# Isolation and Characterization of Some Solid Phases of Fluprednisolone

JOHN K. HALEBLIAN*, ROBERT T. KODA, and JOHN A. BILES

**Abstract** □ Seven different solid phases of fluprednisolone were isolated. Six were crystalline and one was amorphous. Of the crystalline phases, three were anhydrous, two were monohydrates, and one was a *tert*-butylamine disolvate. The phases were characterized using IR spectroscopy, X-ray powder diffraction, melting points, and densities. The heats of fusion were determined using differential scanning calorimetry. Heats of solution in water for three phases were also determined.

**Keyphrases** □ Fluprednisolone, solid phases—isolation, characterization □ Polymorphism—fluprednisolone □ IR spectrophotometry—identification, fluprednisolone solid phase □ X-ray powder diffraction—identification, fluprednisolone solid phase □ Differential scanning calorimetry—identification, fluprednisolone solid phase

Many drug molecules have been found to exist in more than one crystalline form. These crystalline modifications may have sufficient differences in their physical and thermodynamic properties that stability and biological availability may be affected. The importance of polymorphism on the therapeutic effectiveness of a drug is well recognized and previously was reported in the literature (1, 2).

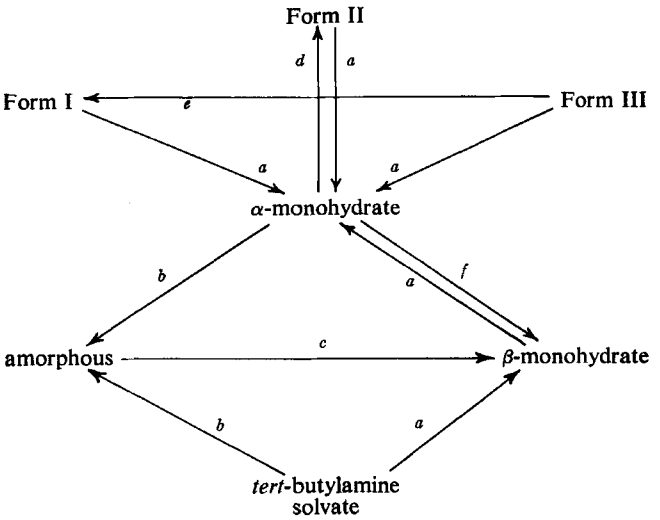
Fluprednisolone[1], 6α-fluoro-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione, was selected as the drug for study since there was evidence that this drug could be isolated in several crystalline phases[2]. This report describes the methods used in the isolation and characterization of some crystalline modifications of fluprednisolone.

## EXPERIMENTAL

All X-ray powder diffractions were run on a spectrometer[3] adapted for X-ray diffraction, using copper Kα radiation[4] with a 1° beam slit and a 0.2° detector slit. All IR spectra were run as mineral oil mulls between sodium chloride windows on a spectrophotometer[5].

Heats of fusion were determined on a differential scanning calorimeter[6], using high temperature operation with a scan speed of 10°/min. Melting points were determined using either the differential scanning calorimeter or a microscope equipped with a micro hot-stage apparatus (Kofler).

The solubility of each crystalline phase was determined in water and *n*-octanol at temperatures between 0 and 60°. An excess of each form of the drug was added to the solvent in a 120-ml. bottle. The stoppered bottle was placed in an apparatus (Wruble) and was allowed to reach equilibrium at constant temperature (±0.1°). Equilibrium was considered established when the concentration of the steroid in solution remained constant for a 48-hr. period. Samples were withdrawn using a preheated syringe and filtered through an adapter (Sweeney), using a 0.22-μ Millipore filter. Aqueous solutions were assayed using a spectrophotometer (Beckman-Gilford) at 241.5 nm. Solutions of the steroid in *n*-octanol were assayed using a spectrophotometer (Beckman DB) at 241.5 nm.




