ON THE RELATIONSHIP BETWEEN THE EFFECT OF PHENOL ON THE OXYGEN UPTAKE AND THE VIABILITY OF *PENICILLIUM NOTATUM* SPORES

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0·05 to 0·2 per cent phenol in Horowitz medium progressively reduces the oxygen uptake of *Penicillium notatum* spores. 0·08 per cent causes fungistasis and fungicidal action is evident at 0·15 per cent and higher concentrations. Exogenous respiration is less sensitive to phenol than spore germination. Measurement of oxygen uptake can not be used for quantitative evaluation of fungicidal action. The concentration exponent for the effect of phenol on oxygen uptake is $-1·5$.

The respiration of cells—which includes all cellular oxidations yielding energy—is usually measured in terms of oxygen uptake or carbon dioxide formed. There is relatively little information correlating the effects of fungicides on the respiration and viability of fungal spores. It was, therefore, of interest to measure the respiration of the spores of *Penicillium notatum* in the presence and absence of phenol and to relate it, if possible, to the fungistatic and fungicidal action.

Experimental

The methods of preparation of spore suspensions containing $37·5 \times 10^6$ spores per ml. and of evaluation of fungicidal activity were those of Chauhan and Walters (1961).

The procedure for measurement of oxygen uptake was essentially that described by Umbreit, Burris and Stauffer (1959), using a Warburg apparatus at 28° and a shaking speed of 100 oscillations per min. To the body of each reaction flask was added 1·5 ml. of double-strength Horowitz (1947) fluid medium (pH 5·3), 0·5 ml. of freshly prepared spore suspension and sufficient sterile water to produce 3 ml., allowing for the volume of 0·5 or 1·0 per cent phenol solution added to the sidearm. The centre well contained 0·2 ml. of 20 per cent potassium hydroxide solution and a 2 cm. square of fluted filter paper. The phenol solution was tipped into the body of the reaction flask, usually 1 hr. after closing the manometer tap, but in some cases after 4, 6 or 9 hr. Readings were taken hourly for 12 hr. and at intervals for a further 12 hr., where practicable. Duplicate reaction flasks and thermobarometers were set up for each experiment and all results are the means of replicate experiments.

To determine whether there was fungistasis a reaction flask was opened after 12 or 24 hr. and a sample of the suspension examined microscopically for swollen spores and germ tubes. Absence of bacterial contamination was confirmed at the same time. The suspension was then filtered, the spores washed, and fungicidal activity evaluated; counts were made after 4 to 48 hr. incubation, according to the phenol concentration and time before addition.

RESULTS

Fig. 1 shows that the oxygen consumption of *P. notatum* spores in Horowitz medium decreased as the phenol concentration was increased from 0·05 to 0·2 per cent; 0·25 per cent produced but little further reduction. Spore germination was not inhibited by 12 hr. contact with 0·05 per cent phenol. In 0·06 per cent phenol large swollen spores were present after 12 hr. and most of these produced germ tubes in 24 hr. Spores were

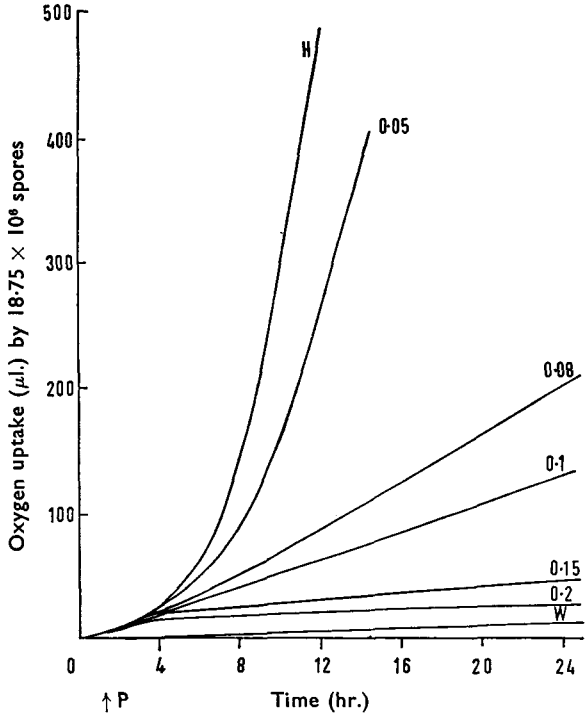

FIG. 1. Oxygen uptake of *P. notatum* spores at 28° in Horowitz medium (H) and with phenol added (P ↑) after 1 hr. to give a concentration of 0·05—0·20 per cent, and in water (W).

only slightly swollen in 0·08 per cent phenol after 12 hr. and there was no change after 24 hr. Above this concentration there was neither swelling nor germination in 24 hr. Less than 20 per cent of spores were killed in 12 hr. by 0·05 to 0·15 per cent phenol, a much higher percentage was killed with higher concentrations or a longer (24 hr.) contact time (Table I).

