Response of Oxidation & Coupled Phosphorylation in Plant Mitochondria to 2,4-Dichlorophenoxyacetic Acid [1, 2]

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The herbicidal action of 2,4-dichlorophenoxyacetic acid (2,4-D) has been the subject of many investigations in the years since this compound was first demonstrated to be a plant toxicant. Almost as many possible mechanisms for this toxicity have been suggested. These include depletion of respiratory substrate, abnormal cellular proliferation, the production of toxic substances in response to 2,4-D application, changes in nitrogen metabolism or ion uptake and metabolism, interference with the level of endogenous auxin, and abnormal phosphatase activity (4, 7, 9). While any of these might be responsible for the death of plants, contrary evidence may be adduced for each of them, and it has become common to think of the herbicidal action of 2,4-D as a complex of many responses with no one response alone producing the toxic effect.

A possible explanation for the phytotoxic effect of 2,4-D may lie in its ability to uncouple phosphorylation from oxidation. The importance of oxidative phosphorylation in growth and maintenance of life is well recognized (1) and severe disruption of cellular metabolism as a result of uncoupling can easily result in the death of cells. In 1952 Brody (3) demonstrated that 2,4-D was an uncoupling agent for phosphorylation associated with pyruvate oxidation in rat liver mitochondria, and later Switzer (5) showed a similar effect in soybean mitochondria using succinate, pyruvate, and α-ketoglutarate as substrates. In intact Chlorella cells 2,4-D inhibits the incorporation of inorganic phosphate into nucleotides while simultaneously stimulating either endogenous or citrate oxidation (8).

This uncoupling effect of 2,4-D has not been investigated as intensively as seems appropriate to its potential as an explanation for the weed-killing attributes of 2,4-D, and many questions regarding the magnitude of this effect and its relation to growth of intact cells remain unanswered. The work reported here will provide a confirmation of the previous reports of uncoupling due to 2,4-D and some additional information on aspects of this effect which have not previously been reported.

Materials & Methods

Heads of cauliflower (*Brassica oleracea*, L.) and roots of red beet (*Beta vulgaris*, L.) were obtained from fresh shipments at a local wholesale market. All other plant materials were harvested from plants growing in the field locally. The procedure used for isolating the particulate fraction (mitochondria) was essentially the same for all plant materials used. Chilled heads of cauliflower were washed with distilled water, broken into sections at the base, and the uppermost 4 to 5 mm of the immature inflorescence removed. The remaining stem tissue was immersed in a distilled water ice bath for 20 to 30 minutes. All subsequent operations were carried out between 0 C and 1 C. The chilled tissue, 200 g, was passed through a stainless steel grater with openings 2 mm in diameter into 240 ml of a solution consisting of 0.6 M sucrose, 0.05 M tris-hydroxymethylaminomethane (tris), and 0.005 M ethylenediaminetetraacetic acid (EDTA) at pH 8.0. This suspension was then stirred at slow speed (ca 30 volts) in a Lourdes homogenizer for 1 minute in 15 second intervals with a 15 second cooling period between stirrings. The homogenate was transferred to a Pexton press and strained through fine muslin. The filtrate was centrifuged at 3,000 $\times$ g for 10 minutes and the residue discarded. The supernatant liquid was centrifuged at 15,000 $\times$ g for 25 minutes and the supernate discarded. The pellet was resuspended in 50 ml of 0.6 M sucrose, 0.05 M tris at pH 7.0 with the aid of a hand-operated Potter homogenizer. The particles were resedimented at 15,000 $\times$ g for 25 minutes and finally suspended in 5 ml of 0.4 M sucrose in 0.05 M tris at pH 7.0.

The nitrogen content of this preparation was determined by direct Nesslerization after digestion of the trichloracetic acid precipitate (6). The protein N determined in this manner varied between 1.0 and 2.0 mg/ml.

In most cases the oxidative and phosphorylative activity of the mitochondria was determined in a Warburg respirometer at 25 C, using substrates and cofactors as indicated in the description of the results. Unless otherwise indicated, the oxygen uptake was determined for a 30 minute period after tipping the mitochondria into the reaction mixture. Then the flasks were rapidly transferred to an ice bath and 1

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or 2 ml aliquots of the contents added to 2 ml of 10 % trichloracetic acid in 10 ml tubes which were then centrifuged for 3 minutes at 1,000 × *g*. Aliquots of the supernate were used for determining inorganic phosphate (Pi) remaining in the solution by the colorimetric assay of reduced phosphomolybdate complex. The inorganic phosphate which disappeared in the presence of a hexokinase-glucose phosphate trap was taken to represent esterified phosphate.

