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THE METABOLIC FATE OF ORALLY ADMINISTERED ^{3}H-NORETHYNODREL AND ^{3}H-NORETHINDRONE IN HUMANS*

D. S. LAYNE,† T. GOLAB, K. ARAI‡ and G. PINCUS

The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., U.S.A.

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Abstract—The excretion of radioactivity in the urine of four women, after oral administration of tritiated 17α-ethynyl-estr-5(10)-ene-3-one-17β-ol (norethynodrel) averaged 38 per cent of the administered dose. Two women receiving oral doses of tritiated 17α-ethynyl-19-nortestosterone (norethindrone) excreted, respectively, 70 and 50 per cent of the dose in their urine, 30 per cent of the dose of ^{3}H-norethynodrel was found in bile collected from one subject. Thirty to sixty per cent of the radioactivity in the urine of subjects receiving either ^{3}H-norethynodrel or ^{3}H-norethindrone was conjugated as glucosiduronate and another 9 to 25 per cent was another conjugate, presumably sulfate. Some evidence was obtained on the nature of the conversion products of norethynodrel in the glucosiduronate fraction of the urine of two women. These were mainly steroid alcohols, all but one of which carried the ethynyl side chain. Only traces of ketosteroids were present, and no evidence was found of major conversion of norethynodrel to phenolic compounds.

STUDIES of the urine of human subjects after the administration of 17α-ethynyl-19-nortestosterone (norethindrone)[1] and its esters[2] have accounted for only a small fraction of the administered dose. The results indicate that 17α-ethynyl-19-norsteroids might be excreted to a large extent by a route other than the urine. Arai *et al.*,[3] however, found that after the oral administration of tritiated 17α-ethynyl-estr-5(10)-ene-3-one-17β-ol (norethynodrel) to rabbits about 50 per cent of the radioactivity was excreted in the urine, although analysis of the bile of cannulated rabbits indicated that there was considerable enterohepatic circulation of the ingested material. The present paper details the results of a study of the excretion of radioactivity in five women receiving tritiated norethynodrel and in two women receiving tritiated norethindrone. A study of the percentage of the excreted radioactivity that was extracted from urine and from bile after hydrolytic procedures is also described, together with an investigation and partial identification of the conversion products of ^{3}H-norethynodrel present as glucosiduronates in the urine of two premenopausal women.

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METHODS

Randomly tritiated norethynodrel (72 μc/mg) and norethindrone (41 μc/mg) were prepared by the Wilzbach[4] method and purified by the procedures of Arai *et al.*[3] One patient receiving norethynodrel was a 67-year-old woman (M.P.) who had undergone cholecystectomy 4 days before the start of the experiment. The other subjects were premenopausal female inmates of an open ward at the Worcester State Hospital who were hospitalized for mental conditions. The steroids were administered orally in gelatin capsules, except in the case of Patient R.B. who received norethindrone by intravenous infusion in saline. The steroids were administered at 9 a.m. and urine collection was begun immediately thereafter. The dosages are shown in Table 1. In all the subjects except S.P. and A.P. the dose of steroid was administered on one day only. In these two subjects a dosage of 100 mg/day of nonradioactive norethynodrel was continued throughout the 7-day collection period.

TABLE 1. DOSAGE AND ROUTE OF ADMINISTRATION OF TRITIATED NORETHYNODREL AND NORETHINDRONE TO WOMEN

Patient	Age	Steroid	Route of administration	Dose (mg/day)	Dose (μc)
S.P.	29	Norethynodrel	Oral	100	10
U.S.	37		Oral	10	5
J.D.	36		Oral	10	10
A.P.	31		Oral	100	10
M.P.	67		Oral	5	2
I.G.	41	Norethindrone	Oral	10	10
R.B.	41		Intravenous	0·7	10

