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The effect of concentration, time and temperature on the viability of *Penicillium notatum* spores exposed to phenols

N. M. CHAUHAN* AND V. WALTERS†

Spores of *Penicillium notatum* exposed to resorcinol, *p*-chlorophenol, *m*-cresol, chlorocresol and hexylresorcinol, give values for the concentration exponent, n, from 7·3 to 3·7 when calculated from first order reaction velocities; higher values are obtained from the times for fixed mortality levels. Values of the temperature coefficient, θ, vary from 1·045–1·331. The log survivor-time curves are convex to the axes for resorcinol and increasingly concave in the order of decreasing water solubility for the other phenols. Equitoxic (99% mortality in 20 min) molar concentrations of the phenols have about a 680-fold range, but Ferguson values, except for resorcinol, vary only from 0·10 to 0·26.

THE viability of *Penicillium notatum* spores exposed to phenol has been investigated by Chauhan & Walters (1961, 1962). We now describe the change in activity resulting from the introduction of various substituents into the phenol molecule.

Experimental

Aqueous solutions of *m*-cresol (Laboratory Reagent) 2%, resorcinol B.P. 10%, *p*-chlorophenol (Laboratory Reagent) 1%, chlorocresol B.P. 0·25%, hexylresorcinol B.P. 0·4%, were stored at 4° and diluted as required. The activity of these compounds at 25° was examined in the same way as that of phenol (Chauhan & Walters, 1961, 1962), using suspensions containing $6{\cdot}25 \times 10^6$ ml^{-1} *P. notatum* spores. Counts were made after incubation at 28° for 12 hr, and, when hyphal growth was not too dense to permit accurate counting, at intervals up to 48 hr, to ensure maximum recovery.

Results

Spores remaining viable after exposure to resorcinol and phenol, germinated within 12 hr, but 14 hr were necessary with the other phenols. When there were few survivors, germination occurred up to 20 hr after exposure. Untreated spores germinated within 10 hr. Spore mortality increased with the concentration of phenols, reaction time and temperature (Figs 1 and 2). The shape of the log survivor curves for spores exposed to 0·08% chlorocresol at 5° increments in temperature from 15–40°, was the same as for the effect of concentration in Fig. 1.

Discussion

The values of the concentration exponent, n, calculated from reaction velocities, are less than those calculated from the times for particular mortalities (Table 1). This discrepancy occurs because of the greater

From the Department of Pharmacy, University of Ife, Ibadan Branch, Ibadan, Nigeria.

* School of Pharmacy, Haile Sellassie I University, Addis Ababa. † The School of Pharmacy, University of London, 29–39, Brunswick Square, W.C.1.

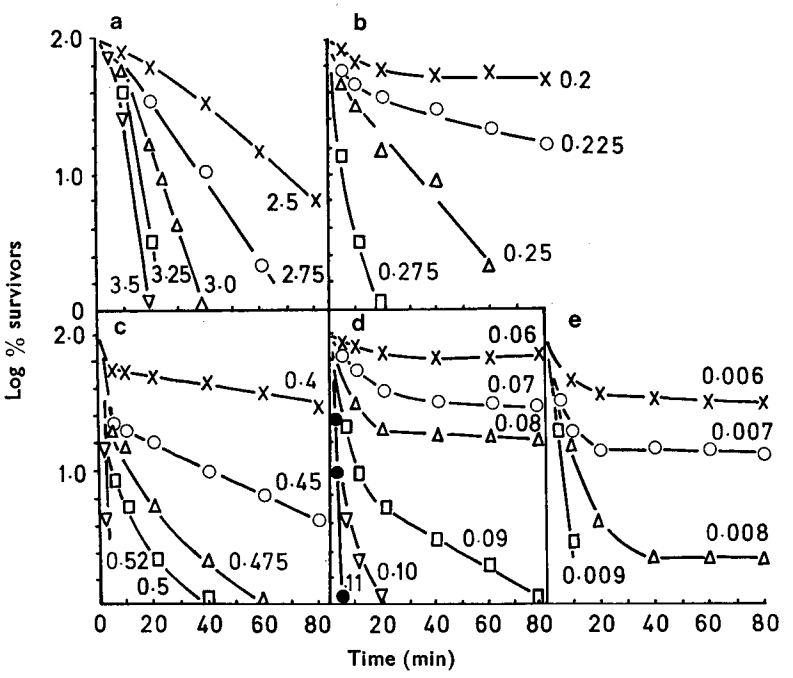

FIG. 1. Log % survivor-time curves for *P. notatum* spores exposed to (a) resorcinol, (b) *p*-chlorophenol, (c) *m*-cresol, (d) chlorocresol and (e) hexylresorcinol. Figures on curves are % concentrations.

divergence of the rate of fungicidal reaction from first order kinetics, over the wider concentration range used in the former method of calculation.

