a delay between disappearance from the lumen and appearance in the blood, are in agreement with previous studies (12), which indicate that the greater a compound's lipophilicity, the longer will be its holdup in the intestinal wall and the less will penetrate to the serosal side of the membrane.

Finally, as is expected with longer holdup, the ester is more extensively metabolized than is the acid, as indicated by TLC. With dinoprost, the main metabolites are 15-ketodinoprost and 13,14-dihydro-15-ketodinoprost. As the experiment progressed, the metabolism decreased (Fig. 7). With the ester, four main metabolites are seen: dinoprost, 15-ketodinoprost, 13,14-dihydro-15-ketodinoprost, and 13,14-dihydrodinoprost. Although the amount of intact ester entering the blood increased slightly as the experiment progressed, the total metabolism increased, as indicated by the increase in 13,14-dihydrodinoprost in the circulation.

In summary, intestinal prostaglandin absorbed appears to have at least three phases. First, prostaglandins appear to diffuse rapidly from the gut lumen into the intestinal wall. Second, once in the gut wall, time is required for the compound to reach the other side of the cell. During this transcellular movement, the compound appears to be extensively, although not completely, metabolized. The duration of this second phase appears to be directly related to lipophilicity. Third, the compound and its metabolites are released into the mesenteric circulation. Prostaglandin esters apparently are released slower and over a longer period than the parent acids; however, they also undergo more extensive metabolism.

This study demonstrated differences in dinoprost and dinoprost methyl ester absorption patterns in the rat. However, these differences were not in agreement with the hypothesis that prostaglandin esters are absorbed faster than the parent prostaglandins in this species.

REFERENCES

(1) W. Magee, S. Armour, and O. Miller, *Biochim. Biophys. Acta*, **306**, 270 (1973).

(2) A Robert, W. Magee, O. Miller, and J. Nezamis, *ibid.*, **348**, 269 (1974).

(3) N. F. H. Ho and W. Higuchi, *J. Pharm. Sci.*, **63**, 686 (1974).

(4) N. Ho, J. Park, W. Morozowich, and W. Higuchi, *J. Theoret. Biol.*, **61**, 185 (1976).

(5) J. Y. Park, thesis, University of Michigan, Ann Arbor, Mich., 1977.

(6) F. Jacobs and M. Luper, *J. Appl. Physiol.*, **11**(I), 136 (1957).

(7) P. Jacobs, F. H. Bothwell, and R. Charlton, *Am. J. Physiol.*, **210**, 694 (1966).

(8) H. Kavin, N. Levin, and M. Stanley, *J. Appl. Physiol.*, **22**, 604 (1967).

(9) J. Lee, *Am. J. Physiol.*, **200**, 979 (1961).

(10) J. Lee and K. Duncan, *Gastroenterology*, **54**, 559 (1968).

(11) K. J. Desai, thesis, University of Michigan, Ann Arbor, Mich., 1977.

(12) V. Houston, D. Upshall, and J. Bridges, *J. Pharmacol. Exp. Ther.*, **189**, 244 (1974).

ACKNOWLEDGMENTS

Special thanks are extended to Dr. N. F. H. Ho, Department of Pharmaceutical Chemistry, University of Michigan, Ann Arbor, Mich., for advice and consultation during this work.

Conjugated Estrogens Bioinequivalence: Comparison of Four Products in Postmenopausal Women

WALLACE P. ADAMS *x, JUN HASEGAWA *, RAYMOND N. JOHNSON ‡, and RONALD C. HARING ‡

Received November 6, 1978, from the **Pharmacy Research and Development Department and the* ‡*Analytical Research and Development Department, Ayerst Laboratories Inc., Rouses Point, NY 12979.* Accepted for publication February 7, 1979.

Abstract □ The bioequivalence of four conjugated estrogens tablets USP was compared by measurement of seven estrogens or estrogen metabolites in the urine during steady-state dosing in postmenopausal women. Two studies compared three generic products with the innovator's product. The urinary excretion of 17α-dihydroequilin, 17α-dihydroequilenin, and 17α-estradiol were significantly greater in all cases with the innovator's product than with the generic products. Statistically significant differences between products were observed occasionally for other components. The generic products thus were bioinequivalent to the innovator's product, although all products essentially met current compendial specifications. A third study observed no significant differences between three batches of the innovator's product for the seven components. Total conjugated estrogens excretion of all products at the steady state was essentially equal and correlated with neither disintegration time nor dissolution half-time. Bioinequivalence between products is discussed in relation to the need for an improved USP conjugated estrogens monograph. Evidence suggesting the metabolism of a fraction of dosed estrone, equilin, and 17α-dihydroequilin to 17β-estradiol, 17β-dihydroequilin, and 17α-dihydroequilenin, respectively, is presented.

Keyphrases □ Estrogens, conjugated—bioinequivalence of generic and proprietary products, postmenopausal women □ Bioequivalence—conjugated estrogens, bioinequivalence of generic and proprietary products, postmenopausal women □ Product substitution—estrogens, conjugated, bioinequivalence of generic and proprietary products, postmenopausal women

Conjugated estrogens of natural origin are composed of at least nine different estrogens or estrogen metabolites, each present in a different amount. The USP monograph for conjugated estrogens (1) contains specifications for total conjugated estrogens and for the two most abundant components, sodium estrone sulfate and sodium equilin sulfate. The sodium estrone sulfate specification is 50–65% and the sodium equilin sulfate specification is 20–35% of the total conjugated estrogens content. The monograph does not contain quantitative specifications for additional components, although an identification test (2) requires the presence of a prominent GLC peak for 17α-dihydroequilin and additional peaks for 17α-estradiol, 17β-dihydroequilin, equilenin, 17β-estradiol, and 9-dehydroestrone.

