Metabolic Effects Related to Fungitoxicity of Carboxin

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ABSTRACT

Concentrations of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) which reduce the rate of growth of *Rhizoctonia solani* and *Verticillium albo-atrum* about 90% inhibit glucose oxidation about 50%. Glucose oxidation by conidia of *Neurospora crassa* and mature sporidia of *Ustilago maydis* is unaffected or only slightly inhibited, whereas acetate oxidation by the two organisms is inhibited 70-90%. Carboxin is about 10 times as toxic to growth of *U. maydis, N. crassa,* and *Saccharomyces pastorianus* on an acetate substrate as on a glucose substrate. The fungicide is detoxified by riboflavin or riboflavin phosphate in the presence of light.

Syntheses of ribonucleic acid, deoxyribonucleic acid, and protein are reduced 60-90% in rapidly metabolizing cells of *R. solani, U. maydis, V. albo-atrum,* and *N. crassa.* In sporidia of *U. maydis,* the high energy nucleotide phosphate is reduced 64%; but the level of free nucleotides is reduced only 21%. Interference with energy generation or with the synthetic aspects of the citric acid cycle appears to be primarily responsible for carboxin toxicity. Phytopathology 60:1422-1427.

A recently developed systemic fungicide, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (carboxin, DCMO, Vitavax, D735), has been investigated as a possible control of several plant diseases (1, 3, 8, 16, 17). The fungicide is primarily selective for basidiomycetes, and is particularly effective in controlling smut diseases (4). Selective fungitoxicity in some cases at least is correlated with uptake of the toxicant (10).

Mathre (11) recently investigated the mechanism of action of carboxin and localized primary effects of the toxicant in the citric acid cycle. The present study considers the mechanism of action of carboxin. While the approach and data obtained in certain experiments differ somewhat from those of Mathre, our results support the conclusion that the primary and possibly the only significant direct action of the compound is on reactions in or closely associated with the citric acid cycle.

MATERIALS AND METHODS.—*Culture methods.*—The test organisms used were *Rhizoctonia solani* Kuehn; *Neurospora crassa* Shear & B. O. Dodge, wild type STA-4; *Saccharomyces pastorianus* Hans.; *Verticillium albo-atrum* Reinke & Berth., ATCC 10833; and *Ustilago maydis* (DC.) Cda., ATCC 14826.

R. solani, N. crassa, and *S. pastorianus* were grown and cell quantity was standardized according to the procedures of Hock & Sisler (9). The following standard preparations were used: *R. solani,* 5 mg dry wt of hyphal discs/ml; *N. crassa,* 0.3 mg dry wt of conidia/ml; and *S. pastorianus,* 0.65 mg dry wt of cells/ml. *Verticillium albo-atrum* was grown at room temp for 10-14 days on a 2% agar nutrient medium (2) supplemented with 2 g yeast extract/liter. Conidia were washed from the cultures with distilled water, and mycelial fragments were removed by filtration through cheesecloth. The conidia were washed twice in distilled water and resuspended in distilled water, and suspensions adjusted to give an initial dry wt of 0.43 mg dry wt/ml. *Ustilago maydis* was grown in liquid nutrient medium (2) supplemented with 2 g yeast extract/liter at 30 C on a water-bath shaker for 18-22 hr. The sporidia were washed twice in 0.02 M phosphate buffer and resuspended in buffer, and the concn adjusted to give an initial dry wt of 0.7 mg/ml.

In all the following studies, with the exception of toxicity tests in which it is stated otherwise, the initial standard cellular concn were those mentioned above. All tests were made in the nutrient medium of Coursen & Sisler (2) or in specified modifications of this medium. Incubations were carried out in a water-bath shaker at 25 C for *R. solani* and *V. albo-atrum* and 30 C for *N. crassa, U. maydis,* and *S. pastorianus.* The toxicant was added in methanol. Final concn of methanol in treated and untreated cultures never exceeded 1%.

