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

Phase	ΔH_s (0–23°)	ΔH_s (37–57°)	ΔH_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 ΔH_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

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

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-

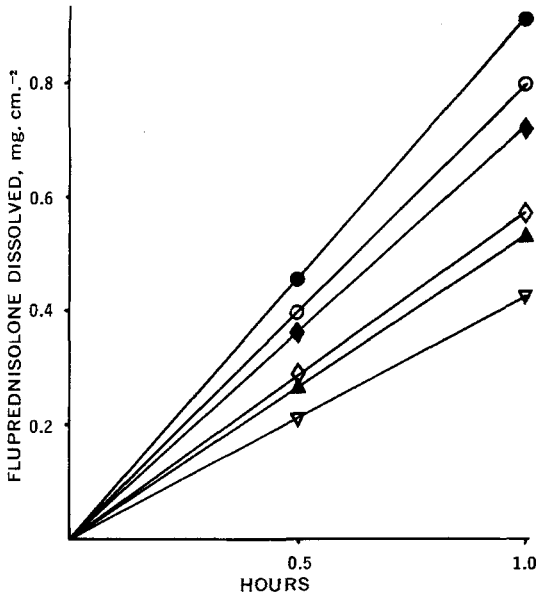

Figure 1—*Dissolution of various phases of fluprednisolone in water at 23°. Key:* ●, *Form I;* ◇, *Form II;* ○, *Form III;* ▽, *α-monohydrate;* ▲, *β-monohydrate; and* ◆, tert-*butylamine disolvate.*

veloped by Ballard and Nelson (3). One to three pellets of pure fluprednisolone phases were implanted subcutaneously in the abdominal region of rats. Sprague-Dawley rats of either sex, weighing between 225 and 430 g., were used in the implantation studies. The animals were anesthetized with ether, and a ventral midline incision was made. The subcutaneous connective tissue lateral to the incision was teased apart, and the pellets were implanted. The incision was closed by suturing after implantation. Five to eight animals were used in each test group. Four additional rats were used as unoperated controls. A third group of four rats were implanted with pellets of pure cholesterol. The test animals were allowed food and water *ad libitum.*