Fig. 2 shows that the rate of oxygen uptake per hr. increased in the absence of phenol. When phenol was added after 1, 4, 6 or 9 hr. to give a concentration of 0·1 per cent, the rate remained almost the same as that before addition but with higher concentrations it decreased markedly with increase in the time before addition. The fungicidal action was much greater when phenol was added after 4 hr. than after 1 hr. but there

TABLE I

EFFECT OF CONCENTRATION OF PHENOL ON OXYGEN UPTAKE AND VIABILITY OF *P. notatum* SPORES

Phenol* concentration (per cent)	Oxygen uptake (μl.) by $18{\cdot}75 \times 10^6$ spores in		Germination† in		Per cent survivors after	
	12 hr.	24 hr.	12 hr.	24 hr.	12 hr.	24 hr.
0	486	–	+	+	95	–
0·05	273·6	–	+	+	95	–
0·06	151·9	–	–S	±S	89	–
0·08	94·2	205·8	–S	–S	89	83
0·1	61·6	132·0	–	–	86	91
0·13	39·5	66·9	–	–	87	–
0·15	32·4	47·1	–	–	80	38
0·176	27·0	33·4	–	–	57	–
0·2	19·7	27·2	–	–	37	13
0·25	18·0	26·0	–	–	14	0·002
Control	7·0	12·3	–	–	95	97

* Spores suspended in Horowitz medium and phenol solution added after 1 hr. to give concentration shown. Control = spores in water only.

† Suspension examined for germination and swelling (S) of spores, then filtered, spores washed and incubated in Horowitz medium to determine per cent survivors. (+ = all spores germinated, ± = many germinated, – = none germinated).

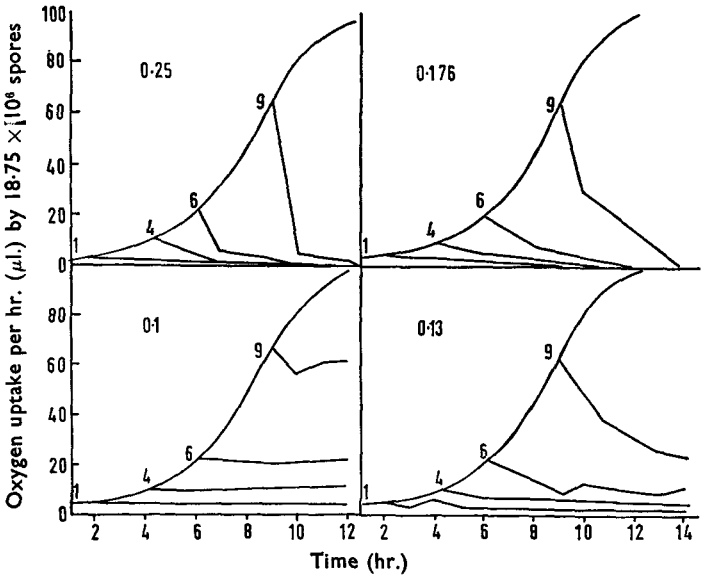

FIG. 2. Effect of adding phenol after 1, 4, 6 and 9 hr. to give a concentration of 0·1, 0·13, 0·176 or 0·25 per cent on the oxygen uptake of *P. notatum* spores in Horowitz medium at 28°.

was little further increase in activity when it was added after 6 or 9 hr. (Table II).

Spores in Horowitz medium without phenol showed no change in size after 1 hr. but after 4 hr. they were swollen and after 6 hr. about 10 per cent had produced small germ tubes. All spores appeared to have germinated in 9 hr. The production of germ tubes was accompanied by an increased rate of oxygen uptake (Figs. 1 and 2).

TABLE II

EFFECT OF TIME OF ADDITION OF PHENOL ON THE VIABILITY OF *P. notatum* SPORES

Phenol* concentration (per cent)	Per cent survivors after 12 hr.† when phenol was added after			
	1 hr.	4 hr.	6 hr.	9 hr.
0·1	88	83	79	63
0·13	87	68	65	67
0·15	80	32	30	27
0·176	57	4	1	<1
0·25	15	*c.* 0·007	*c.* 0·001	*c.* 0·001

* Spores suspended in Horowitz medium and phenol solution added after 1, 4, 6 or 9 hr. to give concentration shown.

† Suspension filtered 12 hr. after commencement, spores washed and incubated in Horowitz medium to determine per cent survivors.

DISCUSSION

The mean results of duplicate manometric experiments carried out on different occasions with and without phenol, were satisfactory when compared by the *t* test (Table III). For 10 different results the mean oxygen uptake of spores in Horowitz medium in 12 hr. was 486 μl. with a coefficient of variation of 4·2 per cent, indicating good reproducibility.

