Secretion of Ethynylestradiol and Its 3-Cyclopentyl Ether in the Milk of Lactating Rats (34107)

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Few studies have investigated the appearance of oral contraceptives in milk after their administration to nursing mothers. Pincus *et al.* (1) have shown that up to 0.13% of the radioactivity from administered norethynodrel and smaller amounts of radioactivity from ethynodiol diacetate appear in milk within 4 days after their administration to human patients. In a smaller study (2), between 0.45% and 1.5% of the administered radioactivity from norethynodrel appeared in the milk of lactating mothers over a 5-day period. In neither case was the radioactivity in the milk characterized.

In the present study, the excretion of ethynylestradiol (EE) in the milk of lactating rats was compared with that of its 3-cyclopentyl ether (quinestrol, EECPE).

Materials and Methods. Ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether (sp act 0.67 μg/μg) and ethynylestradiol-3-cyclopentyl-1-^{14}C-ether (sp act 0.0392 μCi/μg) were mixed to give an ^{3}H to ^{14}C ratio of approximately 7:1 and a sp act of 0.197 μCi/μg ^{3}H and 0.0275 μCi/μg ^{14}C. Ethynylestradiol (sp act 0.83 μCi/μg) was diluted with unlabeled material to give a specific activity approximately the same as that of EECPE (*i.e.*, 0.197 μCi/μg ^{3}H).

Doses for administration (22.7 μCi ^{3}H or 115 μg EECPE and 18.1 μCi ^{3}H or 102 μg EE) were suspended in 0.5 ml of an aqueous vehicle (3). Aliquots of the final dosage form were always taken for liquid scintillation counting to establish the exact amount of radioactivity.

General protocol. Two days postpartum, the litters of nursing mothers were either reduced or increased to ten suckling pups. Groups of three mothers then received orally either EE or EECPE. The pups were allowed to suckle and five from each mother killed at 4 hr and 24 hr after administration of the drug to the mother.

At 24 hr, pups of approximately the same age, which had suckled untreated mothers, were transferred to the treated mothers (nine pups per mother). Pups from each group were killed 24 hr later (*i.e.*, 48 hr after drug administration).

Extraction of radioactivity from rat pup brains and bodies. The brains from each group of pups were removed, pooled, and transferred to a 15-ml Tenbroek grinder. They were ground with successive aliquots of a 1:1 mixture of methylene chloride and absolute ethanol up to a total volume of 50 ml. After filtration, the filtrate was evaporated to dryness, taken up with 15 ml of liquid scintillation cocktail,[4] transferred to a counting vial, and counted. The remaining bodies from each group were pooled and homogenized in a Waring Blendor with successive aliquots of the above methylene chloride–absolute ethanol mixture up to a total volume of 250–300 ml. After filtration, 10 ml of the filtrate were transferred to a counting vial, evaporated to dryness, the residue dissolved in 15 ml of liquid scintillation cocktail, and the vial transferred to a liquid scintillation spectrometer for counting.

Isolation of radioactivity from lipid extracts. The following samples of either ex-

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[4] Counting medium: 7 g PPO, 0.3 g dimethyl POPOP, and 100 g napthalene in 1 liter of redistilled dioxane.

tracts of homogenates of whole pups were examined: (1) pups fed for 4 hr or (2) pups fed for 24 hr by mothers treated with EECPE and (3) pups fed for 4 hr by mothers treated with EE. Radioactivity was separated from the crude lipid extracts by reversed phase column chromatography. The resulting radioactive components were further characterized by TLC and crystallization to constant specific activity.

Column partition chromatography. Celite partition chromatography using the reversed-phase system water–methanol–*n*-propanol–xylene–isooctane (2:1:1.5:1:3) was used (4) to separate EECPE [eluted in the second holdback volume (HBV)] from nonpolar constituents in the lipid extract. Any metabolites, more polar then EECPE, including EE, are eluted at the solvent front in this system.