Radioactivity in the excreta was assayed in a Packard liquid scintillation spectrometer and was corrected for quenching as previously described.[3, 5] An extraction with chloroform was carried out on the first 24-hr urine samples from all the subjects, on all the subsequent urine samples from Subjects S.P. and A.P., and on the first 24-hr bile sample from Subject M.P.; and the extraction was repeated after incubating the urine or bile for two separate 24-hr periods with 500 units/ml of β-glucuronidase (Ketodase, Warner–Chilcott) as described previously.[3] The aqueous residue from these extractions was saturated with sodium chloride, adjusted to pH 1, and an extraction with ethyl acetate was carried out.[6] The radioactivity present in each extract was assayed.[5] The glucosiduronate extracts from Subjects S.P. and A.P. were pooled and washed with 0·1 of their volume of 5% sodium bicarbonate and three times with 0·1 of their volume of water. The extract was dried and was then separated into phenolic and nonphenolic fractions by partition between N NaOH and ether as described by Brown.[7] The nonphenolic fraction, which contained more than 95 per cent of the total radioactivity, was chromatographed on a 2-cm-diameter column containing 70 g Celite mixed with 35 ml 70% (v/v) aqueous methanol. Elution was carried out successively with hexane, increasing concentrations of benzene in hexane, benzene, ethyl acetate, and methanol.

RESULTS

Table 2 shows the percentage of the administered radioactivity recovered in each 24-hr urine sample for each subject. The percentages in the feces of Subjects A.P. and

M.P. and in the bile of M.P. are also listed. The recovery in the urine of the four subjects without bile cannulae who received ^{3}H-norethynodrel ranged from 32 to 40 per cent (mean 38 per cent) of the administered dose. In Subject M.P. only 28 per cent of the radioactivity appeared in the urine. In this subject 30 per cent was accounted for in the

TABLE 2. PERCENTAGE OF THE ADMINISTERED DOSE OF ^{3}H-NORETHYNODREL OR ^{3}H-NORETHINDRONE PRESENT IN 24-HR SAMPLES OF EXCRETA

	Norethynodrel								Norethindrone	
Subject	S.P.	U.S.	J.D.	A.P.		M.P.			I.G.	R.B.
Day	Urine	Urine	Urine	Urine	Feces	Urine	Bile	Feces	Urine	Urine
1	19	14·2	20·9	12·4	9·6	21·7	22·4	4·6	25·0	40·2
2	13·5	10·4	5·9	8·6	23·9	4·1	4·3	*	24·4	19·1
3	4·0	4·6	3·4	4·5	1·2	2·0	3·3	14·6	0·4	7·4
4	3·2	3·3	2·8	2·1	2·4	*	*	6·4	0·6	3·5
5	0·0	0·0	3·3	2·1	*	*	*	1·5	0·0	0·0
6	*	*	1·6	1·3	*				*	*
7	*	*	2·1	1·6	*				*	*
Total	39·7	32·5	40·0	32·6	37·1	27·8	30·0	27·1	50·4	70·2

* No measurable radioactivity.

TABLE 3. RADIOACTIVITY EXTRACTED AFTER HYDROLYTIC PROCEDURES FROM THE EXCRETA OF WOMEN RECEIVING ^{3}H-NORETHYNODREL AND ^{3}H-NORETHINDRONE

The figures are percentages of the total radioactivity present in the first 24-hr collection.

Procedure	Subjects* S.P. Urine	U.S. Urine	J.D. Urine	A.P. Urine	M.P. Bile	M.P. Urine	I.G. Urine	R.B. Urine
Chloroform extraction of unconjugated steroid	1·3	1·5	4·9	1·1	3·2	2·6	2·6	0·8
Extraction with chloroform after hydrolysis for 48 hr with β-glucuronidase	40·2	31·1	60·3	43·3	74·1	39·3	54·5	36·5
Extraction with ethyl acetate after saturation with NaCl at pH 1	20·1	9·3	19·2	25·3	5·3	22·1	19·3	26·5
Extraction with chloroform after boiling 1 hr with HCl	4·8	10·3	1·2	6·8	3·2	4·1	7·3	2·0
Total	66·4	52·2	85·6	76·5	85·8	68·1	83·7	65·8

* Subjects S.P., U.S., J.D., A.P., and M.P. received ^{3}H-norethynodrel; Subjects I.G., and R.B. received ^{3}H-norethindrone.

bile and 27 per cent in the feces. The two subjects receiving ^{3}H-norethindrone excreted, respectively, 70 per cent and 50 per cent of the dose in their urine.

