Table I—Enatiomeric Binding Data of Pseudoracemic Warfarin (10 μg·mL^{-1}) in the Absence and Presence of Sulfinpyrazone (15 μg·mL^{-1})

	Control	Sulfinpyrazone
(*R*):(*S*) Unbound fraction (f_u) ratio	1.58 (*SD* ± 0.15; *n* = 11)	2.25 (*SD* ± 0.26; *n* = 11)
t test		$p < 0.001$
Percentage bound[a]		
(*R*)	99.15 (*SD* ± 0.24) (f_u% = 0.85)	98.73 (*SD* ± 0.14) (f_u% = 1.27, increase of 49.41%)
(*S*)	99.47 (*SD* ± 0.16) (f_u% = 0.53)	99.42 (*SD* ± 0.10) (f_u% = 0.58, increase of 9.43%)
pesudoracemate	99.31 (f_u% = 0.69)	99.08 (f_u% = 0.92, increase of 34.06%)

[a] Calculated as the mean of the individual enantiomer binding data.

tially complicating role that stereoselective changes in protein binding play in the interaction is evident from a consideration of the equation for total body clearance which pertains to poorly extracted drugs (6):

$$CL = f_u \cdot CL'$$

Without acknowledging the stereoselective change in f_u between the two warfarin enantiomers brought about by concomitant sulfinpyrazone administration, one might incorrectly conclude that any inequivalent change in *CL* between (*R*)- and (*S*)-warfarin has its origins at the enzymatic level (as measured by the intrinsic clearance *CL'*), a result of metabolic induction or inhibition. In light of our present observations, it is apparent that stereoselective protein binding displacement of a poorly extracted drug may manifest itself as an inequivalent change in the *CL* of the two enantiomers in the absence of any dynamic change in metabolism. Sulfinpyrazone is metabolized in humans, and the plasma-protein binding of the parent drug and its metabolites have been investigated (17). Obviously, sulfapyrazone metabolites could complicate the protein binding interaction with warfarin *in vivo*; however, our evidence with volunteers who concomitantly receive warfarin and sulfinpyrazone (15) indicate this is not the case. Interestingly, sulfinpyrazone has been reported to induce the metabolism of (*R*)-warfarin while simultaneously inhibiting the metabolism of the (*S*)-enantiomer (18, 19). These conclusions were drawn from the sulfinpyrazone-induced changes in enantiomeric clearance.

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Thermodynamic Studies of Tolbutamide Polymorphs

Keyphrases □ Tolbutamide—crystalline polymorphs, solubility as a function of temperature, polymorphic conversion, transition temperature

To the Editor:

Aqueous suspensions of tolbutamide were found to thicken to an unpourable state after several weeks of occasional shaking (prior to daily dosing). Samples of the same suspensions that were not shaken showed excellent stability after years of storage at ambient and elevated temperature. Microscopic examination revealed that the thickening was due to partial crystalline conversion of the original plate-like tolbutamide crystals to very fine needle-shaped crystals, which tend to form a highly flocculated structure. The crystals were identified as a polymorphic form rather than a solvate or change in habit. IR spectra and X-ray diffraction confirmed that the acicular form is identical with Burger's form III (1). The polymorphic conversion was unexpected since published solubility data suggest that form I is the more stable polymorph at room temperature (1, 2). However, a transition temperature, above which form III converts to form I, has been reported by several investigators to be somewhere between 98.5°C and 118°C (1, 3, 4). An inconsistency here is clearly apparent: form I cannot be the more stable polymorph at temperatures both above and below a transition temperature. Furthermore, the reported solubility data (1) indicating that form I is less soluble than form III at 37°C conflict with suspension stability data generated in this laboratory, which indicate that form I converts to form III at room temperature. To resolve the discrepancy between the published literature and recent observations in this laboratory, the aqueous solubilities of polymorphs I and III were determined as a function of temperature.

The form I tolbutamide used in this study was prepared for commercial use by a final recrystallization from methanol–water[1]. Form III was made by stirring an aqueous suspension

[1] Lot No. 445HS; The Upjohn Co., Kalamazoo, Mich.

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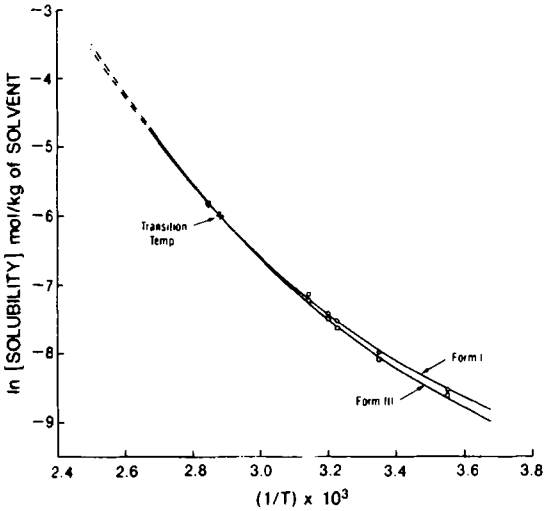