Oxygen uptake determinations in some experiments were made with a recording Beckman oxygen electrode apparatus utilizing a magnetic stirrer and operated in a water bath at 25 C.

Those solutions which were not made up fresh daily were stored at −13 C when not in use. The 2,4-D, recrystallized four times from hot water, had a mp of 137 to 138 C.

For experiments on the growth of cauliflower stem tissue, disks 2 mm thick and 9 mm in diameter were cut from the same type of tissue as was used for the preparation of mitochondria. These disks were washed three times with distilled water, blotted, weighed in groups of 10 on a rapid-weighing analytical balance, rinsed with 0.75 % Purex solution and transferred to covered 50 ml flasks containing 3 ml of sterile half strength Hoagland's nutrient solution and 1 % sucrose in 0.05 M tris buffer at pH 7.0 and incubated with shaking for 72 hours at 25 C. At the end of this time they were removed, blotted, and the change in fresh weight determined.

Results

► Effect of 2,4-D on Oxidation & Phosphorylation: The response of cauliflower stem mitochondria to 2,4-D over the range from 1×10^{-4} to 3.2×10^{-3} M is illustrated in figure 1. Concentrations up to 5.6×10^{-4} M have relatively little effect on either oxygen uptake using malate as a substrate, or on the phosphorylation which is associated with this oxidation. However, concentrations of 1×10^{-3} M, and higher, cause a progressive inhibition of both processes. The effect is relatively greater on the phosphorylation reaction with the result that the P/O ratio is reduced. A concentration of 3.2×10^{-3} M 2,4-D causes about a 70 % inhibition of oxygen uptake, higher concentrations completely suppress the oxidation of malate in cauliflower mitochondria. In cases where oxygen uptake is completely eliminated, an increase in the free Pi of the solution is found as has been reported with soybean mitochondria (5).

The reduction in net phosphate esterification in the presence of 2,4-D could result from an interference with ATP synthesis or the activation of a hydrolytic process by the 2,4-D. In order to determine whether 2,4-D was in fact activating an adenosine triphosphatase in the mitochondria an investigation of the breakdown of both ATP and ADP in these preparations was made. The results are summarized in table I, which shows that there are active systems for hydrolyzing ATP and ADP, but that there is no effect of either 2,4-D or 2,4-dinitrophenol (DNP) on the rate of Pi production from ATP. Over a 30 minute period an amount of Pi which is equal to the terminal phosphate of approximately half of the added ATP is produced, but since the adenosine triphophosphatase in the cauliflower mitochondria is presumably one which produces pyrophosphate from ATP followed by hydrolysis by a pyrophosphatase, this quantity of Pi probably represents the hydrolysis of only one-fourth of the ATP supplied. In other experiments we have found an active pyrophosphatase in the cauliflower mitochondria.

Table I

Production of Inorganic Phosphate From Added ATP or ADP by Cauliflower Stem Mitochondria*

Time, min	Treatment	ATP, μmoles Phosphate	ADP, μmoles Phosphate
0	Control	0.38	0.20
	2,4-D—10^{-3} M	0.34	...
	DNP—10^{-4} M	0.52	...
5	Control	1.72	1.92
	2,4-D—10^{-3} M	1.66	...
	DNP—10^{-4} M	1.86	...
15	Control	4.28	3.48
	2,4-D—10^{-3} M	4.60	...
	DNP—10^{-4} M	4.46	...
30	Control	5.34	5.49
	2,4-D—10^{-3} M	5.30	...
	DNP—10^{-4} M	5.40	...

* Each flask contained 0.1 μmole cytochrome c, 1.0 μmoles DPN, 5 μmoles $MgCl_2$, 0.4 to 0.8 mg of mitochondrial N, 12 μmoles ADP or ATP in 3 ml of 0.27 M sucrose, 0.1 M tris at pH 7.0 with additions of 2,4-D or DNP as shown below. Incubated for indicated time at 25 C.

