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Duodenal Glucuronide Synthesis

II. Identification of Estradiol Glucuronide as a Conjugation Product of Estradiol by the Rat Duodenal Mucosa. Quantitative Studies

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Estradiol was incubated in the presence of duodenal mucosal specimens. Free estradiol was extracted with benzene. Thereafter the product, extractable from the supernatant by ethylacetate, was hydrolyzed by β-glucuronidase. The product liberated was then extracted with benzene. Analyses of this product with the Kober reagent revealed that 53 % of the original estradiol appeared as a glucuronide formed during the incubation with duodenal mucosa.

As has been indicated in previous studies [1] estradiol, when incubated with surviving duodenal tissue slices, is conjugated to a compound identified by paper chromatography as estradiol glucuronide. In order to produce further evidence quantitative studies have been performed.

EXPERIMENTAL

The incubation procedures were exactly the same as described in the first part of these experiments. Also the extraction procedures were the same. In order to obtain conditions, comparable to the previous chromatographic procedures, incubates from 2 Warburg flasks were first combined.

First the free estradiol was extracted with benzene. After extraction of the conjugated estradiol-compound with ethylacetate this product was hydrolyzed with β-glucuronidase. The liberated estradiol was then extracted with benzene, the extract was evaporated to dryness and the residue was then transferred to absolute ethanol. From the mixture proteins were removed by centrifugation and the supernatant was transferred to Kober-tubes. The ethanol was then evaporated.

Quantitative estimation of estradiol. This was performed according to the method described by Brown[2], making use of the Kober reaction. To the evaporated residue were added 3 ml of the Kober reagent (2 g chinol/100 ml 60 % H_2SO_4, allowed to stand in the dark for one day before use). The tubes were heated on a boiling water bath for 20 min and brought to room temperature; 0.2 ml aq. dest. was added and the tubes were rewarmed for 10 min and cooled again. The mixture was then diluted for the photometric reading. The brown-yellow colour was read in a spectrophotometer at 518 mμ which was found to be the absorption maximum for pure estradiol and for the experimental samples.

Table 1. Quantitative determination of the amount of estradiol conjugated by duodenal mucosa and hydrolysable by β-glucuronidase. Original amount of free estradiol in the incubation mixture 490 μg.

Samples (1–4) during different runs (I–V)	Tissue dry weight mg	Estradiol conjugated μg	Estradiol conjugated per 10 mg tissue μg
I			
1	10.6	265	251
2	10.8	270	248
3	12.8	285	222
4	13.8	*	
II			
1	11.6	255	220
2	8.2	*	
3	7.0	285	305
4	8.3	275	330
III			
1	11.2	265	238
2	9.2	275	288
3	12.0	*	
4	7.4	262	353
IV			
1	13.5	263	195
2	14.6	*	
3	11.1	270	243
4	9.6	*	
V			
1	11.2	*	
2	9.2	*	
3	12.0	262	218
4	7.4	*	

* Specimens stained brown after the addition of the Kober reagent.

The extinction curve was obtained by preparing a stock solution from estradiol (5 mg/10 ml) and a series of dilutions (containing 15–55 μg estradiol/ml). The colour produced from these samples with the Kober reagent was rectilinear in the 25–50 μg range. According to this a 1:10 dilution was necessary for the later incubation samples. Estradiol was incubated in the Ringer solution but without the tissue slices. This mixture was then treated in the same way as the actual samples. After extraction and hydrolysis these samples should be free of estradiol and serve well as blanks for the Kober reagent readings. The slight colour developed by the blanks was subtracted in the final calculations.

RESULTS

Table 1 presents the results obtained in 5 parallel experiments. The results are expressed as μg of estradiol conjugated per 10 mg of duodenal tissue. The maximum was 353 μg and the minimum 195, mean 256 μg. The amount of estradiol of two Warburg incubates was 490 μg. It appears then that a maximum of 71 and a minimum of 40 % of estradiol was conjugated to glucuronide. Excluding the extreme values the conjugated estradiol amounts to 53 % of the total steroid material.

DISCUSSION

These studies support our previous observations that the duodenal mucosa possesses the ability to carry out active glucuronide synthesis in the presence of suitable material. The chromatographic studies showed no signs of free estradiol in the ethylacetate extract. The present quantitative analyses on the other hand gave high readings for estradiol after hydrolysis of the extracted material with an enzyme specific for the β-glucuronides.

The quantitative studies meet with some technical difficulties. The Kober reaction involves the use of H_2SO_4 which causes a darkening of the samples in the presence of some organic compounds. Therefore the presence of, *e. g.*, glucuronic acid in the conjugated samples does not allow analyses of the non-hydrolyzed material by this method. The results are, however, in good agreement with some of our other similar studies. As shown with phenolphthalein which can be detected by relatively simple chemical means the amount conjugated under similar experimental conditions amounts to 60 % [3].

These studies do not reveal in what form the remaining portion of the original material after the incubation is. Since the incubation mixture contains sulphate ions it is also possible that a certain amount of estradiol-sulphate is formed.

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