Studies on the Transport of Estrogens by the Rat Small Intestine *in Vivo* (33463)

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Using the steady state perfusion technique of Shanker *et al.* (1), Schedl and co-workers showed that percentage of absorption of corticoids from the small intestine of both rats (2) and humans (3) is independent of concentration over a wide range and suggested that steroids are absorbed by diffusion.

Incubation of several corticoids with everted sacs of rat intestine likewise indicated diffusion as their absorption mechanism (4). However, under similar experimental conditions, it was shown that estradiol-17β, testosterone, and Δ^4 -androstene-3, 17-dione undergo metabolic transformation prior to being transferred by an active mechanism from the mucosal to the serosal side (4, 5). Such an active transfer seems to be dependent upon the formation of glucuronide conjugates.

The observation that corticosteroids were absorbed *in vitro* by a process similar to that occurring *in vivo* and the lack of data on intestinal transport of estrogens and androgens *in vivo* casts some doubt on the statement that steroids are in general absorbed by a simple diffusion process (2, 3). It seemed therefore worthwhile to determine the mechanism responsible for the transport of estrogens by the rat intestine *in vivo*. We were interested in comparing a natural hormone (estradiol-17β) with two synthetic estrogenic steroids, ethynylestradiol and its 3-cyclopentyl ether.

Materials and Methods. General. Ethynylestradiol-6, 7-^{3}H-3-cyclopentyl ether (EECPE, specific activity [spact.] 0.67 μCi/μg) and ethynylestradiol-3-cyclopentyl-1'^{14}C ether (spact. 0.0392 μCi/μg) were mixed to give an ^{3}H/^{14}C ratio of approximately 8/1 and a spact. of 0.213 μCi/^{3}H/μg and 0.0267 μCi of ^{14}C/μg. The ^{3}H–14 labeled EECPE was prepared in order to gain information quickly about cleavage of the ether linkage. In other words, loss of ^{14}C (i.e., increase in ^{3}H/^{14}C ratio) is indicative of cleavage of the ether linkage. Ethynylestradiol-6,7^{3}H (EE, spact. 0.83 uCi/μg) and 17β-estradiol 6, 7^{3}H[4] (E, spact. 1.56, μCi/μg) were diluted with unlabeled material to give a spact. approximately the same as that of EECPE (i.e., 0.213 μCi of ^{3}H/μg).

Aliquots of the final dosage forms were always taken for liquid scintillation counting to establish the exact amount of radioactivity. Doses for administration (5.29 μCi of ^{3}H or 24.8 μg of EECPE, 4.85 μCi of ^{3}H or 22.8 μg of EE and 3.91 μCi of ^{3}H or 18.1 μg of E) were suspended in an aqueous vehicle (6). Crude data were then corrected in proportion to a constant dose of 5 μCi of ^{3}H and 0.625 μCi of ^{14}C where applicable. This correction was considered legitimate since actual values did not vary by more than $\pm$20% of this value. This correction normalizes values in the same sense as calculating percentage of administered dose.

A. Intestinal absorption. Male albino rats, single strain, weighing 350–400 g were used. Under ether anesthesia, the small intestine was exposed through a midline incision of the abdomen. Ligatures were placed at the iliac end and about 2 cm below the entry of the ductus choledocus. The labeled compounds were introduced directly into the small intestine by means of a soft catheter through a small incision 3–4 cm below the entry of the ductus choledocus. Groups of 3 animals per each compound were killed 7.5, 30, and 120 min after administration. Bile ducts were cannulated only in the animals to be killed at the 120-min interval. This was done to prevent accumulation of bile in the upper seg-

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[4] The estradiol-6,7-^{3}H was purchased from New England Nuclear Corporation.

ment of intestine.