The appearance of Pi in mitochondrial preparations exposed to high concentrations of 2,4-D can be assumed to result from the hydrolysis of the added ADP, since as may be seen from the last column of table I, these mitochondria can also hydrolyze added ADP. In this case the amount of Pi produced in 30 minutes is equal to approximately half of the terminal phosphate of the added ADP.

Of the substrates tested in these studies malate was most severely uncoupled by 2,4-D, but other TCA cycle substrates and reduced diphosphopyridine nucleotide (DPNH) are also affected as indicated in figure 2. In this case only the P/O ratios are shown. Here malate shows essentially the same response as in figure 1, and citrate gives a similar, although less pronounced, uncoupling response to 2,4-D. It is of considerable interest that while the rate of both malate and citrate oxidation is inhibited by 2,4-D in the concentrations used here, this is not true of succinate or DPNH. Even 3.2×10^{-3} M 2,4-D has no significant effect on oxygen uptake by cauliflower mitochondria with these substrates, while phosphorylation is reduced to an extent which results in a 40 to 60 % decrease in P/O at this concentration of 2,4-D. Al-

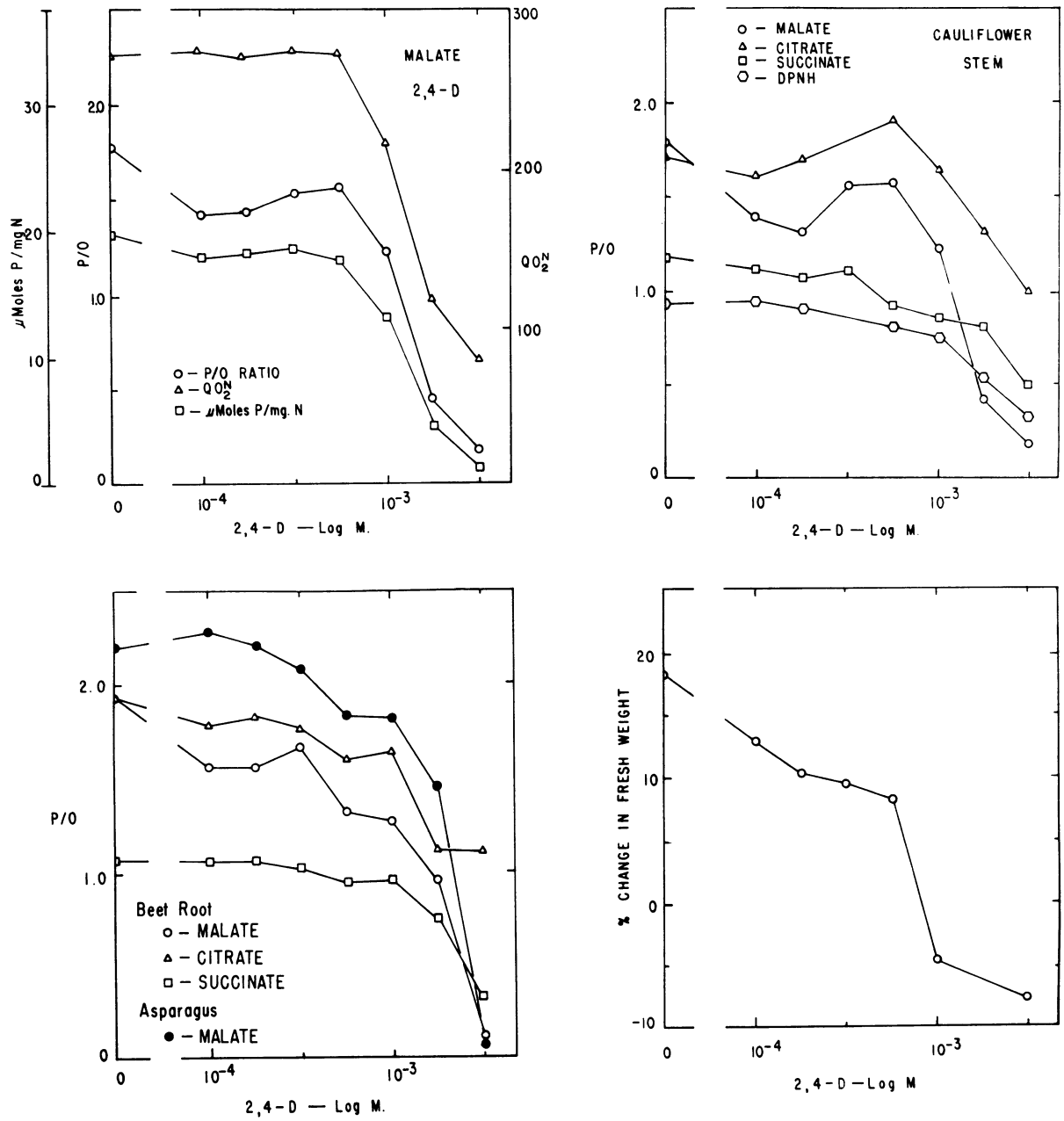

Fig. 1 (*Upper left*). 2,4-D Effects on malate oxidation and coupled phosphorylation by cauliflower stem mitochondria. Reaction mixture contained 60 μmoles Na malate, 1 μmole DPN, 0.1 μmole cytochrome c, 6 μmoles ADP, 5 μmoles $MgCl_2$, 24 μmoles KH_2PO_4, 75 KM units hexokinase, 10 μmoles glucose, 0.4 to 0.8 mg mitochondrial nitrogen, and the appropriate 2,4-D concentration in 3 ml, with a final concentration of 0.27 M sucrose, 0.1 M tris, pH 7.0. Mitochondria suspended in 0.4 ml of 0.4 M sucrose, 0.05 M tris were tipped into the reaction mixture at zero time, and phosphorylation was measured after 30 minutes oxygen uptake at 25 C. The points represent an average of four separate determinations.

Fig. 2 (*Upper right*). 2,4-D Effects on P/O ratios obtained with the oxidation of various TCA cycle substrates using cauliflower stem mitochondria. Reaction mixture the same as in figure 1, using 60 μmoles of the appropriate substrate. DPN was omitted with succinate. DPNH oxidation was measured in 1 ml total volume, using one-third as much of each cofactor and 10 μmoles DPNH as substrate. Experimental conditions as in figure 1. Each point is the average of two or more determinations.