Toxicity tests.—Toxicity of carboxin to *R. solani* was determined by the method of Hock & Sisler (9). The effect of the toxicant on growth of *N. crassa* was determined by making measurements of mycelial extension. Mycelial plugs cut with a cork-borer from cultures grown on Difco potato-dextrose agar were used as inocula. Cultures were incubated in petri plates at room temp in nutrient medium containing 2% agar and various concn of toxicant. When acetate was used as the carbon source, 2.5 g/liter of sodium acetate were substituted for glucose in the nutrient medium. Sensitivity of *V. albo-atrum* to carboxin was determined by making dry-wt measurements after a 24-hr incubation with several concn of the toxicant in nutrient medium. The toxicity of carboxin to *S. pastorianus* (initial inoculum, 0.02 mg dry wt/ml) and *U. maydis* (initial inoculum, 0.03 or 0.7 mg dry wt/ml) were determined in the nutrient medium and in a modified nutrient medium containing 5 g/liter of sodium acetate rather than glucose as a carbon source. The acetate medium also contained 0.01 M citrate, and was adjusted to the desired pH (6.0 or 6.4) with KOH. Cultures were agitated at 30 C, and growth was mea-

sured by increase in absorbance at 450 mμ. Toxicity to *S. pastorianus* was also determined under anaerobic conditions. Throughout these experiments, N_2 passed over hot copper was bubbled into stoppered test tubes containing *S. pastorianus* cells in the nutrient medium. The sensitivity of *U. maydis* to carboxin was determined at several pH values. The nutrient medium for these tests was modified so that it contained 0.05 M phosphate and 0.02 M citrate. The desired pH was obtained by the addition of KOH.

Auxanographic tests were made by the method of Coursen & Sisler (2) using various chemicals as possible antagonists of carboxin toxicity. Effects of riboflavin on carboxin toxicity to *V. albo-atrum* were studied further. Riboflavin phosphate was dissolved in distilled water and the solution sterilized by filtration through a membrane filter. The sterilized riboflavin phosphate solution was then mixed with autoclaved nutrient medium (×2) to give a final riboflavin concn of 2×10^{-3} M. Carboxin was added in methanol. All the above steps were carried out in the dark. Samples of the final solution were incubated at 28 C for 24 hr in a growth chamber with light intensity of about 40 ft-c. Samples of the solution were also incubated for the same period at 28 C in the dark. The riboflavin phosphate solutions were then mixed at 40 C with an equal volume of autoclaved nutrient medium containing 2% melted agar and conidia of *V. albo-atrum*. The mixtures were poured into petri plates and incubated at 25 C in the dark for 48 hr.

Respiratory measurements.—Conventional manometric techniques were used to study effects of carboxin on the oxidation of glucose and acetate by standardized preparations of test organisms. In all experiments, the final fluid volume in the vessel excluding the KOH was 2 ml. Toxicant and substrate were added to the cells at the initiation of the experiment. Oxidation of substrates was studied in the nutrient medium minus a N source at 30 C with *U. maydis* and *N. crassa* and at 25 C with *V. albo-atrum* and *R. solani*. In acetate oxidation studies, 2.5 g/liter sodium acetate replaced glucose in the medium. Respiratory quotients were determined for *U. maydis* in the glucose medium by the direct method of Umbreit et al. (14). Measurement of succinate (0.1 M) and pyruvate (0.2 M) oxidation by sporidia of *U. maydis* was made with a Gilson oxygen cathode (Oxygraph) under conditions similar to those used in manometric studies. In order to facilitate penetration of the substrates into the sporidia, the pH of the medium was adjusted to pH 4.0 with HCl.

Uridine and thymidine incorporation.—Cells of *R. solani, N. crassa, V. albo-atrum,* and *U. maydis* were incubated in the nutrient medium containing from 1×10^{-5} M to 1×10^{-4} M uridine-2-^{14}C (specific activity from 1 to 51.5 mc/mmole). Samples taken at various intervals were washed twice with cold 0.01 M citrate buffer adjusted to pH 6.4 with KOH. Mycelial discs of *R. solani* and conidia of *N. crassa* were extracted 3 times with cold 5% trichloroacetic acid (TCA). Extracts were combined, and a sample was added to toluene-methanol scintillation fluid (9). The TCA insoluble residue was suspended in scintillation solution, and radioactivity in the two fractions was determined with a liquid scintillation spectrometer. Samples of *V. albo-atrum* and *U. maydis* were extracted 3 times with 50% ethanol. Extracts were combined, samples added to scintillation solution, and counts determined. Cellular residue of *V. albo-atrum* was suspended in scintillation solution and counted.

