enhancing effect could be such that most of the effect is elicited within a few minutes. Alternatively, the surfactant may promote better interfacial contact and thus increase the effective surface area of the membrane. Studies are now being initiated to determine if immersion of the fish in surfactant solutions for various periods of time will affect the rate of absorption of secobarbital upon subsequent immersion of the fish in secobarbital solutions without surfactant. This should establish whether or not the surfactant promotes drug absorption by modifying the barrier properties of the biologic membranes. However, the present investigation has shown already that the effect of polysorbate 80 on drug absorption is a function of the drug and of surfactant concentrations, and that an effect of polysorbate 80 concentrations above the CMC can represent the net result of absorption enhancement and retardation.

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Comparative Absorption of Micronized and Nonmicronized Medroxyprogesterone Acetate in Man

By DAVID L. SMITH, ALBERT L. PULLIAM, and ARLINGTON A. FORIST

A specific, sensitive method has been developed for the analysis in urine of microgram quantities of the principal urinary metabolite of medroxyprogesterone acetate. The method consists essentially of the following steps: hydrolysis with β-glucuronidase, extraction with chloroform, Florisil column chromatography, thin-layer silica gel chromatography, and measurement of either ultraviolet absorption or fluorescence resulting from sulfuric acid treatment. The method has been used to compare the gastrointestinal absorption of medroxyprogesterone acetate from tablets containing either 10 mg. of micronized or nonmicronized medroxyprogesterone acetate. The 8-hr. excretion of metabolite following oral ingestion of the tablets by normal adult humans was employed as the measure of absorption. The increased metabolite output, resulting from the tablet prepared from micronized medroxyprogesterone acetate, was very highly significant ($p < 0.001$). Ten subjects in a crossover study excreted an average of 2.23 ± 0.19 (S.E.M.) times as much metabolite in 8 hr. after ingesting the micronized formulation as they did after ingesting the nonmicronized one.

THIS STUDY was undertaken to develop an analytical method for the purpose of determining whether a tablet prepared from 10 mg. of micronized medroxyprogesterone acetate[1] (I) would afford a significant increase in absorption compared to a tablet prepared from nonmicronized material. Since medroxyprogesterone acetate has very low solubility in water (~0.3 mg./100 ml. at 37°), its gastrointestinal absorption may be limited by its gastrointestinal dissolution rate; reducing its particle size, therefore, might be expected to increase its physiologic availability (1–4). Helmreich and Huseby (5), who employed doses of 50–200 mg. of medroxyprogesterone acetate, have already noted that particle size reduction might influence its absorption efficiency.

Helmreich and Huseby (6) identified the principal urinary metabolite of medroxyprogesterone acetate as 6β,17α,21-trihydroxy-6-methyl-pregn-4-ene-3,20-dione,17-acetate (II). Others (7) have reported it to be the 21-acetate (III). This metabolite, which is excreted in the human as a glucuronide, accounts for approximately one-half of the total drug-related material excreted in the urine (5). The 24-hr. urinary output in the human was found to range from about 4–8% of a 200-mg.

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[1] Marketed as Provera by The Upjohn Co., Kalamazoo, Mich.

dose with more than 80% of medroxyprogesterone acetate-related material excreted in the feces (5). These data alone do not indicate poor absorption, however, since biliary excretion of medroxyprogesterone acetate and/or its metabolites is extensive even after intravenous administration of medroxyprogesterone acetate (8).

The approach of Helmreich and Huseby (5), who measured the amount of the major urinary metabolite excreted in the urine, seemed to be the most practical measure of absorption. A comparison of the absorption of medroxyprogesterone acetate at the 10-mg. dose level by measurement of the metabolite excreted in the urine required a very sensitive analytical method. The method developed is an extension of previously described procedures (5, 9, 10) and consists essentially of the following steps: (*a*) hydrolysis with β-glucuronidase, (*b*) extraction with chloroform, (*c*) Florisil[2] column chromatography, (*d*) thin-layer silica gel chromatography, and (*e*) measurement of either the specific ultraviolet absorption or fluorescence resulting from sulfuric acid treatment.

