DOSAGE/RESPONSE RELATIONSHIPS IN MOULD INHIBITION

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WHILE in the inhibition of several moulds by alkyl *p*-hydroxybenzoates, the expression :

$$y = a\,x^b, \qquad (1)$$

where y is proportionate reduction in rate of increase in colony diameter (inhibition), x is concentration of inhibitor, a and b are constants ($b < 1$), provides a fair statement of results and permits a reasonable comparison of substances[1], considerations raised by Hinshelwood in connexion with antibacterial action[2] have suggested the desirability of re-examining data for agreement with the form of the Langmuir adsorption isotherm.

The experimental points for the inhibition of *Aspergillus niger* by the alkyl *p*-hydroxy- and *p*-aminobenzoates is fitted rather better by the logarithmic (equation 1) than by the simple adsorption relationship. For these cases the former remains the better statement of results, although it throws no light on possible mechanism of inhibitor action. There is, however, little to choose between the two curves over a fair part of the inhibitory range, and even with this organism the adsorption form might serve as a first approximation. The alkyl *p*-aminobenzoate inhibition of two other organisms (*Penicillium roqueforti* and *Byssochlamys fulva*) fits the adsorption equation as well as, or rather better than, the logarithmic form. The detail of these investigations will be reported elsewhere, but certain implications of the equation based on the adsorption phenomenon seem to merit attention.

From the adsorption isotherm :

$$k_1 x(1-A\theta) = k_2 A\theta, \qquad (2)$$

where k_1 and k_2 are constants of adsorption and desorption respectively, θ is number of molecules adsorbed per unit area, A is effective area occupied by each molecule adsorbed at the surface, x is molar concentration of substance being adsorbed, it follows that :

$$A\theta = B.x/(1 + Bx), \qquad (3)$$

where $B = k_1/k_2$.

We can now look at two ways in which inhibition may be related to adsorption per unit area :

(i) as proportional to the *area* occupied by the adsorbed molecules, when

$$y = K_1 A\theta, \qquad (4)$$

where y is inhibition, K_1 is a constant expressing the relative effectiveness per unit area occupied by adsorbed molecules ;

(ii) as proportional to the *number* of molecules adsorbed, when

$$y = K_2\theta, \qquad (5)$$

where K_2 is a constant expressing the relative inhibitory effectiveness per molecule adsorbed.

Regarding the first possibility and combining (3) and (4),

$$y = K_1 B.x/(1 + Bx). \qquad (6)$$

The corresponding equation for the second postulate would be :

$$y = K_2 B.x/A(1 + Bx). \qquad (7)$$

Equations (6) and (7) can then be written in the linear forms :

$$x/y = 1/K_1 B + x/K_1. \qquad (6a)$$

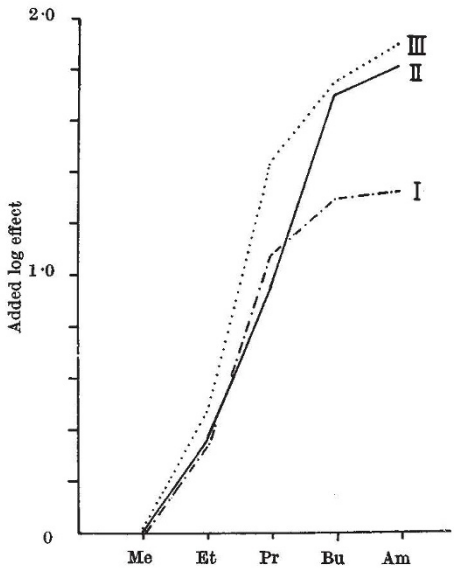
$$x/y = A/K_2 B + Ax/K_2. \qquad (7a)$$

If then the experimental data be plotted as x/y against x, the slope of the line (generalized as $1/K$) represents $1/K_1$ or A/K_2 according to the supposition being examined.