The cellular residue of *U. maydis* sporidia was processed further to separate ribonucleic acid (RNA) from deoxyribonucleic acid (DNA). The ethanol-insoluble cellular residue was incubated overnight at 37 C in 0.5 N NaOH (5). Samples were acidified to pH 1 with HCl at 4 C and centrifuged. The pellet was washed twice at 4 C with H_2O adjusted to pH 1 with HCl. Supernatants were combined and radioactivity was determined as described above for ethanol extracts. Radioactivity in DNA remaining in the residue was determined by suspending the residue in scintillation solution and counting. Tests showed that after this residue was extracted with 4% perchloric acid (PCA) at 90 C for 15 min, less than 1% of the radioactivity remained in the residue.

Incorporation of thymidine-2-^{14}C into mycelial discs of *R. solani* followed the methods and chemical concn used by Hock & Sisler (9).

Phosphate metabolism.—*Verticillium albo-atrum* and *U. maydis* were used in measuring the effects of carboxin on incorporation of ^{32}P into TCA soluble and insoluble fractions. Cultures were incubated in the nutrient medium, which was modified so that phosphate concn ranged from 1×10^{-5} M to 1×10^{-3} M. The medium was buffered with 0.01 M citrate, and the pH adjusted to 6.4 with KOH. The solutions contained ^{32}P ranging from 0.1 to 0.6 μc/ml. Samples were removed at various intervals, and the cells washed twice with cold 0.01 M citrate buffer adjusted to pH 6.4 with KOH. Cells were extracted and radioactivity determined by procedures described for uridine incorporation into *R. solani*.

Nucleotides extracted from *U. maydis* cells with 4% PCA were purified by passage over Norit (13), and acid labile phosphorus was released from the nucleotides by treatment with 1 N HCl at 100 C for 7 min (14). Inorganic phosphate released was determined by the method of Fiske & Subbarow (6).

Phenylalanine incorporation.—Cells of *V. albo-atrum* and *U. maydis* were incubated in the nutrient medium containing 5×10^{-5} M L-phenylalanine-^{14}C (uniformly labeled) with a specific activity of 2 mc/mmole. Samples were removed at 30-min intervals and washed twice in cold 0.01 M citrate buffer, pH 6.4. Cells were extracted 3 times with 50% ethanol, and radioactivity in the cellular residue was determined by the method described for uridine incorporation.

RESULTS.—*Toxicity tests.*—Concentration of carboxin normally used in metabolic investigations involving the standard cellular preparation were: *R. solani,* 10 μg/ml; *N. crassa,* 50 μg/ml; *V. albo-atrum,* 10 μg/ml; and *U. maydis,* 2 μg/ml. These concn of toxicant produced strong (> 90%) but not complete inhibition of growth. Carboxin was equally toxic to sporidia of *U. maydis* at various pH values between 5 and 7.

TABLE 1. Effect of carboxin on oxidation of glucose by sporidia of *Ustilago maydis*, mycelium of *Rhizoctonia solani*, and conidia of *Neurospora crassa* and *Verticillium albo-atrum*

Organism	μg/ml Toxicant	% Inhibition[a]
U. maydis (sporidia)		
12 hr old	2	38
19 hr old	2	5
R. solani (mycelia)	10	48
N. crassa (conidia)	50	0
	100	0
V. albo-atrum (conidia)	10	46

[a] Based on total O_2 consumed in 4 hr by *U. maydis* and *R. solani* and in 3 hr by *N. crassa* and *V. albo-atrum*.

Among the metabolites screened in auxanographic tests for reversal of carboxin toxicity to *V. albo-atrum*, only riboflavin and riboflavin phosphate were effective. Further studies showed that light was required for the detoxication. Carboxin solutions incubated with riboflavin phosphate in the light and subsequently assayed were not toxic to *V. albo-atrum*. Toxicity was not reduced in similar solutions incubated in the dark or in solutions without riboflavin phosphate incubated in the light.

Respiratory and growth measurements.—The effect of carboxin on glucose oxidation varied according to the organism (Table 1). The toxicant did not inhibit glucose oxidation in conidia of *N. crassa*, but reduced the rate of oxidation in cells of *R. solani* and *V. albo-atrum* about 50%. Respiration of young sporidia of *U. maydis* (12 hr) was moderately inhibited over a 4-hr period. In older sporidia (18-22 hr), inhibition was sometimes as high as 25% initially, but then declined until there was little or no effect after 4 hr.