EXPERIMENTAL

Test Tablets.—Both tablets contained 10 mg. of medroxyprogesterone acetate and 0.05 mg. of ethinyl estradiol,[3] except that in one of them the medroxyprogesterone acetate was micronized. The micronized medroxyprogesterone acetate, which was used in the preparation of the compressed tablet, had a size distribution so that 99.9% of the particles were smaller than 10 μ diameter. Micronized and nonmicronized medroxyprogesterone acetate possessed specific surface areas, prior to preparation of the tablets, of approximately 7.4 $M.^2$/Gm. and 1.2 $M.^2$/Gm., respectively. Both of the formulations contained the other excipients of the commercial tablet.

Subjects and Conduct of Absorption Tests.—The tablets were ingested orally by normal female (postmenopausal) volunteers after an overnight fast; no food was taken until 2 hr. after administration of the tablet, and fluid intake was controlled. The ages and weights of the subjects are given in Tables II and III. Two tests were conducted: 1 tablet nonmicronized *versus* 1/2 tablet nonmicronized and, several months later, 1 tablet micronized *versus* 1 tablet nonmicronized. Both tests were of the crossover type, and required 2 weeks each. On day 1 of each week, control urine (8 a.m. to 4 p.m.) was collected and on day 2 one of the test tablets was ingested at 8 a.m. and urine collected from 8 a.m. to 4 p.m. The urine samples from each subject were randomly coded, but were assayed in parallel to avoid complicating the results with possible day-to-day assay variations.

Analytical Procedure.—The procedure used for the determination of the 6,21-dihydroxy metabolite of medroxyprogesterone acetate differs from the Helmreich and Huseby procedure (5) primarily in that it employs thin-layer chromatography.

Six milliliters of 0.5 *M* pH 4.7 acetate buffer was added to 60 ml. of the urine to be examined in a 100-ml. volumetric flask, and 15 ml. of 5000 units β-glucuronidase/ml.[4] added. The flask was placed in a constant-temperature bath at 47° for 18 hr. The hydrolyzed urine was transferred to a 250-ml. separator and extracted twice with 100 ml. of $CHCl_3$ as follows. The funnel was shaken vigorously for 1 min. and then allowed to stand for 1 hr.; the remaining emulsion was broken by gently swirling the separator. The $CHCl_3$ extracts were drained into a second 250-ml. separator, and the combined extracts washed with 20 ml. of 0.1 *N* NaOH and 20 ml. of 0.1 *N* HCl. Five minutes were allowed for each wash. The washed extract was drained into a 250-ml. beaker and evaporated to dryness under nitrogen at temperatures below 45°. The residue was dissolved in 5 ml. of $CHCl_3$ and transferred quantitatively to a previously prepared Florisil column (see below). The column was eluted with 25 ml. of $CHCl_3$, 25 ml. of 2% CH_3OH in $CHCl_3$, and 50 ml. of 25% CH_3OH in $CHCl_3$. The 25% CH_3OH–$CHCl_3$ fraction was collected in a 50-ml. wide-mouth centrifuge tube and evaporated to dryness under nitrogen at temperatures below 45°. The centrifuge tube was washed down with 5 ml. $CHCl_3$, 3 ml. $CHCl_3$, and 1 ml. $CHCl_3$, evaporating to dryness after each wash. The residue was dissolved in a small volume of $CHCl_3$ and quantitatively transferred to a fluorescent, 20 × 20 cm. Silica Gel G thin-layer plate of 250 μ thickness. The plate was developed to its full length with $CHCl_3$–C_2H_5OH (17:3), and the metabolite zone located by fluorescence quenching. The silica gel corresponding to the location of the metabolite was removed from the plate and transferred to a 35-ml. centrifuge tube. The metabolite was eluted from the silica gel by shaking with two 10-ml. portions of absolute methanol; after centrifuging, each eluate was decanted into a 50-ml. beaker. The combined eluates were evaporated to dryness under nitrogen at temperatures below 45°. Exactly 0.5 ml. of absolute methanol was added to the residue followed by exactly 4.5 ml. of 70% H_2SO_4. After the solution had stood for 30 min., the ultraviolet spectrum was recorded from 340–380 mμ with a Cary spectrophotometer.