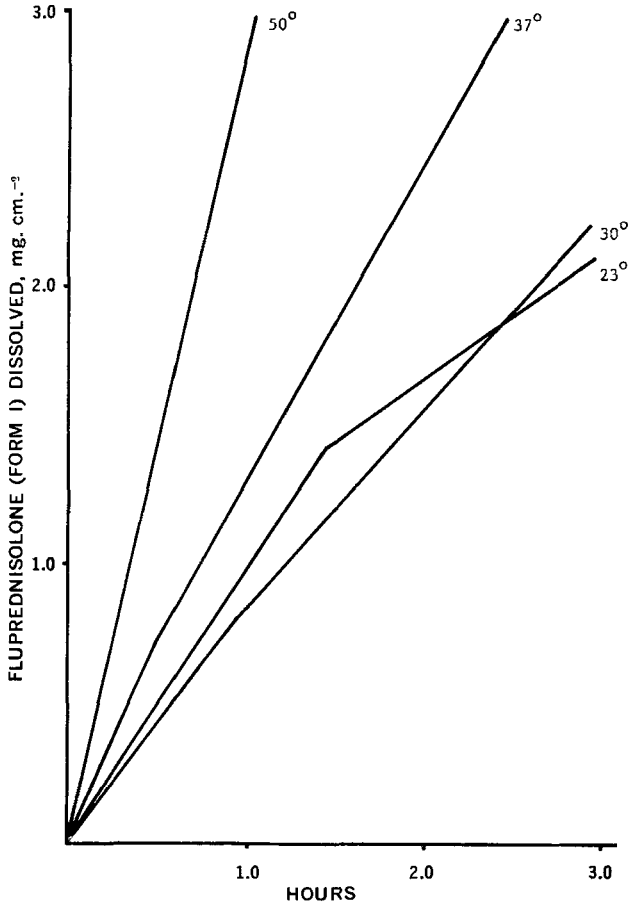

Figure 2—*Dissolution of Form I in water at various temperatures.*

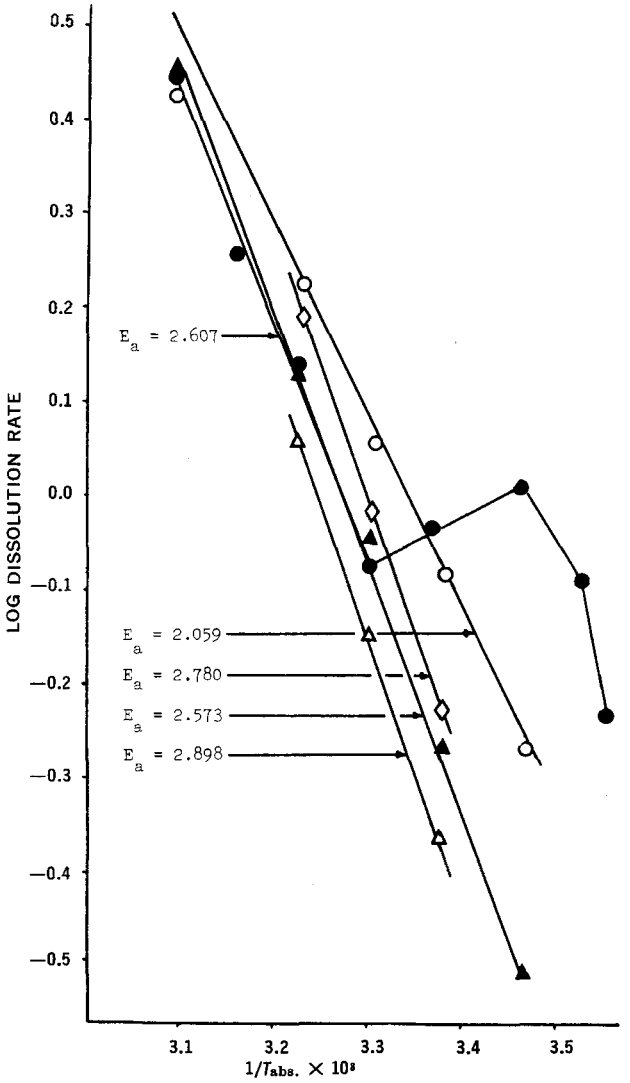

Figure 3—*Dissolution rates of various phases of fluprednisolone in water as a function of temperature. Key:* ●, *Form I;* ◇, *Form II;* ○, *Form III;* △, *α-monohydrate; and* ▲, *β-monohydrate.*

After 72 hr., the pellets of fluprednisolone were removed, cleaned of extraneous organic material, dried, and weighed. The average surface area of the pellets was also determined. The mean absorption rate per surface area of pellet was calculated for each crystalline phase.

The biological response to the absorbed drug was observed by determining its effect on animal weight loss and adrenal gland atrophy. The animals were weighed daily, and the percent loss in body weight was calculated. At the end of 72 hr., the pellets of fluprednisolone were removed, the animal was sacrificed, and the adrenal glands were removed. The whole gland was freed of surrounding lipid and connective tissue, and its wet weight was determined. The adrenal gland atrophy was compared to standard adrenal gland weights (9). From these data, the weight loss of adrenal gland per animal weight per hour was calculated.

