an index of griseofulvin bioavailability in humans. Recently, Greenblatt *et al.* (12) suggested that the single-dose bioavailability of digoxin preparations could be assessed in humans from 1-day, rather than 6-day, urinary excretion data.

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Epicillin and Ampicillin: Crystalline Modifications and Their Physicochemical Differences

Keyphrases □ Epicillin—crystalline modifications, physicochemical properties □ Ampicillin—crystalline modifications, physicochemical properties □ Antibiotics—epicillin and ampicillin, crystalline modifications and their physicochemical differences

To the Editor:

Epicillin (I), a new broad spectrum semisynthetic penicillin, is formed by the coupling of 6-aminopenicillanic acid with D-α-amino-1,4-cyclohexadiene-1-acetic acid (1). Its bacteriology (2, 3), pharmacology, and clinical applications have been reported (4–8). Epicillin is similar to ampicillin (II) in antimicrobial activity, although epicillin may exert a significantly greater bactericidal activity *in vitro* against certain Gram-negative bacteria than does ampicillin (2).

Although the molecular structure of epicillin differs only slightly from that of ampicillin, the difference being confined to the side chain, some physicochemical properties of epicillin differ significantly from those of ampicillin; these differences are reported herein.

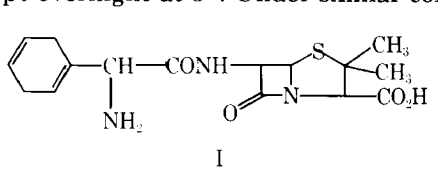
Table I—X-Ray Powder Diffraction Patterns of Epicillin Anhydrate and Epicillin Trihydrate

Epicillin Anhydrate		Epicillin Trihydrate	
d^a	$I/I_1{}^b$	d	I/I_1
11.3	0.17	11.8	0.21
10.7	0.49	8.0	0.11
10.2	0.09	7.8	0.14
6.55	0.06	7.2	0.88
6.15	0.25	5.82	1.00
5.92	0.16	5.44	0.21
5.62	0.22	5.13	0.25
5.48	0.80	5.07	0.21
5.10	0.28	4.90	0.89
4.57	0.04	4.56	0.56
4.41	0.24	4.47	0.28
4.32	0.11	4.40	0.24
4.22	0.20	4.10	0.36
4.11	0.45	4.01	0.41
3.95	0.62	3.95	0.21
3.85	1.00	3.82	0.50
3.75	0.12	3.76	0.59
3.51	0.14	3.60	0.17
3.45	0.12	3.48	0.32
3.40	0.24	3.45	0.52
3.35	0.12	3.32	0.61
3.13	0.01	3.21	0.19
3.07	0.38	3.17	0.15
2.96	0.12	3.08	0.62
2.90	0.12	3.03	0.29
2.85	0.09	2.97	0.24
2.81	0.12	2.91	0.14
2.76	0.11	2.80	0.34
2.69	0.11	2.74	0.18
2.62	0.21	2.69	0.18
2.58	0.12	2.66	0.16
2.48	0.11	2.60	0.21
2.41	0.05	2.58	0.25
2.30	0.11	2.51	0.20
2.25	0.19	2.26	0.23

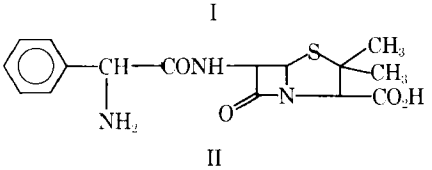
[a] Interplanar spacing in angstrom unit. [b] Relative intensity visual estimation, radiation; Cu/Ni (Norelco, model IC 2000, Philips Electronic Instruments, Mount Vernon, N.Y.).

At room temperature (25°), epicillin trihydrate is about four times more soluble in water than is the anhydrate, 17.8 and 4.1 mg/ml, respectively. In contrast, ampicillin trihydrate is less soluble than is the anhydrate (9, 10), 7 and 10 mg/ml, respectively. In a study of ampicillin solubility at different temperatures, the solubility curves for the anhydrous and trihydrate forms intersected at about 50°, the transition temperature of these two crystalline forms (11); in a similar study, the transition temperature was 42° (10). No clearcut transition temperature is obtainable for the epicillin anhydrate–epicillin trihydrate system.