Broad content ranges are specified for sodium estrone sulfate and sodium equilin sulfate. The content of the third major component, sodium 17α-dihydroequilin sulfate, which by GLC is 15% of the total estrogens in the innovator's product, is unspecified; the specification for this component is an imprecise and nonquantitative identification test. A similar requirement is made for minor components.

Pharmaceutical equivalents are defined (3) as "drug products that contain identical amounts of the identical active drug ingredient. . . ." Drug product compliance with compendial standards is generally assumed to assure pharmaceutical equivalence (4). A consequence of the broad or nonquantitative specifications for both major and minor components is that compendial standards may not

0022-3549/79/0800-0986$01.00/0

Table I—Estrogens Content Determined by USP XIX Assay Method, Disintegration Times, and Dissolution Half-Times of Conjugated Estrogens Tablets USP [a]

Product or Batch	Sodium Estrogen Sulfate Content, mg/tablet			Disintegration Time, min		Dissolution Half-Time, min	
	Estrone	Equilin	Total Conjugated Estrogens	Mean	Range	Mean	Range
			Study I				
P1	0.70	0.38	1.29	23.0	19–29	24.5	23.4–25.9
A	0.82	0.34	1.24	28.2	25–31	55.5	48.4–64.7
			Study II				
P2	0.69	0.35	1.31	41.3	36–49	119	101–138
B	0.74	0.33	1.18	22.5	18–30	89.1	54.3–123
C	0.75	0.34	1.20	74.5	69–81	—[b]	—[b]
			Study III				
P3	0.66	0.36	1.27	56.2	49–67	224	186–287
P4	0.67	0.38	1.28	38.9	33–44	164	148–175
P5	0.69	0.37	1.30	41.8	38–52	241	199–284

[a] The assay for estrogens content, disintegration times, and dissolution half-times used 20, six, and three tablets, respectively, except the disintegration times for P3, P4, and P5, which used 24 tablets. [b] Tablets were unavailable for dissolution half-time determination.

assure the pharmaceutical equivalence of conjugated estrogens products.

A previous publication (5) presented a sensitive and specific method to quantitate seven estrogens or estrogen metabolites in the urine of postmenopausal subjects after dosing to the steady state. The usefulness of the urinary excretion profile in assessing the bioinequivalence of conjugated estrogens products from multiple sources was discussed. The present investigation examined the bioinequivalence of four conjugated estrogens products based on their urinary excretion profiles. The innovator's product and three generic products, all of which meet or nearly meet current compendial specifications, were studied. The purpose of this study was to investigate the extent to which adherence of these products to compendial specifications assures their bioequivalence.

EXPERIMENTAL

The results of Studies I–III, conducted at intervals of 1 year, are reported.

Subjects—Eleven, 12, and eight postmenopausal women were selected for Studies I, II, and III, respectively. A physical examination and medical history were obtained for each subject. The postmenopausal syndrome was established by documented physiological or surgical menopause of long duration and anestrogenism was established by colpocytogram. Each subject in Studies II and III was requested to take no medication other than the conjugated estrogens. Each subject signed a voluntary informed consent form.

Study I—The two physiologically menopausal subjects' ages were 51 and 54 years, and the nine surgically menopausal subjects' average age was 50 years (range 44–61). The average weight of all subjects was 65 kg (range 54–91). Serum biochemistry tests[1] and urinalyses[2] (pH, glucose, and protein) were conducted to assure normal liver and kidney function. Nine subjects were concomitantly receiving thyroid replacement hormones, digoxin, or an oral hypoglycemic agent.

Study II—The eight physiologically menopausal subjects' average age was 52 years (range 50–57), and the four surgically menopausal subjects' average age was 44 years (range 37–54). The average weight of all subjects was 62 kg (range 54–73). All subjects were in good health, established by physical examination, serum biochemistry tests[1], and urinalyses[2] (pH, glucose, and protein).

Study III—The four physiologically menopausal subjects' average age was 53 years (range 51–56), and the four surgically menopausal subjects' average age was 35 years (range 29–38). The average weight of all subjects was 65 kg (range 51–94). The good health of the subjects was established as in Study II.

[1] SMA 12/60.
[2] Combistix, Ames Co., Elkhart, Ind.

Protocol—*Study I*—The study was divided into four consecutive phases of 28, 21, 14, and 21 days. The first phase was a washout period to permit urinary estrogen levels of those subjects previously taking estrogen medication to return to baseline levels. At the end of this phase, a 24-hr urine sample was collected from each subject.

During the second phase, each subject received a 2.5-mg dose (as two 1.25-mg tablets) of conjugated estrogens daily for 21 days. Each subject received Treatments P1[3] and A (generic product) in a randomized two-way crossover design. Each subject was instructed to select a time for dosing each morning and to take the dose at the same time each morning. On any day between Days 16 and 20 inclusive, a 24-hr urine sample was collected from each subject beginning at the time of dosing. The third phase was a washout period; a 24-hr urine sample was collected on any day between Days 11 and 13 inclusive. The fourth phase was the second half of the crossover design.

Diet was unrestricted throughout the three studies.