RESULTS AND DISCUSSION

The dissolution rates of six phases of fluprednisolone in water were determined using the Wruble apparatus at 6 r.p.m. The results at 23° are presented in Fig. 1 as milligrams of fluprednisolone dissolved per square centimeter of surface area *versus* time. Over the initial

Table I—Adrenal Gland Weight Loss Produced by Various Crystalline Phases of Fluprednisolone

Phase	Number of Animals	Expected Mean[a] Adrenal Weight, mg.	Adrenal Gland Weight at End of Experiment, mg.	Adrenal Gland Weight Loss, mg.	Hours	Gram Atrophy per Gram Rat Weight per Hour × 10^{-7}
Form I	5	38.18 (36.9–39.5)	24.49 (23.05–25.95)	13.69 (12.75–14.85)	71.70	8.05 ± 0.26
Form II	7	44.96 (41.7–48.0)	28.41 (27.60–33.20)	12.10 (12.15–14.80)	71.35	6.57 ± 0.17
Form III	6	43.38 (39.3–45.8)	28.61 (26.55–30.40)	14.76 (12.75–16.85)	72.37	7.09 ± 0.12
β-Monohydrate	8	43.97 (42.0–45.8)	30.96 (29.05–33.25)	13.00 (10.95–14.35)	72.65	6.11 ± 0.19
α-Monohydrate	8	45.21 (43.0–48.4)	33.08 (29.85–36.20)	12.12 (9.60–14.90)	71.83	5.52 ± 0.27
tert-Butylamine disolvate	6	52.83 (50.9–57.31)	33.33 (29.95–39.15)	19.50 (11.75–26.15)	81.75	5.85 ± 0.57

[a] Reference 9.

Table II—Summary of *In Vitro* and *In Vivo* Dissolution Rates, Animal Weight Loss, and Adrenal Gland Weight Loss

Phase	*In Vitro* Dissolution Rate at 23°[a]	*In Vitro* Dissolution Rate at 37°, mg. $hr.^{-1}$ $cm.^{-2}$	*In Vivo* Dissolution Rate, mg. $hr.^{-1}$ $cm.^{-2}$	Percent Body Weight Loss (72 hr.)	Adrenal Gland Weight Loss[a] (72 hr.)
Form I	0.9167 ± 0.0129	1.3517 ± 0.0037	0.237 ± 0.010	17.0	8.05 ± 0.26
Form II	0.5714 ± 0.0079	1.5216 ± 0.0046	0.186 ± 0.004	12.6	6.57 ± 0.17
Form III	0.8038 ± 0.0063	1.6596 ± 0.0067	0.209 ± 0.011	11.2	7.09 ± 0.12
β-Monohydrate	0.5273 ± 0.0020	1.3312 ± 0.0041	0.162 ± 0.004	15.8	6.11 ± 0.19
α-Monohydrate	0.4096 ± 0.0038	1.1366 ± 0.0037	0.147 ± 0.005	14.7	5.52 ± 0.27
tert-Butylamine disolvate	0.7403 ± 0.0056	1.6363 ± 0.0071	0.204 ± 0.009	12.9	5.85 ± 0.57

[a] Adrenal atrophy per rat weight per hour × 10^{-7}.

60-min. period, the dissolution of each of the phases followed apparent zero-order kinetics.

Several phase transitions were observed during the dissolution studies. The *tert*-butylamine disolvate was rapidly converted to the β-monohydrate form, and Form I was converted to the α-monohydrate. The dissolution behavior of Form I in water at various temperatures is presented in Fig. 2. The rate of dissolution at 50° appears to be more rapid than the rate of transition. However, at 23, 30, and 37°, the transition rate of Form I to the α-monohydrate may be of the same order as the dissolution rate of Form I. It was previously reported (8) that the heat of solution of Form I from 0 to 23° differs from that determined at 37–57°. This difference in heat

Table III—Equations Correlating *In Vitro* and *In Vivo* Dissolution Rates[a] with Biological Effects