The animals were exanguinated by heart puncture and the small intestine with the exception of the tract above and immediately below the entry of the ductus choledocus was carefully removed. The intestinal lumen was washed consecutively with 50 ml of absolute ethanol and 50 ml of methylene chloride. The washes were combined for analysis (referred to as intestinal washings). The intestine was thereafter transferred to a container and homogenized in a Vir Tis "45" homogenizer with successive aliquots of a 1:1 mixture of absolute ethanol and methylene chloride up to a total volume of 100 ml; the final homogenate was filtered.

1. *Analysis of intestinal washings.* An aliquot (0.5 ml) of each sample was added directly to the dioxane scintillation mixture (6) and counted. The remainder of the washings from each time period were respectively combined and evaporated to dryness.

The residue from each wash was transferred quantitatively by means of 6 alternate washed with water (total of 10 ml) and ether (total of 20 ml) to a 50-ml glass-stoppered centrifuge tube. The phases were shaken together and the ether layer was transferred to a clean 50-ml graduated centrifuge tube. The aqueous phase was shaken with an additional 20 ml of ether. The ether extracts were combined and adjusted to 50 ml. Ten ml of the ether extract (free fraction) were evaporated in a counting vial. After addition of 15 ml of dioxane scintillation mixture, the vials were transferred to a liquid scintillation spectrometer for counting (6). The remaining ether extracts were evaporated to dryness and analyzed as described in paragraph D. One ml of the aqueous phase (total conjugates) was transferred to a counting vial containing 15 ml of dioxane scintillation fluid and counted.

2. *Analysis of intestinal walls.* Each filtrate was evaporated to dryness and extracted with alternate washes of water and ether as described in paragraph A. 1 Radioactivity in the water and ether fractions was likewise determined as described under A. 1. The remaining ether extracts of the 7.5-min samples from EE or E treated animals as well as the 30-min samples from EECPE treated rats were respectively combined, evaporated to dryness and analyzed as described in paragraph D.

B. Analysis of peripheral plasma and perirenal fat. Samples of plasma and perirenal fat were obtained at each time period and processed for determination of radioactivity as previously described (6).

C. Analysis of Portal Plasma. Sex and size of the animals as well as surgical procedure were similar to those described in Sect. A. Portal blood collection started 2 min after the intraduodenal administration of either EE or E and 2 and 30 min after that of EECPE. The choice of these time intervals was determined on the basis of data on rate of intestinal absorption (6) and/or concentration in the intestinal wall.

Blood was collected up to the limits of feasibility (about 3.5 min) and averaged about 7–8 ml/animal. Groups of 3 animals for each compound were used. After centrifugation, the plasma samples of each group were respectively combined and analyzed. Aliquots of 0.1 or 0.5 ml from each sample were added directly to the liquid scintillation fluid to establish total radioactivity present. Five ml of each sample were diluted to 10 ml with distilled water and shaken with one 20-ml and two 15-ml portions of ether. The ether extracts were combined and adjusted to 50 ml. Ten ml of the ether extract (free fraction) were evaporated in a counting vial. After addition of 15 ml of liquid scintillation fluid, the vials were transferred to a liquid scintillation spectrometer for counting (6). Each remaining ether extract was evaporated to dryness and analyzed as described in paragraph D.

One ml of the aqueous phase (total conjugates) was transferred to a counting vial containing 15 ml of liquid scintillation cocktail and counted. Four vol of ethanol were added to each remaining aqueous phase and the plasma protein removed by filtration on a Buchner filter. The filtrates were flash evaporated and the residues taken up in 20 ml distilled water. A 0.25-ml portion of each 20-ml sample was added to the scintillation mixture and counted to determine total radi-

oactivity present. The remaining aqueous phase of each sample was then divided into two aliquots and further processed to determine the nature of the conjugates.

1. *Glucuronide conjugates.* A 10-ml portion of each aqueous phase was adjusted to pH 4.5 and 1 ml of Ketodase (5000 units of β-glucuronidase) and 1 ml of 0.1*M* acetate buffer (pH 4.5) were added. The samples were then incubated at 37° for 48 hr and the steroids liberated extracted with one 20-ml and two 15-ml portions of ether. The ether extracts were combined and adjusted to 50 ml. Ten ml of the ether extract were evaporated in a counting vial. After addition of 15 ml of scintillation fluid each vial was transferred to the liquid scintillation spectrometer and counted.