Whatever the detailed factors represented by the slope, B can be determined by substitution in equations (6*a*) and (7*a*). This constant would then, by definition, provide a measure of relative adsorbability at a biological surface.

It is possible by these means to examine dosage/response data in order to analyse inhibitory action in terms of : 'biological' adsorbability (B) and relative effectiveness per unit area occupied by adsorbed molecules (K_1 or K_2/A). The technique seems likely to have a use in the more critical study of toxic substances and has obvious similarities to the Lineweaver and Burk manipulation of the Michaelis–Menten equation[3].

The application of the method is illustrated in the case of the relative inhibitory action of successive alkyl *p*-aminobenzoates on *B. fulva* (see graph).



INCREASED EFFECT WITH INCREASING CHAIN-LENGTH OF ALKYL *p*-AMINOBENZOATES

Curve I : Logarithm of concentration causing 50 per cent inhibition of *B. fulva*. Methyl = −0·43.

Curve II : Logarithm of lowering of surface tension (data of Adams, Rideal *et al.*[4]). Methyl = 0·28.

Curve III : Logarithm of 'biological adsorption' (B) as defined in text. Methyl = −1·0.

Curve I shows the increase with chain-length of overall inhibitory effectiveness as gauged by − log (concentration giving 50 per cent inhibition). This should be compared with surface activity data (Curve II)[4] from Adams, Rideal *et al.* Up to propyl, there is good agreement between this biological effect and the simple physical property of lowering surface tension at an air–water interface. Beyond propyl, the biological effect falls markedly below the surface tension data. If, however, K and B are dissected out from the biological data, there are two interesting consequences. The slope of the line increases, that is, K decreases, with chain-length :

Ester	K
Methyl	20·4
Ethyl	18·5
Propyl	12·4
Butyl	11·4
Amyl	9·8

At the same time, the logarithmic increase in 'biological' adsorption (B), plotted against chain-length (Curve III), agrees very much better with the simple physical determination (Curve II).

Since inhibition seems likely to be more closely related to the number of molecules adsorbed than to the area they occupy, it would seem profitable to examine the slope values regarded as A/K_2 (equation 7a). Without losing sight of the possibility of decreased K_2/A being due to a decrease in effectiveness per molecule (K_2), a more reasonable proposition for such a homologous series would be that it results from increasing A due to extra molecular bulk or a different disposition relative to the adsorbed surface.

It needs to be observed at this point, however, that other inhibitor/organism relationships need not be so straightforward as that quoted above. At least two reasons can contribute to this: (i) the accurate determination of the constants is often difficult; (ii) the dosage/response relationship is, to a varying degree, only approximated by the simple adsorption form, and it might be necessary to restrict the determination of constants to a relatively small portion of the curve such that permits valid comparison between substances. With these reasonable provisos, data for *P. roqueforti* and *A. niger* seem to show at least the same trends as those seen clearly in *B. fulva*. Their detailed examination will be dealt with elsewhere in collaboration with Mr. G. W. K. Cavill.

In conclusion, we should like to express our indebtedness to Prof. C. N. Hinshelwood for his critical comment on an earlier draft of the ideas set out in the present communication. Needless to say, although the present form has been influenced by such comments, Prof. Hinshelwood cannot be held responsible for any of its residual imperfections.

[1] Vincent, J. M., *J. Soc. Chem. Ind.*, **66**, 149 (1947); Cavill, G. W. K., and Vincent, J. M., *ibid.*, **66**, 175 (1947).
[2] Hinshelwood, C. N., "The Chemical Kinetics of the Bacterial Cell" (Clarendon Press, Oxford, 1946).
[3] Wilson, P. W., chapter in "Respiratory Enzymes" (University of Wisconsin Biochemists, Burgess Publishing Co., 1939).
[4] Adams, R., Rideal, E. K. *et al.*, *J. Amer. Chem. Soc.*, **48**, 1758 (1926).