If the fluorescence end point was to be used, the sulfuric acid solution was allowed to stand for 3 hr., and then with a precalibrated spectrophotofluorometer the fluorescence intensity at 535 mμ[5] resulting from activation at 465 mμ[5] was recorded using an Aminco-Bowman spectrophotofluorometer. The metabolite concentration in the urine and the total output were calculated from the appropriate standard curve, taking into consideration the total urine volume and dilution and yield factors. In the present study, an assay yield of 65.7 ± 2.2% was obtained. Correction for the "blank" was

[2] Florisil is a magnesium silica gel adsorbent manufactured by the Floridin Co., Tallahassee, Fla.

[3] This combination of medroxyprogesterone acetate and ethinyl estradiol is marketed as Provest by The Upjohn Co., Kalamazoo, Mich.

[4] Marketed as Ketodase by Warner-Chilcott.

[5] The fluorescence and activation wavelengths are uncorrected. (*Cf. Reference 15*, Chap. 4, p. 121.)

TABLE I.—FLUOROMETRIC AND ULTRAVIOLET RESPONSE OF THE 21-ACETATE (III) UPON TREATMENT WITH 63% SULFURIC ACID

Concn., mcg./ml.	Florescence at 535 mμ (F)[a] × Scale Rdg. × Sens. Factor	F/mcg./ml.	Absorbance 340 mμ	Absorbance 360 mμ	Absorbance 380 mμ	A_{Allen}	A_{Allen}/mcg./ml.
0.000	0.0000		0.000	0.000	0.000	0.000	
0.005	0.0075	1.50					
0.010	0.0155	1.55					
0.025	0.0369	1.48					
0.035	0.0556	1.59					
0.050	0.0769	1.54					
0.100	0.174	1.74					
0.250	0.435	1.74					
0.350	0.568	1.62					
0.500	0.747	1.49					
1.070	1.77	1.65	0.043	0.068	0.030	0.031	0.0290
3.250	5.35	1.65	0.135	0.202	0.082	0.093	0.0286
5.370	8.59	1.60	0.220	0.340	0.140	0.160	0.0298
7.490	11.0	1.47	0.305	0.470	0.190	0.222	0.0296
10.700	16.5	1.54	0.438	0.670	0.272	0.315	0.0294
	Mean	1.58					0.0293
	S.D.	±0.09					±0.00048
	% S.D.	±5.7%					±1.6%

[a] Activation: 465 mμ. Corrected for a blank of 0.015.

made by subtracting the result obtained with a pretreatment urine sample.

Preparation of the Florisil Column.—The Florisil was washed and activated as follows: 1 Kg. of Florisil (60–100 mesh) was washed by shaking sequentially with 2 L. of 95% ethanol for 1–2 hr., 2 L. of 25% CH_3OH—$CHCl_3$ for 2 hr., and 2 L. of fresh 25% CH_3OH–$CHCl_3$ overnight. The Florisil was then filtered and washed twice with 1-L. portions of absolute ethanol, dried, and then activated at 600° for 4 hr. The Florisil columns were prepared by packing 25-ml. burets (11 mm. diameter), plugged with glass wool, to a height of 7 cm. with gentle tapping. The columns were eluted with 50 ml. of $CHCl_3$ before adding the samples.

RESULTS

Analytical End Points for the 6,21-Dihydroxy Metabolite.—Since the 17-acetate (II) was not available for calibration at the outset of this study, the 21-acetate (III) was used. When II was synthesized, it was shown to have a response equivalent to III and IV in the end points used. This is in agreement with the results of Zaffaroni (11), who found that free steroids and their acetates gave identical sulfuric acid-induced absorption spectra.

