Comparative Action of Some Dinitrophenols on Fungus Spore Respiration.* (18871)

HAROLD G. SHIRK AND HOPE E. BYRNE. (Introduced by L. R. Kuhn.)

From the Prevention of Deterioration Center, National Research Council, Washington, D. C. and Department of Bacteriology, University of Maryland, College Park.

Respirational and metabolic reactions to nitrated phenols by various biological systems were reviewed by Edsall(1), Clifton(2), and von Oettingen(3). The authors indicate that nitrated phenols may exert either a significant stimulatory or inhibitory action, dependent upon the concentration applied. Field *et al.* (4), in comparing the stimulatory capacity of 6-cyclopentyl-, 6-cyclohexyl-, 6-methyl-2,4-dinitrophenol (DNOC) and 2,4-dinitrophenol (DNP) on yeast respiration found that concentrationwise their efficiency was in the order named, and the total excitatory effect exerted by each was essentially the same. Qualitative similarities for DNOC and DNP in stimulating as well as in inhibiting the respiratory intensity of yeast suspension were demonstrated by Field and Tainter(5).

Evidence is here presented in the form of dosage-response data to show that certain substituents, when introduced into the DNP molecule, materially influence the ability of the resultant derivative to inhibit the oxygen uptake by the spores of the strongly cellulolytic fungus, *Myrothecium verrucaria*. The similarity of the degree of poisoning effected by 12 of the 16 dinitrophenols† considered here is pointed out. Correlative relationships between chemical structure and biological response are indicated, with the limited number of derivatives available.

Method. The solubility characteristics of many of the nitrophenols considered here necessitated first a means whereby complete solution of each derivative was assured at every dosage level to be considered. Although many organic solvents proved suitable, methyl cellosolve proved most practical since it had no effect upon spore respiration at concentrations needed for solubilization. A requisite quantity of a given derivative, previously verified by melting-point determination, was dissolved in the organic solvent, then added to a quantity of the buffered nutrient solution and subsequently made to volume. The reverse procedure of adding nutrient solution to the organically solubilized nitrophenol derivative occasionally caused flocculation of the compound. A buffered nutrient media, pH 6.5, was composed as follows: NH_4NO_3, 3.0 g; KH_2PO_4, 2.59 g; K_2HPO_4, 2.21 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; sucrose, 50 g; distilled water to 1,000 ml. Preliminary experiments with this same spore population indicated that the buffering capacity of this medium was sufficient to maintain a pH 6.5 for at least a 24-hour period. The freshly prepared spore suspensions used in each determination were made from 4-day-old cultures of the fungus grown at 30°C in Petri dishes containing Difco nutrient agar overlaid with a circle of Whatman No. 5 filter paper. The mineral salt and carbohydrate portions of this medium were the same as those used for preparing the spore suspensions. Spores were harvested by flooding the surface of the culture with nutrient solution and freeing the spores with a glass rod when necessary. This suspension was filtered through cheesecloth and subsequently adjusted to a population of 50×10^6 spores per ml. The cus-

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1. Edsall, G., *New Eng. J. Med.*, 1934, v211, 385.

2. Clifton, C. E., Advances in Enzymology, Interscience Publishers, Inc., New York, 1946, v6, 287.

3. von Oettingen, W. F., *Nat. Inst. of Health Bull.*, No. 190, 1949, 237.

4. Field, J., Martin, A. W., Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 388.

5. Field, J., and Tainter, E. G., *Arch. Intern. Pharmacodynamie*, 1936, v54, 184.

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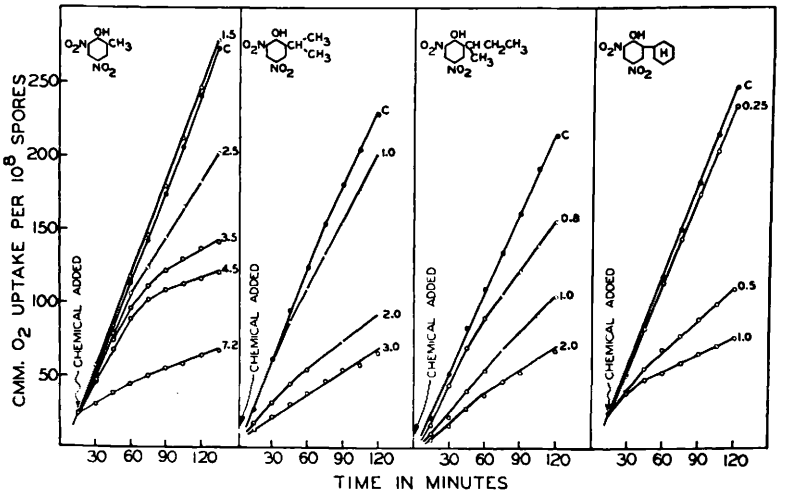

FIG. 1. Effect of several concentrations of 6-substituted 2,4-dinitrophenols on rate of oxygen uptake of a 10^8 spore population of *Myrothecium verrucaria*. Values at the ends of the curves indicate the number or fraction of 10^{-4} moles per liter final concentration and C indicates respiratory rate of untreated controls.

