



Figure 2—Plasma codeine levels after an oral dose of two tablets containing 65 mg of codeine.

for GLC analysis in an 8-hr day. The chromatography of the samples requires approximately the same time. Precision for codeine determination was 7% for levels of 50 ng/ml or greater.

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## Aqueous Solubility of <sup>14</sup>C-Triamcinolone Acetonide

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**Abstract** □ The blue tetrazolium colorimetric assay has often been employed in the analysis of corticosteroids. However, the reaction between blue tetrazolium and corticosteroids partly depends on the apparent pH of the system. In an earlier study of the temperature dependence of the solubility and dissolution of triamcinolone acetonide in aqueous dissolution media (distilled water and 0.2–1.0 M potassium chloride solutions), pH was not considered. To determine the reliability of the data previously reported, the solubility of <sup>14</sup>C-triamcinolone acetonide was determined by a radioisotopic method. The results of the analyses, using a liquid scintillation counting system, indicate that the steroid solubility is generally about 20% lower than that reported previously.

**Keyphrases** □ Triamcinolone acetonide, <sup>14</sup>C-labeled—synthesized, aqueous solubility determined, radioisotopic method □ Radioisotopic methods—determination of aqueous solubility of <sup>14</sup>C-triamcinolone acetonide □ Glucocorticoids—<sup>14</sup>C-triamcinolone acetonide, synthesis, aqueous solubility determined, radioisotopic method □ Solubility, aqueous—<sup>14</sup>C-triamcinolone acetonide, radioisotopic method

In a previous report from these laboratories (1), data were presented for the dissolution and solubility of triamcinolone acetonide in aqueous media. The data were obtained from the colorimetric determination of steroid concentration. The pH of the aqueous dissolution systems employed was not considered.

Graham and Kenner (2) studied the parameters affecting the chemical reaction involved in the colorimetric assay of triamcinolone acetonide and indicated a dependence of absorbance on the apparent pH

of the system. Thus, the colorimetric process employed might have involved some error, particularly when the measured absorbance was relatively low. To define more precisely the solubility of the steroid in aqueous media, <sup>14</sup>C-labeled triamcinolone acetonide was synthesized and its aqueous solubility was determined by measuring the associated radioactivity.

## EXPERIMENTAL

**Materials**—The following were used as received: concentrated perchloric acid (70%)<sup>1</sup>, spectrophotometric grade acetone<sup>2</sup>, anisole<sup>2</sup>, ethanol<sup>2</sup>, ether<sup>2</sup>, dioxane<sup>2</sup>, 1,4-bis[2-(5-phenyloxazolyl)]benzene<sup>2</sup>, potassium chloride<sup>2</sup>, 2,5-diphenyloxazole<sup>2</sup>, sodium bicarbonate<sup>2</sup>, 1,3-<sup>14</sup>C-acetone<sup>3</sup>, <sup>14</sup>C-benzoic acid<sup>4</sup>, triamcinolone acetonide<sup>5</sup>, triamcinolone alcohol<sup>5</sup>, 1,2-dimethoxane<sup>6</sup>, recrystallized blue tetrazolium<sup>2</sup>, chloroform<sup>2</sup>, toluene<sup>2</sup>, sodium hydroxide<sup>2</sup>, and methanol<sup>2</sup>.

**Solutions**—A stock solution of 1,3-<sup>14</sup>C-acetone was diluted with acetone to yield a solution with a specific activity of 3.02 × 10<sup>7</sup> dpm/ml. A liquid scintillation solution was prepared by dissolving 12 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 750 ml of dioxane, along with 125 ml of anisole and 125 ml of 1,2-dimethoxane. A standard solution of <sup>14</sup>C-benzoic acid was prepared to provide suitable specific activity.

<sup>1</sup> Allied Chemicals, General Chemical Division, Morristown, N.J.

<sup>2</sup> Fisher Scientific Co., Fair Lawn, N.J.

<sup>3</sup> ICN Chemical and Radioisotope Division, Irvine, Calif.

<sup>4</sup> New England Nuclear Corp., Boston, Mass.

<sup>5</sup> Squibb Institute for Medical Research, E.R. Squibb and Sons, New Brunswick, N.J.

<sup>6</sup> Eastman Organic Chemicals, Rochester, N.Y.

**Table I—Solubility of <sup>14</sup>C-Triamcinolone Acetonide in Dissolution Media<sup>a</sup>**

Temperature	Distilled Water	Potassium Chloride Solution		
		0.2 M	0.5 M	1.0 M
28°	17.5 (±0.05)	9.8 (±0.05)	6.6 (±0.03)	2.5 (±0.05)
37°	20.7 (±0.13)	13.0 (±0.08)	9.6 (±0.05)	4.2 (±0.06)
50°	26.5 (±0.17)	21.0 (±0.01)	15.9 (±0.01)	8.4 (±0.05)

<sup>a</sup> Results (in micrograms per milliliter) are the averages of eight determinations (±1 SD).