*a* = suspension in water
*b* = 138° under vacuum (2 mm. Hg)
*c* = pressure
*d* = 180° for 5 min.
*e* = standing in air
*f* = aqueous suspension at 80° and seeded with β-monohydrate

*Scheme I—Phase interconversions of the various polymorphs of fluprednisolone*

The amount of solvent bound to the various crystalline phases of fluprednisolone was determined by either the Karl-Fischer method (for hydrates), weight loss studies, or the determination of molar absorbancies.

Densities of the crystalline phases were determined by the method of Bernal and Crowfoot (3). A few crystals of the steroid were suspended in mineral oil. Carbon tetrachloride was carefully added to the mineral oil–steroid mixture until a stable suspension of the crystalline phase existed, even after prolonged centrifugation. At this point, the density of the crystalline material was assumed to be identical to the mineral oil–carbon tetrachloride mixture. The density of the liquid phase was determined using a pycnometer.

**Isolation of Phases**—Fluprednisolone was crystallized from solutions of various solvents under different conditions. For example, Form I was isolated using the following procedures: (*a*) evaporation of a solution of fluprednisolone in ethyl acetate at room temperature and collection of the columnar crystals adhering to the sides of the beaker, (*b*) evaporation of an acetone solution of fluprednisolone on a hot plate, and (*c*) crystallization by cooling a hot saturated solution of fluprednisolone in anhydrous methanol in a sealed flask. Form II was obtained by heating the α-monohydrate at 180° for 5 min. on the stage of a melting-point apparatus (Fisher-Johns). Cooling of a hot saturated solution of fluprednisolone in acetone in a closed flask gave Form III. After crystallization occurred, the supernatant layer was decanted and the crystals were allowed to air dry at room temperature. If, on the other hand, an acetone solution of fluprednisolone was allowed to evaporate at room temperature in an open system, a mixture of Form III and the β-monohydrate was obtained. The anhydrous nature of Forms I, II, and III was verified by: (*a*) heating each of the phases in a fluid[7] and observing for the absence of effervescence, (*b*) absence of

[1] Marketed as Alphadrol by The Upjohn Co., Kalamazoo, Mich.
[2] Preliminary investigation by J. A. Biles.
[3] General Electric XRD-5.
[4] λ = 1.54051 Å.
[5] Perkin-Elmer Infracord, model 137.
[6] Perkin-Elmer, model DSC-1B.
[7] 555, Dow Corning Corp., Midland, Mich.

Table I—The $d$ Distances of $I/I_1$ Values of Various Crystalline Phases of Fluprednisolone Using Copper K$\alpha$ Radiation

| Form I | | Form II | | Form III | |
|---|---|---|---|---|---|
| $d$, Å | $I/I_1$ | $d$, Å | $I/I_1$ | $d$, Å | $I/I_1$ |
| 10.3940 | 11.67 | 8.8378 | 20.00 | 8.9624 | 38.76 |
| 9.0173 | 28.46 | 7.8584 | 18.18 | 8.4181 | 17.98 |
| 8.3784 | 39.05 | 7.1608 | 55.45 | 6.3203 | 39.55 |
| 7.1320 | 9.49 | 6.4114 | 48.18 | 6.1035 | 44.94 |
| 5.9604 | 33.94 | 6.0619 | 98.18 | 5.5004 | 100.00 |
| 5.4835 | 100.00 | 5.6041 | 100.00 | 5.0348 | 91.01 |
| 5.1510 | 12.04 | 5.2111 | 56.36 | 4.7036 | 19.10 |
| 4.7410 | 19.85 | 5.0348 | 38.18 | 4.5026 | 14.83 |
| 4.4577 | 20.80 | 4.4801 | 57.27 | 4.2168 | 21.91 |
| 4.2168 | 19.49 | 4.0367 | 34.54 | 4.0550 | 10.11 |
| 3.7666 | 10.22 | 3.9783 | 20.91 | 3.9309 | 4.49 |
| 3.5448 | 9.49 | 3.5587 | 24.54 | 3.6746 | 19.10 |
| 3.4112 | 11.68 | 3.4502 | 16.36 | 3.5309 | 12.92 |
| 3.0557 | 7.66 | 3.2348 | 18.18 | 3.4241 | 15.17 |
| | | | | 3.2406 | 20.22 |
| | | | | 3.0455 | 21.35 |

