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METABOLISM OF ETHYNYLOESTRADIOL AND OESTRADIOL IN THE GUINEA-PIG

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SUMMARY

The metabolism and tissue localization of the synthetic oestrogen, ethynyloestradiol, was studied in female guinea-pigs. After oral or intraperitoneal administration of [4-^{14}C]-ethynyloestradiol, 26·7% of the dose was recovered in urine over a 7-day period and 52·4% in the faeces. The major compound present in both urine and faeces was ethynyloestradiol. Metabolites were present in urine as glucuronides and were unconjugated in faeces. After administration of [4-^{14}C]-oestradiol, most of the radioactivity was excreted in urine within 24 h.

From 2 to 4 h after administration of ethynyloestradiol, most of the radioactivity was found in the gastrointestinal tract with up to 9·2% in bile and liver. Only small amounts were found in the reproductive tract. Mean values for the amount of dose in other tissues were: total plasma volume, 0·7%; adipose tissue, 2·2% and muscle, 1·7%.

INTRODUCTION

The metabolism of naturally-occurring and synthetic oestrogens in a number of species has recently been reviewed [1, 2]. In view of the fact that the guinea-pig, like humans, appears to excrete metabolites of the naturally-occurring oestrogens mainly in the urine [3–6], that glucuronides appear to be the main urinary oestrogen conjugates [6] and that oestrogens appear to undergo an enterohepatic circulation [5], it was of interest to study the metabolism of the synthetic oestrogen, ethynyloestradiol, in the guinea-pig. Since no previous investigation of the metabolism of this oestrogen has been carried out in the guinea-pig, in addition to measuring urinary and faecal excretion, the tissue distribution of the steroid was also studied and the results compared with those obtained in humans [7, 8].

MATERIALS AND METHODS

Mature female albino guinea-pigs of the Dunkin–Hartley strain (body weight 420–640 g) were used. Each animal received approximately 0·25 μCi [4-^{14}C]-ethynyloestradiol (S.A. 40 μCi/mg) or 0·25 μCi [4-^{14}C]-oestradiol (S.A. 184 μCi/mg) either by intraperitoneal injection or orally by intubation. For administration the steroid was dissolved in 0·5 ml ethanol to which 1·5 ml 0·9% NaCl was added. Before administration, 0·1 ml of the solution was used to determine the total amount of radioactivity in the dose and after administration the syringe and needle were washed with ethanol to determine the amount of radioactivity not injected. Two of the animals (nos. 8 and 9) were pre-treated with ethynyloestradiol (2 μg twice weekly injected intramuscularly in oil) for 2 and 7 months respectively before administration of labelled ethynyloestradiol. The animals were allowed free access to food and water and they were kept in metabolic cages which allowed urine and faeces to be collected separately.

The methods used to determine radioactivity in urine, faeces, plasma and tissues were described previously [7, 8]. To estimate radioactivity in bile, as much bile as possible was removed from the gall-bladder and the volume made up to 10 ml with ethanol. Samples (1 ml) of the ethanolic solution were evaporated to dryness and the residue dissolved in 0·1 ml ethanol for measurement of radioactivity.

RESULTS

Radioactivity in urine and faeces after administration of [4-^{14}C]-*ethynyloestradiol*

Values for the daily excretion of radioactivity in urine and faeces of guinea-pigs after administration of [4-^{14}C]-ethynyloestradiol are shown in Table 1. Up to 43% of the dose could be recovered in urine over a 5 day period and approximately half of the total radioactivity recovered in urine was excreted within the first 24 h of administration of ethynyloestradiol. Small amounts of radioactivity were still detectable for up to 7 days after administration. There was no significant difference in excretion between those animals which received the dose orally and those which received it by injection. The biological half-life of the radioactivity associated with the administered ethynyloestradiol and its metabolites in urine was similar after intraperitoneal (20·9 h) and oral (22·2 h) administration. Much larger amounts of radioactivity were excreted in faeces than in urine. The mean value ($\pm$S.E.M.) for the percentage of the dose excreted in urine was 26·7 $\pm$ 3·5% and in faeces 52·4 $\pm$ 3·2%. The mean biological half-life for the excretion of radioactivity in faeces was about 23 h.

Table 1. Urinary (U) and faecal (F) excretion of radioactivity after administration [4-^{14}C]ethynyloestradiol.