Study II—The study was divided into four consecutive 28-day phases. The first phase was a washout period; a 24-hr urine sample was collected from each subject during the latter part of the 4th week. Each subject received two 1.25-mg tablets of conjugated estrogens daily for 21 days during the last three phases. Each subject received Treatments P2[4], B (generic product), and C (generic product) in a randomized three-way crossover design. Each subject was instructed to select a time for dosing each morning and to take the dose at the same time each morning. On Days 17–19, three consecutive 24-hr urine samples were collected from each subject, beginning at the time of dosing. In accord with the recommended cyclic dosing schedule, no medication was administered during the last 7 days of the last three phases.

Study III—The study design was similar to that of Study II. Six subjects were common to Studies II and III. A shorter washout phase of at least 18 days was allowed for these six subjects. Each subject received Treatments P3[5], P4[6], and P5[7] in a randomized three-way crossover design.

***In Vitro* Tests**—The content and disintegration times of each product and batch were determined for conformity to USP specifications. Sodium estrone sulfate, sodium equilin sulfate, and total conjugated estrogens were determined by the spectrophotometric method described in the USP. Disintegration times were determined by the procedure for plain coated tablets described in the USP (6). The content of nine individual sodium estrogen sulfates was determined by a GLC method (7).

Dissolution half-times were determined utilizing USP Dissolution Method II (8). The dissolution medium was simulated gastric fluid TS, 1000 ml, and the stirring speed was 150 rpm. Based upon preliminary studies, at least six 10-ml samples were withdrawn at times above and below the dissolution half-times for each product or batch. The dissolution medium was not replaced after sampling. Results were determined

[3] Premarin conjugated estrogens tablets USP, batch 108984, Ayerst Laboratories, Montreal, Quebec, Canada.
[4] Premarin conjugated estrogens tablets USP, batch D403 WXA, Ayerst Laboratories, New York, N.Y.
[5] Premarin conjugated estrogens tablets USP, batch E124 BFA, Ayerst Laboratories, New York, N.Y.
[6] Premarin conjugated estrogens tablets USP, batch A176 AFP, Ayerst Laboratories, New York, N.Y.
[7] Premarin conjugated estrogens tablets USP, batch J203 CLB, Ayerst Laboratories, New York, N.Y.

Table II—GLC Analysis of Conjugated Estrogens Tablets USP

Sodium Estrogen Sulfate	Product or Batch, μg/tablet							
	P1	P2	P3	P4	P5	A	B	C
Estrone	715	730	720	687	720	846	806	853
Equilin	308	352	326	327	314	351	348	371
Equilenin	42	37	29	31	38	46	40	46
17α-Estradiol	45	48	46	60	59	11	11	11
17β-Estradiol	11	10	8	10	10	3	2	3
17α-Dihydroequilin	211	220	216	220	214	37	54	53
17β-Dihydroequilin	35	27	31	33	29	4	7	5
17α-Dihydroequilenin	29	20	19	24	23	4	8	12
17β-Dihydroequilenin	9	8	7	9	8	—[a]	—[a]	—[a]
Total	1405	1452	1402	1401	1415	1302	1276	1354

[a] Approximately 1 μg of 17β-dihydroequilenin was present in Products A–C; accurate quantitation was prevented by an interfering peak in the chromatogram.

by interpolation after correction for the drug and dissolution medium removed by sampling.

Assay—Seven estrogens or estrogen metabolites in the urine were determined using enzyme hydrolysis of urine samples, extraction and purification, and GLC (5). An initial version of the method, in which urine samples were frozen prior to assay and the GLC derivatization reagent contained 1% trimethylchlorosilane catalyst, was used for Study I. Studies II and III used the published method, which specifies refrigerated sample storage and a 10% catalyst level.

Aliquots of each 24-hr urine sample from the three studies were assayed for creatinine (9, 10).

Calculations—Urinary excretion differences between treatments were examined based on the 1-day levels (Study I) or on the 3-day mean levels (Studies II and III) of each estrogen (11–13). Attainment of the steady state in Studies II and III was examined by multivariate analysis of the Day 18 minus Day 17 and Day 19 minus Day 18 differences (14). When significant treatment differences were observed in Study II, differences between individual treatments were evaluated by a Student t test, with the Bonferroni adjustment for multiple comparisons.

RESULTS AND DISCUSSION

Tablet Assays—The total conjugated estrogens content of all products (Table I) was within USP specifications (6) and ranged from 1.18 to 1.31 mg/tablet or from 94.4 to 104.8% of the labeled amount. Sodium estrone sulfate ranged from 52.0 to 62.7% of the total conjugated estrogens content in seven products or batches; Product A exceeded the USP specification range (1) of 50.0–65.0% by 1.1%. Sodium equilin sulfate was within USP specifications (1) in all cases and ranged from 26.7 to 29.7% of the total conjugated estrogens content.

The content in all products of nine individual sodium estrogen sulfates and their total was determined by GLC analysis (Table II).

Disintegration and Dissolution—The disintegration times of all products (Table I) were within the 90 min specified by the USP and ranged from 22.5 to 74.5 min. No correlation was observed between the disintegration times and the amounts of total conjugated estrogens excreted per 24 hr at the steady state.

The USP conjugated estrogens tablets monograph does not contain a dissolution rate requirement. A study was performed to investigate the possible relationship between dissolution rate and estrogens excretion. Mean dissolution half-times ranged from 24.5 to 241 min (Table I); a correlation between these values and mean disintegration times was observed ($r = 0.84$, $p < 0.025$). No correlation between dissolution rate and estrogens excretion was observed.