Respiratory quotients were more affected by carboxin in young sporidia of *U. maydis* than in older sporidia (Table 2). In the latter the RQ for treated and control cells was essentially the same after the first hr of incubation; neither CO_2 production nor O_2 uptake was inhibited by the toxicant. High RQ was characteristic of young sporidia, but this was reduced appreciably by carboxin. Both O_2 uptake and CO_2 production were inhibited, but the former was reduced less than the latter.

Tests were made to determine whether 2,4-dinitrophenol (DNP) would relieve respiratory inhibition caused by carboxin. DNP increased the initial rate of glucose oxidation by *R. solani* more than 40% above that of the control in the presence or absence of carboxin. But the rate of oxygen uptake by cells treated with DNP plus carboxin declined to 80% of the control after 4 hr; whereas that of cells treated with DNP alone remained at the initial level of stimulation. Respiration of cells treated with carboxin only was about 50% of the control.

The general effects of DNP on respiration of *U. maydis* somewhat resembled those described for *R. solani*. Although DNP initially increased oxygen uptake of carboxin-treated sporidia slightly, the respiration declined in 4 hr to a rate less than half that of sporidia treated with carboxin alone (Fig. 1-A). DNP decreased carboxin inhibition of glucose oxidation in conidia of *V. albo-atrum* from about 50% to 25%, but did not stimulate respiration of untreated cells.

Effects of oligomycin on glucose oxidation were compared with those of carboxin. In conidia of *V. albo-atrum*, oligomycin (10 μg/ml) inhibited glucose oxidation 49% (after 3 hr), an inhibition similar to that produced by carboxin (46%). Oligomycin (5 μg/ml) inhibition of glucose oxidation in sporidia of *U. maydis* was stronger than that caused by carboxin, and increased with time rather than decreased as did carboxin inhibition (Fig. 1-A, B).

DNP, which is reported to relieve respiratory inhibition caused by oligomycin in some organisms (12), gave only a transient partial relief of inhibition in *U. maydis*. DNP plus oligomycin affected respiration much in the same manner as DNP plus carboxin (Fig. 1-B).

Although carboxin did not inhibit glucose oxidation in *N. crassa* and affected that of mature sporidia of *U. maydis* only slightly, the toxicant (at the same concn used in glucose studies) strongly inhibited acetate oxidation in both organisms (Table 3). Oxidation of pyruvate and succinate in *U. maydis* was also strongly inhibited by carboxin. Oligomycin (5 μg/ml) inhibited acetate oxidation in *U. maydis* to about the same extent (95%) as did carboxin.

The difference in sensitivity of glucose and acetate oxidation to carboxin suggested that sensitivity of growth on the two substrates might also differ. Growth tests made with three organisms showed ca. 10-fold increase in toxicity of carboxin when acetate replaced glucose as the substrate. The ED_{50} value for *S. pastorianus* was decreased from 140 to 20 μg/ml. For *U. maydis* (0.003 μg dry wt/ml) the value was decreased from 0.3 μg/ml to 0.02 μg/ml. A similar substrate-

TABLE 2. Respiratory quotients of young (12 hr) and old (19 hr) sporidia of *Ustilago maydis* oxidizing glucose in the presence or absence of 2 μg/ml carboxin

Hr	Control	Treated	% Inhibition oxygen uptake
Young cells			
1	1.16	0.90	34
2	1.28	0.96	46
3	1.32	1.08	45
4	1.34	1.10	25
Old cells			
1	1.21	1.10	21
2	1.18	1.14	1
3	1.01	1.02	0
4	0.88	0.93	0

TABLE 3. Effect of carboxin on rate of acetate oxidation by sporidia of *Ustilago maydis* and conidia of *Neurospora crassa*