The percentages of the excreted radioactivity recovered by extraction of the first 24-hr samples of urine and bile after hydrolytic procedures are shown in Table 3.

The results of the partial identification of the conversion products of ^{3}H-norethynodrel in the glucosiduronate fraction of the urine of Subjects S.P. and A.P. are shown in Table 4. Very small amounts of radioactivity were eluted from the Celite column by hexane or mixtures of hexane and benzene. Examination of these fractions by chromatography in ethyl acetate–cyclohexane on silica gel plates, followed by spraying with antimony trichloride in chloroform,[8] suggested the presence of small amounts of the following three steroids: 17α-ethynyl-19-nortestosterone; 17α-ethynyl-estr-5(10)-ene-3ϵ, 17β-diol; and 17α-ethynyl-10β-hydroxy-19-nortestosterone. These compounds are numbered I, II, and III in Table 4. Four peaks of radioactivity were eluted with benzene. These fractions were designated A, B, C, and D in order of increasing polarity.

TABLE 4. STRUCTURES TENTATIVELY ASSIGNED TO THE CONVERSION PRODUCTS OF ^{3}H-17α-ETHYNYL-ESTR-5(10)-ENE-3-ONE-17β-OL (NORETHYNODREL) IN THE GLUCOSIDURONATE FRACTION OF THE URINE OF SUBJECTS S.P. AND A.P.

Compound	Structure
I	17α-ethynyl-19-nortestosterone
II	17α-ethynyl-estr-5(10)-ene-3ϵ,17β-diol
III	17α-ethynyl-10β-hydroxy-19-nortestosterone
IV	No structure assigned
V	17α-ethynyl-19-nor-5α-androstane-3α,10β,17β-triol
VI	17α-ethynyl-19-nor-5α-androstane-3β,10β,17β-triol
VII	17α-ethynyl-19-nor-5β-androstane-3α,10β,17β-triol
VIII	17α-ethynyl-19-norandrost-4-ene-3β,10β,17β-triol

Fraction A consisted of 6·4 mg of crude crystalline material. Recrystallization from acetone–hexane gave 3·4 mg, m.p. 185 to 195°, $\nu_{max.}^{KBr}$ 1089 (*sec.* alcohol), 1475 (CH_2), 2930, 2961 (C—H), and 3453 (OH) cm^{-1}. This material was designated compound IV.

Fraction B weighed 8·6 mg. Recrystallization from methanol–ether gave 3·5 mg, m.p. 190 to 210°, $\nu_{max.}^{KBr}$ 1019, 1068, 1294 (*sec.* alsohol), 1382 (*tert.* alcohol), 2891, 2966 (C—H), 3340 (C≡CH), and 3400 (OH) cm^{-1}. This material was designated compound V.

Fraction C consisted of a very small amount of material (Compound VI).

Fraction D weighed 10·8 mg. Four recrystallizations from acetone–ether gave 2·2 mg, m.p.p. 203 to 207°, $\nu_{max.}^{KBr}$ 1018, 1040, 1062, 1299 (*sec.* alcohol), 1380 (*tert.* alcohol), 1446, 2886, 2956, 3300 (C≡CH), 3399, 3455 (OH) cm^{-1}. This material was designated compound VII. The mother liquors from the recrystallization of this substance yielded a small amount of another material (compound VIII).

The structures tentatively assigned in Table 4 are supported by the following evidence.