2. *Sulfate conjugates.* A 9-ml portion of each aqueous phase was adjusted to pH 6 and 1 ml of pH 6.0 acetate buffer (0.1 *M*) and 100 mg of Mylase P were added. Incubations and extractions were performed as in the case of glucuronides.

D. Characterization of compounds present in the ether extracts from intestinal lumen, *intestinal wall, and portal plasma.* The radioactivity of these oily extracts was separated from the lipid constituents by reversed phase celite partition chromatography. The radioactive components were then separated by further celite chromatography and thin-layer chromatography. The celite partition chromatography was carried out as described by Siiteri *et al.* (7). The reversed phase system, water–methanol–*n*-propanol–xylene–isooctane (2:1:1.5:1:3) was used to isolate the radioactive components from the lipid material. In this particular system, the more polar materials such as estrone, estradiol-17β and ethynylestradiol are eluted at the solvent front whereas the less polar ethynylestradiol-3-cyclopentyl ether is eluted in the second to the third hold-back volume (HBV).

Radioactive peaks containing estrone, estradiol-17β, and ethynylestradiol were further purified by chromatography on an isooctane–*t*-butanol–methanol–water (50:20:16:14) system. In this system estrone is eluted in the second HBV while estradiol-17β and ethynylestradiol are both in the fourth HBV. Radioactive peaks were further characterized by thin-layer chromatography (TLC) on silica gel G using benzene–ethyl acetate (3:1) as developing solvent. The mobility of radioactive peaks was compared with that of known standards run on the same plate. The standards were visualized by exposure to iodine vapors. Radioactivity on the TLC plate was detected by a Packard Strip Scanner (model 7201 B). For final characterization, the radioactive peak was recovered from the silica gel plate, mixed with the appropriate cold standard and recrystallized to constant specific activity.

The ether extracts from samples of portal plasma were streaked across 5-cm TLC silica gel G plates and developed in the system benzene–ethyl acetate (3:1). In certain cases radioactive peaks were eluted from the gel and rerun in two other solvent mixtures, chloroform–methanol–water (485:15:1) and cyclohexane–acetone–ethyl acetate (13:6:1). Appropriate standards were run with each plate and final characterization of the radioactive material was done by addition of the cold standard and recrystallization to constant specific activity.

Results. A. Intestinal absorption. Analysis of intestinal lumen and wall. The data are shown in Table I. More than 80% of the administered dose of E or EE had disappeared from the intestinal lumen after 7.5 min. The amount of ether extractable material (free fraction) present in the intestinal wall at 7.5 and 30 min was 4.3 and 1.6% of the administered dose, respectively, for E; and 10 and 2.9%, respectively, for EE. The amount of ether nonextractable material (total conjugates) present in the intestinal wall at 7.5 and 30 min was slightly more than 1% of the administered dose in the case of EE and even less in the case of E. The rate of absorption of EECPE was considerably slower. The $^{3}H/^{14}C$ ratio of the material present in the intestinal lumen at 7.5 and 30 min was similar to that of the administered dose indicating that the compound had undergone little or no alteration up to this time. A significantly higher $^{3}H/^{14}C$ ratio of the material present in the intestinal lumen at 120 min might indicate some loss of the ^{14}C-labeled

TABLE I. Distribution of Radioactivity in the Small Intestine of Rats Following Intraduodenal Administration of Radioactive Estradiol (E), Ethynylestradiol (EE) or Ethynylestradiol-3-cyclopentyl Ether (EECPE).