Animal no.	1	2	12		14		7		10		11		13	
Route of administration	intraperitoneally								orally					
Days after administration	% of administered dose excreted													
	U	U	U	F	U	F	U	F	U	F	U	F	U	F
1	15.8	26.0	7.9	20.6	17.9	24.5	5.3	32.3	17.3	22.1	13.3	19.2	11.0	29.7
2	9.7	11.3	4.5	23.9	13.4	16.6	3.2	10.1	5.7	14.6	9.2	15.2	5.9	14.1
3	3.5	5.6	2.9	11.2	3.7	6.2	2.6	9.9	2.2	5.0	4.0	6.4	3.2	6.9
4	1.2	-	1.3	4.8	1.2	1.5	0.7	5.6	0	2.2	1.1	3.3	0.7	1.3
5-7	0.8	-	0	2.1	0	0.5	1.7	2.8	0	0.5	0	0.8	0	0.2
Total	31.0	42.9	16.6	62.6	36.2	49.3	13.5	60.7	25.2	44.4	27.6	44.9	20.8	52.2

Almost all the radioactivity excreted in urine and faeces could be extracted. The amounts extracted before and after application of various hydrolytic procedures are shown in Table 2. For most animals only small amounts of the metabolites were excreted in urine in the unconjugated state and the largest amounts of radioactivity were extractable after enzymic hydrolysis suggesting that the metabolites were present as glucuronide conjugates. Up to 17·8% of the urinary radioactivity could be extracted by using the pyridinium sulphate extraction procedure suggesting that some metabolites were excreted as sulphates. In contrast more than 67% (mean value 81%) of the radioactivity in faeces could be extracted without any hydrolysis suggesting that most of the faecal metabolites were excreted in the unconjugated state possibly due to hydrolysis by intestinal microorganisms or by the enzymatic content of the gastrointestinal tract. Only small amounts were present as glucuronides and sulphates.

Paper chromatography of extracts of enzyme-hydrolysed urine from four animals and extracts of faeces from three animals, using the formamide–chloroform solvent system, showed that ethynyloestradiol itself was the major metabolite present and accounted for 48–62% of the urinary radioactivity (6·5–23·4% of the dose) and 48–62% of the faecal radioactivity (18·3–27·4% of the dose).

Radioactivity in tissues after administration of [4-^{14}C]-*ethynyloestradiol*

The amounts of radioactivity found in tissues and body fluids 2 and 4 h after administration of [4-^{14}C]-ethynyloestradiol are shown in Table 3. Most of the administered dose (59–79%) was found in the gastrointestinal tract. The bile and liver of some animals also contained large amounts of radioactivity. Only small amounts of radioactivity were found in tissues of the reproductive tract, most of it being present in the uterus. This was particularly noticeable in animal no. 9 which had been pretreated for 7 months with ethynyloestradiol and in which the uterus was enlarged. Negligible amounts of radioactivity were found in tissues of animals no. 2 and 9 which were killed 7 days after administration of ethynyloestradiol.

The concentration of radioactivity in adipose tissue obtained from different parts of the animal showed considerable variation (see Table 4). The highest concentration was found in adipose tissue from the mesentery in some animals and from around the uterus in others.

The amount of the administered dose of ethynyloestradiol present in total plasma volume, total adipose tissue and skeletal muscle is shown in Table 5. These values have been calculated assuming that skeletal muscle [9] and plasma [10] account for 42·8 and 3·9%

Table 2. Extraction of radioactivity from urine (U) and faeces (F) of guinea-pigs receiving [4-^{14}C]ethynyloestradiol.

Animal no.	2	12		14		7		10		11		13	
Route of administration	intraperitoneally							orally					
Hydrolysis procedure	% of radioactivity extracted												
	U	U	F	U	F	U	F	U	F	U	F	U	F
None	4.0	21.1	67.4	1.7	69.9	28.9	89.3	4.0	85.1	16.3	89.8	0	84.8
Enzyme	87.8	81.3	6.5	69.3	7.8	44.4	14.7	78.2	9.0	61.6	5.0	79.3	8.7
Pyridinium sulphate	9.6	10.8	10.2	6.1	11.3	17.8	8.1	10.3	5.2	9.4	6.2	5.8	12.3
Acid	82.3	72.9	18.1	71.3	30.7	51.9	6.9	65.4	9.2	58.3	8.1	76.4	17.1
Total (none + enzyme)	91.8	102.4	73.9	71.0	77.7	73.3	104.0	82.2	94.1	77.9	94.8	79.3	93.5

Table 3. Radioactivity in tissues and body fluids of guinea-pigs receiving [4-^{14}C]ethynyloestradiol.