| $\alpha$-Monohydrate | | $\beta$-Monohydrate | | *tert*-Butylamine Solvate | |
|---|---|---|---|---|---|
| $d$, Å | $I/I_1$ | $d$, Å | $I/I_1$ | $d$, Å | $I/I_1$ |
| 9.9270 | 58.12 | 7.8584 | 50.76 | 15.3580 | 100.00 |
| 7.4305 | 31.11 | 7.1897 | 14.36 | 7.6220 | 7.69 |
| 6.4816 | 99.49 | 6.4022 | 35.90 | 6.8042 | 6.25 |
| 6.2317 | 68.38 | 5.6041 | 100.00 | 6.0209 | 16.34 |
| 5.6041 | 72.65 | 5.3358 | 49.74 | 5.2789 | 36.06 |
| 4.8834 | 100.00 | 4.9511 | 51.28 | 4.6962 | 8.65 |
| 4.6068 | 50.43 | 4.5950 | 4.10 | 4.3287 | 10.09 |
| 3.9481 | 24.78 | 4.3496 | 13.33 | 4.1486 | 8.65 |
| 3.7354 | 35.04 | 4.2069 | 10.25 | 4.0008 | 3.85 |
| 3.5378 | 31.62 | 4.0008 | 14.87 | 3.7903 | 5.96 |
| 3.2406 | 27.35 | 3.8885 | 21.54 | 3.5309 | 4.61 |
| 3.0660 | 14.53 | 3.7509 | 15.38 | | |
| | | 3.5657 | 30.76 | | |
| | | 3.3857 | 16.41 | | |
| | | 3.2406 | 14.36 | | |
| | | 3.1078 | 4.10 | | |

weight loss when the phases were dried at 138° under vacuum, and (*c*) comparison of the molar absorbancy at 241.5 nm.

The $\alpha$-monohydrate phase was obtained by the evaporation of a saturated solution of fluprednisolone in methanol at room temperature in an open system. It was subsequently found that this phase could also be obtained by the evaporation of saturated solutions of fluprednisolone in ethanol and *n*-propanol. The $\beta$-monohydrate was obtained by evaporating a saturated solution of the steroid in water to approximately 5% of the original volume. The solution was filtered while hot, and the crystals were air dried at room temperature. The degree of hydration for both the $\alpha$- and $\beta$-monohydrate phases was determined by Karl-Fischer methods, weight loss studies, and a comparison of their molar absorbancies with that of anhydrous Form I.

The *tert*-butylamine solvate was obtained by the crystallization of fluprednisolone from a solution in *tert*-butylamine. A comparison of the molar absorbancy of this solvated form with anhydrous Form I indicated that two molecules of *tert*-butylamine were bound to each molecule of fluprednisolone.

The amorphous phase of fluprednisolone was obtained by the lyophilization of a solution of the steroid in *tert*-butanol, as suggested by Shell (4).

The proof of the chemical nature of each polymorph was established by converting each phase to Form I. This was accomplished by dissolving each polymorphic phase in acetone and evaporating the acetone solution to dryness on a hot plate. In each case, the phase obtained was Form I of fluprednisolone.

## RESULTS AND DISCUSSION

Seven different phases of fluprednisolone were isolated. Three were anhydrous, three were solvates, and one was amorphous. The anhydrous phases were designated Forms I, II, and III, with Form I being the most stable at room temperature. The dimorphic monohydrates were designated $\alpha$- and $\beta$-monohydrates. The third solvate was the *tert*-butylamine disolvate.

