EFFECT OF CONJUGATION TO GLUCOSE ON THE UPTAKE OF 17β-[6,7-^{3}H]ESTRADIOL BY RABBIT LIVER SUBCELLULAR FRACTIONS

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SUMMARY

Homogenates and nuclear fractions from rabbit liver were incubated with 17β-[6,7-^{3}H]estradiol and with the 3-glucoside and 3-glucuronide of this steroid. Localization of 17β-estradiol in the cell nucleus was enhanced when the glucoside rather than the free steroid was incubated with homogenates. It is suggested that conjugation to glucose may facilitate the transport of the steroid into the liver cell, or may protect it from metabolism. The effect was not observed in uterine tissue fractions.

The formation of the 3-glucosides of estrone, 17α-estradiol and 17β-estradiol can be effected by a glucosyltransferase present in rabbit liver microsomes (1, 2). These glucosides are not urinary metabolites, but are extremely good substrates for a β-D-glucosidase present in the cytosol of rabbit liver, kidney and small intestine (3, 4). In exploring the possibility that glucoside formation and breakdown might have some importance in intracellular transport of the steroid, we measured the uptake of radioactivity into cell nuclei of liver and uterus following in vitro incubation of tissue homogenates or of isolated nuclei with 17β-[6,7-^{3}H]estradiol-3-glucoside, and compared the results with those found when either of these tissue fractions were incubated with 17β-[6,7-^{3}H]estradiol or 17β-[6,7-^{3}H]estradiol-3-glucuronide.

EXPERIMENTAL PROCEDURE

17β-[6,7-^{3}H]estradiol (specific activity 40 Ci/mmol) was purchased from Searle Corporation (Toronto, Ont.) 17β-[6,7-^{3}H]estradiol-3-glucoside (specific activity 40 Ci/mmol) and 17β-[6,7-^{3}H]estradiol-3-glucuronide (specific activity 40 Ci/mmol) were prepared by methods described previously (1-4).

Virgin female New Zealand white rabbits were killed by cervical dislocation and the liver and uterus were removed and immersed in ice-cold 0.15 M potassium chloride. Tissue fractions were prepared essentially as described by Brecher et al. (5). Each tissue was homogenized in a Sorvall omnimixer with ten volumes of 10 mM Tris-HCl-5 mM $MgCl_2$-10 mM KCl, pH 8.0, (TMK buffer) and the homogenate was filtered through cheesecloth. Nuclear fractions were prepared by centrifuging homogenates at 1,500xg for 15 minutes, and mitochondrial-microsomal fractions by centrifuging the resultant supernatant at 105,000xg for 60 minutes. Each pellet was then resuspended in TMK buffer to the volume of the original homogenate.

Incubation mixtures contained the following: steroid or glucoside, 150,000 dpm; tissue homogenate or tissue fraction, 0.5ml; TMK buffer, 0.5ml. Samples were incubated with constant shaking at 25 ℃ for one or for three hours. Incubations were carried out in triplicate. Nuclear or microsomal mitochondrial pellets were precipitated or re-precipitated as described above, washed with TMK buffer, and re-centrifuged. They were then assayed for radioactivity by one of two methods. In the first method (Table 1) each pellet was extracted successively with ethanol (2ml) and

chloroform-methanol, 2:1 (2ml). The extracts were combined, evaporated to dryness under nitrogen and redissolved in scintillation fluid made up of toluene containing 4.0g of PPO and 100ml of Biosolve BBS-3(Beckman) per liter. In the second method (Table 2) the washed pellets were suspended in 0.1ml of water and solubilized by incubation at 37 ℃ with 1ml of NCS solubilizer (Amersham-Searle). Scintillation fluid was then added and the sample assayed for radioactivity in a Nuclear Chicago Unilux II counter operating at a tritium efficiency of 30%.

RESULTS AND DISCUSSION

The results of a representative experiment are shown in Table I. Similar results were obtained in each of four other experiments. They indicate that tritium is more readily incorporated into the nuclear fraction of rabbit liver homogenates from 17β-estradiol-3-glucoside than from either 17β-estradiol or 17β-estradiol-3-glucuronide. This is in contrast to the results of parallel incubations with rabbit uterus, in which tissue the tritium is most readily incorporated into the nuclear fraction from 17β-estradiol. Careful chromatographic examination of the material in the nuclear fraction indicated that, in all cases, both in liver and uterus, the tritiated compound incorporated was predominantly free 17β-estradiol. No evidence was found in any of the experiments for the presence in the nuclear fraction of other than a small proportion of unhydrolysed glycoside, either glucoside or glucuronide. The results in Table 2 confirm that when 17β-estradiol or its 3-glucoside are incubated with liver homogenates, the nuclear fraction obtained by subsequent fractionation of the tissue

TABLE I Uptake of 17β-estradiol and conjugates by the nuclear fraction of rabbit tissues. Figures are the average of three determinations.

Tissue	Substrate	dpm in nuclear fraction (x 10^{-2}) Incubation time at 25 °C	
		1 hr	3 hr
Liver	17β-Estradiol	18	26
Liver	17β-Estradiol 3-glucoside	77	240
Liver	17β-Estradiol 3-glucuronide	21	46
Uterus	17β-Estradiol	300	270
Uterus	17β-Estradiol 3-glucoside	21	23
Uterus	17β-Estradiol 3-glucuronide	8	7

shows a higher uptake of tritiated steroid originally present as the glucoside. The same effect was observed in the fraction containing mitochondria and microsomes. When, however, the tissue fractions were first separated from the homogenate and then incubated individually with the substrates, the steroid was taken up to a greater extent when present in the free form.

Two possible explanations of these results are:

a) That the passage through the cell wall of 17β-estradiol-3-glucoside in liver cells is more facile than that of free 17β-estradiol. The possibility exists of an active trans-

TABLE 2. Uptake of 17β-estradiol and 17β-estradiol 3- glucoside by rabbit liver subcellular fractions. Figures are the average of three determinations. Incubations were for 1 hr at 25° C.

Subcellular Fraction	dpm incorporated (x 10^{-2})	
	17β-Estradiol	17β-Estradiol 3-glucoside
A. Incubation of homogenate followed by subcellular fractionation:		
nuclei	95	150
mitochondria-microsomes	37	68
B. Incubation of subcellular fractions:		
nuclei	490	180
mitochondria-microsomes	400	230

port (6) of the glucoside into the cell in a manner analagous to that known to occur for some glycosides in kidney cortex (7). The steroid glycoside, once in the cell, would be rapidly hydrolysed to 17β-estradiol by the very active cytoplasmic β-glucosidase (3, 4). This hypothesis is tenable only if it is supposed that the mechanism is absent from cells in the homogenates of uterus.

b) That the free 17β-estradiol added to the liver homogenates is rapidly metabolized. This metabolism might be oxidative, or consist of conjugation to glucuronide by the glucuronyl transferase of the endoplasmic reticulum (8). The glucoside, on the other hand, would be protected from

metabolism, and after passage through the membrane, would be hydrolysed by β-glucosidase to yield free steroid for uptake by nuclear protein. The nuclear and mitochondria-microsomal fractions prepared by prior fractionation of homogenates would be devoid of UDP-glucuronic acid, the soluble cofactor required for glucuronide synthesis, although they would still contain demonstrable amounts of β-glucosidase (9). The results obtained with uterine tissue are in accord with the very low content in this tissue of glucuronyl transferase (10), and of β-glucosidase (3).

Further work will be needed to examine these possible interactions, but the results strongly indicate that conjugation of estrogens with glucose may have a regulatory effect on their metabolism and localization in liver tissue.

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