Thin-layer chromatography. Radioactive peaks eluted from the reversed phase partition columns were further characterized by TLC on silica gel G using benzene:ethyl acetate (3:1) as developing solvent. Known standards were run simultaneously.

Radioactivity measurements. Samples to be assayed were dissolved in dioxane scintillation counting fluid, and the ^{3}H and ^{14}C counted in a Tri-Carb spectrophotometer (Model 3365). Quenching was measured using an automatic external standard in connection with a set of predetermined quench curves.

Results and Discussion. Appearance of radioactivity in brain and body of pups nursed by mothers administered EE or EECPE. As only half a litter from any group was killed at any time interval, each value has been multiplied by 2 so that the data reported in Table I are disintegrations per minute per litter. The total radioactivity in any one litter is assumed to reflect the amount of radioactivity secreted into the rat milk over the time period during which the litter suckled.

Table I shows the accumulation of radioactivity in the pup brains and bodies at various time intervals after administration of EE ir EECPE to the mothers.

Very little radioactivity appears in the brain of pups after EECPE administration at any time interval; after EE administration virtually none appears. These results agree with previous observations on the storage of these two compounds in the adult rat brain (5).

The whole bodies of the pups from others treated with EECPE contained more radioactivity than those fed by mothers receiving EE.

The pups nursing for 24 hr contained slightly more radioactivity than those which suckled for 4 hr after administration of the drug to the mother. There was a significant fall-off in the amount of radioactivity present in the pups fed from 24–48 hr. These findings indicate that in the lactating rat given a dose of EECPE, maximum secretion of the drug and its metabolites into the milk occurs in the first 4 hr after administration. This is further supported by the sharp decrease in radioactivity secreted into the milk at 24–48 hr.

TABLE I. Amount of Radioactivity Present in Brain and Body of Suckling Pups Following the Administration of Radio-labeled EE or EECPE to Lactating Rats.

Compound admin. to mothers	Time interval in hr	Body—DPM × 10^3				Brain—DPM × 10^3			
		H^3	C^{14}	H^3/C^{14}	% of dose[a]	H^3	C^{14}	H^3/C^{14}	% of dose[a]
EECPE	0–4	523.0 ± 52.0[b]	73 ± 6.0	7.2	1.04	1.5 ± 0.4	0.2 ± .06	7.5	0.003
EECPE	0–24	723.0 ±120.0	104 ±16.0	6.9	1.45	11.5 ± 2.6	1.9 ± .3	6.0	0.023
EECPE	24–48	155.0 ± 21.0	21 ± 3.2	7.4	0.31	3.2 ± 0.7	0.48 ± .11	6.7	0.006
EE	0–4	23.2 ± 1.9	—	—	0.058	0.31 ± 0.10	—	—	—
EE	0–24	20.7 ± 1.7	—	—	0.051	0.19 ± 0.10	—	—	—
EE	24–48	16.7 ± 1.9	—	—	0.041	0.22 ± 0.18	—	—	—

[a] Calculated on the basis of administered H^3.

[b] Average ± standard error.

At no time is there a significant amount of radioactivity secreted into the milk from EE-treated mothers.

Characterization of radioactivity in the milk. Table II gives the chromatographic data from the three extracts studied. Ninety-three percent of the radioactivity present in homogenates of whole pups fed for 4 hr by mothers receiving EECPE corresponded to the unaltered EECPE. The remaining 7% was constituted by more polar metabolite(s). The 24-hr sample showed that as much as 24% of the radioactivity was in the form of more polar metabolites. Failure of these polar materials to move from the origin when analyzed by TLC suggests that polyhydroxylated metabolities of EECPE are the major components. The higher $^{3}H/^{14}C$ ratio also indicates cleavage of the ether linkage.

The EECPE-like material collected from each column was later shown by TLC to have the same R_f value as authentic EECPE. Final identification was obtained by mixing with cold standard and crystallizing to constant specific activity. On the other hand, analyses of radioactivity present in homogenates of whole pups nursed by EE-treated mothers, indicated the presence of one component corresponding to authentic EE.

