even this case doubtful.

In the present work about 75% of spore respiration was inhibited by actidione levels (2 p.p.m.) which did not interfere with germination. At higher levels (above 3.6 p.p.m.), where germination was completely inhibited, the maximum inhibition of respiration was about 85%. This may indicate that only roughly 10% of respiration is required for growth. A similar conclusion was reached by Commoner and Thimann(13) for the effects of iodoacetate on *Avena coleoptile*. However, the present manometric data alone can not establish a direct connection between inhibition of growth and respiration. Until this is done other interpretations of the per cent of respiration linked with growth are possible.

Conclusions. The effect of actidione on the germination and the spore and mycelial respiration of *Myrothecium verrucaria* was measured by dosage-response curves and LD50 values and by change in per cent inhibition with time. Spore respiration was about 4 times as sensitive as germination and 10 times as sensitive as mycelial respiration. Only a small fraction of respiration seemed to be required for growth. Inhibition of spore and mycelial respiration decreased with time, in the spores because of decreasing sensitivity and possibly also because of detoxication.

1. Leach, B. E., Ford, J. H., and Whiffen, A. J., *J. Am. Chem. Soc.*, 1947, v69, 474.

2. Whiffen, A. J., *Mycologia*, 1950, v42, 253.

3. Wallen, V. R., Sutton, M. D., and Skolko, A. J., *Phytopath.*, 1950, v40, 156.

4. Brownlee, G., *Ann. Rev. Microbiol.*, 1951, v5, 197.

5. Sindon, J. W., Mix, A. J., and Siu, R. G. H., *Res. Rpt. Quartermaster General Lab.*, Microbiol. Ser. No. 8, 1948.

6. Mandels, G. R., and Norton, A. B., *Res. Rpt. Quartermaster General Lab.*, Microbiol. Ser. No. 11, 1948.

7. Darby, R. T., and Goddard, D. R., *Am. J. Bot.*, 1950, v37, 379.

8. Dixon, M. *Manometric Methods*, New York, Macmillan, 1943.

9. Walker, A. T., *Thesis*, Iowa State College, 1952.

10. Ter Horst, W. P., and Felix, E. L., *Ind. Eng. Chem.*, 1943, v35, 1255.

11. Klomparens, W., *Phytopath.* (Abstr.), 1951, v41, 22.

12. Klopping, H. L., *Thesis*, Utrecht University, 1951.

13. Commoner, B., and Thimann, K. V., *J. Gen. Physiol.*, 1941, v24, 279.

Received October 29, 1952. P.S.E.B.M., 1952, v81.

Effects of 2,4-Dinitrophenol Concentrations on Rates of Respiration and Fermentation of Yeast.* (19940)

ASER ROTHSTEIN AND HARRY BERKE.

From the Division of Pharmacology, Department of Radiation Biology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Most of the interest in the action of 2,4-dinitrophenol (DNP) on metabolism has stemmed from its ability to stimulate respiration(1,2), to prevent assimilation and other synthetic processes(3), and to interfere with the coupling of phosphorylation and respiration(4-6). It has also been noted that higher concentrations of DNP inhibit respiration of glucose(1) and acetate(7) and, under anaerobic conditions, the fermentation of glucose(8). However, the observations concerning inhibition of metabolism were incidental to studies pertaining to other actions of DNP. In the present paper quantitative observations are presented concerning marked differences in the inhibitory action of DNP on respiration as compared to fermentation, with glucose as substrate. Comparisons are also made with the stimulating actions of DNP on endogenous and exogenous respiration.

