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Short Communications

Microcalorimetric studies of the interaction of *m*-hydroxybenzoates with *E. coli* and with *S. aureus*. Demonstration of a Collander relationship for biological response

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Summary

A linear relationship exists between the microcalorimetrically observed biological response to challenge of a sensitive organism by a series of drugs and the number of methylene groups present in the alkyl chains of these same drugs. A Collander type relationship is shown to exist for microcalorimetric response data obtained for interaction of alkyl *m*-hydroxybenzoates with *Escherichia coli* and *Staphylococcus aureus*.

Recent papers from this laboratory (Beezer et al. 1986a, 1987a) have demonstrated the existence of group additivity schemes for biological response upon interaction of *p*-hydroxybenzoates and *o*-, *m*- and *p*-alkoxyphenols with *Escherichia coli*. These data are the biological equivalent to linear free energy relationships (LFER) and indicate that such relationships do indeed exist for, at least, the initial interaction of these metabolic modifiers with *Escherichia coli*. If such biologically based direct LFERs could be shown to be general for biological responses it would obviate some of the need to establish structure–activity relationships (QSAR) by indirect (inferential) methods such as through partition coefficients, fragmental constants or other physicochemical parameters (Taylor and Kennewell, 1981).

Typically, measurement of partition coefficients for solutes partitioned between aqueous phase and different non-aqueous solvents are correlated via the Collander equation (Kubinyi, 1978). This equation has recently been subject to a thermodynamic analysis (Beezer et al., 1987b) which indicated the thermodynamic basis of the linear regression parameters.

The slope of the Collander equation was also shown to be of use in establishing a reference solvent for use in partitioning studies. Just as the original Collander equation established a relation-

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TABLE 1

Log(dose)$_{max}$ data for interaction of alkyl m-hydroxybenzoates upon interaction with Escherichia coli NCTC 10418 and with Staphylococcus aureus NCTC 4135

	E. coli	*S. aureus*
Methyl	1.4276	1.4903
Ethyl	0.3336	0.9971
Propyl	−0.1761	0.8669
Butyl	−0.0989	0.3749

ship between K_d for partition of a solute between different solvents, so the biologically based LFER data should allow the derivation of a Collander type equation relating the interaction of a solute with a variety of organisms.

Shown in Table 1 are the data for $\log(\text{dose})_{max}$ (derived as described previously, Beezer et al. 1986a, 1987a) for interaction of a series of *m*-hydroxybenzoates with *Escherichia coli* (NCTC 10418) and with *Staphylococcus aureus* (NCTC 4135). These data when plotted in the form of the Collander equation i.e.

$$\log(\text{dose})_{max}(E.\ coli) = a + b\ \log(\text{dose})_{max}(S.\ aureus)$$

yield the following regression parameters: all compounds: $a = 0.732$, $b = 0.540$, $r = 0.8707$. Excluding the butyl derivative; $a = 0.908$, $b = 0.399$, $r = 0.9932$.

Thus, there appears to exist, as expected, a formal relationship between the biologically determined values of $\log(\text{dose})_{max}$ for each organism, the regression parameters indicating the relative sensitivities of the organisms toward the applied compounds (see Beezer et al., 1987b, for a thermodynamic analysis of the Collander equation). As shown previously (Beezer et al., 1987b) the value of b can be a sensitive discriminator between solvent systems used in partitioning studies. Here, no doubt, it reflects the differing solvent/barrier properties of the cell walls of the organisms studied. The wall of *E. coli* has an outer lipid layer and is typical of Gram-negative organisms, while the *S. aureus* wall is typical of Gram-positive organisms, comprised mainly of a matrix of peptidoglycans and teichoic acids. However, although the cell walls of most bacteria may be considered to be either of the Gram-positive or -negative type there exists wide variation in structure and composition with these types. For example the walls of mycobacteria (a Gram-positive group which includes a number of important pathogens) may contain large amounts of lipid (up to 60% of the dry weight). Because of the limited number of compounds studied it is not possible to determine whether the Collander form applies to all compounds in the series or whether there are two such relationships, one extending only to the propyl derivative and one extending from the butyl derivative onwards. Such a "break" in properties around the butyl derivative in various homologous series is not unusual (Yalkowsky et al., 1972; Smith et al., 1975; Beezer et al., 1986b, 1987c and d).

Apart from confirming the generality of the Collander relationships the demonstration here of such a relationship for two different organisms may be useful for: (i) prediction of activity of drug substances toward micro-organisms other than those under test, thus reducing the need to evaluate directly the response of pathogens; (ii) introducing more detail and information into discussions of the relative sensitivities and mechanisms of action of different organisms toward a given drug substance.

We believe this is the first direct demonstration that the absorption of compounds from aqueous solution by cell walls is exactly analogous to the partition process into non-aqueous solvents. Such a Collander relationship emphasises the role of cell walls as *solvents;* the possibility of predicting that role has interesting implications in drug action.

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