Animal no.	3	4	8*	9*	5	6
Time of killing (h after injection)	2	2	2	2	4	4
Tissue or fluid	% of administered dose in tissue or fluid					
Stomach	1.1	5.0	15.3	14.2	16.7	17.8
Small intestine	35.2	43.8	39.4	4.0	14.4	14.2
Large intestine	30.5	14.9	24.2	40.8	27.8	44.2
Uterus	0.026	0.019	0.014	0.086	0.024	0.045
Vagina	0.016	0.012	0.003	0.021	0.014	0.014
Ovary	0.01	0.003	0.017	0.004	0.012	0.004
Brain	0.06	0.014	0.004	0.023	0.014	0.009
Adrenals	0.009	0.005	0.003	0.017	0.004	0.005
Spleen	0.01	0.029	0.005	0.054	0.003	-
Lungs	0.09	0.017	0.008	-	0.007	0.025
Liver	1.0	3.5	0.76	0.42	1.2	1.5
Heart	0.02	0.014	0.007	0.016	0.005	0.01
Kidney	0.14	0.13	0.13	0.20	0.14	0.43
Bladder	0.02	0.012	0.008	0.015	0.026	0.026
Bile	0.15	5.7	0.96	0.61	2.8	7.6
Urine	0.51	0.42	0.53	1.3	1.3	0.12

* Animals 8 and 9 were pretreated for 2 and 7 months respectively with 2 μg ethynyloestradiol twice weekly. All animals received [4-^{14}C]ethynyloestradiol orally except no.3.

of body weight respectively. No values are available for the total amount of adipose tissue present in the guinea-pig. However for comparison with the studies in humans [8], a value of 18·8% of body weight has been used and the values in Table 5 were calculated from estimations performed on abdominal fat.

The proportion of radioactivity in the various tissues present in a freely extractable or conjugated form is shown in Table 6. Most of the radioactivity in stomach, large intestine, muscle and adipose tissue was in a freely extractable state whereas in the small intestine, bile and plasma, most of the metabolites were in a conjugated form. In plasma there was a decrease in freely extractable radioactivity and an increase in conjugated radioactivity between 2 and 4 h after administration of the dose.

Radioactivity in urine and faeces after administration of [4-^{14}C]-*oestradiol*

Values for the daily excretion of radioactivity in urine and faeces after administration of [4-^{14}C]-oestradiol are shown in Table 7. In contrast with the

Table 4. Radioactivity in adipose tissue from different parts of the guinea-pig.

Animal no.	3	4	8	9	5	6
Time of killing (h after injection)	2	2	2	2	4	4
Source of adipose tissue	% of administered dose per g tissue					
Abdominal	-	-	0.08	0.38	0.21	0.19
Mesenteric	0.86	0.48	0.39	0.10	0.20	-
Uterine	-	-	0.08	0.73	-	0.48
Perianal	-	-	0.08	0.56	0.12	0.20
Renal	0.43	0.28	0.21	0.53	0.13	0.29
Ovarian	-	-	0.25	0.59	-	-
Subcutaneous	0.27	0.14	0.04	0.42	-	-

Table 5. Radioactivity in total plasma volume, total adipose tissue and total muscle tissue after administration of [4-^{14}C] ethynyloestradiol to guinea-pigs.

Animal no.	3	4	8	9	5	6
Tissue or fluid	% of administered dose in total tissue or fluid					
Plasma	1.5	0.2	0.3	0.9	0.4	0.7
Adipose tissue	-	-	0.8	4.6	1.7	1.6
Muscle	1.6	3.7	1.1	1.6	0.7	1.4

results obtained with ethynyloestradiol most of the dose (>68%) was excreted in the urine with only small amounts (<14%) in the faeces and excretion was usually completed within 24 h.

DISCUSSION

No previous investigation of the metabolism or tissue distribution of ethynyloestradiol in the guinea-pig has been reported.

As with the naturally-occurring oestrogens there appear to be similarities between the metabolism of ethynyloestradiol in the guinea-pig and in humans. Up to 43% of the dose may be excreted in urine, the main urinary conjugate appears to be the glucuronide, the biological half-life of the radioactivity associated with the administered steroid was 21·6 h in the guinea-pig and 27 h in humans [7], and unchanged ethynyloestradiol was the major steroid present in urine. Faecal excretion appears to be more important in the guinea-pig than in humans. It seems likely that the large amounts of the dose excreted in faeces result from biliary excretion and not from lack of absorption of the dose. Sandberg *et al.*[5] recovered 58% of a dose of oestrone and oestriol within 4 h in the bile of animals with a bile fistula suggesting that an entero-hepatic circulation of oestrogens occurred. In the present investigation up to 7·6% of the dose was found in bile of animals killed at 2–4 h after administration of ethynyloestradiol. Even up to 4 h after administration most of the dose was present in the gastrointestinal tract.