Sulfuric Acid-Induced Ultraviolet Absorption.—Like many steroids (12), II, III, and their hydrolysis product (IV) exhibit an induced ultraviolet absorption upon treatment with concentrated sulfuric acid. Sulfuric acid-induced absorption has comparable sensitivity to the method of Porter and Silber (13), and is more specific for II and III. The 63% sulfuric acid-induced ultraviolet absorption of III at 360 mμ reaches a maximum value within 30 min. and is stable for at least 2 hr. When corrected for background by the Allen method (14), the ultraviolet absorption is linear from 1–10 mcg./ml. with a relative standard deviation of 1.6% (Table I). The Allen absorbance is defined by the equation:

$$A_{Allen} = A_{360m\mu} - {}^1/_2 (A_{380m\mu} + A_{340m\mu}) \quad \text{(Eq. 1)}$$

This correction, which is designed to eliminate the effects of linear background absorption due to impurities, yields a blank which is essentially independent of the urine volume and increases the selectivity of the end point. The induction of ultraviolet absorption by concentrated sulfuric acid in a solution of the metabolite, which was isolated from urine of the medroxyprogesterone acetate-treated subject (*vide infra*), was found to have a relative standard deviation of 1.7% at the 3 mcg./ml. level.

Sulfuric Acid-Induced Fluorescence.—Concentrated sulfuric acid also induces in compounds II, III, and IV an intense fluorescence maximum at 535 mμ when activated at 465 mμ. In this respect, they are similar to corticosterone (15). The 63% sulfuric acid-induced fluorescence response of III is linear from 0.005–10.7 mcg./ml. with a relative standard deviation of 5.7% (Table I). Replicate determinations of the production of fluorescence by the metabolite, which was isolated from the urine of a medroxyprogesterone acetate-treated subject, showed a relative standard deviation of 4.9% at the 3 mcg./ml. level. The fluorescence reaches a maximum in about 2 hr. and is stable for at least an additional 2 hr. The Allen correction could not be employed using the fluorescence end point because the impurities showed a nonlinear wavelength response.

Isolation of the Metabolite from Urine.—Various amounts of III were added to the urine of a normal, nontreated, female subject and carried through the procedure of Helmreich and Huseby (5). III could not be detected in the 25% CH_3OH–$CHCl_3$ fraction from the Florisil column; instead, all of it appeared in the 2% CH_3OH–$CHCl_3$ fraction in which the fluorescence response was directly proportional to the amount of III added to the urine. This result indicated that the metabolite was not III, as reported by Castegnaro and Sala (7), since the 25%

CH_3OH–$CHCl_3$ fraction contains by far the major portion of urinary metabolite.

Since III did not behave like the metabolite in the Helmreich and Huseby isolation procedure, it obviously could not be used to test or modify this procedure. Consequently, a 24-hr. urine collection from a female patient, who had been receiving 200 mg. of medroxyprogesterone acetate daily for 1 week, was obtained as a source of the metabolite.[6] This urine will be referred to as the "medroxyprogesterone acetate urine." Aliquots (0, 1, 2, 4, 6, and 10 ml.) of this urine were added to aliquots of a urine sample from a normal, nontreated female to give a total volume of 30 ml. Using the Helmreich and Huseby procedure, both fractions from the Florisil column were examined for the metabolite. By both the fluorescence and ultraviolet end points, the response of both the 2 and 25% CH_3OH–$CHCl_3$ fractions were proportional to the volume of medroxyprogesterone acetate urine added. The major portion of the responding material (90% by ultraviolet; 80% by fluorescence) was found in the 25% CH_3OH–$CHCl_3$ fraction. The results of these preliminary experiments with the Helmreich and Huseby procedure were so encouraging it was decided to test the procedure by assaying the 8-hr. urine samples from 5 subjects who had received either 1 or one-half tablet of the nonmicronized formulation. Employing duplicate assays, the increased urinary output of the metabolite resulting from 1 tablet over and above that from one-half tablet was just significant at the 95% confidence level. Although these results were encouraging, the Allen absorbance averaged only 0.022 at the 10-mg. medroxyprogesterone acetate dose level. These results indicated that although the Helmreich and Huseby procedure was applicable to the determination of the 6,21-dihydroxy metabolite at the low levels resulting from 10 mg. of medroxyprogesterone acetate, its sensitivity would have to be increased in order to statistically demonstrate small absorption differences.