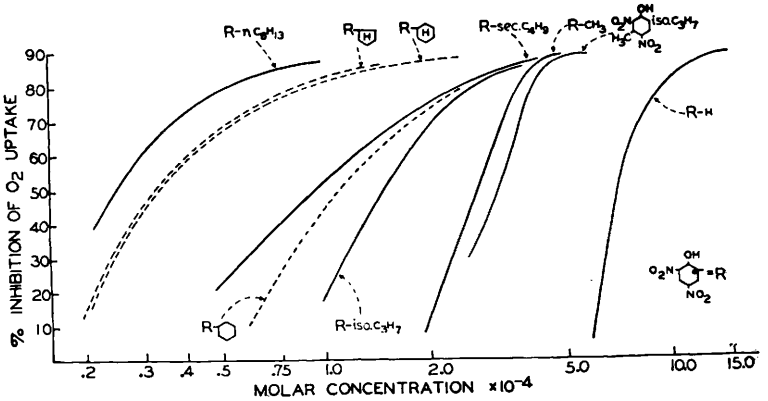

FIG. 2. Inhibitory effect of 6-C alkyl-, aryl- and cycloalkyldinitrophenols on oxygen respiration of *M. verrucaria* spores including the 2,4-dinitrophenol effect.

tomary manometric technics as described by Umbreit *et al.*(6), were applied to determine effects of the various nitrophenols on the oxygen uptake mechanism of the fungus spores.

Results. The time effect on oxygen uptake of several concentrations of four dinitrophenol derivatives included in this report is illustrated by the curves presented in Fig. 1. Generally, the behavior displayed here typifies the inhibitory action both as to its rapidity and type. In all cases with active derivatives the maximal effect on the oxidative mechanism is fully detectable within one hour after applying the poison. The action produced by DNOC was slightly retarded as compared with that caused by the other related chemicals here displayed. Inhibitory activity was calculated by comparing the slopes of the time-rate curves of the untreated controls with those of the treated spore suspensions, choosing slopes of the latter only when full poisoning had been effected. The comparative potency as well as the final common poisoning

6. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Technics and Related Methods for the Study of Tissue Metabolism; Burgess Publishing Co., Minneapolis, 1946.

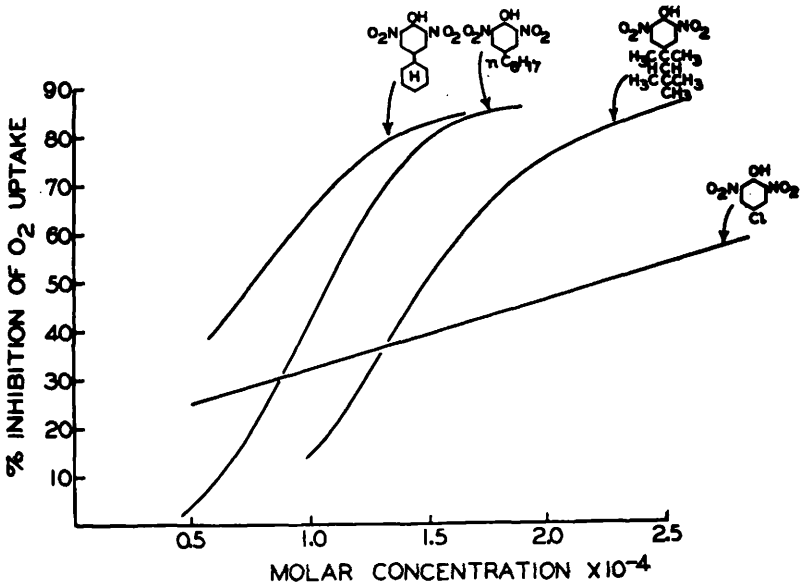

FIG. 3. Comparative inhibitory response of some 2,6-dinitrophenol derivatives on the respiration of *M. verrucaria* spores.

action of nine 2,4-DNPs on the oxidative mechanism of these fungus spores is shown by the dosage-response data in Fig. 2. Increase in the length of the alkyl chain of substituents located in the sixth position of the 2,4-DNP nucleus, generally increases effectiveness in reducing the rate of oxygen consumption, with the 6-*n*-hexyl-2,4-DNP molecule exercising the most toxic reaction.

Utilizing the ionization constant data as gathered by Krahl and Clowes(7), a calculation was made of the dissociated and undissociated concentrations for six of the derivatives included in the above figure. The calculations indicate that the responses shown in Fig. 2 are caused by the structural relationships rather than possible difference in undissociated concentrations formed by each derivative.