Comparable values of n and the temperature coefficient, θ (Table 2), for phenols have been obtained with bacteria (Chick, 1908; Tilley, 1939, 1942; Withell, 1942; Jordan & Jacobs, 1945, 1946) and bacteriophage (Brown, Cook & Oduro-Yeboah, 1965). The values of n increased with temperature for chlorocresol and hexylresorcinol, but showed little change

TABLE 1. CONCENTRATION EXPONENTS (n) FOR THE ACTIVITY OF PHENOLS ON *P. notatum* SPORES

Compound	n	
	a	b
Phenol	6·9 (0·5–1·25)*	11·6 (1·0–1·25)
Chlorocresol	5·6 (0·06–0·11)	13·8 (0·09–0·11)
m-Cresol	7·0 (0·4–0·5)	37·0 (0·475–0·52)
p-Chlorophenol ..	7·3 (0·2–0·275)	13·7 (0·25–0·275)
Resorcinol	6·2 (2·5–3·5)	5·2 (2·75–3·5)
Hexylresorcinol ..	3·7 (0·006–0·009)	7·1 (0·007–0·009)

Mean values of n calculated using Watson's (1908) equation:

(*a*) from first order reaction velocities, k min^{-1}, for each concentration;

(*b*) from times for 99% mortality (75% for hexylresorcinol) read from survivor curves. Values of n from the calculated slopes of log time for 75% mortality—log concentration regressions are similar to (b).

* Range of concentrations.

TABLE 2. TEMPERATURE COEFFICIENTS (θ) FOR THE ACTIVITY OF PHENOLS ON *P. notatum* SPORES

Compound	Concentration %	Temperature range ° C	θ
Chlorocresol ..	0·07	15–40	1·045
	0·08	15–40	1·060
	0·09	15–35	1·081
m-Cresol ..	0·4	15–40	1·075
	0·45	15–35	1·045
p-Chlorophenol	0·25	15–30	1·120
Resorcinol ..	3·25	15–30	1·331
	3·50	15–25	1·276
Hexylresorcinol	0·005		1·055
	0·006	15–40	1·055
	0·007		1·095

θ calculated by substitution of reaction velocities in equation of Phelps (1911).

TABLE 3. EFFECT OF TEMPERATURE ON CONCENTRATION EXPONENTS OF PHENOLS ON *P. notatum* SPORES

Compound	Concentration range %	n					
		Temperature ° C					
		15	20	25	30	35	40
Chlorocresol ..	0·07–0·09	3·1	2·7	4·0	5·6	5·5	6·4
m-Cresol	0·4–0·45	9·6	6·9	6·9	6·7	6·9	—
Resorcinol ..	3·25–3·5	11·2	2·9	6·4	—	—	—
Hexylresorcinol ..	0·005–0·007	2·5	2·9	4·2	5·5	5·4	5·5

θ calculated from reaction velocities.
Experiment not done with *p*-chlorophenol.

for *m*-cresol, and a variable response for resorcinol (Table 3). With bacteria, Berry & Michaels (1950) found a decrease with ethylene glycol ethers, whereas Tilley (1939) noted both an increase and a decrease with phenols. The activity of the phenols used was not modified by ionization, since their pK_a values are about 9–10 (Kortum, Vogel & Andrussow, 1961) and the pH values of the reaction systems were about 5·5. The effect of substituting a *m*-hydroxy group in the phenol molecule was to decrease its relative per cent activity about threefold, but a *m*- Me, *p*- Cl, *m*- Me and *p*- Cl, or *p*- $CH_2(CH_2)_4Me$, increased it about 2, 4, 11 and 125 times respectively (Table 4). The order of molar solubility of the phenols in water correlates with this increase in activity and approximates to the change in shape of the survivor curves, that is, convex to the axes for the very soluble resorcinol, linear for phenol and concave to the axes for the other phenols. The distal portions of the curves for the latter phenols become increasingly flatter in the order of decreasing solubility. The lipid solubility of phenol is increased by a substituent -Cl or alkyl group whereas an -OH decreases it. The cell membrane being of a lipoprotein is penetrated more readily by neutral molecules possessing lipophilic groups (Davson & Danielli, 1952; Albert, 1963, 1965). It is likely, therefore, that the initial rate of reaction, and hence the shape of the proximal portion of the log survivor-time curve, is determined by the lipid solubility of the compound. Thus Richardson & Reid (1940), Fogg & Lodge (1945) and Bean, Heman-Ackah & Thomas (1965) have related the