Treatment	Time (min)	Intestinal lumen (dpm × 10^3)				Intestinal wall (dpm × 10^3)							
						Free fraction				Total conjugates			
		^3H	^{14}C	% of dose[a]	$^3H/^{14}C$[c]	^3H	^{14}C	% of dose[a]	$^3H/^{14}C$	^3H	^{14}C	% of dose[a]	$^3H/^{14}C$
E	7.5	1397 ± 155[b]	—	12.7	—	473 ± 68	—	4.3	—	42.9 ± 6.9	—	0.39	—
E	30	726 ± 167	—	6.6	—	176 ± 30	—	1.6	—	37.4 ± 7.7	—	0.34	—
EE	7.5	1705 ± 324	—	15.5	—	1111 ± 216	—	10.1	—	153 ± 29	—	1.4	—
EE	30	583 ± 79	—	5.3	—	319 ± 25	—	2.9	—	121 ± 30	—	1.1	—
EECPE	7.5	4257 ± 429	519 ± 58	38.7	8.2	5313 ± 455	672 ± 54	48.3	7.9	18.7 ± 3.8	2.2 ± 0.6	0.17	8.5
EECPE	30	2695 ± 467	328 ± 63	24.5	8.2	4048 ± 198	519 ± 25	36.8	7.8	36.3 ± 11	5.8 ± 1.8	0.33	6.3
EECPE	120	759 ± 222	82 ± 29	6.9	9.2	814 ± 49	96 ± 5	7.4	8.5	45 ± 4.2	7.2 ± 0.5	0.41	6.2

[a] Calculated on the basis of an administered 5-μCi ^3H dose.

[b] Average ± SE.

[c] $^3H/^{14}C$ ratio of administered dose = 8.

TABLE II. Distribution of Radioactivity in Body Fat and Plasma Following Intraduodenal Administration of Radioactive Estradiol (E), Ethynylestradiol (EE) or Ethynylestradiol-3-cyclopentyl Ether (EECPE).

Treatment	Time (min)	Total body fat[a] (dpm × 10^3)				Peripheral plasma (dpm × 10^3/ml)							
						Free fraction				Total conjugates			
		^3H	^{14}C	% of dose[b]	$^3H/^{14}C$[d]	^3H	^{14}C	% of dose[b]	$^3H/^{14}C$	^3H	^{14}C	% of dose[b]	$^3H/^{14}C$
E	7.5	48 ± 12.6[c]	—	0.44	—	0.77 ± 0.45	—	0.007	—	3.96 ± 1.05	—	0.036	—
E	30	87 ± 18	—	0.79	—	0.44 ± 0.1	—	0.004	—	3.3 ± 0.89	—	0.030	—
EE	7.5	125 ± 25.6	—	1.14	—	5.9 ± 2.2	—	0.054	—	10.0 ± 0.2	—	0.091	—
EE	30	550 ± 172	—	5.0	—	1.98 ± 0.5	—	0.018	—	22.0 ± 1.68	—	0.200	—
EECPE	7.5	66 ± 13.2	8.9 ± 1.7	0.6	7.4	0.66 ± 0.07	0.07 ± 0.009	0.006	9.7	0.44 ± 0.04	0.046 ± 0.005	0.004	9.5
EECPE	30	627 ± 259	80.4 ± 33	5.7	7.8	3.2 ± 0.23	0.33 ± 0.025	0.029	9.7	3.08 ± 0.6	0.55 ± 0.08	0.028	5.6
EECPE	120	4026 ±1098	479 ±132	36.6	8.4	3.6 ± 0.41	0.35 ± 0.08	0.033	10.3	8.03 ± 0.7	1.0 ± 0.1	0.073	8.0

[a] Estimated to be 25% of body weight.

[b] Calculated on the basis of an administered 5-μCi ^3H dose.

[c] Average ± SE.

[d] $^3H/^{14}C$ ratio of administered dose = 8.

cyclopentyl group had occurred.