Fig. 3 (*Lower left*). Effects of 2,4-D on P/O ratios of beet root and asparagus tip mitochondria oxidizing various substrates. Reaction mixture and conditions as outlined in figure 1.

Fig. 4 (*Lower right*). Growth of cauliflower stem disks in the presence of 2,4-D. Change in fresh weight of 10 disks shaken three days at 25 C in sterile medium consisting of half strength Hoagland's solution, 0.1 M tris, 1 % sucrose and the desired 2,4-D concentration, pH 7.0. Points are the average of four determinations.

though the relative effectiveness of 2,4-D as an uncoupler is less with succinate and DPNH, it is apparent that the effect on phosphorylation is common to several substrates. Since only that portion of the electron transport system which follows DPN reduction would be common to all these materials (1), it is probable that at least part of the effect of 2,4-D is being exerted on phosphorylative reactions coupled with flavoprotein and cytochrome oxidations.

The effect of 2,4-D on the P/O ratios obtained with beet root mitochondria metabolizing malate, citrate and succinate is summarized in figure 3. Although there are some differences, such as the relative resistance of citrate oxidation by beet mitochondria to uncoupling, the general response is of the same type found with cauliflower mitochondria. Mitochondria prepared from other dicotyledoneous plant materials including the floral portions of cauliflower heads (the portion discarded in preparing the stem mitochondria), avocado fruit, cucumber fruit, and snap bean fruit, have all shown similar sensitivity to uncoupling of malate or citrate oxidation by 2,4-D with essentially the same effect on P/O as is shown in figures 2 and 3 for cauliflower and beet root mitochondria. The question of whether the resistance to 2,4-D injury shown by monocots might be related to a greater resistance of monocotyledonous mitochondria to uncoupling by 2,4-D is to some extent answered by the results obtained with asparagus mitochondria as shown in the line with filled circles in figure 3 which indicates that the oxidation of malate by these mitochondria is uncoupled by 2,4-D in the same way as was found with cauliflower and beets.