Equation	n	r	$s_{x \cdot y}$	Equation No.
In vitro dissolution rate at 23° and *in vivo* dissolution from subcutaneous pellet implants:				
$y = 0.169x + 0.079$	6	0.983	0.007	(1)
where x = *in vitro* dissolution rate at 23°, and y = *in vivo* dissolution rate				
In vitro dissolution rate at 37° and *in vivo* dissolution from subcutaneous pellet implants:				
$y = 0.119x + 0.009$	5	0.991	0.004	(2)
where x = *in vitro* dissolution rate at 37°, and y = *in vivo* dissolution rate				
Percent body weight loss and *in vitro* dissolution rate at 23°:				
$y = -8.936x + 18.894$	5	0.790	1.2848	(3)
where x = *in vitro* dissolution rate at 23°, and y = percent body weight loss				
Percent body weight loss and *in vitro* dissolution rate at 37°:				
$y = -6.674x + 23.166$	5	0.814	1.218	(4)
where x = *in vitro* dissolution rate at 37°, and y = percent body weight loss				
Adrenal gland weight loss and *in vitro* dissolution rate at 23°:				
$y = 4.519x + 3.1749$	5	0.976	0.2416	(5)
where x = *in vitro* dissolution rate at 23°, and y = adrenal gland weight loss				
Adrenal gland weight loss and *in vitro* dissolution rate at 37°:				
$y = 1.895x + 3.466$	5	0.681	0.5212	(6)
where x = *in vitro* dissolution rate at 37°, and y = adrenal gland weight loss				
In vivo dissolution rate and adrenal gland weight loss:				
$y = 0.030x + 0.004$	6	0.8379	0.0201	(7)
where x = adrenal gland weight loss, and y = *in vivo* dissolution rate				

[a] mg. $hr.^{-1}$ $cm.^{-2}$.

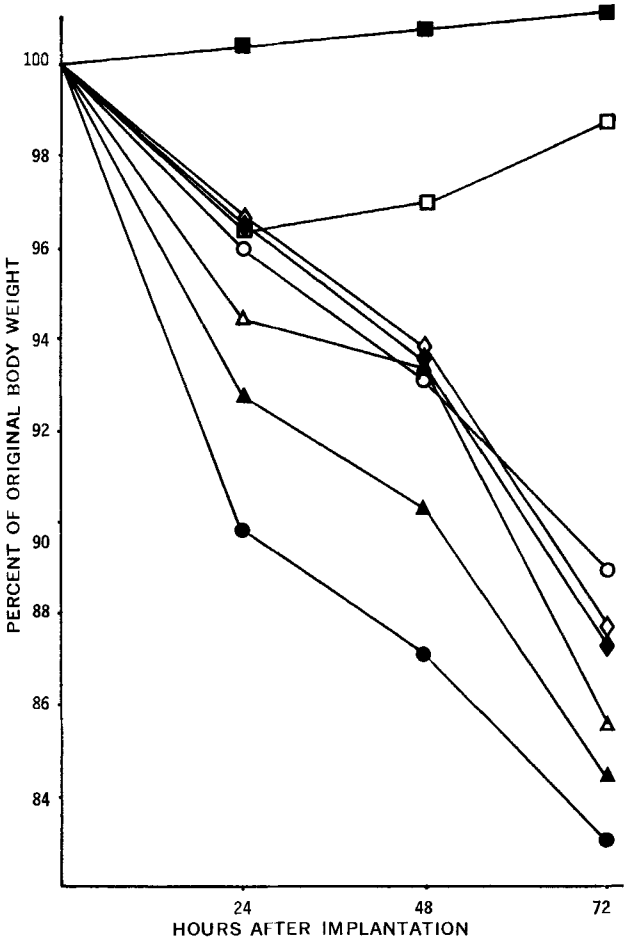

Figure 4—*Percent of original body weight remaining after fluprednisolone implantation. Key:* ■, *unoperated control;* □, *sham-operated control;* ●, *Form I;* ◇, *Form II;* ○, *Form III;* △, *α-monohydrate;* ▲, *β-monohydrate; and* ◆, tert-*butylamine disolvate.*

of solution was found to be due to a phase transition of Form I to the α-monohydrate.