There is no evidence that epicillin anhydrate is converted to the trihydrate in an acidic slurry, even when kept overnight at 5°. Under similar conditions,



I



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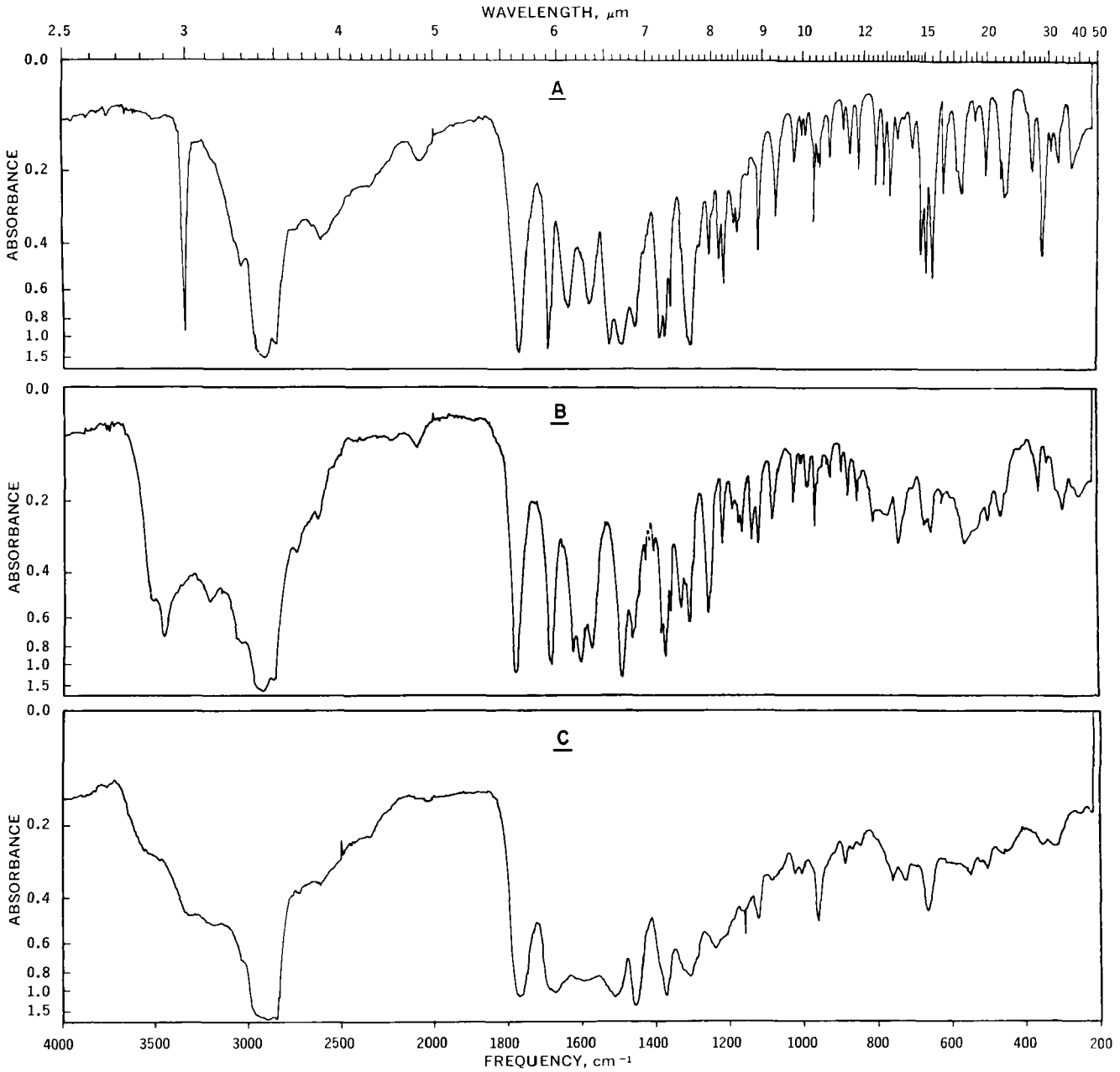

Figure 1—*IR spectra of polymorphic forms of epicillin: Key: A, anhydrate; B, trihydrate; and C, amorphous form.*

however, ampicillin anhydrate is converted to the crystalline needle-like trihydrate (12, 13) within 12 hr. Conversely, an aqueous slurry of epicillin trihydrate is converted to the anhydrate at ambient temperatures, and the conversion is accelerated if the slurry is heated to about 40°. In the case of ampicillin trihydrate, an aqueous slurry can be converted to the anhydrate only at 80–100° (11). Evidently, ampicillin trihydrate is more stable than the anhydrate, whereas the reverse is true for epicillin.

Epicillin is isolated more easily in the anhydrous form than as the trihydrate, although pure crystals of the latter can be obtained under controlled conditions, *i.e.*, acid–base recrystallization from an acidic aqueous solution when the pH is raised to between 3.5 and 4.5, preferably at 0–5°. If the crystals are dried without heating and under reduced pressure, their trihydrate form is maintained. The anhydrate is obtained when the temperature of an aqueous suspension of epicillin trihydrate is raised to 25–40° or higher or, alternatively, when the pH of an aqueous alkaline solution of epicillin is adjusted to about 5 by the addition of acid.