Although these measurements were made manometrically over a 30 minute period, determinations with the oxygen electrode show that the effect of 2,4-D on the oxidation of malate is manifested almost instantaneously and that there is no change in the effect over a 30 minute period. In one such experiment 1×10^{-3} M 2,4-D decreased the rate of malate oxidation by cauliflower stem mitochondria by 35 % within 30 seconds after its addition, with the rate thereafter remaining essentially constant for 30 minutes. In another oxygen electrode experiment where concomitant phosphorylation was measured, the relative inhibition of the P/O ratio was essentially the same at 5, 10, 15, and 30 minutes after adding the 2,4-D.

► Effect of 2,4-D on Growth: To illustrate the relationship of growth inhibition to the maximum uncoupling effect of 2,4-D a series of experiments was carried out to determine the effect of 2,4-D on the growth of cauliflower stem disks as measured by water uptake. These disks were incubated for 3 days in nutrient solution in tris buffer at pH 7.0 with additions of 2,4-D over the same concentration range employed in the previous experiments and the change in fresh weight determined. Three of these experiments are summarized in figure 4 which shows that although disks incubated with 2,4-D from 1×10^{-4} M to 5.6×10^{-4} M gained slightly less weight during the 3 day period than control disks, the sharp break in the growth curve occurs at 1×10^{-3} M 2,4-D, where there is an actual loss of weight rather than an increase. This is the same concentration at which the maximum uncoupling response is shown in mitochondrial preparations of this tissue.

Disks of beet root tissue prepared and incubated in the same manner showed a similar response, with those exposed to 10^{-3} M 2,4-D or higher losing weight while those in lower concentrations increased in fresh weight, although not as much as untreated controls.

► Effect of Indoleacetic Acid on Oxidation & Phosphorylation: To determine whether the uncoupling effect of 2,4-D might be common to auxins in general or might prove to be an important difference between 2,4-D and natural auxin, the effect of indoleacetic acid (IAA) over the same concentration range used with 2,4-D on the oxidation of malate and coupled phosphorylation in cauliflower stem mitochondria was tested. Figure 6 shows that although there is some inhibition of malate oxidation by the higher concentrations of IAA, the decrease in oxygen uptake is accompanied by a corresponding decrease in phosphorylation so that there is no effect on the P/O ratio. This result is in conformity with those of previous workers (3, 5) who found no uncoupling response to the natural auxin.

► Comparison With Uncoupling Effect of Dinitrophenol: As a means of evaluating the relative effectiveness of 2,4-D as an uncoupler, a study of DNP effects on oxidation and phosphorylation in cauliflower stem mitochondria was undertaken. As is shown in figure 7, DNP is much more effective in reducing the P/O ratio since 1×10^{-4} M DNP has brought it essentially to zero. Over this concentration range DNP inhibits oxygen uptake only slightly with a maximum of about 30 % inhibition, but its effect on phosphorylation is substantial with a resultant decrease in P/O. As with 2,4-D, concentrations of DNP higher than those required for complete inhibition of phosphorylation result in an increase in the free inorganic phosphate in the preparation. Concentrations of DNP higher than 10^{-3} M completely inhibit oxygen uptake with malate in these mitochondria.

Other experiments in which 2,4-D and DNP were added simultaneously to cauliflower mitochondria showed that the combined effect on P/O's with malate as a substrate was strictly additive.

► Dinitrophenol Effects on Growth: The effect of DNP over the same concentration range used in the mitochondria experiments on the growth of cauliflower stem disks is illustrated in figure 5. The concentration which produces a maximum uncoupling has resulted in a loss of fresh weight by the disks while lower concentrations have reduced the fresh weight increase of the disks over the 3 day period.