Phase transitions occurred under a variety of conditions. Al crystalline phases were converted to the $\alpha$-monohydrate upon suspension in water. Thus, aqueous suspensions of Forms I, II, and III

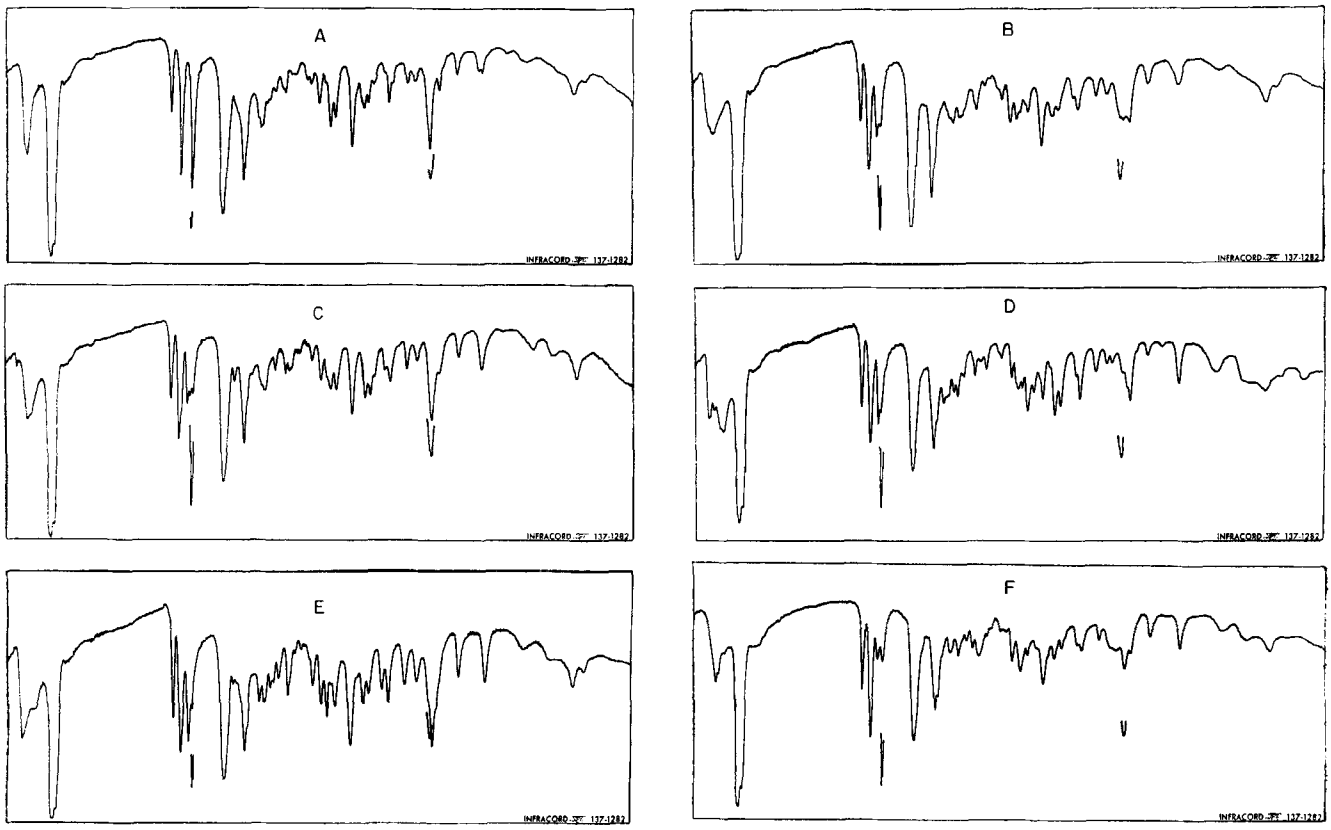



**Figure 1**—*IR spectra of the crystal modifications of fluprednisolone. Key: A, Form I; B, Form II; C, Form III; D, α-monohydrate; E, β-monohydrate; and F,* tert*-butylamine disolvate.*