A considerable amount of ether extractable material (free fraction with an $^3H/^{14}C$ ratio similar to that of the administered dose was present in the intestinal wall at 7.5 and 30 min. A smaller but significant amount of ether extractable material could still be detected in the wall at 120 min. This material had an $^3H/^{14}C$ ratio higher than that of the administered compound. This change in $^3H/^{14}C$ ratio presumably reflected cleavage of the cyclopentyl ether group which had occurred in the intestinal lumen at this time. The amount of ether nonextractable material present in the intestinal wall at any time interval was negligible.

B. Analysis of body fat and peripheral plasma. The data are shown in Table II. As determined on the basis of the ^3H present in the perirenal fat, very little radioactivity accumulated in the body fat of E-treated animals. On the other hand as much as 5% of the administered ^3H was present at 30 min in the fat depots of EE-treated rats. At this time a similar percentage of ^3H had accumulated in the fat depots of EECPE-treated animals. However, up to this time, more than 60% of EECPE was still in the process of being either absorbed or released into the blood whereas EE had virtually disappeared from both intestinal lumen and wall. By 120 min 36% of the administered ^3H was present in the fat depots of EECPE-treated rats. These results confirm previous findings (6), that EECPE is stored in body fat.

Peripheral plasma concentrations of free steroids and their respective conjugates were likewise similar to those described in a previous publication (6). In the case of E and EE, plasma conjugated radioactivity was greater than plasma free (ether extractable) radioactivity, whereas in the case of EECPE more free steroid was observed in plasma at 7.5 and 30 min.

C. Analysis of portal plasma. The data are shown in Table III. Regardless of the compound administered, at the time intervals during which portal blood samples were collected, most of the radioactivity was ether extractable (free fraction). Most of the ether-nonextractable radioactivity (total con-

TABLE III. Distribution of Radioactivity in Portal Vein Plasma Following Intraduodenal Administration of Radioactive Estradiol (E), Ethynylestradiol (EE) or Ethynylestradiol-3-cyclopentyl Ether (EECPE).

Treatment	Time of collection (min)	Total plasma (dpm × 10^3/ml)				Free fraction (dpm × 10^3/ml)				Conjugates fraction (dpm × 10^3/ml)											
										Total				Glucuronides				Sulfates			
		^3H	^{14}C	% of dose[a]	$^3H/^{14}C$[b]	^3H	^{14}C	% of total plasma	$^3H/^{14}C$	^3H	^{14}C	% of total plasma	$^3H/^{14}C$	^3H	^{14}C	% of total conjugates	$^3H/^{14}C$	^3H	^{14}C	% of total conjugates	$^3H/^{14}C$
E	2– 5.5	77	—	0.7	—	55	—	71.4	—	16.5	—	21.4	—	9.4	—	57	—	3	—	18	—
EE	2– 5.5	176	—	1.6	—	143	—	81.2	—	26.8	—	15.2	—	14.6	—	55	—	5.4	—	20	—
EECPE	2– 5.5	7.7	0.67	0.07	11.5	5.94	0.55	71.4	10.8	1.76	0.10	14.3	17.6	0.88	0.065	50	13.6	0.85	0.074	48	11.5
EECPE	30–33.5	33	3.8	0.3	8.6	22	2.3	66.7	9.4	11	1.4	33.3	7.6	5.8	0.6	53	9.5	7.8	0.89	71	8.8

[a] Calculated on the basis of an administered 5 μCi ^3H dose.
[b] $^3H/^{14}C$ ratio of administered dose = 8.9.

TABLE IV. Nature of the Compounds Present in the Ether Extract from Intestinal Lumen or Wall and Portal Plasma Following Intraintestinal Administration of E, EE, or EECPE.