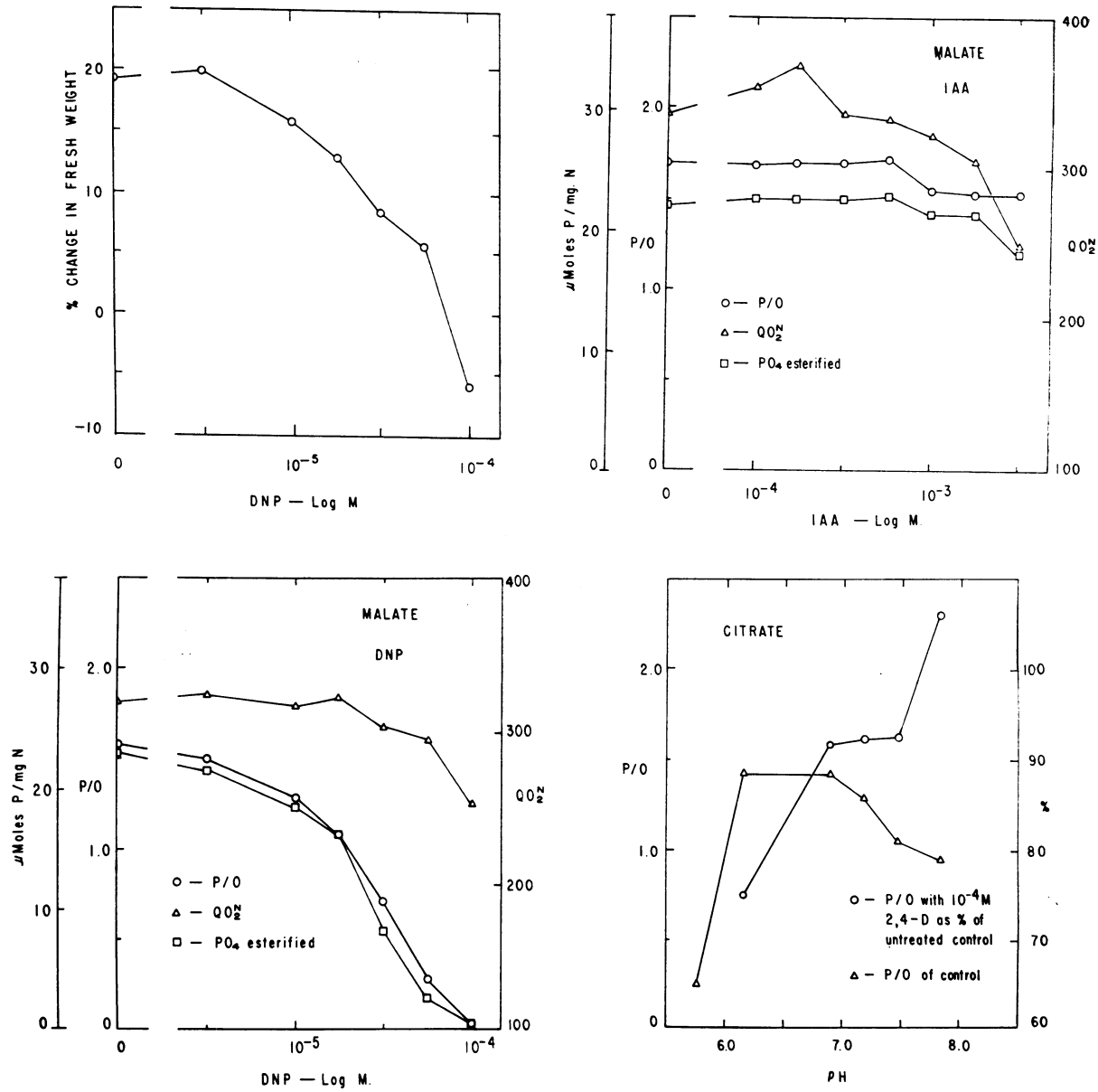

Fig. 5 (*Upper left*). Growth of cauliflower stem disks in the presence of DNP. Conditions as outlined in figure 4. Points are the mean of four determinations.

Fig. 6 (*Upper right*). Oxidation of malate and coupled phosphorylation by cauliflower stem mitochondria in the presence of various concentrations of indoleacetic acid. Experimental conditions and reaction mixture as outlined under figure 1.

Fig. 7 (*Lower left*). Oxidation of malate and coupled phosphorylation by cauliflower stem mitochondria in the presence of various concentrations of 2,4-dinitrophenol. Reaction mixture and experimental conditions as outlined under figure 1.

Fig. 8 (*Lower right*). Influence of pH on P/O ratio of cauliflower stem mitochondria oxidizing citrate and response to 10^{-4} M 2,4-D. Conditions as under figure 1 except that tris buffer containing citrate and KH_2PO_4 was adjusted to the desired pH with HCl or NaOH. Final pH determined on aliquots of reaction mixture at end of experiment.

This relationship of growth inhibition to uncoupling is of the same type found with 2,4-D.