**Table II**—Densities, Melting Points, and Heats of Fusion for Crystalline Modifications of Fluprednisolone

| Crystalline Phase | Density | Melting Point—Micro Hot Stage | Melting Point—Scanning Calorimeter | Heat of Fusion, kcal./mole |
|---|---|---|---|---|
| Form I | 1.318 | 199–200° | 199° | 4.616 |
| Form II | 1.350 | 193–194° | 215° | 5.245 |
| Form III | 1.388 | 208–210° | 215° | 5.476 |
| α-Monohydrate | 1.357 | 212°[a] | 215° | 5.245 |
| β-Monohydrate | 1.347 | 205° | 219° | 7.135 |
| *tert*-Butylamine disolvate | 1.177 | 105° | 88° | 5.689 |

[a] The α-monohydrate melted at 145°, resolidified at 157°, and finally melted at 212°.

and the β-monohydrate were transformed at various rates to the α-monohydrate. The *tert*-butylamine solvate in aqueous suspension, however, was initially converted to the β-monohydrate, which in turn was transformed into the α-monohydrate. Form III, in contact with air at room temperature, was slowly transformed to Form I.

The α-monohydrate and the *tert*-butylamine solvate were converted to an amorphous phase upon drying at 138° under vacuum (2 mm. Hg). The α-monohydrate was also converted to Form II when heated to 180° for 5 min. on the stage of a melting-point apparatus (Fisher-Johns). When an aqueous suspension of the α-monohydrate phase at 80° was seeded with a crystal of the β-monohydrate, the suspension was transformed into the β-monohydrate phase.

The amorphous phase was converted to the β-monohydrate when subjected to pressures of 7750 p.s.i. in a die having 50 mm.$^2$ faces in a press (Carver). Compression of the amorphous phase was done without evacuation of the die. It is possible that atmospheric and adsorbed moisture could account for the formation of a hydrated phase from the anhydrous amorphous form.

A summary of phase interconversions is shown in Scheme I. The phases were identified using X-ray powder diffraction, IR spectroscopy, and differential scanning calorimetry. Partial conversions and mixtures could be detected by differential scanning calorimetry.

It was found that X-ray powder diffraction was the preferred method for the determination of various crystalline phases. The results of the X-ray powder diffraction patterns obtained on the XRD-5 spectrometer for six crystalline phases of fluprednisolone are shown in Table I. The *d* distances are given, and the relative intensities of each reflection were calculated as $I/I_1$ ($I_1$ being the most intense reflection).

The IR spectra for each crystalline polymorph are shown in Fig. 1. Although only small differences in IR spectra can be observed, these changes are of sufficient magnitude to be of value in phase identification. It is our feeling, however, that IR spectra are only useful in conjunction with other methods for the identification of phases.

The physical properties for each crystalline polymorph were studied. The densities, melting points, and heats of fusion are shown in Table II. The melting points for each phase were determined by using either a microscope equipped with a micro hot-stage apparatus

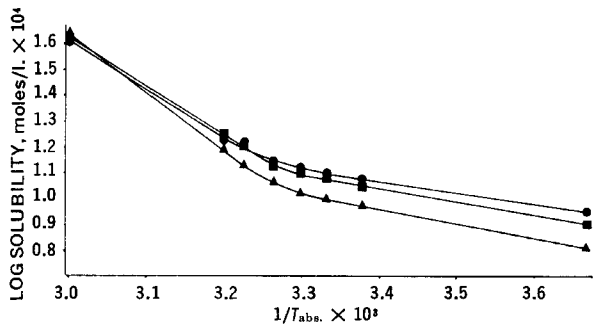



**Figure 2**—*Solubility of three phases of fluprednisolone in water as a function of temperature. Key:* ■, *Form I;* ▲, *α-monohydrate; and* ●, *β-monohydrate.*