Addition of Thin-Layer Chromatography (TLC) to the Helmreich and Huseby Procedure.—In an attempt to reduce the response of the blank, TLC was added to the procedure following the Florisil chromatography. After the 25% CH_3OH–$CHCl_3$ eluate was evaporated to dryness, the residue was dissolved in a small volume of $CHCl_3$, quantitatively transferred to a thin-layer plate, and developed with $CHCl_3$–C_2H_5OH (17:3). With this developing system the metabolite has an R_f of about 0.7 and moves ahead of most of the urinary components in the extract. In order to locate the position of the metabolite on the TLC plate and to study the effect of TLC on the procedure, aliquots of the medroxyprogesterone acetate urine were added to aliquots of a urine sample from a normal, nontreated female to give a total volume of 30 ml. These spiked samples were assayed essentially as before but with the addition of TLC to the procedure. Although at least ten TLC zones were observed in the 25% CH_3OH–$CHCl_3$ fraction, the density of one zone clearly increased with the volume of the medroxyprogesterone acetate urine added. Sections of the TLC plate were eluted with methanol, and assayed by measuring the ultraviolet absorption at 360 mμ following addition of 63% sulfuric acid. The metabolite zone produced 94% of the total absorption at 360 mμ. Another zone, whose R_f corresponded to the alcohol (IV), accounted for the remaining 6%. The addition of TLC to the procedure increased its specificity and lowered the background considerably, but decreased the over-all yield by about 20%.

Further Efforts to Increase Sensitivity and Assay Yield.—Further experiments were carried out with the medroxyprogesterone acetate urine in an attempt to improve the sensitivity of the procedure, *e.g.*, the extracting solvent, its volume, extraction time, number of extractions, and agitation method were varied in an attempt to increase yield and precision. It was found, for example, that an extraction method employing vigorous shaking by hand followed by allowing the emulsion to stand for 1 hr. gave higher yields and better precision than the use of an automatic wrist-action shaker.

Florisil chromatography decreases considerably the blank contribution from nonspecific background material (determined by the fluorescence end point or total ultraviolet absorption at 360 mμ) but does not remove any of the fluorescence-quenching TLC zones, *i.e.*, the chloroform extract and the 25% CH_3OH–$CHCl_3$ fraction show the same TLC pattern.

The yield of metabolite was increased by decreasing the contact time between the extracting solvent and the NaOH and HCl wash solutions. In the final procedure, the contact time of these wash steps was strictly controlled and minimized.

A relatively high concentration of β-glucuronidase was used in the present studies to insure complete hydrolysis. The 18-hr. incubation with β-glucuronidase at 47° was sufficient for complete hydrolysis, *e.g.*, 18 and 42 hr. incubation gave the same end point response.

The use of the total ultraviolet absorption at 360 mμ was investigated in an attempt to increase sensitivity. As shown by the data of Table I, the use of the Allen correction decreases the sensitivity by one-half. Because of variation in the absolute ultraviolet absorbance of the blanks at 360 mμ, however, the ultraviolet results were poor when the Allen correction was not used.

Washing and activating the Florisil decreased the total ultraviolet absorption of the blank considerably, but did not affect the assay yield.

The addition of TLC to the procedure and the optimization of other variables resulted in a three to fourfold increase in the sensitivity of the procedure. The assay yield was determined by adding the metabolite to water and performing the entire procedure. The yield determined in this manner was 65.7 ± 2.2%. Considering the complexity of the procedure, the yield and precision were considered quite satisfactory.

Stability of the Metabolite.—Originally, it had been intended to determine the yield every day that a set of assays were run by the use of a parallel yield determination, *i.e.*, by adding aliquots of a "standard solution" of the metabolite in methanol to control urine. In fact, this procedure was followed for some time until it was discovered that the yield was actually decreasing with time. TLC of the methanol solution of metabolite, which had been standing for some time, showed three zones,

[6] Dr. R. A. Huseby, American Medical Center, Denver, Colo., supplied this sample.