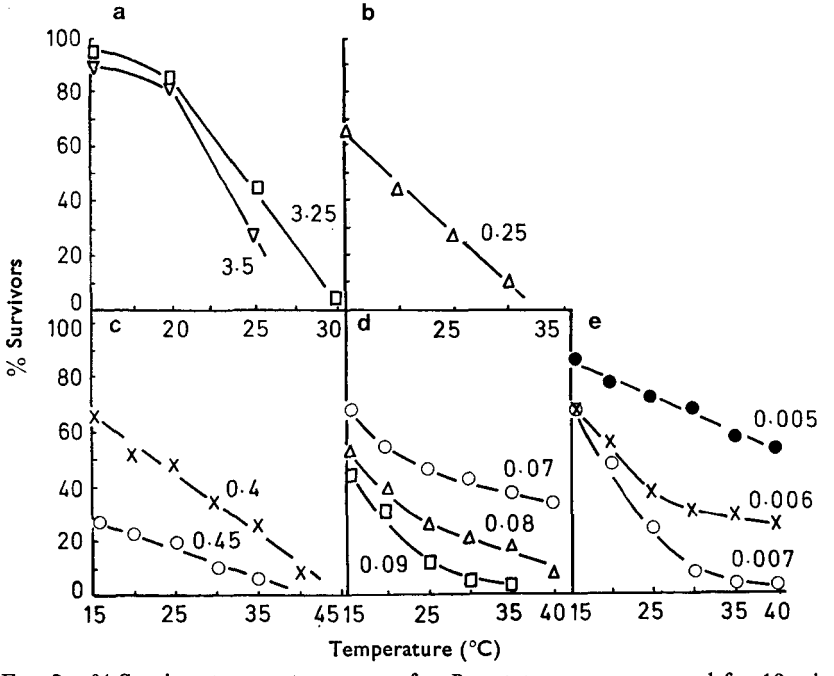

FIG. 2. % Survivor-temperature curves for *P. notatum* spores exposed for 10 min to phenols as in Fig. 1.

antibacterial activity of phenols to their o/w partition coefficients. Presumably, with the highly polar compound, resorcinol, penetration of the lipid layer of the cell membrane occurs slowly with a consequent lag in action. With phenol there was no evident lag while the more lipid soluble compounds act rapidly initially, because adsorption and penetration of the lipid barrier to reaction sites can occur more readily. It is likely that an equilibrium distribution, as reported by Bean & Das (1966), is set up in the systems. Unless more cell receptor sites can be occupied

TABLE 4. RELATIVE ACTIVITIES OF PHENOLS ON *P. notatum* SPORES

	Resorcinol	Phenol	*m*-Cresol	*p*-Chlorophenol	Chlorocresol	Hexylresorcinol
Conc (%) for 99% mortality in 20 min ..	3·5	1·125	0·515	0·275	0·1	0·009
Molarity (St)	0·318	0·119	0·0476	0·0214	0·007	0·00046
Relative activity (%) ..	0·32	1	2·2	4·1	11·3	125
Relative molar activity ..	0·38	1	2·5	5·6	17	258
Approx. solubility in water 1 in	1	12	42	37	260	2000
Solubility in mole/litre (So)	9·083	0·885	0·22	0·21	0·027	0·0026
Ferguson value $\frac{St}{So}$ ..	0·035	0·14	0·22	0·1	0·26	0·18

by partitioning of phenol, through increasing the concentration of the aqueous phase, mortality ceases or at least is much reduced, as, for example, with hexylresorcinol. Thus the apparent resistance of the spore population would appear to be relative to the toxic agent, giving rise to survivor curves of different shape.

Table 4 shows that when equitoxic molar concentrations are compared, there is about a 680-fold range in activity. When the thermodynamic activity is calculated (Ferguson, 1939) the variation is only about 7·4-fold, and, except for resorcinol, the aqueous phase is toxic at about 1/10 to 1/4 phenol saturation. The latter values indicate that the lipid solubility of the phenol molecule could be increased, by substitution, beyond that of hexylresorcinol, to produce equitoxic compounds active in greater dilution, since the aqueous phase saturation of the toxic concentration of hexylresorcinol is only 0·18.

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