As in humans only small amounts of the dose (<0·1%) were found in the reproductive tract. The concentration of radioactivity in guinea-pig uterus (1·9% of dose/100 g tissue) was higher than that of human myometrial tissue (0·32% of dose/100 g tissue). There was considerable variation in the concentration of radioactivity in the various fat depots of the body; a finding which makes difficult the calculation of the proportion of the dose localising in adipose tissue. Steinetz *et al.*[11] found variations in the amount of ethynyloestradiol localising various fat compartments of the rat. Based upon the amount of radioactivity in abdominal adipose tissue in humans 3 h after administration of [^{14}C]-ethynyloestradiol it was calculated that 12·5–38·6% of the dose was present in total adipose tissue [8]. A similar calculation for the guinea-pig gives a value of less than 5% suggesting that adipose tissue in this species is even less important than it is in humans as a store for the steroid. Meli *et al.*[12] did not find ethynyloestradiol to be stored in body fat in rats. The amount of the dose present in the total blood volume was also much lower in guinea-pigs than in humans.

In contrast with our results showing that faecal excretion was more important than urinary excretion for the metabolites of ethynyloestradiol, other investigations [3–6] suggested that metabolites of the naturally-occurring oestrogens were excreted predo-

Table 6. Radioactivity in tissues in a freely extractable or conjugated form after administration of [4-^{14}C] ethynyloestradiol.

Animal no.	4		8		9		5		6	
Time of killing (h after injection)	2		2		2		4		4	
Tissue or fluid	% of tissue radioactivity in a freely extractable (F) or conjugated (C) form									
	F	C	F	C	F	C	F	C	F	C
Stomach	91.7	8.3	78.5	21.5	44.6	55.4	92.7	7.3	95.0	5.0
Small intestine	20.9	79.1	26.2	73.8	18.2	81.8	50.1	49.9	42.2	57.8
Large intestine	82.5	18.5	92.2	7.8	85.6	14.4	87.3	12.7	84.3	15.7
Bile	21.4	78.6	9.1	90.9	4.5	95.5	7.1	92.9	4.9	95.1
Plasma			16.7	83.3	25.0	75.0	5.0	95.0	3.0	95.0
Leg muscle			84.7	15.3	100.0	0.0	33.3	66.7	100.0	0.0
Abdominal fat			92.1	7.9	97.0	3.0	100.0	0.0	75.0	25.0
Liver			78.1	21.9	29.4	70.6	28.6	71.4	58.7	41.3

Table 7. Excretion of radioactivity in urine (U) and faeces (F) after administration of [4-^{14}C]oestradiol.

Animal no.	13		14		13		14	
Route of administration	intraperitoneally				orally			
Days after administration	U	F	U	F	U	F	U	F
1	75.6	10.7	87.0	7.4	80.5	5.1	68.8	13.6
2	0	0	4.0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Total	75.6	10.7	91.0	7.4	80.5	5.1	68.8	13.6

minantly in the urine. In order to exclude the possibility that this difference was accounted for by differences in the strain of guinea-pig used, two of the animals which had received ethynyloestradiol in the present study were also administered [^{14}C]-oestradiol. Whether the oestradiol was administered orally or intraperitoneally, metabolites of this steroid were excreted predominantly in the urine with only small amounts in the faeces. Similarly in rabbits oestradiol is excreted predominantly in the urine whereas ethynyloestradiol is mainly eliminated in the faeces [2]. Moreover in the guinea-pig excretion of metabolites was much quicker after giving oestradiol than after giving ethynyloestradiol. Almost all (82·4–92·4% of the dose) of the metabolites of oestradiol were excreted within 24 h compared to the much slower excretion of metabolites of ethynyloestradiol. Støa and Borjesson[6] suggested that the rapid metabolism and excretion of metabolites of oestradiol in the guinea-pig were due to rapid conjugation with glucuronic acid with consequent rapid excretion of the conjugate. Glucuronides of oestrone and oestradiol are readily formed by perfusion of the steroid through guinea-pig liver [13]. The slower excretion of ethynyloestradiol may be due to a slower rate of conjugation of the metabolites with glucuronic acid. Whether the slower rate of excretion of metabolites of ethynyloestradiol is also associated with the formation of sulphate conjugates, which are readily produced in humans, remains to be determined.

These preliminary results suggest a certain amount of correlation between the metabolism of ethynyloestradiol in the guinea-pig and the human but more detailed investigations are necessary to see how far the similarity extends.

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