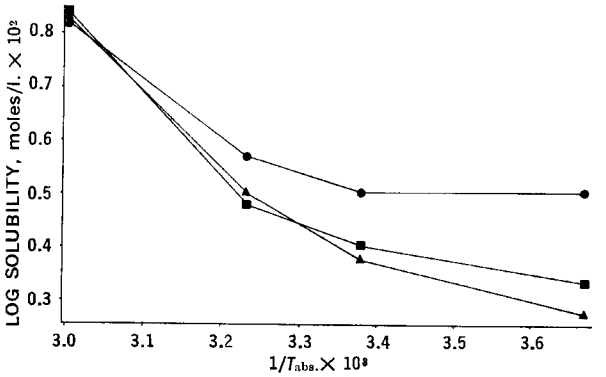



**Figure 3**—*Solubility of three phases of fluprednisolone in* n-*octanol as a function of temperature. Key:* ■, *Form I;* ▲, *α-monohydrate; and* ●, *β-monohydrate.*

(Kofler) or the differential scanning calorimeter. The heats of fusion were also determined on the differential scanning calorimeter using an indium standard. The differential scanning calorimetry thermogram for the α-monohydrate showed an endothermic peak at 130°, which was indicative of the loss of water of crystallization. This was followed by an exothermic peak at 150° where Form II precipitated from the supercooled melt after losing water. An exothermic peak followed at 215°, which was in agreement with the melting point of Form II. To confirm the findings, crystals were isolated after reaching the 150° peak. X-ray diffraction indicated that conversion of the α-monohydrate to Form II had occurred.

The equilibrium solubilities in water and *n*-octanol were determined for Form I, the α-monohydrate, and the β-monohydrate phases at temperatures varying between 0 and 60°. The plots of the logarithm of the molar solubility *versus* the reciprocal of the absolute temperature in water and *n*-octanol are presented in Figs. 2 and 3, respectively. The plots indicate that the slope of the best straight line through the points from 0 to 23° is significantly different from the slope of the line from 37 to 60°. This may be indicative of: (*a*) a crystalline phase change, or (*b*) alterations in the degree of association of fluprednisolone with solvent at various temperatures. It was previously found (Scheme I) that when Form I or the β-monohydrate was suspended in water at room temperature, phase transition occurred, with the formation of the α-monohydrate form. However, when the α-monohydrate was suspended in water above 37° and below 23°, phase transition was not observed.

Figure 4 presents the solubility data for the α- and β-monohydrate forms in water and *n*-octanol as the logarithm of the solubility ratio *versus* $T^{-1}$. From the slope of the best straight line drawn through the points, one may calculate the enthalpy of conversion ($\Delta H_{\alpha,\beta}$) of the β-form to the α-monohydrate form according to the method of Higuchi *et al.* (1). The transition temperature may be obtained by extrapolating the line to the point where the solubility ratio is unity ($s_\beta/s_\alpha = 1$). The transition temperature ($T_t$) at this point was 57.5°, and the enthalpy of conversion ($\Delta H_{\alpha,\beta}$) was 209 cal. mole$^{-1}$. Since the free energy of transition ($\Delta F_{\alpha,\beta}$) at the transition temperature is zero and $\Delta F_{\alpha,\beta} = \Delta H_{\alpha,\beta} - T\,\Delta S_{\alpha,\beta}$, the entropy of

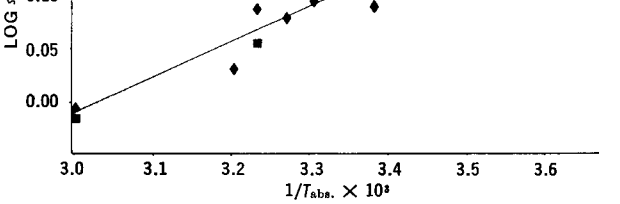



**Figure 4**—*Plot of the logarithm of the solubility ratios of the β-monohydrate to α-monohydrate phases of fluprednisolone as a function of temperature. Key:* ■, *in n-octanol; and* ◆, *in water.*

Table III—Heats of Solution and Fusion in Kilocalories per Mole for Three Polymorphic Forms of Fluprednisolone

| Phase | $\Delta H_s$ (0–23°) | $\Delta H_s$ (37–57°) | $\Delta H_f$[a] |
|---|---|---|---|
| Form I | 0.510 | 2.106 | 4.616 |
| α-Monohydrate | 0.669 | 2.301 | 5.245 |
| β-Monohydrate | 0.469 | 2.092 | 7.135 |