* This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

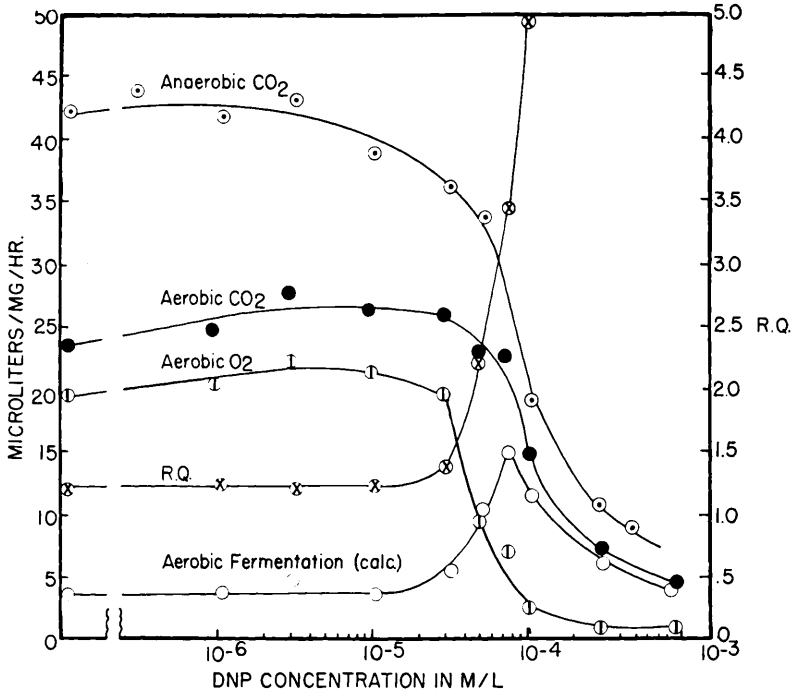

FIG. 1. Effects of various concentrations of DNP on rates of respiration, anaerobic and aerobic CO_2 production, and aerobic fermentation, with glucose as a substrate. Each Warburg flask contained 10 mg of yeast in 3 ml suspension at pH 3.5, at 30°C. Initial glucose conc. was .2 M.

Methods. Fresh Baker's yeast (Standard Brands, Inc.) was thoroughly washed. In washing, centrifugation was carried out for only 3 minutes at about 1000 X gravity, so that only the heavy viable cells were carried down. Colloidal materials and cell debris were thereby discarded. The cells were then starved for 2 to 3 hours with aeration in order to attain the steady-state condition in regard to endogenous metabolism(9). All measurements of CO_2 production and oxygen consumption were carried out by standard Warburg technic. The pH of the yeast suspension and of all solutions was adjusted to pH 3.5. At this pH, fermenting yeast is self-buffering(10) and the action of DNP is maximal(1).

Results. DNP was found to be a potent inhibitor of respiration. Concentrations as low as 2 to 3 x 10^{-5} M produced a definite inhibition; 5 x 10^{-5} M produced 50% inhibition; and 2 x 10^{-4} M produced virtually 100% inhibition (Fig. 1). On the other hand, if CO_2 production rather than O_2 consumption was measured, also under aerobic conditions and with glucose as a substrate, the inhibition curve was not the same as for respiration, but was shifted considerably toward higher DNP concentrations. Thus, no inhibition was observed until the DNP concentration was at least 5 x 10^{-5} M. An inhibition of 50% required 1 x 10^{-4} M. Even at 1 x 10^{-3} M DNP,† there was only 80% inhibition. In terms of the concentration of DNP necessary to produce 50% inhibition, O_2 consumption was twice as sensitive to the inhibitor as was aerobic CO_2 production.

Aerobic CO_2 production by yeast from glucose is the sum of the CO_2 associated with respiration and that associated with aerobic fermentation. If it is assumed that the R.Q. for respiration of glucose is 1.0, then respiratory CO_2 is equal to the O_2 consumption. The CO_2 associated with aerobic fermentation can then be calculated by subtracting the respiratory CO_2 from the total aerobic CO_2. Normal-

† Concentrations of DNP higher than 1 x 10^{-3} M could not be used because of the limited solubility of this compound at pH 3.5.

TABLE I. Effect of Glucose Concentration on Stimulating Action of DNP on Respiration. Temp., 30°C; pH, 3.5; yeast conc., 10 mg/flask; DNP conc., 1×10^{-5} M. All determinations were in duplicate.