	% Inhibition	
Hr	*U. maydis*	*N. crassa*
1	28	42
2	71	75
3	87	75
4	91	78

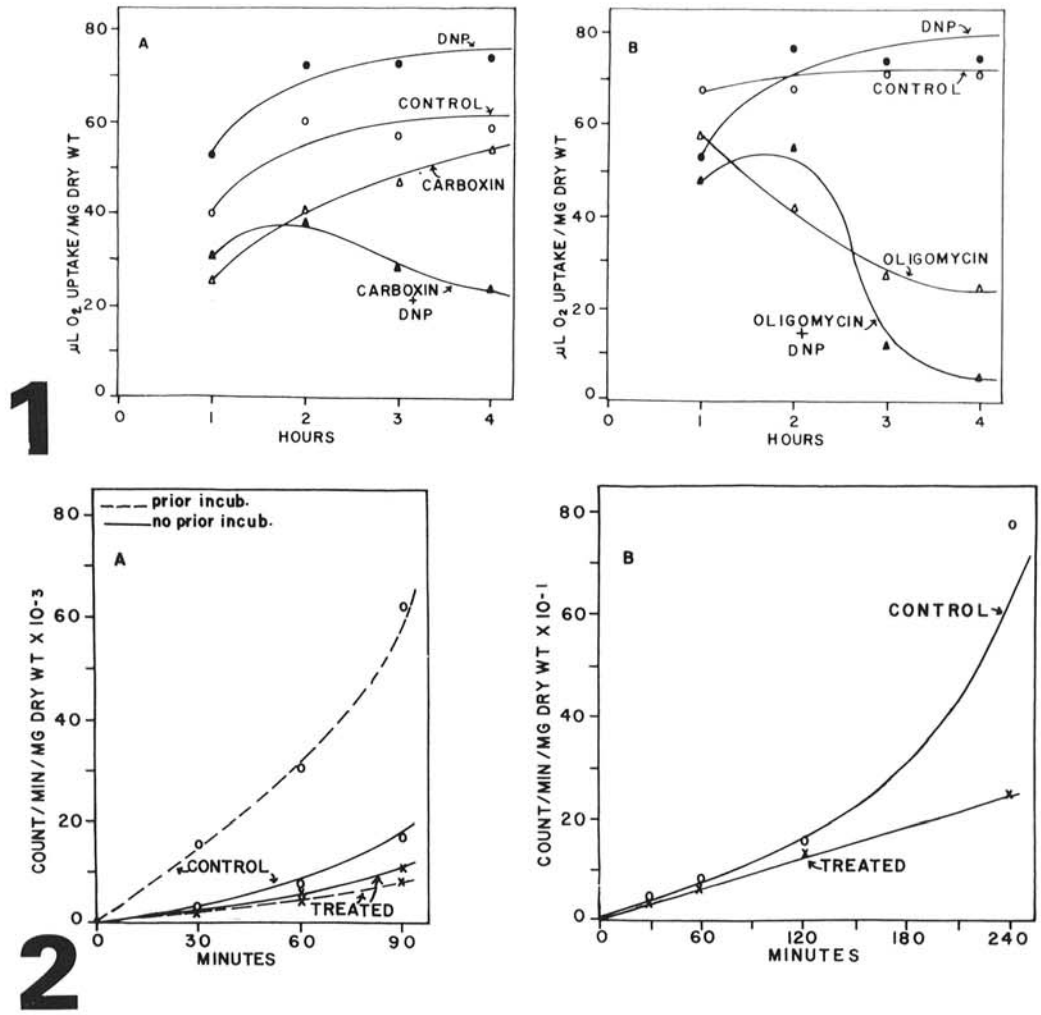

Fig. 1-2. 1) (A) Effects of 2 μg/ml carboxin and 3.16×10^{-4} M 2,4-dinitrophenol (DNP) on glucose oxidation by sporidia of *Ustilago maydis*. (B) Effects of 5 μg/ml oligomycin and 3.16×10^{-4} M DNP on glucose oxidation by sporidia of *U. maydis*. **2)** (A) Effect of 2 μg/ml carboxin on incorporation of uridine-2-^{14}C (specific activity, 1 mc/mmole) into the ethanol-insoluble fraction of *U. maydis* sporidia. (B) Effect of 10 μg/ml carboxin on incorporation of uridine-2-^{14}C (specific activity, 1 mc/mmole) into the trichloroacetic acid insoluble fraction of *Rhizoctonia solani* mycelial discs.

toxicity relationship was evident with *N. crassa*, but precise ED_{50} values were not determined.