[a] From differential scanning calorimetry studies.

transition ($\Delta S_{\alpha,\beta}$) for the β-monohydrate to α-monohydrate form may be calculated. By using this relationship, $\Delta S_{\alpha,\beta} = 0.632$ e.u. was obtained. It is difficult to extrapolate the results of the entropy changes of the β- to α-monohydrates to alterations in molecular arrangement in the crystal lattice. A larger $\Delta S_{\alpha,\beta}$ would indicate a greater degree of freedom of the molecules in the crystal lattice. The small $\Delta S_{\alpha,\beta}$ observed may represent minor alterations in bonding occurring with the interconversion of the α-form.

The heats of solution for Form I, the α-monohydrate, and the β-monohydrate were determined from 0 to 23° and from 37 to 57°. These values are compared with the heats of fusion determined from differential scanning calorimetry data and are shown in Table III. The difference in $\Delta H_s$ from 0 to 23° and from 37 to 57° from each phase was a constant value.

The β-monohydrate form exhibits 25% greater solubility than the α-monohydrate phase at room temperature. Although the differences in solubility are small, with a judicious choice of the proper polymorphic phase for use in pharmaceutical suspensions of fluprednisolone, physical stability of such preparations can be optimized.

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# Comparison of Dissolution Rates of Different Crystalline Phases of Fluprednisolone by *In Vitro* and *In Vivo* Methods


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**Abstract** □ The *in vitro* dissolution rates of six crystalline phases of fluprednisolone were determined and compared with *in vivo* dissolution rates from pellet implants in rats. The dissolution studies were correlated with animal weight loss and adrenal gland atrophy. Excellent correlations were obtained between the *in vitro* and *in vivo* dissolution rates; however, correlation with animal weight loss and adrenal gland atrophy was only fair.



**Keyphrases** □ Fluprednisolone, crystalline phases—dissolution rates comparison, *in vivo*, *in vitro* □ Dissolution rates, fluprednisolone phases—*in vivo*, *in vitro* comparison


It was demonstrated that factors such as the total surface area of the dosage form and the solubility of the drug in surrounding biological fluids have a profound influence on the rate of absorption from subcutaneous pellet implants (1–3). The site of implantation, animal activity, substances used in the preparation of the dosage form, and crystalline phase may also influence the absorption rate (4, 5).

The purpose of this study was to correlate the *in vitro* rates of dissolution of several solvated and nonsolvated phases of fluprednisolone[1] with *in vivo* rates of absorption from solid pellet implants and with two biological responses, namely, animal weight loss (6) and adrenal gland atrophy (7).

Six crystalline phases of fluprednisolone were selected for this study. Of these forms, three were anhydrous, two were monohydrates, and one was a *tert*-butylamine disolvate. The isolation and characterization of the polymorphic forms were previously described by Haleblian *et al.* (8).

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

## EXPERIMENTAL

Cylindrical pellets, 8 mm. in diameter, of six pure fluprednisolone phases were prepared by compression in a Carver press at 7750 p.s.i. for 15 sec. The *in vitro* dissolution rates of the pellets were determined by placing each pellet in a polyethylene holder which secured the pellet in the center of a 120-ml. bottle. Water was added to each bottle, and the bottles were placed in a Wruble apparatus. For each separate run of the experiment, either the solvent, the temperature, or the velocity of agitation of the Wruble apparatus was modified. At specific intervals of time, samples of solution were withdrawn from each bottle using a preheated syringe; the solution was passed through a Millipore filter (0.22 μ) using a Swinney adapter. Each sample was assayed spectrophotometrically at 241.5 nm. in a Beckman DU or Beckman DB spectrophotometer.

The *in vivo* dissolution rates of the six crystalline phases of fluprednisolone were determined by pellet implantation techniques de-