Glucose conc., M/L	Rate of respiration: No DNP, μl/mg/hr	Rate of respiration: DNP, μl/mg/hr	Increase due to DNP, μl/mg/hr	Stimulation, %
.0	.40	2	1.6	500
.001	2.8	5.7	2.9	103
.003	11.2	15.8	4.6	41
.005	14.6	19.8	5.2	36
.01	16.3	20.5	4.2	25
.1	19.1	23.3	4.2	22

ly baker's yeast has a significant level of aerobic fermentation even with maximal oxygenation. For example, in the experiment of Fig. 1 the R.Q. was 1.2. The rate of aerobic fermentation was 3.5 μl/mg/hr, or somewhat less than 10% of the rate of anaerobic fermentation. In the presence of DNP, in concentrations above 3×10^{-5} M, there was a dramatic increase in the R.Q. The rate of aerobic fermentation increased five-fold, from a normal of 3.5 μl/mg/hr to a peak at 15 μl/mg/hr at 8×10^{-5} M DNP.

The inhibition curve for anaerobic fermentation was somewhat parallel to that for aerobic CO_2 production, with 50% inhibition in each case requiring a DNP concentration of 1×10^{-4} M, twice as high as that required for 50% inhibition of respiration. The appearance of an elevated rate of aerobic fermentation seemed to be related to this relative insensitivity to DNP of fermentation as compared to respiration. For example, those concentrations of DNP (3 to 8×10^{-5} M) which gave an appreciable inhibition of respiration but which had little effect on fermentation induced a high rate of aerobic fermentation. In fact, the increase in the rate of aerobic fermentation was roughly proportional to the decrease in the rate of respiration. On the other hand, those concentrations of DNP (above 8×10^{-5}) which were associated with a marked inhibition of anaerobic fermentation, resulted in a parallel diminution in the rate of aerobic fermentation.

Concentrations of DNP lower than those required to inhibit respiration, resulted in a small but definite stimulation of respiration. In Fig. 1, this may not be too apparent, but in other experiments, a consistent stimulation of about 20% was observed. The stimulation of respiration on a percentage basis was much more evident when the concentrations of glucose were lower (Table I), with by far the greatest effect, a 500% increase, in the complete absence of glucose. However, on an absolute basis, the increased oxygen consumption was greater in the presence of higher concentrations of sugar. Thus the stimulation of respiration by DNP seems to be in part due to an increased endogenous rate and in part due to an increased respiration of glucose.

The effects of DNP on endogenous respiration are shown in more detail in Fig. 2. A definite stimulation was observed at concentrations of DNP as low as 3×10^{-6} M, but the maximal effect, a 700% increase, was found at 5×10^{-5} M DNP. Higher DNP concentrations were associated with decreasing rates of respiration, with complete disappearance of any stimulating action at 1×10^{-3} M. In terms of the concentrations of DNP required, the inhibition of the elevated endogenous respiration (Fig. 2) is markedly similar to the inhibition of the respiration of glucose (Fig. 1).

The stimulation of endogenous respiration by DNP was further characterized by studies with carbon monoxide (CO). Respiration of glucose by yeast is sensitive to CO, with reversal of the inhibition by light(11). On the other hand, endogenous respiration of starved yeast is normally insensitive to CO (12). Fig. 3 indicates that the increment of endogenous respiration induced by DNP is sensitive to CO, with reversal by light, re-