The action of 2,4-D and DNP in uncoupling phosphorylation in our preparations appears to be generally similar, with the major difference being the greater effectiveness of the DNP. This may be an indication that DNP has a greater affinity for some reactive site at which the uncoupling of phosphorylation by both compounds occurs. It is probable, however, that at least part of the difference is due to the location of the reactive site behind a diffusion barrier in the mitochondrion which permits more ready access of the uncharged molecules of each to the point of reaction. The concentration of 2,4-D required to produce the same percentage inhibition of the P/O ratio is 18 times as great as that of DNP. This difference is of the same order of magnitude as the difference in the concentration of undissociated molecules, which is 11-fold greater for DNP at the same pH due to the difference in their pK's (2.96 for 2,4-D as against 4.00 for DNP).

► Relationship of pH to Uncoupling Effect: Some evidence for the location of the reactive sites attacked by the 2,4-D and DNP behind a diffusion barrier may be obtained from a study of the effect of pH on the uncoupling effect of these materials. An experiment of this type with 2,4-D is illustrated in figure 8. The upward-trending line with open circles shows the effect of 1×10^{-4} M 2,4-D on the P/O ratio of cauliflower stem mitochondria metabolizing citrate. The results are expressed as percentage of the untreated control at each pH. At pH 5.7 the 2,4-D causes about a 25% reduction in the P/O, while at pH 7.8 the P/O in the presence of this amount of 2,4-D is slightly higher than the control. As the pH decreases, with a resultant increase in the concentration of undissociated 2,4-D molecules, there is an increase in the uncoupling effect of the same total concentration of 2,4-D. The other line in figure 8 shows the effect of pH on the P/O ratios of the untreated controls and indicates an optimum coupling between pH 6.5 and 7.0.

Similar experiments performed with DNP have shown this compound is also more effective in uncoupling at a lower pH. At pH 5.6 a 1×10^{-5} M concentration of DNP completely inhibits phosphorylation, but at pH 7.6 the same concentration only reduces the P/O 5% below the control level.

► Inhibition of Purified Malic Dehydrogenase by 2,4-D: Experiments with mitochondria indicated that 2,4-D was inhibiting the oxidation of malate and citrate but had no effect on the oxidation of succinate or DPNH although phosphorylation was uncoupled with all of these substrates. This observation raised the possibility that in addition to interfering with the phosphorylative process, the 2,4-D was also specifically inhibiting the initial dehydrogenation of those substrates from which electrons are transferred directly to DPN. To test this possibility, studies were made of the effects of 2,4-D on the activity of purified porcine malic dehydrogenase (obtained from California Corporation for Biochemical Research). The activity of the enzyme was measured at 340 mμ in a recording spectrophotometer by the reduction of DPN associated with the oxidation of malate, or the reverse reaction by the oxidation of DPNH associated with the reduction of oxalacetate.

The activity of malic dehydrogenase measured in this way and the influence of 2,4-D on this activity is summarized in table II. Increasing increments of 2,4-D cause a progressive diminution in the enzyme activity, with a concentration of 1×10^{-2} M causing a 60 to 80% inhibition regardless of the direction in which the reaction is run. To test whether this inhibition might be simply a response to the acid moiety of the 2,4-D, similar experiments were carried out with acetic acid. With this acid the same range of concentrations caused a 10 to 30% stimulation of either malate oxidation or oxalacetate reduction with no indication of an inhibitory effect.

Discussion

The data presented here, together with previous observations of uncoupling of mitochondrial phosphorylation by 2,4-D suggests that this effect of the synthetic auxin may be an important factor in the toxic responses of plants to 2,4-D applications in the weed killing range. Support for this conclusion may be obtained from three different types of evidence:

► I. The uncoupling effect of 2,4-D on isolated mitochondria is severe, and with malate as a sub-

Table II

Effects of 2,4-D on Oxidation of Malate & Reduction of Oxalacetate by Purified Malic Dehydrogenase*

Treatment	Malate oxidation		Oxalacetate reduction	
	ΔAbsorbance/min	% Inhibition	ΔAbsorbance/min	% Inhibition
Control	0.064		0.070	
3.2×10^{-4} M 2,4-D	0.059	7.8	0.061	12.9
1.0×10^{-3} M 2,4-D	0.056	12.5		
3.2×10^{-3} M 2,4-D	0.043	32.8	0.051	27.2
1.0×10^{-2} M 2,4-D	0.023	64.1	0.016	77.2