The correlation was based on data obtained from separate disintegration time and dissolution rate experiments. A previous study[8] of the disintegration times of two batches of the innovator's product and four generic products in simulated gastric fluid TS and assay of conjugated estrogens dissolved in this fluid during tablet disintegration demonstrated no significant difference between disintegration times and times to 90% dissolution. These results, combined with those of the present study, indicate that, for the representative products examined, water-soluble sodium estrogen sulfate dissolution is rate limited by disintegration. Since the total conjugated estrogens excretion of all products was essentially equal, as will be discussed in a subsequent section, and individual tablet disintegration times ranged only up to 81 min, the validity of the 90-min USP disintegration time limit (6) in assuring equal extent of absorption could not be examined.

[8] R. N. Johnson and R. P. Neil, Ayerst Laboratories, Rouses Point, N.Y., unpublished results.

Bioinequivalence Method—Bioequivalent drug products are defined (3) as "pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference . . . ." The present experimental design precluded rate measurements. Equal extent of urinary excretion is a necessary, but not sufficient, condition for bioequivalency. However, unequal urinary excretion is a sufficient condition for bioinequivalence. Therefore, the present method permits assessment of bioinequivalence of conjugated estrogens products if those products differ in the extent of absorption.

Improvements in sample storage and analytical procedure, which resulted in the improved recovery values reported previously, were made after Study I. The subject population was different in all three studies, although six subjects were common to Studies II and III. Therefore, absolute values between studies must be compared cautiously. Mean endogenous urinary estrogen levels (Tables III–V) generally were near, but greater than, the estimated minimum quantifiable levels reported previously.

The frequent occurrence of endogenous estrogens below the minimum quantifiable levels in some subjects increased the uncertainty in their estimation. However, the mean values were considered sufficiently reliable for each study to estimate true increases in excretion by subtraction

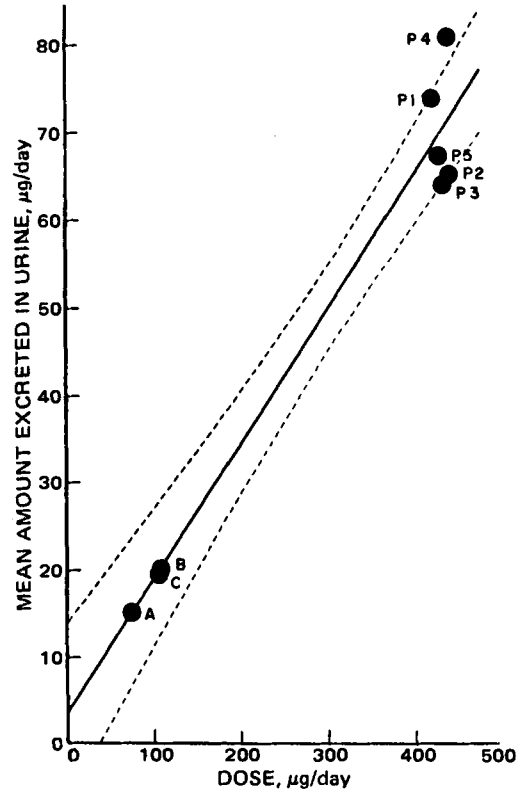

Figure 1—*Correlation between the daily sodium 17α-dihydroequilin sulfate dose and the amount of 17α-dihydroequilenin, expressed as the sodium estrogen sulfate, excreted in the urine per day* ($r = 0.98$, $p < 0.001$) *for each product or batch. Values corrected for endogenous levels. Key: P1–P5, five batches of the innovator's product; A–C, three generic products; ---, 95% confidence limits about the regression line.*

Table III—Mean Steady-State Urinary Estrogen Levels Over 24 hr for 11 Subjects Receiving Treatments P1 and A Conjugated Estrogens Tablets (Study I)

	Sodium Estrogen Sulfate[a]							
	17α-ED	17β-ED	17α-DHEQ	17β-DHEQ	17α-DHEQN	ES	EQ	Total
Mean endogenous levels[b], μg/24 hr	7.0	10.9	1.5	2.0	3.5	10.0	5.9	40.8
Treatment means[b,c], μg/24 hr								
P1	22.8	102.2	23.0	32.3	77.5[d]	339.1	25.0	621.9
A	9.2	86.9	1.5	25.2	18.7[d]	406.7	28.1	576.3
Probability of difference between treatment averages	$p = 0.064$	N.S.[e]	$p < 0.01$	N.S.	$p < 0.001$	$p < 0.05$	N.S.	—

[a] Abbreviations are as follows: 17α-ED, 17α-estradiol; 17β-ED, 17β-estradiol; 17α-DHEQ, 17α-dihydroequilin; 17β-DHEQ, 17β-dihydroequilin; 17α-DHEQN, 17α-dihydroequilenin; ES, estrone; and EQ, equilin. [b] Values are uncorrected for analytical losses (Tables III–V). [c] Values are uncorrected for endogenous levels (Tables III–V). [d] Values represent the data of 10 subjects only. [e] Not statistically significant at $p < 0.05$ (Tables III–V).