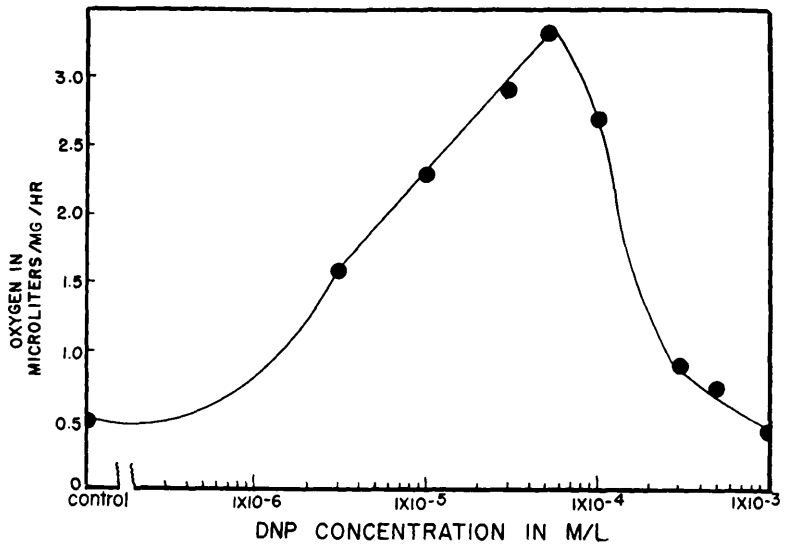

FIG. 2. Effect of DNP on endogenous aerobic metabolism. Each Warburg flask contained 40 mg of yeast in 3 ml suspension at pH 3.5, at 30°C.

sembling the respiration of glucose in this respect, rather than the normal endogenous respiration.

Discussion. The action of DNP on the rates of metabolism is complex, and obviously involves an interaction of DNP with several different enzyme sites at different concentrations of DNP. Low concentrations (1×10^{-6} to 1×10^{-5}) stimulate both endogenous and exogenous metabolism. Such concentrations are also associated with the inhibition of assimilation(3), phosphate uptake(13), potassium uptake(10) and many other synthetic activities. These effects are undoubtedly associated with the action of DNP in "uncoupling" phosphorylation from respiration(4-6). The experiments with carbon monoxide indicate that the endogenous respiration, which normally proceeds by a CO-insensitive system, is not only stimulated to a marked degree, but is shifted into CO-sensitive pathways, presumably a pathway involving the cytochrome system.

Higher concentrations of DNP (3×10^{-5} M to 1×10^{-4} M) inhibit respiration probably by acting on cytochrome reductase(14) but not the cytochrome system(15). Parallel to the inhibition of respiration, there was a diminished Pasteur effect and a marked increase in aerobic fermentation. DNP does not differentiate between respiration and the Pasteur effect, the inhibition in each case being about the same (the Pasteur effect was quantitated in terms of the Meyerhof Quotient)(16).

The highest concentrations of DNP (8×10^{-5} M to 1×10^{-3} M) inhibit both the aerobic and anaerobic fermentation of glucose, but at the same time induce a fermentation of endogenous stores(17). It is not known by what mechanism these effects are produced.

Summary. Low concentrations of DNP stimulate the respiration of living yeast cells in the presence or absence of glucose. Respiration in the absence of substrate is normally insensitive to CO, but the increment increase due to DNP is sensitive to CO. Higher concentrations of DNP inhibit both respiration and fermentation of glucose. However, the respiratory pathway is considerably more sensitive to the inhibitor than is the fermentation pathway. In consequence, at certain concentrations of DNP, the Pasteur effect is depressed and there is a striking increase in the rate of aerobic fermentation.