NITROGENOUS CONSTITUENTS OF THE POTATO

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OSBORNE and Campbell[1] isolated a globulin from the expressed sap of minced potatoes. By extracting the crushed potato residue with 10 per cent sodium chloride, they were able to obtain an additional amount of protein which they considered was identical with the globulin contained in the press juice. When a solution of this protein in 10 per cent sodium chloride was gradually heated, a flocculent coagulum was formed at about 60° C., and further precipitates were obtained at higher temperatures up to 80° C. At this temperature coagulation of all the protein was complete, although a minute amount of proteose material appeared to remain in solution. The authors concluded that the only true protein present in potato was a globulin, with rather indeterminate heat-coagulation properties, for which they proposed the name of 'tuberin'. Neuberger and Sanger[2] extended this work and found that the potato residue, after removal of the press juice, contained a small amount of nitrogen (about 8 per cent of the total potato-nitrogen) which was insoluble in all the usual protein solvents. In more recent studies on tuberin, Groot, Janssen, Kentie, Oosterhius and Trap[3] have shown that it can be separated electrophoretically into two components.

Determinations of the amino-acid content of tuberin were carried out by Sjollema and Rinkes[4] and by Groot[5]. The results of the earlier workers are probably not very reliable owing to the inadequacy of methods at that time. It is noteworthy, however, that Groot's recent figure for the lysine content of tuberin is of the same low order, 3·6 per cent, as that obtained by the earlier workers.

Using Gale's decarboxylase method[6], I have found that a sample of tuberin, prepared from King Edward potatoes by heat coagulation of the press juice at 80° and *p*H 4·5, had a lysine content of 7·7 per cent, a value that is more consistent with the high nutritive value of tuberin reported by Kon[7] and by Chick and Cutting[8]. Estimations of the other essential amino-acids of tuberin were made by the methods of Tristram[9] for valine, leucines, tyrosine and phenylalanine, of Macpherson[10] for arginine and histidine, of Lugg[11] for cystine and methionine, of Hess and Sullivan[12] for tryptophan, and of Rees[13] for threonine. The results show that the only amino-acid present in inadequate amount is methionine (see accompanying table). In addition to tuberin, the potato contains a variety of other nitrogenous substances present in considerable amount, the actual ratio of protein to non-protein nitrogen varying appreciably with different samples. In the case of the King Edward potatoes used in the present work, about half to two-thirds of the total nitrogen was non-protein.

AMINO-ACID COMPOSITION OF TUBERIN AND OF WHOLE POTATO

Amino-acid	Amino-acids (gm.) per 16 gm. nitrogen in Whole potato	Tuberin
Phenylalanine	5·4	6·6
Leucines	11·3	17·5
Valine	4·8	6·1
Tryptophan	0·8	1·6
Threonine	3·7	5·9
Arginine	4·4	6·0
Histidine	1·7	2·2
Lysine	5·0	7·7
Cystine	1·7	2·1
Methionine	1·6	2·3

Neuberger and Sanger[2] and Chick and Cutting[8] examined the non-protein fraction and found that about 50 per cent of the nitrogen was present in the form of amides (glutamine and asparagine), and about 30 per cent in the form of nitrogenous bases precipitable by phosphotungstic acid. Only about 10–20 per cent of the non-protein nitrogen was found to be present in the form of α-amino-acids. In the present work, this α-amino fraction has been separated by the procedure of Vickery[14] and analysed for the essential amino-acids by the methods specified for tuberin above. By combining the values obtained for the amino-acid content of the non-protein fraction with those found for tuberin, and assuming that all the protein present in potato has the same composition as tuberin, it is possible to calculate the amino-acid composition of the 'crude-protein' (N × 6) of the whole potato. The result is given in the table, and shows that the latter is poorer in its content of all the essential amino-acids than tuberin, from which it might be predicted that its nutritive value would also be inferior to that of tuberin.