Table IV—Mean Steady-State Urinary Estrogen Levels over Three Consecutive 24-hr Periods for 12 Subjects Receiving Treatments P2, B, and C Conjugated Estrogens Tablets (Study II)

	Sodium Estrogen Sulfate[a]							
	17α-ED	17β-ED	17α-DHEQ	17β-DHEQ	17α-DHEQN	ES	EQ	Total[b]
Mean endogenous levels, μg/24 hr	10.0	9.2	1.6	1.3	3.9	8.1	3.4	37.5
Treatment means, μg/24 hr								
P2 Day 17	32.7	65.8	26.6	28.1	72.2	330.2	15.0	549.3
Day 18	30.3	69.1	20.2	25.5	66.5	341.0[c]	13.3[c]	565.7
Day 19	32.0	67.6	25.2	28.5	69.4	319.9[c]	14.5[c]	545.1
Mean	31.7	67.5	24.0	27.4	69.4	330.4	14.3	553.4
B Day 17	19.8	68.4	6.2	26.5	21.5	349.6	11.0	507.5
Day 18	18.2	69.4	3.9	26.8	26.7	353.7	13.0	507.6
Day 19	15.5	78.6	4.1	28.3	24.2	357.5	9.3	523.1
Mean	17.8	72.1	4.7	27.2	24.1	353.6	11.1	512.7
C Day 17	20.0	89.2	6.6	30.4	26.3	427.5	10.0	608.8
Day 18	20.0	85.4	3.4	27.8	24.5	390.2	12.6	566.4
Day 19	21.3	82.0	13.2	25.8	20.0	330.2	9.4	503.1
Mean	20.4	85.5	7.7	28.0	23.6	382.6	10.7	559.4
Probability of differences among treatment averages	$p < 0.01$	$p < 0.01$	$p < 0.01$	N.S.	$p < 0.01$	N.S.	N.S.	—
Student t test[d]								
P2 *versus* B	$p < 0.01$	N.S.	$p < 0.01$	—	$p < 0.01$	—	—	—
P2 *versus* C	$p < 0.01$	$p < 0.05$	$p < 0.01$	—	$p < 0.01$	—	—	—

[a] See footnote *a* of Table III. [b] Totals for the treatment means represent the data of 11 subjects only. [c] Values represent the data of 11 subjects only. [d] With the Bonferroni adjustment for multiple comparisons.

Table V—Mean Steady-State Urinary Estrogen Levels over Three Consecutive 24-hr Periods for Eight Subjects Receiving Treatments P3, P4, and P5 Conjugated Estrogens Tablets (Study III)

	Sodium Estrogen Sulfate[a]							
	17α-ED	17β-ED	17α-DHEQ	17β-DHEQ	17α-DHEQN	ES	EQ	Total
Mean endogenous levels, μg/24 hr	24.6	30.9	—[b]	—[b]	5.1	7.7	5.4	—
Treatment means, μg/24 hr								
P3 Day 17	41.9	81.0	26.5	32.3	70.3	375.4	11.5	639.0
Day 18	46.4	74.7	24.9	27.8	66.5	341.7	12.4	594.3
Day 19	43.4	85.5	24.8	30.0	70.9	379.2	13.6	647.4
Mean	43.9	80.4	25.4	30.0	69.2	365.4	12.5	626.9
P4 Day 17	53.3	82.6	36.7	36.2	85.9	382.9	15.4	692.9
Day 18	45.6	70.2	31.3	31.4	80.0	360.5	10.9	629.8
Day 19	45.6	76.6	32.9	32.8	92.5	369.0	11.7	661.1
Mean	48.2	76.5	33.6	33.5	86.1	370.8	12.7	661.3
P5 Day 17	47.5	89.2	31.1	32.0	73.4	384.6	11.3	669.1
Day 18	48.5	93.2	32.8	33.0	75.1	375.3	12.1	669.9
Day 19	47.1	82.1	33.5	34.7	69.4	401.3	10.5	678.6
Mean	47.7	88.2	32.5	33.2	72.6	387.1	11.3	672.5
Probability of differences among treatment averages	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	—

[a] See footnote *a* of Table III. [b] The 17α-DHEQ region was masked in six of eight Phase I samples; the 17β-DHEQ region was masked in five of eight samples. Correction for endogenous levels is made using the values obtained in Study II.

of endogenous levels. In this manner, absolute comparisons of excreted estrogens as a function of dose could be made for the combined studies. The absence of information about the specific conjugate estrogen forms excreted does not allow determination of pertinent percentage recovery data. Therefore, the values reported in Tables III–V and subsequent values calculated from these data are not corrected for analytical losses.

The primary advantage of steady-state bioinequivalence studies for the conjugated estrogens is the ability to obtain the highest possible estrogen levels in the biological samples with normal doses while adhering to the recommended cyclic drug dosing schedule. Inadequate knowledge of the elimination pathways and their rates for each estrogen prohibited estimating the time of attainment of the steady state upon daily dosing. Therefore, samples were collected late in the normal 21-day dosing cycle, on a single day between Days 16 and 20 inclusive (Study I) or on Days 17–19 (Studies II and III).

The daily changes in excreted amounts of each estrogen or estrogen metabolite (Day 18 minus Day 17 and Day 19 minus Day 18) were not significantly different from zero. Therefore, the steady state was reached by Day 17, and each study was performed under steady-state conditions.