1. Field, J., Martin, A. W., and Field, S. M., *J. Cell. Comp. Physiol.*, 1934, v4, 405.

2. Peiss, C. N., and Field, J., *J. Biol. Chem.*, 1948, v175, 49.

3. Clifton, C. E., *Adv. Enzymol.*, 1946, v6, 269.

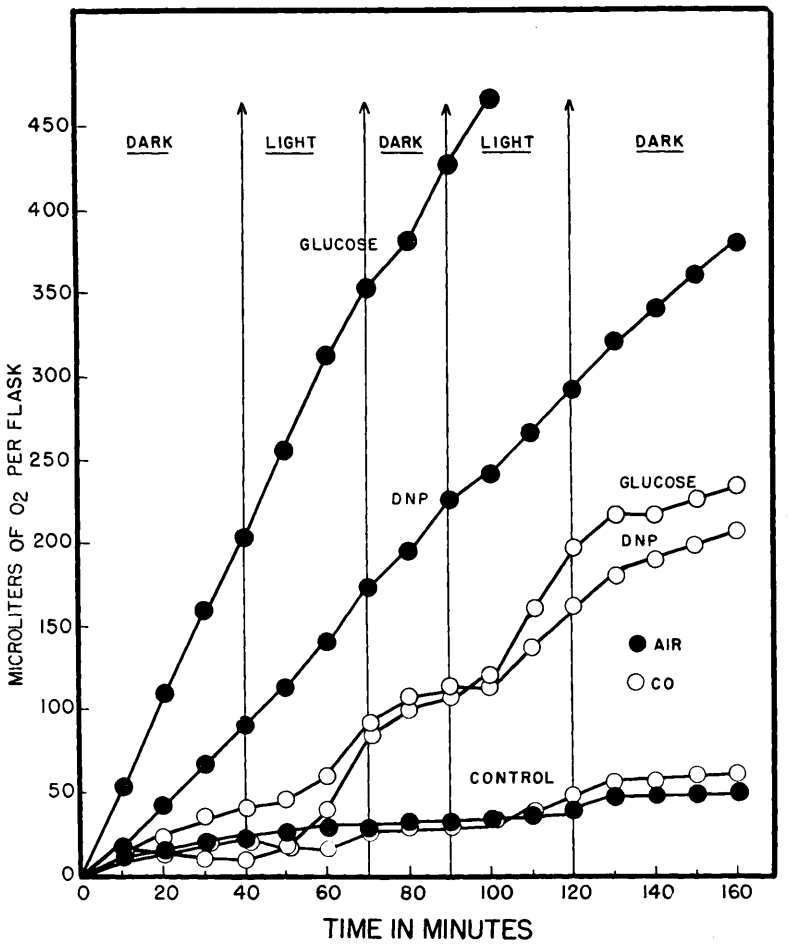

FIG. 3. Effect of CO on DNP-stimulated respiration in the dark and in the light. Each Warburg flask contained 40 mg of yeast in 3 ml of suspension at pH 3.5, at 30°C. Initial glucose conc. was .1 M and the DNP, 5×10^{-5} M. Gas mixtures were air or 95% CO – 5% O_2. Lights were 3 #2 photofloods approximately 50 cm from flasks.

4. Judah, J. D., and Williams-Ashman, H. G., *Biochem. J.*, 1951, v48, 33.

5. Loomis, W. F., and Lipmann, F., *J. Biol. Chem.*, 1948, v173, 807.

6. Cross, R. J., Taggert, J. V., Covo, G. A., and Green, D. E., *J. Biol. Chem.*, 1949, v177, 655.

7. Stoppani, A. O. M., *Nature*, 1949, v164, 1096.

8. Martin, A. W., and Field, J., PROC. SOC. EXP. BIOL. AND MED., 1933, v31, 56.

9. Stier, T. J. B., and Stannard, J. N., *J. Gen. Physiol.*, 1936, v19, 479.

10. Rothstein, A., and Enns, L., *J. Cell. Comp. Physiol.*, 1946, v28, 231.

11. Warburg, O., *Biochem. Z.*, 1927, v189, 354.

12. Stannard, J. N., *J. Cell. Comp. Physiol.*, 1940, v16, 389.

13. Hotchkiss, R. D., *Adv. Enzymol.*, 1944, v4, 153.

14. Haas, E., Harrer, C. J., and Hogness, P. R., *J. Biol. Chem.*, 1942, v143, 341.

15. Ames, S. R., Ziegenhagen, A. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, v165, 81.

16. Burk, D., *Cold Spring Harbor Symp. Quant. Biol.*, 1939, v7, 420.

17. Rothstein, A., and Berke, H., *Arch. Biochem.*, 1952, v36, 195.

Received November 5, 1952. P.S.E.B.M., 1952, v81.