Saccharomyces pastorianus has the capacity to grow under either anaerobic or aerobic conditions; therefore, tests were made to determine whether carboxin affects growth under conditions in which molecular oxygen is not utilized. Although growth of *S. pastorianus* under anaerobic conditions was only 50% of that under aerobic conditions during an 8-hr interval, carboxin (100 μg/ml) was equally inhibitory (30%) under either condition.

Uridine incorporation.—The effect of carboxin on incorporation of uridine into ribonucleic acids (RNA) of *R. solani*, *U. maydis*, and *V. albo-atrum* was strongly dependent on the metabolic state of the cells. The inhibitor had little effect when added to newly harvested cells in which the rate of RNA synthesis was low. Cells of *V. albo-atrum* or *U. maydis* incubated for 2-3 hr in nutrient medium prior to adding uridine subsequently incorporated uridine into RNA at a high rate. Carboxin strongly inhibited incorporation in these cells. The inhibited rate of incorporation was about the same as that in treated cells not incubated prior to addition of uridine and toxicant (Fig. 2-A). The inhibitory pattern in *R. solani* resembled that seen in *U. maydis* and *V. albo-atrum*. The rate of uridine incorporation by treated cells was nearly equal to that of untreated cells during the first 2 hr. After 2 hr, the rate of uridine incorporation in untreated cells rose sharply, but the rate in treated cells remained constant. This resulted in about 80% inhibition during the 2-4 hr period (Fig. 2-B). In conidia of *N. crassa* the initial rate of uridine incorporation was fairly rapid, and appreciable inhibition was evident during the first hr of exposure to carboxin. Inhibition of uridine incorporation was 80-90% after 3-4 hr.

Oligomycin (10 μg/ml) inhibited uridine incorporation into RNA in *V. albo-atrum* more effectively than carboxin. Incorporation in oligomycin-treated conidia was inhibited 98% as compared with 78% in carboxin-treated conidia.

The effects of carboxin on synthesis of DNA in *U. maydis* (^{14}C uridine incorporation) or in *R. solani* (^{14}C thymidine incorporation) was similar to the effects of the toxicant on uridine incorporation into RNA of these organisms.

Phosphate metabolism.—Carboxin and ^{32}P were added to suspensions of *U. maydis* or *V. albo-atrum* cells following an incubation period of 2-3 hr in citrate buffered nutrient medium. During the subsequent 90 min, carboxin inhibited incorporation of ^{32}P into the TCA soluble and insoluble fractions about 50% and 70%, respectively. The level of 7-min acid labile phosphorus in purified free nucleotides from *U. maydis* was measured colorimetrically to determine the effect of carboxin. This fraction (representing high energy phosphates) was reduced 64% in sporidia incubated 90 min in nutrient medium containing carboxin. The free nucleotide level in the same samples, measured by absorption at 260 mμ, was reduced only 21%.

Phenylalanine incorporation.—The effect of carboxin on incorporation of phenylalanine into protein of *U. maydis* and *V. albo-atrum* resembled that of the toxicant on uridine incorporation into RNA of these organisms. Phenylalanine incorporation was inhibited only 20% after 1 hr in cells not previously incubated in nutrient medium; whereas, inhibition was 60% during 1 hr after adding toxicant and ^{14}C phenylalanine in cells previously incubated 2-3 hr in nutrient medium.

DISCUSSION.—Glucose oxidation in *R. solani, V. albo-atrum, U. maydis* and *N. crassa* is proportionally less inhibited by carboxin than growth. In the last two organisms the rate of glucose oxidation is usually unaffected or only slightly depressed by growth-inhibiting levels of the toxicant, but acetate oxidation is strongly inhibited. Sensitivity of substrate oxidation to carboxin was studied most thoroughly in sporidia of *U. maydis*. Not only is oxidation of acetate strongly inhibited in these cells, but also that of succinate and pyruvate. The magnitude of the inhibition is sufficient to account for toxicity of the compound to cell growth. The marked difference in sensitivity of glucose and organic acid oxidation to carboxin implies that the above organic acids are not intermediates in the oxidation of glucose in sporidia of *U. maydis* treated with the toxicant. Gottlieb & Caltrider (7) presented evidence that a citric acid cycle operates in *U. maydis*. Presumably, pyruvate, succinate, and acetate are oxidized via this cycle. Carboxin must therefore inhibit some reaction in the cycle or in the associated electron transport system.