Figure 1—*Aqueous solubilities of tolbutamide forms I and III as a function of temperature.*

of form I tolbutamide (173 g in 20 L of water containing 10 g of polysorbate 80) for several days. The suspension was seeded initially with a small amount of the formulated suspension that contained some of the needle-like crystals of form III. Heating the suspension to ~40°C hastened the conversion of the plate-like form I crystals to the needle-shaped form III crystals. When conversion appeared complete by microscopic observation, the suspension was filtered on a coarse sintered glass filter, washed with deionized water, and dried at 40°C under reduced pressure. The converted crystal form was identified as form III and differentiated from form I by X-ray diffraction patterns and IR spectra which conformed with those published by Burger and others (1, 3, 4). Thermogravimetric analysis and melt solvate assays were negative for solvents. Melting points and differential scanning calorimetric thermograms corresponded to those published previously (1, 3, 4).

The aqueous solubilities of each of the two forms were obtained in the same way as reported by Burger (1). Samples of the stirred suspensions were taken periodically and assayed by UV spectrophotometry or reverse-phase HPLC at 229 nm until a constant value was obtained for several days. The solubility in octanol was determined in the same way, except that no HCl or polysorbate 80 was added. Samples were taken every hour until 4 or 5 h after apparent equilibrium.

The aqueous solubility at 37°C for form I was found to be 14.61 mg/100 mL, and that of form III was 13.03 mg/100 mL. These values are significantly different at the 95% confidence level. The aqueous solubility data in the molal units are plotted against $1/T$ in Fig. 1 (van't Hoff plot). The nonlinearity of the data is due to the wide temperature range covered, as pointed out by Grant (5). Using an iterative least-squares regression program, the solubility data for each of the forms was fit to a modified van't Hoff equation (6) as follows:

$$\ln m_{T_2} = \ln m_{T_1} + \frac{(\Delta\overline{H}_{T_1} - T_1\Delta\overline{C}p)(T_2 - T_1)}{RT_1T_2} + \frac{\Delta\overline{C}p}{R} \cdot \ln\frac{T_2}{T_1} \qquad \text{(Eq. 1)}$$

where T_2 is the temperature of interest and T_1 is an arbitrarily chosen reference temperature. In this study, T_1 was fixed at 128°C, the melting point of form I. $\Delta\overline{H}$ and $\Delta\overline{C}p$ are the differential heat of solution and the difference in heat capacity of dissolved and crystalline solute, respectively, for either form. $\Delta\overline{C}p$ is assumed constant with an arbitrary value of 84 cal/deg·mol, while the heat of solution is dependent on temperature as follows (6):

$$\Delta\overline{H}_{T_2} = \Delta\overline{H}_{T_1} + \Delta\overline{C}p(T_2 - T_1) \qquad \text{(Eq. 2)}$$

Table I—Thermodynamic Parameters Obtained from the Solubility *versus* Temperature Data (Eq. 1)

Polymorph	ln m(128°C)[a]	$\Delta\overline{H}$ (128°C), kcal/mol	$\Delta\overline{C}p$, cal/deg·mol
I	−3.52	15.0	84[b]
III	−3.44	15.4	84[b]

[a] Hypothetical value of the aqueous solubility at 128°C in units of molality. [b] Assumed to be equal in the least-squares fitting routine.

The two unknowns (heat of solution at 128°C and ln m at 128°C) are obtained from the iterative solution of Eq. 1 and are listed in Table I. The heat of solution of form I at 25°C is calculated to be 6.3 cal/deg·mol which is in good agreement with previously published data (2). The two polymorphs are enantiotropic since the solubility curves cross below the melting point. The transition temperature is estimated to be ~75°C. Below 75°C, form III has the lower solubility and is the most stable. Since the aqueous solubilities of the two forms are so close, form I may appear to be quite stable in suspensions at low temperatures, but given enough time, it will transform to the lower energy form. Above 75°C, form I has the lowest solubility and is the more stable of the two in that region.

A qualitative experiment was done to illustrate the transition point. A small quantity of form III crystals was mixed with mineral oil on a microscope slide. The mixture was heated to 100°C on a hot stage for several hours and periodically agitated by pressing and rotating the cover slip. When the temperature was reduced to 95°C and maintained, prismatic plate-like crystals, typical of form I, began to grow throughout the oil mixture while the form III clusters slowly dissolved. Then, the slide was removed from the hot stage and allowed to cool quickly to room temperature. The plates stopped growing, but fine needle-like crystals, typical to form III, began to grow. Obviously, there is a transition temperature between 25°C and 95°C.