TABLE II.—8-hr. URINARY EXCRETION OF 6,21-DIHYDROXY METABOLITE OF MEDROXYPROGESTERONE ACETATE FOLLOWING INGESTION OF EITHER 1 OR 0.5 TABLET OF THE NONMICRONIZED FORMULATION (BOTH END POINTS USED)

Volunteer Code	Week 1 Tablet	Week 1 mcg. Excreted/8 hr. U.V.	Week 1 mcg. Excreted/8 hr. Fluor.	Week 2 Tablet	Week 2 mcg. Excreted/8 hr. U.V.	Week 2 mcg. Excreted/8 hr. Fluor.	Ratio 1 Tablet/0.5 Tablet U.V.	Ratio 1 Tablet/0.5 Tablet Fluor.
G(49, 80)[a]	1	247	204	0.5	79	63	3.13	3.24
J(63, 57)	1	325	298	0.5	134	134	2.43	2.22
C(61, 83)	1	286	357	0.5	115	124	2.49	2.88
S(60, 55)	0.5	87	180	1	298	570	3.43	3.17
L(51, 56)	0.5	192	170	1	270	257	1.41	1.51
						Mean	2.58	2.60
						S.E.M.	±0.35	±0.32
						95% C.I.	±0.97	±0.89

[a] Age and weight (Kg.), respectively.

whereas the fresh solution had shown only one. In four TLC systems the decomposition products of the metabolite possessed R_f values identical to III and IV. The sum of the absorbances of the three zones, as determined by sulfuric acid-induced ultraviolet absorbance at 360 mμ, remained constant, *i.e.*, the appearance of the zones corresponding to III and IV was accompanied by a corresponding decrease in II. The half-life of disappearance of the metabolite in methanol at room temperature is estimated to be about 3 days. No significant decomposition of II takes place during the analytical procedure used in the present study.

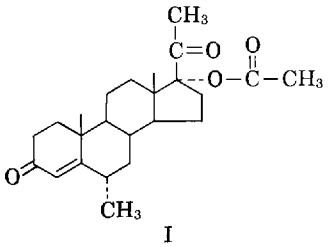
Evaluation of Metabolite Excretion as a Measure of Absorption (One Tablet versus One-Half Tablet).—To test the modified analytical procedure and the hypothesis that the amount of metabolite excreted in the urine is directly related to the amount of medroxyprogesterone acetate absorbed, urine samples were assayed from 5 subjects who had received 1 tablet and one-half tablet of the nonmicronized formulation in a crossover design (Table II). The assay easily distinguished between no drug, one-half tablet and one tablet. Both fluorescence and ultraviolet absorption were employed as the end points. With the modified procedure and using only single determinations, both the ultraviolet and fluorescence end points gave an 8-hr. output of metabolite for every subject in the order: $0 < 0.5 < 1$ tablet. The increased urinary output of the 6,21-dihydroxy metabolite resulting from 1 tablet over and above that from one-half tablet was statistically significant at the 99% confidence level by both end points, when calculated by averaging the ratios obtained for each subject. The average amount of metabolite excreted in the urine in 8 hr. following one-half tablet of the nonmicronized formulation was 121 ± 20 mcg. and 134 ± 64 mcg. (±S.E.M.) by the ultraviolet and fluorescence end point, respectively. The average amount of metabolite excreted in the urine in 8 hr. following 1 tablet of the nonmicronized formulation was 285 ± 13 and 337 ± 21 mcg. (±S.E.M.) by the ultraviolet and fluorescence end points, respectively.

Comparison of Excretion of Metabolite after Ingesting the Micronized and Nonmicronized Formulations.—Each of the 10 subjects excreted a larger amount of metabolite after ingestion of the micronized tablet (Table III). Only the ultraviolet end point was employed, since the sensitivity of the fluorescence end point was not required. There was a very highly significant difference ($p < 0.001$) between the nonmicronized and micronized formulations. The average micronized/nonmicronized ratio ±95% confidence limits of metabolite excreted was 2.23 ± 0.43, when calculated by averaging the individual ratios. The micronized/nonmicronized ratio ±95% confidence limits, calculated from the average amount of metabolite excreted in 8 hr., was 2.12 ± 0.57. The average amount of metabo-

TABLE III.—8-hr. URINARY EXCRETION OF 6,21-DIHYDROXY METABOLITE OF MEDROXYPROGESTERONE ACETATE FOLLOWING INGESTION OF MICRONIZED AND NONMICRONIZED TABLETS