Compound given	Time interval (min)	Source of extract	Compound isolated	% of total	Method of isolation
E (1)	7.5	Intest. lumen	Estrone	20	Reversed phase in A; partition chromatography in C; TLC in B
			17β-Estradiol	80	
E (2)	7.5	Intest. wall	Estrone	70	Reversed phase chromatography in A; TLC in B
			17β-Estradiol	30	
E (3)	2–5.5	Portal plasma	Estrone	63	TLC in B
			17β-Estradiol	27	
EE	7.5	Intest. lumen	Ethynylestradiol	>90	As in (1)
	7.5	Intest. wall	Ethynylestradiol	>100	As in (2)
	2–5.5	Portal plasma	Ethynylestradiol	100	As in (3)
EECPE	30	Intest. lumen	EECPE	>90	Reversed phase in A; TLC in B
			More polar material	<10	
	30	Intest. wall	EECPE	≅90	As in (2)
			More polar material	≅10	
	2– 5.5	Portal plasma	EECPE	≅80	As in (3)
			More polar material	≅20	
	30–33.5	Portal plasma	EECPE	≅50	As in (3)
			More polar material	≅50	

[a] System A = water:methanol:propanol:xylene:isooctane (2:1:1.5:1:3); B = benzene:ethyl acetate (3:1); and C = isooctane:*tert*-butanol:methanol:water (75:25:16:14).

jugates) corresponded to the glucuronide fraction in the case of E or EE. Glucuronide and sulfate conjugates were present in equivalent amount in the 2–5.5-min samples from EECPE-treated animals with a somewhat greater amount of the sulfate fraction in the 30–33.5-min sample. The $^{3}H/^{14}C$ ratio of the free fraction was higher than that of the administered dose in the 2–5.5 min sample but virtually unchanged in the 30–33.5 min sample. The amount of the free fraction present in the portal blood from EECPE-treated rats was similar in the samples obtained at 2–5.5 or 30–33.5 min postadministration.

D. Characterization of compounds present in the ether extracts from intestinal lumen or wall and portal plasma. The data are shown in Table IV. Analysis of the content of the intestinal lumen 7.5 minutes following the administration of E, indicated that 20% of the free fraction was estrone with the remainder corresponding to unaltered E. On the other hand, analysis of the intestinal wall showed that most of the free fraction (70%) was estrone with only 30% remaining as E. The free fraction of the 2–5.5-min portal blood sample had a composition similar to that of the intestinal wall. No conjugates were found. Only EE was found in the three compartments of EE-treated rats.

Analysis of the 30-min samples from either the intestinal lumen or wall or the 2–5.5-min samples from portal vein following the administration of EECPE showed the presence of more polar material indicating that this compound had undergone some metabolic transformation. The 30–33.5-min portal blood sample showed that as much as 50% of the total radioactivity was associated with more polar material none of which corresponded to EE.

Discussion. When aqueous suspensions of the steroids were injected directly into the small intestine in the absence of bile, the rate of disappearance of EECPE was consider-

ably slower than that of either E or EE. Although the presence of bile is necessary to increase the rate of absorption of EECPE to a level comparable to that of the other steroids (Cargill and Meli, unpublished results), bile was prevented from reaching the intestine in order to reproduce as closely as possible the *in vitro* experimental conditions (4, 5). Furthermore, radioactivity recycled in the bile would not be distinguishable from unabsorbed radioactivity remaining in the intestine.

More than 80% of the administered dose of E or EE had disappeared from the intestinal lumen after 7.5 min whereas a much smaller but significant amount was still present in the intestinal wall. In each case, the water extractable radioactivity was a small fraction of the administered dose. By 30 min both E and EE had virtually disappeared from the intestinal lumen and wall.

The radioactivity recovered from portal blood samples was in each case a small percentage of the administered dose. Most of the portal blood radioactivity was ether extractable (free fraction). The ether nonextractable radioactivity was located primarily in the glucuronide fraction. Because of the method of collection of portal blood, it is impossible to state whether the glucuronides had been formed during intestinal transport or were contaminants reentering the portal circulation from the systemic blood.

