cube root law (2) and log normal[1] (3). So that a meaningful comparison could be made between the three dissolution laws, it was arranged that $dis_j(t)$ for the fastest dissolving formulation reached 90% at the same time for each law and that the slowest dissolving formulation had reached 10% at this time. The rate constants k_2 and k_3 were fixed at 5 and 0.5 in one trial and at 5 and 0.1 in another. The time units are arbitrary.

The dissolution profiles for the three laws are shown in Fig. 1, and the values of $H(t)$ calculated using Eq. 1 are listed in Tables I and II. To obtain a better appreciation for the numbers, the plasma profile appropriate to the slowest dissolving formulation was calculated using the $H(t)$ appropriate to the fastest dissolving formulation and compared to the correct profile for each dissolution law. These values are displayed in Fig. 2.

Discussion—Although extensive parameter searches were not carried out, general trends revealed themselves. First, it is surprising how well the method works in these simulations at early times, *i.e.*, when dissolution is not complete. Vaughan and Leach (1) stressed this finding, and it is only reasonable that the method must break down when the drug in the fastest dissolving formulation has completely dissolved. As mentioned earlier, the method will be of most use when the dissolution rate limits input to the body. This condition was ensured in these simulations by having the drug pass rapidly through compartment B such that the ratio of $A/(A + B)$ was nearly 1 (0.98 for the linear case). Reducing k_3 increased the time over which there was close agreement between the predicted and correct values, but this agreement still only occurs on the rising phase of the plasma profile.

However, two important parameters are badly predicted by this method: the peak plasma concentration and the time required to reach it. The peak plasma concentration averages 30–40% in error, while the time required to reach it is over 100% out. The results for the log normal dissolution are particularly bad, but this function is probably "too nonlinear." To be of use to the pharmaceutical industry or the clinician, both parameters would need to be predicted better. Thus, we warn against noncritical use of the method, but the method can be very useful in certain instances. To obtain reliable prediction, the mechanism of dissolution between formulations should be the same both *in vitro* and *in vivo* (as emphasized by Vaughan and Leach). Since the time over which the predictions are reliable is limited by the fastest dissolving formulation to be of use, the dissolution profiles of the formulations to be compared must be similar.

In the present analysis, we took the optimal situation in which the *in vitro* dissolution profile has been assumed to be exactly the same as occurs *in vivo*. If the time scales, however, are different—and this cannot be predicted *a priori*—the difficulties that are created may invalidate the method.

[1] The dissolution profile for the log normal law was calculated using Wagner's (3) method:

$$\frac{dA}{dt} = \frac{e^{-x^2/2}}{\sqrt{2\pi}\sigma t \log_{10} e}$$

and:

$$x = \frac{\log_{10} t - x \text{ mean}}{\sigma}$$

where x mean and σ are the mean and standard deviation of the distribution, respectively. However, rather than fixing x mean and letting σ vary as Wagner did, we fixed the ratio of x mean/σ (= 5), thus giving a more realistic spread of dissolution profiles.

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Screening Procedure for Phorbol Esters Using Brine Shrimp (*Artemia salina*) Larvae

Keyphrases □ Phorbol esters, various—toxicity to brine shrimp larvae related to cocarcinogenic potency □ Brine shrimp larvae—toxicity of various phorbol esters related to cocarcinogenic potency □ Toxicity—various phorbol esters to brine shrimp larvae, related to cocarcinogenic potency □ Cocarcinogenic potency—various phorbol esters, related to toxicity to brine shrimp larvae

To the Editor:

In the continuing search for anticancer agents of natural origin, the recently discovered antileukemic property of phorbol 12-tigliate 13-decanoate (1), from *Croton tiglium* (Euphorbiaceae), is interesting since phorbol esters are better known as irritant cocarcinogens (2). Unlike other naturally occurring anticancer compounds, intensive work has been performed on the activity of phorbol esters in two-stage carcinogenesis experiments, including their interactions with nucleic acids and their effect on the uptake of precursors of these and other macromolecules by the mammalian cell (3). Isolation of further phorbol and related esters from the plant kingdom could, in addition to providing possible new antileukemic agents, yield unique structure–activity information on the complex relationships between irritancy, cocarcinogenicity, and antileukemic activity at the cellular level.

During the fractionation of anticancer compounds from plant sources, there is often a considerable delay between isolation and receipt of biological data. Several screening

Table I—ED_{50}'s of Phorbol Esters and Two Piscicides to *A. salina* Larvae (Brine Shrimp)

Compound	ED_{50}, μg/ml	$f_{ED_{50}}$	95–100% Confidence Limits, μg/ml
Phorbol 12-tetradecanoate 13-acetate	3.8	1.96	1.9–7.4
Phorbol 12,13-didecanoate	6.8	1.96	3.5–13.3
Phorbol 12,13-dibenzoate	11.8	1.96	6.0–23.1
Phorbol	>1000	—	—
4α-Phorbol 12,13-didecanoate	>1000	—	—
Rotenone	0.5	1.71	0.3–0.9
Picrotoxin	2510	1.75	1430–4400

procedures to hasten this time period previously were employed, including the use of goldfish (1) and killifish (4), acute toxicity to frogs (2), and irritant activity on the mouse ear (2). All of these methods require some cost in the provision of specialized laboratory personnel and in animal housing and maintenance. We have found the toxicity to brine shrimp (*Artemia salina*) larvae to be selective between phorbol esters of different cocarcinogenic potencies (1) and also to be sensitive when compared with the piscicides rotenone (4) and picrotoxin (5). This method not only allows the recovery of samples and requires little capital outlay of laboratory space but is capable of being performed by persons with little formal training in biology.

In the laboratory procedure, brine shrimp nauplii were hatched in artificial sea water and separated from unhatched eggs 2 days after seeding. Since phorbol esters are unstable even in the solid form (6), their purity was affirmed in several TLC systems (7). Solutions prepared in methanol were pipetted into numbered 30-ml beakers, with each dilution varying from the last by a factor of log 2. The solvent was removed, the residue in each beaker was dissolved in 0.2 ml of 1% polysorbate 60, and 2.8 ml of a brine shrimp suspension was added so that 30–50 *A. salina* were contained in 3.0 ml.

Forty-eight hours after setting up the experiment, counts were made of dead shrimp. The total population count was determined after sacrifice of survivors with 37.5% formaldehyde solution. Spontaneous mortality in polysorbate controls was <1% and was not considered in subsequent statistical evaluation. Mean values of percent dead animals were plotted on probability paper against log doses, and 95–100% confidence limits of the effective mortality dose (ED_{50}) were obtained in micrograms per milliliter by the method of Litchfield and Wilcoxon (8). Satisfactory statistical limits were obtained when eight replicates per compound were employed.

The potentiality of *A. salina* as an organism for monitoring phorbol esters is clearly shown in Table I. The highly cocarcinogenic compounds phorbol 12-tetradecanoate 13-acetate and phorbol 12,13-didecanoate and the weaker cocarcinogen phorbol 12,13-dibenzoate approached the high lethality of rotenone, approximately in proportion to their tumorigenic potencies (3). The low toxicity of picrotoxin demonstrated that the test system was not generally sensitive to potent phytotoxins. Phorbol, an inactive irritant cocarcinogen, did not affect the brine shrimp. The differential response of this Crustacean branchiopod to phorbol 12,13-didecanoate and its 4α-isomer, which is not cocarcinogenic, shows for the first time structural specificity about the C-4 hydroxyl of the tigliane skeleton in an invertebrate system.

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