TABLE III

RESULTS OF COMPARISON OF OXYGEN UPTAKE DURING 12 HR. BY *P. notatum* SPORES IN HOROWITZ MEDIUM AND WITH ADDED PHENOL

Phenol concentration (per cent)*	0	0·05	0·06	0·08	0·10	0·13	0·15	0·176	0·2	0·25
t	0·163	0·095	0·772	1·669	0·943	0·257	1·597	1·184	0·057	1·448
P	0·8–0·9	>0·9	0·4–0·5	0·1–0·2	0·3–0·4	0·7–0·8	0·1–0·2	0·2–0·3	>0·9	0·1–0·2

* As for Table I.

The ratios of exogenous to endogenous oxygen uptakes in 6, 9 and 12 hr. were 38·7, 40·8 and 69·4 respectively, showing that Horowitz medium is a sensitive growth medium for examining the effect of phenol on the oxygen uptake and viability of *P. notatum* spores. The addition of increasing concentrations of phenol to the growth medium progressively *reduced* these ratios until in Horowitz medium containing 0·2 per cent phenol they were 8·5, 3·4 and 2·8. The opposite effect has been noted and McCallan, Miller and Weed (1954) report that the oxygen consumption of the spores of *Neurospora sitophila, Aspergillus niger* and some other fungi was *increased* by phenol and other fungicides. Fig. 3 shows that the log-log relationships are linear between phenol concentrations of 0·05 to 0·2 per cent and oxygen uptake by *P. notatum* spores in 9, 12 and 24 hr.: they are also linear between concentrations of 0·05 to 0·1 per cent, and times (from Fig. 1) for an uptake of 120 or 200 μl. The calculated slopes of the lines in Fig. 3B are 1·3 and 1·7 giving a mean concentration exponent of $-1{\cdot}5$ for the effect of phenol on oxygen uptake.

Absence of germination in 0·08 per cent phenol in 24 hr. shows that this is the minimum fungistatic concentration. A large amount of oxygen is, however, taken up during this period, indicating that inhibition of

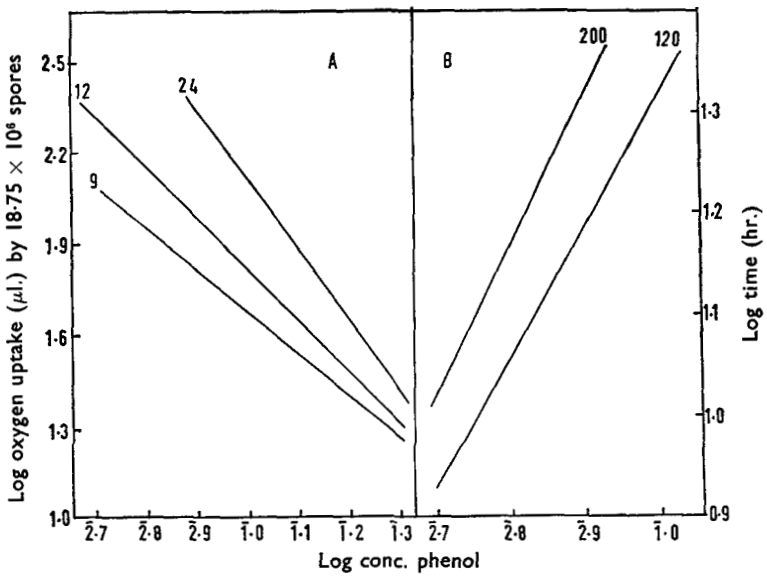

FIG. 3. (A) Relationship between log oxygen uptake in 9, 12 and 24 hr. by *P. notatum* spores and log phenol concentration. (B) Log time—log phenol concentration relationship for oxygen uptakes of 120 and 200μl. by *P. notatum* spores.

germination is a more sensitive criterion of the toxicity of phenol than is inhibition of respiration. A similar conclusion for different fungi and fungicides has been reported whereas claims have been made that inhibition of oxygen uptake parallels growth inhibition (see Cochrane, 1958).

Although oxygen uptake is considerably reduced by fungicidal concentrations of phenol, the change in uptake with concentration is very small compared with the change in the viable spore population. Oxygen uptake thus cannot be used as a quantitative measure of the fungicidal activity of phenol and in this respect our views agree with those of McCallan and others (1954). It should be noted, however, that with bacteria, correlation between bactericidal activity and reduction of oxygen uptake has been reported (Sykes, 1958).

Swollen and germinated spores are more susceptible to phenol than unswollen spores. This is presumably because the former have cell walls which are more permeable to nutrients and hence also to phenol.

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