group of 17 α -oestradiol 3-glucuronide.

Experimental. Mature virgin female New Zealand rabbits weighing 1.2–1.8 kg. were injected subcutaneously with steroids in 1 ml. of Steroid Suspending Vehicle supplied by the National Institutes of Health, Bethesda, Md., U.S.A. Control animals received 1 ml. of the vehicle. All animals received a daily dose for 8 days and were killed on the ninth day. The UDP-*N*-acetylglucosaminyltransferase activity and the UDP-glucuronyltransferase activity of tissue homogenates were determined with [6,7-³H]17 α -oestradiol 3-glucuronide and [6,7-³H]17 α -oestradiol as substrates respectively (Collins *et al.* 1968). The amount of tissue homogenate used for each incubation was 0.05 ml., except in the determination of kidney glucuronyltransferase activity, when 0.5 ml. was used. The protein content of the homogenates was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). All results were calculated in terms of the number of μ moles of substrate conjugated/30 min./g. of protein in the tissue homogenate.

When individual rabbits were injected daily with 2 mg. of 17 α -oestradiol, 17 β -oestradiol or diethylstilboestrol/kg. body wt., an increased *N*-acetylglucosaminyltransferase activity in kidney tissue was observed. No effect was evident on the activity of this enzyme in liver tissue, nor was there any demonstrable effect on the glucuronyltransferase activity in either liver or kidney. Treatment with similar amounts of cortisol, testosterone or progesterone did not affect the activities of either

transferase in liver or kidney. A series of dosages of 17 β -oestradiol ranging from 20 μ g. to 5 mg./kg. body wt. were administered to groups of three animals each. In this experiment the steroid was injected in the form of the benzoate to increase its absorption from the injection site. The dose was, however, calculated in terms of steroid. The results showed that the effect on kidney *N*-acetylglucosaminyltransferase was not evident until a dose of 1.5 mg./kg. body wt. was used. The full effect was attained at 2.5 mg./kg., and was not enhanced by further increase of the dose. Even at the largest dose used, namely 5 mg. of steroid/kg. body wt., no effect of the treatment was evident on kidney glucuronyltransferase, or on either of the transferases in liver tissue. Female rabbits were spayed under Pentothal anaesthesia and allowed to recover for 10 days. After treatment with oestradiol benzoate, kidney *N*-acetylglucosaminyltransferase activity was enhanced over that of control rabbits from the same spayed group. As with intact rabbits no effect of the oestrogen on the activities of liver *N*-acetylglucosaminyltransferase or liver or kidney glucuronyltransferases was apparent.

To confirm the above preliminary findings, six female rabbits were each treated with 2.5 mg. of oestradiol benzoate/kg. body wt./day, and the transferase activities in liver and kidney tissue were compared with those in a control group. The results are shown as Expt. A in Table 1. The *N*-acetylglucosaminyltransferase activity in the kidney was increased by oestrogen treatment, and this effect was statistically significant. The activities of this enzyme in liver, and those of glucuronyltransferase in both liver and kidney, did not differ significantly in the treated animals from those in the controls.

To compare the effect of 17 α -oestradiol and of the non-steroidal oestrogen stilboestrol on kidney *N*-acetylglucosaminyltransferase with that of 17 β -oestradiol, groups of four animals each were treated with each of these compounds, and the transferase activities were compared with those of a control group (Expt. B in Table 1). The kidney *N*-acetylglucosaminyltransferase activity was enhanced to a similar extent by treatment with each of the three compounds. The effect was statistically significant in each case. The means of the uterine weights of

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Table 1. *Effect of treatment for 8 days with oestrogens on steroid glycosyltransferases of rabbit tissues*

Transferase activities are expressed as μ moles of substrate conjugated/30 min./g. of protein in tissue homogenate, and are given as means $\pm$ S.E.M. The assay method was that described by Collins *et al.* (1968).

Expt. A: six animals/group; steroid dose 2.5 mg./kg./day				
	$10^2 \times N$ -Acetylglucosaminyltransferase activity		$10^2 \times$ Glucuronyltransferase activity	
	Liver	Kidney	Liver	Kidney
Control	5.50 $\pm$ 0.51	2.46 $\pm$ 0.24	13.22 $\pm$ 2.98	5.13 $\pm$ 0.56
17 β -Oestradiol benzoate	6.27 $\pm$ 0.41	4.54 $\pm$ 0.75*	14.71 $\pm$ 2.25	6.83 $\pm$ 0.97
Expt. B: four animals/group; dose 1.5 mg./kg./day				
	$10^2 \times$ Kidney <i>N</i> -acetylglucosaminyltransferase activity		Mean uterine wt. (g.)	
Control	1.97 $\pm$ 0.11		2.77	
17 α -Oestradiol	3.36 $\pm$ 0.32*		3.88	
17 β -Oestradiol	3.02 $\pm$ 0.43*		4.04	
Diethylstilboestrol	2.96 $\pm$ 0.34*		3.68	

* Significantly different from controls ($P < 0.05$).

the test animals, which give a rough estimate of the oestrogenic effect of the treatment with each compound, are included in Table 1.

Discussion. The results establish that, after treatment of female rabbits with large doses of oestrogen, the steroid *N*-acetylglucosaminyltransferase activity of kidney homogenates is increased over that of control animals. Although significant effects of the treatment on liver *N*-acetylglucosaminyltransferase or on the phenolic steroid glucuronyltransferases of either liver or kidney were not observed, the results in Expt. A (Table 1) suggest that even larger doses of oestrogen might show significant effects on these enzymes. However, such doses would be massive, and there is no doubt that the kidney *N*-acetylglucosaminyltransferase is much more sensitive to oestrogen treatment than are the other transferases assayed. The results appear to be independent of the endogenous endocrine status of the animals, since they were the same in spayed females as in those with functional ovaries.

Both 17 α -oestradiol and 17 β -oestradiol give rise *in vivo* to 17 α -oestradiol 3-glucuronide, which is a specific substrate for the *N*-acetylglucosaminyltransferase of rabbit tissues (Collins *et al.* 1968). Stilboestrol, however, is excreted by the rabbit mainly as a monoglucuronide (Smith & Williams, 1948) and neither stilboestrol nor its glucuronide formed an *N*-acetylglucosaminide with rabbit liver microsomes *in vitro* (Collins *et al.* 1968). Since stilboestrol was as effective as either 17 α -oestradiol or 17 β -oestradiol in increasing the *N*-acetylglucosaminyltransferase activity in rabbit kidney (Table 1), the effect of all three of these compounds may be

due to their action as oestrogens, rather than as specific substrates for the enzyme. It cannot, however, be considered proved that stilboestrol is not a substrate *in vivo* for *N*-acetylglucosaminyltransferase in the rabbit.

It should be emphasized that the increased kidney *N*-acetylglucosaminyltransferase activity has been demonstrated in these experiments, not simply in the amount of substrate conjugated by homogenates of treated animals, but as the ratio of enzyme activity to total protein in the homogenate. The oestrogenic compounds therefore seem to have stimulated enzyme synthesis, although they may also have produced an activation of the enzyme. Further study of this point requires purification of the microsomal steroid *N*-acetylglucosaminyltransferase. Attempts in this laboratory to achieve solubilization of this enzyme and its separation from glucuronyltransferase have so far resulted in complete loss of activity.

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