Analysis of the ether extractable material from intestinal lumen or wall and portal blood indicated that, unlike EE, E had undergone very rapid metabolic transformation. Following E administration, estrone accounted for 20% of the total radioactivity present in the intestinal lumen and 70% of the radioactivity in the intestinal wall. The concentration of estrone in the portal plasma was similar to that observed in the intestinal wall. The much greater concentration of estrone in the intestinal wall as compared with that in the intestinal lumen could be the result of either, (a) further metabolic transformation of E to estrone taking place in the intestinal wall, and/or, (b) a difference in retention time so that estrone could be released from the intestinal wall at a rate significantly slower than that of E.

Only 15–20% of the total radioactivity present in the portal plasma following E administration of the various estrogens was associated with the conjugation fraction. This suggests that conjugation does not play an important role in the absorption of estrogens by the rat intestine *in vivo* in contrast to the findings of others using *in vitro* techniques (4, 5). Therefore it is possible that estrogens are absorbed *in vivo* by simple diffusion rather than by an active process but our data do not provide a definitive answer to this question. Our findings do suggest that the reduced biological activity of orally administered E may be due to its transformation to the less potent estrone during absorption through the intestinal wall. EE, on the other hand, was not metabolized in the intestinal lumen or wall. EECPE did appear to be partially degraded in the intestinal luman. All three estrogens were metabolically attacked (presumably by the liver) as evidenced by the high proportion of conjugates in the systemic plasma.

The absorption of EECPE deserves further comment. At 7.5 min as much as 85% of the administered dose was still present in the intestine, the greatest amount being in the intestinal wall. Thereafter the compound was slowly released from the intestine so that at 120 min there was still a small but significant amount present. The process by which EECPE is bound to and then released from the intestinal wall is not known and cannot be fully explained on the basis of the data available at the present time. The compound may be bound to the fatty material present in the intestinal wall and the rate at which it is released therefrom dependent upon the metabolic turnover of this material. The presence or absence of bile likewise influences absorption of EECPE. The rate at which a substance enters the systemic circulation thus cannot always be estimated by measuring its rate of disappearance from the intestinal lumen. The possibility of binding and storage in the intestinal wall must not be overlooked.

Summary. Rats intraduodenally administered 17β-estradiol-6,7 -^{3}H (E), ethynyles-

tradiol-6, 7 -^{3}H (EE) or ethynylestradiol-6, 7 -^{3}H-3-cyclopentyl-1-^{14}C ether (EECPE) were killed at 7.5-, 30- or 120-min intervals. Intestinal lumen contents, intestinal wall homogenates and portal plasma were analyzed for free and conjugated steroids. After 7.5 min, more than 80% of the administered dose of E or EE had been absorbed. Characterization of the steroids present in the various ether extractable fractions (free steroid) indicated that, unlike EE, E had undergone rapid metabolic transformation with estrone accounting for 70% of the total radioactivity in the intestinal wall and portal blood. This transformation to the less potent estrone could be responsible for the reduced biological activity of orally administered E.

The intestinal absorption of EECPE was markedly different from that of E or EE. The EECPE was slowly absorbed from the lumen through the intestinal wall. At 7.5 min as much as 85% of the administered dose was still present. Thereafter, the compound was slowly transported with almost complete absorption of the free steroid by 120 min. This delay may be due to binding of EECPE to the fatty material present in the intestinal wall. For a truer evaluation of intestinal absorption, an analysis of whole homogenized intestine may be required to determine whether it significantly exceeds the residual amount of drug present in the intestinal lumen.

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Karyotypic Changes in Mycoplasma-Modified Lines of FL Human Amnion Cells* (33464)

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Chromosome changes in mycoplasma-infected FL human amnion cells have included reduction in chromosome numbers, increase in the frequency of aberrations, and the appearance of several new chromosome varieties (1). The very slowly developing changes continued even during the second and third year of cultivation of the infected cultures, as previously reported (2). After elimination of mycoplasma the chromosome numbers did not revert to higher numbers, and one new chromosome, a large telocentric, persisted in practically all metaphase plates (2). There have been correlated changes in morphology, cultivation characteristics, and in resistance to mycoplasma. The tumor-producing capacity was reduced when the cells were tested in the cheek pouch of cor-

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