Metabolic Interconversions—Although metabolism studies were not an objective of the present work, the excretion of three of the seven components in greater than dosed amounts permitted tentative assignment of their conjugated estrogens origin. The high levels of 17β-estradiol in the urine appear to reflect the well-established metabolic reduction of estrone in humans (15). The amount of 17β-dihydroequilin found in the urine was essentially constant among all products tested and independent of the 17β-dihydroequilin content of these products. Also, levels of 17β-dihydroequilin in the urine exceeded the dosed amounts of this component in the generic products and the dosed amounts of 17β-dihydroequilenin in all products. The apparently constant excretion suggests

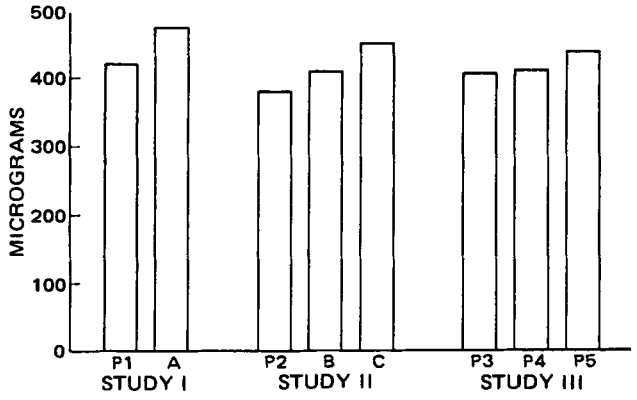

Figure 2—*Summation of estrone and 17β-estradiol, expressed as the sodium estrogen sulfates, excreted in the urine per day at the steady state during daily dosing with two 1.25-mg conjugated estrogens tablets USP. Values are corrected for endogenous levels. Key: see Fig. 1.*

origin from equilin, the tablet content of which was relatively constant for all products. This metabolic conversion is analogous to the reduction of estrone to 17β-estradiol, the only structural difference being the presence of a double bond in the B ring of equilin and 17β-dihydroequilin. The reduction of equilin to 17β-dihydroequilin was observed previously in dogs[9].

The amounts of 17α-dihydroequilenin excreted in the urine are generally greater than the dosed amounts. Possible sources of this compound are 17α-dihydroequilin, equilin, and equilenin. The equilin and equilenin content of all products is relatively constant, whereas the content of 17α-dihydroequilin is much greater in the reference product than in the generic products. A highly significant correlation was observed between the 17α-dihydroequilin dose and the amount of 17α-dihydroequilenin excreted (Fig. 1). Approximately 12% of 17α-dihydroequilin in urine samples is oxidized to 17α-dihydroequilenin during assay (5). This amount would not account for the observed recovery of 17α-dihydroequilenin in the urine and suggests oxidative metabolism of a portion of the dosed 17α-dihydroequilin.

Product Bioinequivalence—The mean endogenous estrogen levels and levels of seven estrogens and estrogen metabolites and their totals excreted during dosing are presented in Tables III–V. Analyses of variance revealed no significant differences in urinary excretion among Treatments P3, P4, and P5 for the seven estrogens (Table V). These three treatments were three batches of the innovator's product manufactured in March, June, and September of 1976 and represented production over a full raw material collection season.

Analyses of variance revealed significant differences in urinary estrogen levels between the innovator's product and the generic products for certain estrogens or estrogen metabolites (Tables III and IV). Levels of

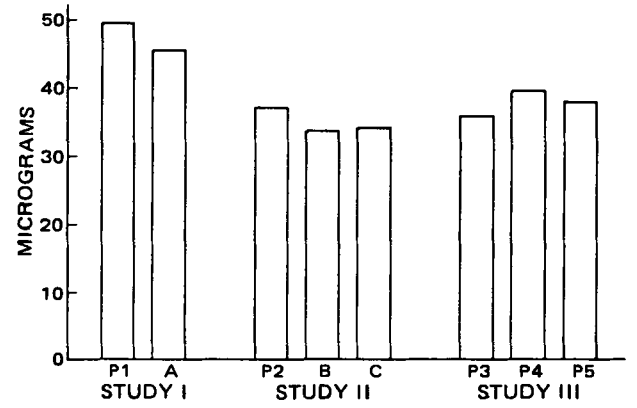

Figure 3—*Summation of equilin and 17β-dihydroequilin, expressed as the sodium estrogen sulfates, excreted in the urine per day at the steady state during daily dosing with two 1.25-mg conjugated estrogens tablets USP. Values are corrected for endogenous levels. Key: see Fig. 1.*

[9] K. Sestanj and D. Dvornik, Ayerst Laboratories, Montreal, Quebec, Canada, unpublished results.

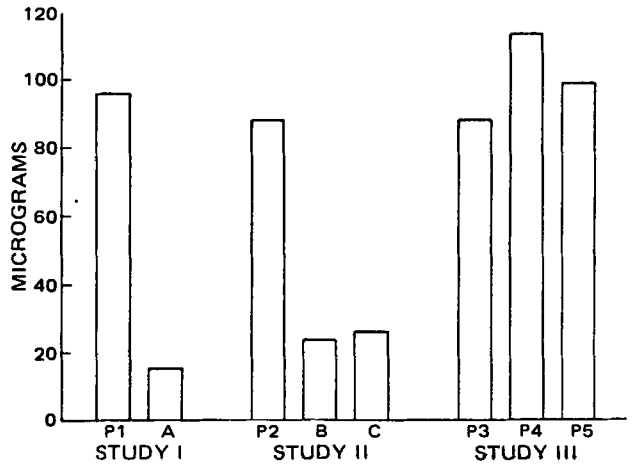

Figure 4—*Summation of 17α-dihydroequilin and 17α-dihydroequilenin, expressed as the sodium estrogen sulfates, excreted in the urine per day at the steady state during daily dosing with two 1.25-mg conjugated estrogens tablets USP. Values are corrected for endogenous levels. Key: see Fig. 1.*

17α-estradiol after dosing with the three generic products were significantly lower than after dosing with the reference product ($p = 0.064$, Treatment A; $p < 0.01$, Treatments B and C). Levels of 17α-dihydroequilin and 17α-dihydroequilenin were also significantly lower with the generic products ($p < 0.01$, Treatments A–C, 17α-dihydroequilin; $p < 0.001$, Treatment A, $p < 0.01$, Treatments B and C, 17α-dihydroequilenin). Levels of estrone were significantly higher after dosing with Treatment A than after dosing with the reference product ($p < 0.05$). Levels of 17β-estradiol were significantly higher after dosing with Treatment C than after dosing with the reference product ($p < 0.05$).