**Table II—Setschenow Equation Data for Triamcinolone Acetonide (Setschenow Ratios<sup>a</sup>)**

Temperature	0.2 M KCl	0.5 M KCl	1.0 M KCl	<i>k</i> <sup>b</sup>
28°	1.77	2.66	6.88	0.746
37°	1.59	2.15	4.90	0.622
50°	1.34	1.67	3.16	0.475

<sup>a</sup> The Setschenow ratio,  $S_0/S$ , represents the solubility of the steroid in pure solvent to its solubility in electrolyte solution of a given molar concentration. <sup>b</sup> "Salting-out" constant.

**Table III—Differential Heats of Solution of Triamcinolone Acetonide in Dissolution Media**

Dissolution Media	$\Delta H_{\text{solution}}$ Values, cal/mole	
	This Study	Previously Reported
Distilled water	3,736	4207
0.2 M KCl	6,760	8340
0.5 M KCl	7,840	8722
1.0 M KCl	10,549	9855

**Synthesis of <sup>14</sup>C-Triamcinolone Acetonide**—The method reported by Heller *et al.* (3) was modified, and the following procedure was employed. Eight hundred milligrams of triamcinolone alcohol was suspended in approximately 15 ml of the stock solution of 1,3-<sup>14</sup>C-acetone, and enough concentrated perchloric acid (about 2 ml) was added to the suspension to dissolve the steroid. The solution was maintained at 25° for 30 min; then 200 ml of distilled water was added, followed by sufficient saturated sodium bicarbonate solution (approximately 250 ml) to make the solution alkaline.

The acetone was allowed to evaporate at ambient temperature, and a white crystalline solid precipitated. The solid, washed with distilled water, was dried at 35° to yield 750 mg of <sup>14</sup>C-triamcinolone acetonide, mp 272–276°. Its melting point, chromatographic behavior<sup>7</sup>, and UV and IR spectra were identical to those of an authentic sample of triamcinolone acetonide<sup>5</sup>. The molar absorptivities in absolute alcohol of the <sup>14</sup>C-triamcinolone acetonide and of the authentic sample of the steroid were 14,690 and 14,714, respectively. Bernstein *et al.* (4) reported a molar absorptivity for the steroid of 14,600. The steroid's specific activity, in comparison with standard <sup>14</sup>C-benzoic acid, was  $6.53 \times 10^3$  dpm/mol.

**Assay of Aqueous Triamcinolone Acetonide Solutions**—One milliliter of the steroid-containing solution was transferred to a polyethylene vial<sup>8</sup> and was followed by the addition of 10 ml of the liquid scintillation solution. The mixture was shaken well. A blank was prepared by adding 1.0 ml of the dissolution medium and 10 ml of the liquid scintillation solution. The activity of the steroid-containing solution relative to the background was measured in a

liquid scintillation analyzer<sup>9</sup> over 20 min. Counting efficiency was determined with standard samples of <sup>14</sup>C-benzoic acid.

**Solubility Determination**—The solubility of <sup>14</sup>C-triamcinolone acetonide in distilled water and in 0.2, 0.5, and 1.0 M potassium chloride solutions was determined as a function of temperature, as in the previous study (1).

## RESULTS AND DISCUSSION

Although concentration plateaus were reached within 4–6 days, as in the previous study (1), investigations were continued for 3–4 additional days to confirm the development of saturation equilibria. The increased sensitivity of the radioisotopic method, compared to the colorimetric method previously employed (1), leads us to accept the lower triamcinolone acetonide solubility values reported in Table I. A minimum of eight determinations of triamcinolone acetonide concentration was made after equilibrium had been achieved.

This procedure had, in fact, been followed in the earlier study (1). The "two determinations" evaluated by Block and Patel were actually two separate experimental runs, each of which involved the collection and analysis of at least four samples postequilibrium. It was possible, therefore, to subject the data to statistical analysis (5). A three-way analysis of variance indicated that the results of the two studies were distinctly different ( $p < 0.01$ ).

The recalculated values of the Setschenow ratios and the "salting-out" constants are listed in Table II. The differential heats of solution are listed in Table III. The constants tabulated in Tables II and III, based on the more reliable data presented in this study, can thus be considered to reflect more accurately the solubility of triamcinolone acetonide in water and in electrolyte solutions.

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<sup>7</sup> The steroids were chromatographed on Eastman Chromagram sheets (cellulose, without fluorescent indicator, No. 6064), using chloroform-toluene (1:3) as the solvent system. The plates were sprayed with 0.1% blue tetrazolium in methanol–16% sodium hydroxide in methanol (1:1).

<sup>8</sup> Polyethylene Prima Vials, No. 40-227, Nuclear Associates.

<sup>9</sup> Uniflux II liquid scintillation system, Nucleac Chicago Corp., Chicago, Ill.