Although the existence of ampicillin monohydrate has been reported (14), crystalline epicillin monohydrate has not yet been isolated.

Prolonged drying of epicillin trihydrate at 50° under reduced pressure, in the presence of a dehydrating agent, results in formation of the amorphous form. This form of epicillin may be converted to either the anhydrous or trihydrate form by recrystallization.

Crystals of epicillin anhydrate are rectangular or plate-like, whereas those of the trihydrate are rod

shaped or needle-like. The X-ray powder diffraction patterns of the anhydrous and trihydrate forms of epicillin are distinguishable (Table I). The X-ray diffraction patterns of epicillin and ampicillin and of epicillin trihydrate and ampicillin trihydrate, respectively, are similar. The amorphous form of epicillin shows no X-ray diffraction pattern.

Epicillin trihydrate contains 13.3% of total volatiles or water, as determined by thermal gravimetric or Karl–Fischer analysis. Differential thermal analysis of the trihydrate shows characteristic endotherms at 92, 102, and 120° and an exotherm peak at about 213–218°; the anhydrate shows only an exotherm peak at about 215–220°. Differential thermal analysis of the amorphous form of epicillin shows only one inflection near 170°. Thermograms of ampicillin and ampicillin trihydrate resemble those of epicillin and epicillin trihydrate, respectively[1].

The solid polymorphic forms of epicillin may also be distinguished by their IR spectra (Fig. 1). Spectra of epicillin anhydrate (A) and epicillin trihydrate (B) differ in the NH and OH stretching regions. A very sharp isolated band at 2.99 μm is characteristic of the anhydrous form, whereas less sharp peaks at about 2.85 and 2.90 μm are found for the trihydrate. The anhydrate also shows distinct bands at 5.65, 5.92, 6.12, 6.32, 6.55, 6.71, 6.86, 7.20, 7.30, 7.37, 7.68, and 7.99 μm; the trihydrate shows corresponding bands at 5.64, 5.95, 6.15, 6.25, 6.36, 6.71, 6.85, 7.02, 7.08, 7.15, 7.25, 7.30, 7.52, 7.68, and 7.99 μm. There are, in addition, striking differences between the two crystalline forms of epicillin evident in the fingerprint region of the IR spectrum (beyond 8 μm). Spectrum C shows the typically poor resolution characteristic of the amorphous form of epicillin. The IR spectra of ampicillin and its hydrated forms were reported previously (11, 14).

Neither the trihydrate nor the anhydrate of epicillin is hygroscopic at 30° when exposed to an atmosphere of 85% relative humidity for 24 hr or to 100% humidity for 2 hr. The amorphous form of epicillin, however, increases in moisture content by 5–8% within 2 hr in air at ambient temperature.

When the epicillin trihydrate is heated *in vacuo* (dehydrated) at 50° for 4 days, the crystal lattice is ruptured and the compound is converted to the amorphous form; under these circumstances, loss of activity (11%) occurs. Similarly, ampicillin trihydrate and monohydrate showed losses in potency after rupture of their crystal lattices (9).

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[1] Details of the differential thermal analysis results are to be published.

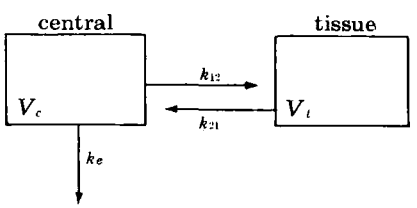
Changes in Pharmacokinetics of Cefazolin due to Stress

Keyphrases □ Cefazolin—pharmacokinetics, changes due to stress □ Pharmacokinetics—cefazolin, changes due to stress □ Stress—effects on pharmacokinetics of drugs, cefazolin

To the Editor:

Several investigators (1–4) have published cefazolin blood level data after intravenous infusion or bolus dose injection. In every case the pharmacokinetics of this drug have been described by a one-compartment model. Inspection of the data indicates that the decline in blood levels is a biexponential process and that the pharmacokinetics of this drug are more appropriately described by at least a two-compartment model, as was confirmed in our laboratories using normal human volunteers.

A 1-g dose of the drug was administered intravenously and serum samples were analyzed for cefazolin concentration as a function of time. The serum samples were assayed by absorbing 20 μl of suitably diluted serum on filter paper disks and placing the disks in bacto antibiotic medium No. 1 agar plates. Nine to 10 ml of agar, previously seeded with *Bacil-*



Scheme I