The discussed metabolic relationships provided a basis for separating the urinary excretion of the conjugated estrogens components into groups (Figs. 2–4). Exceedingly small amounts of 17α-estradiol were observed in the urine of a premenopausal woman (16), possibly as a consequence of estrone metabolism. In the present study, the absence of a correlation with dosed estrone, the good correlation with dosed 17α-estradiol, and the mean recovery of only 24.2% of the dose suggest that dosed 17α-estradiol is the source of the excreted compound. Thus, 17α-estradiol was categorized separately (Fig. 5).

Figure 2 indicates relatively small differences between products in the summation of estrone and 17β-estradiol. Of the six instances in which differences between the reference and generic products are possible for these compounds, only two comparisons were statistically significant. Similarly, relatively small differences between products were observed in the equilin plus 17β-dihydroequilin group (Fig. 3). No significant differences between products were observed within this group. In these two groups, variations between the mean urinary estrogen excretion of the reference product and of the generic product(s) within Studies I and II were less than 20%. Similarly, variations between the mean urinary estrogen excretion of the five batches of the reference product and each generic product were less than 20%.

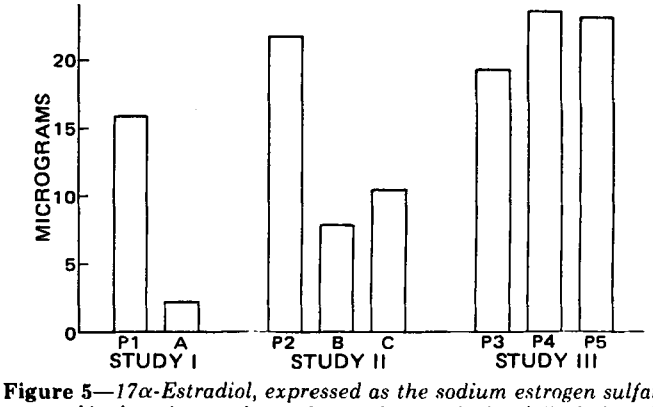

Figure 5—*17α-Estradiol, expressed as the sodium estrogen sulfate, excreted in the urine per day at the steady state during daily dosing with two 1.25-mg conjugated estrogens tablets USP. Values are corrected for endogenous levels. Key: see Fig. 1.*

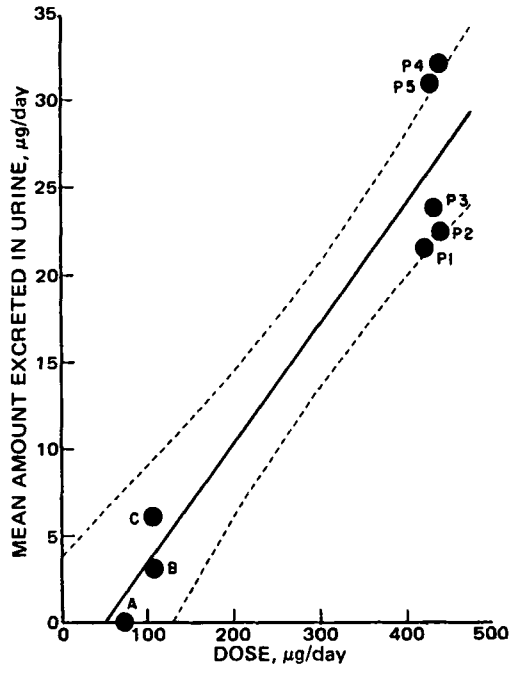

Figure 6—*Correlation between the daily dose of sodium 17α-dihydroequilin sulfate and the amount of 17α-dihydroequilin, expressed as the sodium estrogen sulfate, excreted in the urine per day (r = 0.95, p < 0.001) for each product or batch. Values are corrected for endogenous levels. Key: see Fig. 1.*

Urinary excretion of the 17α-dihydroequilin and 17α-dihydroequilenin group (Fig. 4) and of 17α-estradiol (Fig. 5) was markedly different between the innovator's product and the generic products. In every case, the greater excretion of these components after dosing with the innovator's product was statistically significant compared to dosing with the generic products. The excretion of 17α-dihydroequilin plus 17α-dihydroequilenin of generic Products A, B, and C relative to the reference product was 15.9, 26.5, and 29.4%, respectively. Comparable values for 17α-estradiol excretion from the three generic products relative to the reference product were 13.9, 35.9, and 47.9%, respectively.