This study has demonstrated that as in the case of norethynodrel and ethynodial diacetate, both ethynylestradiol and its 3-cyclopentyl ether are secreted into the milk after administration to lactating female rats. EE appears in the milk unchanged, whereas EECPE appears to undergo some alteration to more polar metabolites. Whether these metabolic alterations occur prior to or after ingestion of milk by the pups was not established. However, as the increase in percentage of polar metabolites occurring between 4 and 24 hr postadministration was not paralleled by a concomitant increase in total radioactivity, we are inclined to believe the metabolic alterations occurred in the pups. This hypothesis is in part supported by the findings that estradiol-17β is metabolized to several polyhydroxy metabolites by the human fetus (6). In other studies (1, 2) investigating the appearance of synthetic estrogens in milk no attempts were made to characterize the metabolites which were present; in one study (1) extracts from human milk were assayed for stimulation of uterine growth in the mouse, but failed to show any activity.

The levels of radioactivity excreted into the milk in this study are similar to those found for other synthetic progestagens and estrogens in the rabbit (1) and human (1, 2). The results from this experiment do not take into consideration any radioactivity

TABLE II. Chromatographic Data on Ether Extracts of Pups Nursed by Mothers Given EE or EECPE.

Compound admin. to mothers	Time period of feeding (hr)	Column fractions combined	Radioactivity in combined fractions		H^3/C^{14}	R_f of radioactivity from column peaks in TLC system benzene-ethylacetate (3:1)
			H^3	C^{14}		
EECPE	0–4	1-5[a]	10,900	2,100	9.9	0.00
		8-35	138,000	36,100	7.6	0.59 (R_f of EECPE = 0.61)
EECPE	0–24	1-4[b]	77,910	19,180	—	0.00
		6-55	245,200	53,350	8.9	0.61 (R_f of EECPE = 0.59)
EE	0–4	1-13[c]	2,210	—	—	0.46 (R_f of EE = 0.48)

[a] Column Size: 56 g celite: Fraction vol: 12 ml.
[b] Column Size: 56 g celite: Fraction vol: 9 ml.
[c] Column Size: 47 g celite: Fraction vol: 10.0 ml.

which may have been excreted by the pups prior to sacrifice, so that the levels found in this work probably signify minimal levels of secretion. However, in this study, milk production was stimulated by sucking, whereas in other studies (1, 2) pipettes or breast pumps were used to obtain the samples for analysis. As the fluid volume produced by this latter method of collection is small, it is not known how this might effect the total excretion of metabolites into the milk.

This study along with those of Pincus *et al.* (1) and Laumas *et al.* (2) demonstrate that the compounds present in oral contraceptives appear in the milk of lactating mothers. The possible effect of these secreted compounds upon the young must be considered when administering these compounds to nursing mothers. Previous reports suggest that sufficient amounts of these compounds or their metabolites could be ingested during breast feeding to cause some effect on the newborn (7).

Summary. Ethynylestradiol-6,7-^{3}H(EE) or its 11-^{14}C-cyclopentyl ether(EECPE) were administered to lactating rats, and the radioactivity ingested by the pups was measured during the following suckling periods: 0–4 hr; 0–24 hr; 24–48 hr. Only very small percentages of the administrered doses were found in the pups during the time intervals studied, the greatest amounts appearing during the first 4 hr after treating the mothers and the least during the 24–48 hr period. Only trace amounts of radioactivity were found in brains of the pups with most of the counts appearing in the carcasses. The radioactive compounds found in the pups were studied by column and thin-layer chromatography. In pups nursed by mothers treated with EE, EE was the only steroid which could be identified, while in pups suckled by EECPE-treated mothers, both EECPE and more polar metabolites were found. Both EE and EECPE extracted from pups were identified by crystallization to constant specific activity.

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