The activation energy (E_a) for the dissolution process for each phase was determined by plotting the log of the dissolution rate against the reciprocal of the absolute temperature. Figure 3 indicates that the activation energy for the dissolution of Form II, Form III, α-monohydrate, and β-monohydrate is constant from 8 to 50°. However, the dissolution rate of Form I was observed to increase from 8 to 15°. At temperatures from 15 to 37°, the rate of conversion of Form I to the α-monohydrate appears to be significant enough to alter the dissolution rate; at temperatures above 37°, the dissolution process is more rapid than the phase interconversion process.

The *in vivo* dissolution rate of various phases of fluprednisolone pellets were determined in rats, using the implantation technique developed by Ballard and Nelson (3). The specific dissolution rate ($\bar{R}/\bar{A}$) was calculated by determining the weight of steroid dissolved per hour ($\bar{R}$) per average surface area of pellet ($\bar{A}$).

The biological effects of the absorbed steroid from pellet implants were determined by following animal weight loss and adrenal gland atrophy. A plot of percent of original body weight remaining *versus* time is shown in Fig. 4. The unoperated controls showed a consistent weight gain, while the sham-operated controls displayed an initial weight loss with a rapid recovery. This initial weight loss was probably due to surgical trauma. The animals that were implanted with pellets of the various phases of fluprednisolone showed a progressive weight loss.

In addition to body weight loss studies, adrenal gland atrophy was observed. At the end of the 72-hr. test period, each animal was sacrificed and the wet weight of the adrenal gland was determined (Table I). No attempt was made to relate the adrenal gland atrophy to any of the specific layers of the adrenal gland.

A summary of the *in vitro* and *in vivo* dissolution rates, animal weight loss studies, and adrenal gland atrophy is presented in Table II. Since three of the phases were solvated, pellet weight loss was converted to an equivalent fluprednisolone weight loss.

Equations correlating the *in vitro* and *in vivo* dissolution rates with animal weight loss and adrenal gland atrophy were determined by the method of least squares and are summarized in Table III, where n = number of data points, $s_{x \cdot y}$ = standard error of estimate, and r = correlation coefficient. From Eqs. 1 and 2, the *in vitro* dissolution rates at 23 and 37° correlate well with the *in vivo* dissolution from subcutaneous pellet implants. This finding supports the work of Hamlin *et al.* (10) who found that *in vitro* dissolution rates determined at low agitation intensities correlated well with *in vivo* dissolution rates from subcutaneous pellet implants. The *in vitro* dissolution rate of Form I at 37° was not included in the regression analysis since it was previously shown that Form I undergoes a phase transition in contact with water to the α-monohydrate form at temperatures below 50°. The *in vivo* dissolution of Form I, therefore, does not reflect the true dissolution rate of the pure phase but is a hybrid rate determined by the relative ratio of Form I and its transformation product (the α-monohydrate) present.

Equations 3 and 4 show the relationship between body weight loss and *in vitro* dissolution at 23 and 37°. As before, Form I was omitted in the calculations because of phase transition. Correlation of body weight and *in vitro* dissolution was only fair (Eq. 3, r = 0.790; Eq. 4, r = 0.814). This finding is not surprising since animal weight loss is not a valid measure of a specific pharmacologic response.

Equations 5 and 6 correlate adrenal gland weight loss and *in vitro* dissolution rates. At 23°, the rate of dissolution gave excellent correlations (r = 0.976) with adrenal gland weight loss, while the *in vitro* dissolution at 37° correlated poorly (r = 0.681). This result may be due to the rapid transition of Form I to the α-monohydrate form at 37°.

In vivo dissolution rate correlated only fairly with adrenal gland atrophy (Eq. 7, r = 0.8379). It is possible that phase transitions may have occurred at the pellet surface in the presence of fluids at the site of implantation.

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