It is of interest to note that in the investigations by Field and Tainter(5) with yeast that the concentration causing 86 and 81% inhibition at pH 6.8 was 65 $\times$ 10^{-4} M for DNP and 15.2 $\times$ 10^{-4} M for DNOC. This indicates that the oxidative mechanism of these spores is significantly more sensitive to the poisoning action of DNP and DNOC than is that of the yeast cell. Generally similar inhibitory responses were reported by Newcomb(8) when investigating the effect of DNP levels ranging from 5 $\times$ 10^{-5} M to 2 $\times$ 10^{-4} M on the respiratory activity of tobacco callus tissue.

Inhibitory influences on the oxygen respiration of these fungus spores by some 2,6-DNP derivatives are indicated in Fig. 3. Except for the reactions demonstrated by the parahalogenated 2,6-DNP, they exerted a generally similar poisoning action as described for 2,4-DNP compounds. Altering the position of the cyclohexyl group to form *p*-cyclohexyl-2,6-DNP resulted in a slight loss of toxicity as compared with the isomeric 6-cyclohexyl-2,4-DNP (Fig. 2).

In contrast to the demonstrated effectiveness of the alkyl-, cycloalkyl-, and phenyldinitrophenols in poisoning the respiratory processes of spores of *Myrothecium verrucaria*, three derivatives formed by the introduction of a carboxyl, amino, or a third nitro group into the 2,4-DNP nucleus (resulting respectively in dinitrosalicylic, picramic, and picric acids) were relatively impotent in affecting this respiratory system (Fig. 4).

7. Krahl, M. E., and Clowes, G. H. A., *J. Cell. Comp. Physiol.*, 1938, v11, 1.

8. Newcomb, E. H., *Am. J. Botany*, 1950, v37, 264.

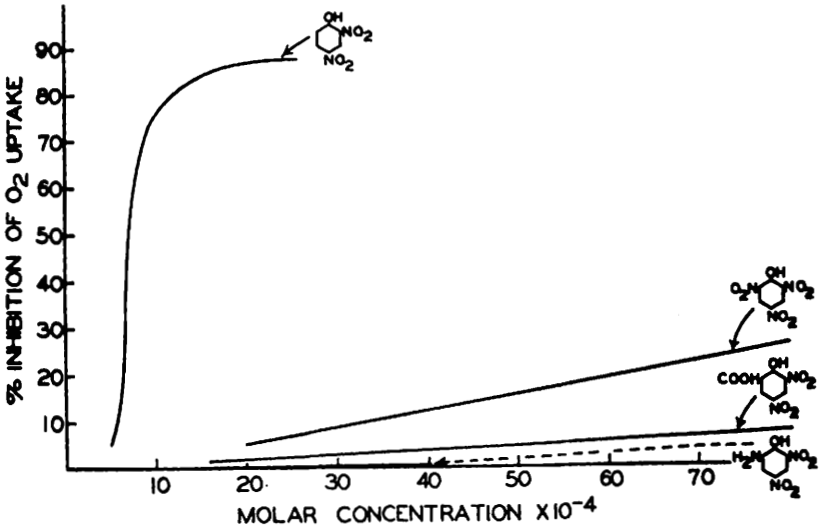

FIG. 4. Relative inhibitory effect on oxygen consumption of *M. verrucaria* spores of 2,4-dinitrophenol and compounds substituted in the 6-position with the nitro, carboxyl or amino group.

Summary. (1) The relative effects of 16 dinitrophenols in inhibiting the oxygen uptake mechanism of spores of *Myrothecium verrucaria* were studied. (2) The alkyl-, cycloalkyl-, or aryl- groups substituted ortho to the hydroxyl group in the 2,4-DNP molecule significantly increased the poisoning effect on this oxidative mechanism as compared to 2,4-DNP. (3) Effective concentrations of all active compounds manifested their full effect within an hour following contact with the spores. (4) The inhibitory effect as caused by all the alkyl substituted 2,4- and 2,6-DNPs averaged 85-90%. (5) Concentrationwise, the 6-*n*-hexyl-2,4-DNP compound proved the most potent, while dinitrosalicylic, picramic, and picric acids were significantly ineffective.

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Primary Amide Groups of Human Hemoglobin.* (18872)

SHERMAN R. DICKMAN AND IRVEN H. MONCRIEF. (Introduced by L. T. Samuels.)
From the Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City, Utah.

Pauling *et al.*(1) observed that the hemoglobin isolated from patients with sickle cell anemia possesses a higher isoelectric point (IEP) than that from normal individuals. Schroeder *et al.*(2) have shown that the amino acid composition of the two proteins is almost identical, and postulated that differences in the folding of the peptide chains were responsible for the electrophoretic results.

An increase in the number of primary amide groups of the sickle cell hemoglobin with a consequent decrease in free carboxyl groups might also be responsible for the higher IEP of this protein. The data of Table I, however, indicate that the number of primary amide groups in normal hemoglobin and in hemoglobins isolated from patients with sickle

* Aided by a research grant from Armour and Co.

1. Pauling, L., Itano, H., Singer, S., and Wells, I., *Science*, 1949, v110, 543.

2. Schroeder, W., Kay, L., and Wells, I., *J. Biol. Chem.*, 1950, v187, 221.