(1) The identification of compounds I, II, and III in Table 4 is based only on chromatographic evidence and on a comparison of the colors obtained by spraying the chromatograms with $SbCl_3$ in chloroform with those given by known reference samples. This technique has been shown to provide a useful preliminary identification of steroids by Neher and Wettstein[9] and has been applied to the study of 19-nor-steroids in this laboratory.[8]

(2) No structure was assigned to compound IV. The Hg complex test[1] was negative on this material and suggests the absence of the ethynyl group. This conclusion is supported by the infrared spectrum, which also indicates the absence of ketonic groups. The compound can be readily oxidized to a ketone with MnO_2 in neutral medium as described for the oxidation of an allylic alcohol by Sondheimer *et al.*[10] This oxidized product is similar chromatographically to 19-nortestosterone but gives a different color on staining with $SbCl_3$ and does not have the ultraviolet spectrum characteristic of an α,β-unsaturated ketone.

(3) The presence of the 17-ethynyl group is indicated in compounds V, VI, VII, and VIII by the results of the Hg complex test.[1] In the case of compounds V and VII, the infrared spectra were obtained and also indicated the presence of the ethynyl group.

(4) Compounds V, VI, VII, and VIII gave a bright green color when sprayed with $SbCl_3$ in chloroform after chromatography on silica gel. This color changed to blue upon standing for 5 min. In a study of over sixty 19-norsteroids in this laboratory[8, 11] this reaction with antimony trichloride has been found only in steroids with a 3,10-dihydroxy grouping. The infrared spectra of compounds V and VI clearly indicate the presence of a tertiary alcohol group, which is in accord with the assignation of a hydroxyl at position 10.

(5) The reduction of a test sample of 17α-ethynyl-10β-hydroxy-19-nortestosterone with sodium borohydride as described by Sondheimer *et al.*[10] gave two materials resembling in polarity compounds VI and VIII. The products of such a reduction would be expected[10, 12] to have the structures assigned to these compounds in Table 4. Micro-oxidation of compound VIII with MnO_2 in chloroform[10] gave a material similar in chromatographic properties and staining reaction to the test sample of 17α-ethynyl-10β-hydroxy-19-nortestosterone. The ultraviolet absorption spectrum of this oxidation product gave a maximum at 241 mμ, confirming the presence of an α,β-unsaturated ketone. This provides evidence for the allylic alcohol structure assigned to VIII.

(6) Compound V (Table 4) showed a peak in the infrared spectrum at 1018 to 1019 cm^{-1}. Rosenkrantz *et al.*[13] have published evidence that this band is characteristic of a 3α,5α configuration in 19-norsteroids. These authors have also suggested that peaks at 1041 and 1060 cm^{-1} (as shown by compound VII) may indicate a 3α,5β grouping.

(7) The structures assigned to compounds V, VI, VII, and VIII are in keeping with their relative polarity[14, 15] Thus the most polar compound (VIII) contains a quasi-equatorial 3-hydroxy group and a double bond. Compound VII has an equatorial 3-hydroxy group, and this compound is more polar than compound VI which also has an equatorial hydroxy group, and in which the α,β configuration is *trans*. This indicates that the α,β configuration in VII is *cis*. The least polar of the four compounds is number V, in which an axial configuration is probable. Since the infrared evidence indicates a 3α,5α grouping,[13] compound V is assigned the structure shown in Table 4.

DISCUSSION

The results in Table 2 indicate that the excretion of radioactivity ingested as norethynodrel is relatively slow. In three subjects the excretion was complete in 4 days, but in Subjects J.D. and A.P. measurable amounts of radioactivity were present in the

urine 7 days after administration. The amount of norethynodrel administered ranged from 5 to 100 mg, but no correlation was observed between dosage and the pattern of excretion of radioactivity. In the one subject (M.P.) from whom the bile was collected, 30 per cent of the administered radioactivity was found in the bile. This result suggests that there is considerable enterohepatic circulation of norethynodrel or its metabolites in the human. The presence of only 27 per cent of the ingested radioactivity in the feces of Subject M.P. indicates that the total absorption of norethynodrel from the gastrointestinal tract in this subject was of the order of 70 per cent. The absorption may, in fact, have been higher since the possibility that part of the radioactivity in the feces of M.P. may have come from bile leakage due to imperfect catheterization cannot be excluded. The two subjects receiving ^{3}H-norethindrone excreted a larger percentage of the administered dose in their urine than did those receiving norethynodrel. However, one of these women (R.B.) received norethindrone intravenously, and this might account for the higher urinary excretion in this patient since no loss of radioactivity would be incurred through nonabsorption of the steroid from the gut.