A comparison of the effects of oligomycin and carboxin on glucose oxidation in *U. maydis* suggests that the latter compound inhibits a reaction in the citric acid cycle. Oligomycin indirectly inhibits electron transport (and O_2 uptake) by interfering with oxidative phosphorylation in coupled systems (12). Oligomycin inhibits both glucose and acetate oxidation appreciably in *U. maydis,* suggesting that the same electron transport system is used for these two substrates. Carboxin inhibits only acetate oxidation to an extent comparable to oligomycin.

The fact that glucose oxidation is practically unaffected by carboxin whereas acetate oxidation is strongly inhibited suggests that the citric acid cycle is not utilized in glucose oxidation, or that different electron transport systems are utilized for the two substrates in carboxin-treated cells. Strong inhibition of acetate oxidation by carboxin could result from interference with oxidative phosphorylation and, as a consequence, the lack of ATP necessary to introduce acetate into the respiratory system. Such a mechanism could explain the different effect of carboxin on glucose and acetate oxidation but not the different effects on oxidation of the former substrate and pyruvate or succinate.

Although carboxin and oligomycin differ in their effects on glucose oxidation in *U. maydis,* the effects of the two compounds are similar in several respects. In addition to having similar effects on acetate oxidation in *U. maydis,* the two compounds inhibit glucose oxidation almost identically (ca. 50%) in *V. albo-atrum*. Inhibition by oligomycin of respiration in some mitochondria and whole cells can be reversed by DNP (12). In sporidia of *U. maydis,* however, there is only a partial reversal of inhibition of glucose oxidation initially, followed by inhibition greater than that produced by oligomycin alone. A similar effect is produced by DNP in carboxin-treated cells. In addition to uncoupling oxidation from phosphorylation, DNP stimulates ATPase that may hydrolyze ATP generated at the substrate level (15); therefore, unusually low levels of ATP may account for strong inhibition of glucose oxidation in cells treated with combinations of DNP and carboxin or oligomycin. Partial relief of carboxin inhibition of respiration in *R. solani* and *V. albo-atrum* by DNP suggests a mechanism of action for the former compound similar to that of oligomycin. Even though the general effects of oligomycin and carboxin are somewhat different, the difference may be mainly quantitative, and a common mechanism of action for the two compounds cannot be excluded. A choice between a site of inhibition of carboxin in the citric acid cycle or in oxidative phosphorylation must await analysis in cell-free systems.

In any case, interference with energy generation or with the synthetic aspects of the citric acid cycle appears to be primarily responsible for carboxin toxicity. This conclusion agrees with that reached by Mathre (11) in a similar study. The rather marked difference in sensitivity of growth on acetate as compared to glucose indicates that ATP generated in glycolysis and possibly during glucose oxidation are responsible for the greater resistance of cells utilizing the latter substrate. Yeast cells growing on glucose under anaerobic conditions derive all energy from glycolytic reactions, and are appreciably more tolerant of carboxin than those growing on acetate in air and deriving their energy from oxidative phosphorylation.

Even though more carboxin is required to inhibit

growth of cells of *U. maydis* on glucose than on acetate, growth on the former substrate is nevertheless quite sensitive to the inhibitor. Toxicity in this case may result from lack of citric acid cycle intermediates required for synthesis or from limited availability of ATP, even though the supply is greater than with acetate as a substrate. The latter mechanism is suggested by the low levels of high energy nucleotide phosphorus present in carboxin-treated cells utilizing glucose. In yeast cells utilizing glucose under anaerobic conditions, the relatively low toxicity of carboxin may result from the inhibition of anaerobically generated citric acid cycle intermediates required for synthesis or from inhibition of metabolism remote from the citric acid cycle.

Inhibition of protein, RNA, and DNA synthesis also appears to result from a primary effect of the toxicant on energy generation and precursor synthesis in the citric acid cycle. During early stages of cell growth, the normally low rate of RNA synthesis is little affected by carboxin. But as growth progresses and the rate of RNA synthesis expands, treated cells are apparently unable to meet the increased demands for energy and precursors, and at this stage strong suppression of RNA synthesis becomes evident.

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