The crystalline conversion was observed in 10 other solvents at room temperature: toluene, hexane, ethanol, chloroform, acetonitrile, methyl propyl ketone, isopropyl alcohol, tetrahydrofuran, dioxane, and octanol. The rate of conversion varied greatly with solubility, the better solvents promoting faster conversion. Since the conversion in octanol is relatively slow, it was chosen for accurate determination of polymorph solubilities. As might be expected, the solubility in octanol of form III at 30°C is significantly lower than that of form I: 19.33 mg/mL for form III and 23.54 mg/mL for form I. IR spectra were obtained on the solids remaining after the solubility study had been completed; the results confirmed the microscopic observation that no polymorphic conversion had taken place in either case during the solubility study.

The data given here present a rational explanation for the observed polymorphic change in suspensions at temperatures of <75°C. The small difference in free energy of the two polymorphic forms and the low solubilities at ambient temperature provide only a small driving force for the conversion.

This allows the preparation of apparently stable suspensions of either form. However, form I suspensions are metastable at ambient temperatures and eventually will change to form III. The conversion may be hastened by catalytic influences such as strong agitation.

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OPEN FORUM

Safety and Efficacy of Dental Sealants in the Prevention of Tooth Decay

In a recent editorial ("Coming of Age" for Device Technology), Dr. Feldmann[1] appears to be unnecessarily harsh on the use of modern dental sealants in preventing caries. The designation of such procedure as "a hoax or even a well-intended but worthless effort by dentists to protect against the ravages of caries" may have had some validity 15 or so years ago, when the early sealant materials were in developmental stages and application techniques were not well understood. Today, however, new sealants have been developed and clinically proven by dental researchers as safe and effective in preventing caries[2]. The use of sealants by dentists in the United States[3] has increased dramatically since 1974 when 37.8% of those surveyed said they offered sealant therapy; in 1982, that figure increased to 57.7%.

The National Dental Caries Prevalence Survey (1979-1980) has shown that among 5- to 17-year-old children, only 16% of the caries incidence occurred in smooth surfaces, but 84% involved chewing surfaces with pits and fissures. It is known that the chewing surfaces of children's teeth are the most susceptible to decay and derive the least benefit from fluorides. The newly developed plastic films are applied to these chewing surfaces to seal the pits and grooves, which prevents food and cariogenic bacteria being trapped and thus offers a new approach to the prevention of dental caries.

The 1983 Consensus Development Conference on the use of dental sealants in the prevention of tooth decay, sponsored by the National Institutes of Health, made the following conclusions[4]:

"The placement of sealants is a highly effective means of preventing pit and fissure caries. It is safe. It is currently underused in both private and public dental health care delivery systems. The reasons for such underuse are complex, but intensive efforts should be taken to increase sealant use. Expanding the use of sealants would substantially reduce the occurrence of dental caries in the population beyond that already achieved by fluorides and other preventive measures. Because dental caries is still a disease common to most young people in the United States and in other countries of the world, such reductions would substantially improve the health of the public and reduce the expenditures for treatment of dental disease. Practitioners, dental health agency directors, and dental educators are urged to incorporate the appropriate use of sealants into their practice and programs."

The American Dental Association Council on Dental Materials, Instruments, and Equipment, which has an acceptance program to evaluate commercially available sealants for safety and efficacy, also considers[5] "pit and fissure sealants are safe and effective as a caries prevention procedure."

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Author's Response:

Apparently, Dr. Rao misunderstood or misinterpreted the message of my June issue editorial. In his letter, Dr. Rao acknowledges that classifying dental sealants as being worthless 15 or so years ago may have had validity; however, he contends that today they are considered by experts in the field to be a highly beneficial technique in protecting against dental caries.

The entire thrust of the editorial, from its title ("Coming of Age" for Device Technology), through its entire text, to the very last paragraph (which stated that ". . . what we are witnessing is truly a medical revolution"), emphasized and reemphasized that many modern medical devices, procedures, and technology now constitute valuable health advances—although this was rarely the case a generation ago. As one dramatic example, we mentioned dental sealants and praised the FDA for keeping an open mind in judging positively the current products and their application. Yes, these modern products have indeed been proven to be a safe and effective caries prevention procedure.

Over twenty-five years ago, I personally was on the ADA staff as Director of the American Dental Association's drug testing laboratory. I well remember that the biggest challenge to ADA at that time was to reverse lay, professional, and scientific thinking regarding the value of fluoridation. Until then, only the toxicity and adverse effects were generally recognized and known. But, by carefully adjusting the daily intake of fluoride, remarkable benefits could be achieved while avoiding any adverse reactions. This was one of the earlier technical revolutions in the dental field—sealants are a more recent advance.

In conclusion, we would respectfully urge Dr. Rao to read again our June editorial. Then, it should be clear to him that we have no disagreement whatsoever as to the current value of dental sealants.

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