The figures in Table 3 show that from 30 to 60 per cent of the radioactivity in urine was extractable after incubation with β-glucuronidase, and this radioactivity probably represents steroid conjugated as glucosiduronate. The smaller percentage of radioactivity extracted after solvolysis at pH 1 (Table 3) probably represents steroid conjugated as sulfate. There is no appreciable difference in the amounts of radioactivity extracted after the various hydrolytic procedures in the urine of subjects receiving norethynodrel and in those receiving norethindrone.

Table 2 shows that only 70 and 85 per cent of the administered radioactivity was accounted for in the two subjects (A.P. and M.P.) in whom the total excreta were collected. It must be borne in mind that the assay of radioactivity in crude excreta is subject to considerable error. In our experience the errors in counting tritium in bile and feces can approach 30 per cent. It is possible, however, that some loss of radioactivity might occur because of instability of the random tritium label in the steroids used although, as has been pointed out previously,[3] similar recoveries of administered radioactivity have been obtained by workers using 4-^{14}C-progesterone. It is therefore possible that degradation of the steroid molecule in the body, particularly in the gastrointestinal tract, could lead to the excretion of some of the tritium by routes such as the breath or sweat. The storage of the steroid in body fat, and its excretion slowly over a period of time, is also a possibility.

The partial identification of the conversion products of norethynodrel is largely based on assumptive evidence. The results accord with the findings of previous workers[1, 2] who have shown that the administration of norethindrone or its esters does not lead to the excretion of significant amounts of ketosteroids or ketogenic steroids in the urine. However, whereas Langecker[1] could not find evidence of the excretion in the urine of large amounts of steroid alcohols or of steroids bearing the ethynyl side chain after the administration of norethindrone, the main conversion products of norethynodrel isolated in the present study appear to be steroid alcohols, all but one of which (compound IV, Table 4) carry the ethynyl side chain. No explanation of this discrepancy can be offered, but the present study probably accounts for only part of the excretion products of the ingested norethynodrel.

The evidence that many of the metabolites possess a 10-hydroxy group is of interest although, in the absence of authentic reference samples, the identification of the 3,10-dihydroxy metabolites remains assumptive. It is known that 17α-ethynyl-10β-hydroxy-19-nortestosterone can be produced artifactually from norethynodrel, and this conversion can be effected to some extent by gastric juice and by blood.[3] It is possible that this compound might then give rise to some of the trihydroxy compounds found in the urine. Another possibility is that 10-hydroxylation of several metabolites of norethynodrel might occur in the gastrointestinal tract during enterohepatic circulation. The metabolites present in bile were not investigated in this study, but Arai *et al.*[3] found that 17α-ethynyl-estr-5(10)-ene-3ϵ,17β-diol was the main metabolite of norethynodrel excreted in the bile of rabbits. This compound might be formed by the liver from norethynodrel and then hydroxylated in the gut.

Less than five per cent of the radioactivity in the urine of Subjects S.P. and A.P. was found in the phenolic fraction. This indicates that norethynodrel is not converted to phenolic metabolites to a major extent in the human, in agreement with results previously obtained in the rabbit.[3] It must be emphasized, however, that these results do not preclude the formation of phenolic metabolites from norethynodrel in amounts that might have physiological effects. In view of the high estrogenicity claimed by Paulsen *et al.*[16] for orally administered norethynodrel, the possible importance of the 10-hydroxy metabolites, either as intermediates in the aromatization of the 19-norsteroid or as estrogens *per se* merits further study.

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