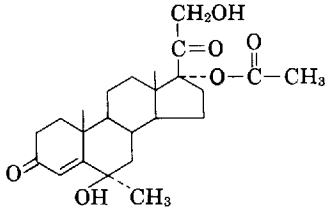
Volunteer Code	Week 1 Tablet	Week 1 mcg. Excreted/8 hr.	Week 2 Tablet	Week 2 mcg. Excreted/8 hr.	Ratio Micronized/Nonmicronized
A(64, 66)[a]	Nonmicronized	244	Micronized	778	3.19
B(61, 54)	...	351	...	915	2.61
C(60, 59)	...	278	...	757	2.72
D(63, 89)	...	176	...	468	2.66
E[62, 80)	...	243	...	596	2.45
F(59, 77)	Micronized	643	Nonmicronized	283	2.27
G(46, 77)	...	532	...	414	1.29
H(52, 65)	...	634	...	515	1.53
I(60, 68)	...	796	...	472	1.69
J(55, 72)	...	478	...	248	1.93
				Mean	2.23
				S.D.	±0.60
				S.E.M.	±0.19

[a] Age and weight (Kg.), respectively.



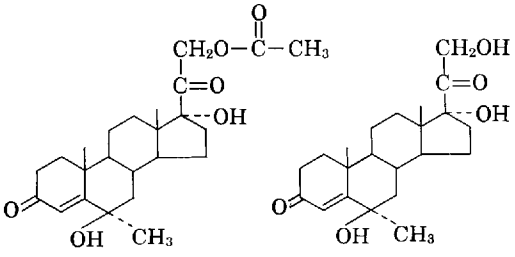
I

Medroxyprogesterone Acetate



II

(17-Acetate)



III IV

(21-Acetate)

lite excreted by the 10 subjects in 8 hr. was 312 ± 30 mcg. (S.E.M.) and 660 ± 47 mcg. following the nonmicronized and micronized formulations, respectively (3.1 and 6.6% of the dose, respectively).

DISCUSSION

Ten subjects were employed in a crossover design in order to minimize the effect of possible week-to-week variables, *e.g.*, a change in the percentage of drug which is converted to the measured metabolite or a change in the percentage of drug absorbed independent of formulation differences. The increase, therefore, in the quantity of metabolite excreted in the urine in 8 hr. after ingesting the micronized formulation undoubtedly results from increased absorption. The applicability of the procedure used was demonstrated by showing that an average of about twice as much metabolite was excreted in 8 hr. after ingesting 1 tablet as after one-half tablet.

The greater urinary excretion of the metabolite during the first 8 hr. following ingestion of micronized medroxyprogesterone acetate must result from the increased rate of absorption of medroxyprogesterone acetate from this formulation. Because of the finite transit time of drug through the gastrointestinal tract, this increased 8-hr. excretion of metabolite very likely reflects an increase in the eventual total amount absorbed. The latter interpretation is based on the data of Helmreich and Huseby, who found that even at doses 20 times greater than used in the present study, an average of about 70% of the 24-hr. excretion of metabolite occurred during the first 8 hr.

The metabolite is not the 21-acetate (III), as reported by Castegnaro and Sala (7), since the latter possesses different TLC R_f values from the metabolite in four systems and, unlike the metabolite, is eluted from the Florisil column in the 2% CH_3OH–$CHCl_3$ fraction. When the 17-acetate (II) was subsequently synthesized by an unambiguous method (16), it was shown to undergo the 17- to 21-acetate migration in methanol and to have the same R_f values as the metabolite in several systems, thus substantiating the 17-acetate assignment of Helmreich and Huseby (6). The observed conversion of II to III probably explains why Castegnaro and Sala identified the metabolite as the 21-acetate. Since the latter workers also found the metabolite in the 25% CH_3OH–$CHCl_3$ fraction of the Florisil column, the acetate group migration must have occurred after Florisil chromatography. This acetate group migration is not unique with the metabolite. Similar behavior has been noted by Gardi *et al.* (17), who observed that corticosteroid 17-monoesters could be rearranged quantitatively to the corresponding 21-monoesters.

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