* For malate oxidation each cuvette contained 60 μmoles malate, 1.0 μmoles DPN, 10 μg enzyme protein, and 2,4-D as shown below in 3 ml of 0.2 M tris buffer at pH 9.0. For oxalacetate reduction each cuvette contained 1.2 μmoles oxalacetate, 0.15 μmoles DPNH, 5 μg enzyme protein, and 2,4-D as shown in 3 ml of 0.2 M tris buffer at pH 7.0. Change in absorbance measured at 340 mμ in a recording spectrophotometer. The rates reported are for the first 2 minutes after adding enzyme.

strate, is essentially complete at 3.2×10^{-3} M (707 ppm). The effect is immediate and at least for short periods, continuous. Phosphorylation with other substrates is also uncoupled and it seems clear that in the presence of concentrations of this order, there would be a very significant reduction in the ATP supply from oxidative metabolism. Whether such concentrations do come in contact with mitochondria in plants sprayed with 2,4-D is unresolved, but the ability of some tissues to accumulate 2,4-D in concentrations exceeding those to which they are exposed (2) argues for the probability that the cytoplasm of treated plants may contain 2,4-D concentrations in this range.

The detrimental effect of a reduction—even if less than complete—of the ATP supply would be most pronounced in those cells where active growth and synthesis of new cellular material was occurring. The death of growing points or production of growth abnormalities could well result from such a decrease in energy supply.

► II. The close coincidence of severe uncoupling in mitochondria with the abolition of growth in tissue sections suggests that there is a causal relationship between the uncoupling effects of 2,4-D and growth inhibition or death of the cell.

► III. Similarities in the effects of 2,4-D and DNP support the probability that 2,4-D is toxic to plants in the same way, and to somewhat the same degree as DNP. On the basis of these studies there is no reason to conclude that 2,4-D is acting to uncouple phosphorylation in a way which is appreciably different from that by which DNP achieves this effect. Each inhibits growth at uncoupling concentrations, both are influenced in the same way by the pH of the external medium, their uncoupling effects are additive, and each is an effective uncoupler with a wide spectrum of substrates and in many species.

One of the interesting aspects of these data is the indication that 2,4-D appears to act on phosphorylation and oxidation in a different fashion, and probably at different locations in the sequence of reactions transferring electrons from TCA substrates to atmospheric oxygen. Succinate and DPNH oxidation are completely unaffected by 2,4-D in concentrations up to 3.2×10^{-3} M, while malate and citrate oxidation are almost completely inhibited by this level of 2,4-D. This points to a probability that the inhibitory effect on oxidation of these compounds is exerted on the initial dehydrogenation reaction and the transfer of electrons to DPN, since this step would be missing in the succinate or DPNH oxidation reactions. Testing of this hypothesis with purified malic dehydrogenase revealed that this enzyme is indeed inhibited by 2,4-D, and further that this inhibition is exerted whether the reaction is going from malate to oxalacetate or in the reverse direction.

Summary

The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) severely uncoupled phosphorylation associated with the oxidation of malate, citrate, succinate, and DPNH by mitochondria isolated from a variety of plants. This uncoupling effect is large enough that it seems adequate to account for the acute toxic responses elicited from plants by applying 2,4-D in the weed-killing range of concentrations.

Concentrations of 2,4-D which profoundly uncouple phosphorylation in isolated mitochondria also completely inhibit growth of disks prepared from the same tissues.

Indoleacetic acid is without an uncoupling effect on these mitochondria in the same concentration range used with 2,4-D. A comparison of the relative effectiveness of 2,4-D and dinitrophenol shows that 2,4-D is about 1/20 as active as an uncoupler as DNP, although part of this difference is accounted for by the differences in undissociated molecules of the two acids. With both compounds the uncoupling effect increases as the pH is lowered, supporting the suggestion that the reactive sites for their action are located behind a diffusion barrier whose permeability to molecules is greater than to ions.

In addition to a decrease in phosphorylation, 2,4-D also causes an inhibition of malate and citrate oxidation, but not with succinate or DPNH. This is probably related to an effect on the DPN-requiring dehydrogenases, since 2,4-D inhibits the oxidation of malate or reduction of oxalacetate by purified malic dehydrogenase.

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