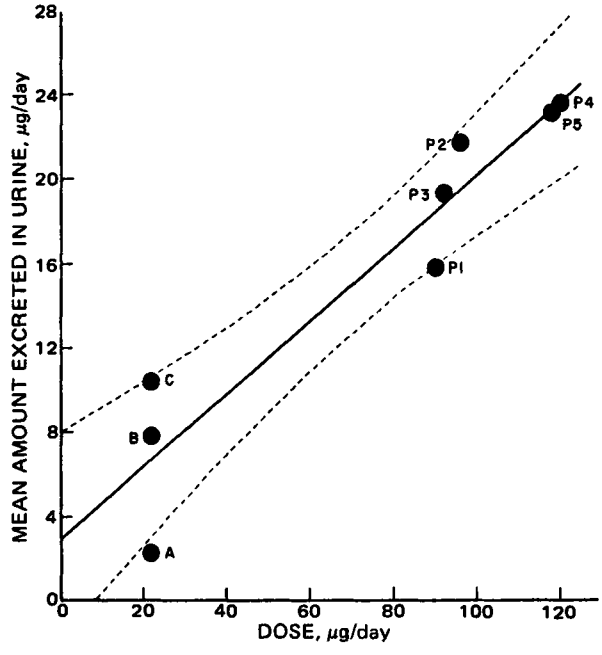

Figure 7—*Correlation between the daily dose of sodium 17α-estradiol sulfate and the amount of 17α-estradiol, expressed as the sodium estrogen sulfate, excreted in the urine per day (r = 0.94, p < 0.001) for each product or batch. Values are corrected for endogenous levels. Key: see Fig. 1.*

If urinary estrogen excretion were a measure of relative extent of absorption, there should be a linear relationship between dose and urinary excretion. This relationship was previously established by demonstrating that at the 1.25- and 2.5-mg dose levels, constant percentages of the individual estrogens were excreted. In the present study, highly significant correlations between the four- to fivefold differences in the dose of 17α-dihydroequilin and 17α-estradiol among the four products and their excretion in the urine were observed (Figs. 6 and 7). These correlations confirm previous findings.

Total estrogen recoveries from all products were similar. Expressed as a percentage of the daily dose and calculated as previously described, the mean total recovery for all treatments was 19.9 ± 1.2 (*SD*) %. The extent of absorption and metabolic fate of the conjugated estrogens are unknown. However, when solutions of 4-^{14}C-estrone sulfate (6-mg dose) and 6,9-^{3}H-equilin sulfate (7-mg dose) were simultaneously ingested by two premenopausal volunteers (21 and 22 years old), a total of 70–88% of the administered radioactivity was recovered in the urine after 5 days[10]. These findings, together with the results of the present study, indicate that these estrogens are both well absorbed and highly metabolized.

The results from Tables III and IV and Figs. 4 and 5 clearly indicate that the three generic conjugated estrogens products studied are bioinequivalent to the innovator's product. This bioinequivalence occurs primarily as a result of large differences in the urinary 17α-hydroxy estrogen levels. As shown in Figs. 6 and 7, the bioinequivalence of the generic conjugated estrogens products relative to the innovator's product is due to differences in estrogens composition among products (Table II). The absence of significant differences between batches of the innovator's product (Table V) is due to the similar composition of each batch. Thus, the bioinequivalence that occurs among conjugated estrogens products is attributable to their pharmaceutical inequivalence. The broad content ranges for sodium estrone sulfate and sodium equilin sulfate and the inadequate specifications for other components suggest the need for more rigorous specifications in the USP conjugated estrogens monograph.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 181, 182.

(2) "Fourth Supplement to USP XIX and NF XIV," Mack Publishing Co., Easton, Pa., Jan. 31, 1978, pp. 74, 75.

(3) *Fed. Regist.*, **42**, 1624 (Jan. 7, 1977), Title 21, subchapter D, part 320.

(4) M. I. Blake, *U.S. Pharmacist*, **3**, 66 (1978).

(5) R. N. Johnson, R. P. Masserano, B. T. Kho, and W. P. Adams, *J. Pharm. Sci.*, **67**, 1218 (1978).

(6) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 182, 183, 650.

(7) R. Johnson, R. Masserano, R. Haring, B. Kho, and G. Schilling, *J. Pharm. Sci.*, **64**, 1007 (1975).

(8) "Fourth Supplement to USP XIX and NF XIV," Mack Publishing Co., Easton, Pa., Jan. 31, 1978, pp. 194, 195.

(9) S. Pino, J. Benotti, and H. Gardyna, *Clin. Chem.*, **11**, 664 (1965).

(10) M. G. Metcalf and E. G. Hunt, *Clin. Biochem.*, **9**, 75 (1976).

(11) J. E. Grizzle, *Biometrics*, **21**, 467 (1965).

(12) P. W. John, "Statistical Design and Analysis of Experiments," Macmillan, New York, N.Y., 1971, pp. 115–117.

(13) B. J. Winer, "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill, New York, N.Y., 1971, pp. 240–244.

(14) D. S. Morrison, "Multivariate Statistical Methods," 2nd ed., McGraw-Hill, New York, N.Y., 1976, chap. 5.

(15) C. Longcope, D. S. Layne, and J. F. Tait, *J. Clin. Invest.*, **47**, 93 (1968).

(16) H. Adlercreutz, M. J. Tikkanen, and D. H. Hunneman, *J. Steroid Biochem.*, **5**, 211 (1974).

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Mr. C. F. Boudreau and Mr. M. T. Gaffney with the statistical analyses, Mr. N. G. Nash with the tablet dissolution studies, Ms. S. S. MackNair and Mrs. I. B. Matott with clinical aspects of the studies, and Dr. J. B. Southwick with medical supervision of the subjects.

[10] K. Sestanj, E. Langenbach, and D. Dvornik, Ayerst Laboratories, Montreal, Quebec, Canada, unpublished results.