STUDIES IN THE METABOLISM OF PLANT CELLS

IX. THE EFFECTS OF 2,4-DINITROPHENOL ON SALT ACCUMULATION AND SALT RESPIRATION

By R. N. ROBERTSON*, MARJORIE J. WILKINS*, and D. C. WEEKS†

[*Manuscript received February* 16, 1951]

Summary

2,4-Dinitrophenol, while increasing the respiration, inhibits the accumulation of ions by carrot cells. Further investigation is necessary to determine whether the inhibition is due to a direct effect of the dinitrophenol on the mechanism or whether the dinitrophenol indirectly prevents the mechanism from operating by causing some disorganization within the cell, possibly in the mitochondria. If the assumption that dinitrophenol inhibits phosphate transfers is justifiable, hypotheses of salt accumulation might require modification to allow for the participation of energy-rich phosphate. This would suggest that the Lundegardh mechanism may be a part of a more complex mechanism.

I. INTRODUCTION

During the last ten years, Lundegardh (cf. Lundegardh 1945) has developed a theory for the mechanism of accumulation of ions by plant cells. He suggests that the accumulation mechanism is dependent on the transfer of electrons by the cytochrome system and the simultaneous liberation of hydrogen ions. He thus connects the accumulation mechanism to the respiration. Robertson and Wilkins (1948) have shown that the accumulation rates are not inconsistent with such a hypothesis, and Weeks and Robertson (1950) have shown that the respiration is undoubtedly dependent on cytochrome oxidase.

Suggestions (cf. Hoagland 1944) have been made that the accumulation mechanism may be dependent on phosphorylation, since the only well-understood energy transfer system involves phosphate transfer. Nance (1949), observing that 2,4-dichlorophenoxyacetic acid (2,4-D) inhibited accumulation without affecting respiration, suggested that this may be due to an inhibitory effect of 2,4-D on phosphate transfers, but it has not been proved experimentally that 2,4-D affects phosphate transfers.

Loomis and Lipmann (1948) have suggested that 2,4-dinitrophenol (DNP) replaces phosphate in the oxidation and thus uncouples phosphorylation, while not lowering or even slightly stimulating the rate of oxidation. An alternative explanation of the effect of dinitrophenol on oxidation rate has more recently been suggested by Teply (1949).

Bonner (1949*a*, 1949*b*), assuming that dilute dinitrophenol would have an inhibitory effect on phosphate transfers in living tissue, investigated its influence on the respiration and growth of *Avena* coleoptiles. He found that growth

* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.
† Botany School, University of Melbourne.

was inhibited while respiration was stimulated. He found also that adenylic acid increased both respiration rate and growth. From this he concluded that high-energy phosphate acceptors normally limit respiration in *Avena* coleoptiles and that dinitrophenol acts in respiration as an "effective substitute for adenylic acid and inorganic phosphate," but does not allow the transfer of energy-rich phosphate to growth processes. Newcomb (1950) has shown that dinitrophenol stimulates oxygen uptake in tobacco callus tissue.

In this paper, experiments designed to examine the effect of dinitrophenol on respiration and accumulation will be described. If the assumption that dinitrophenol can prevent transfer of phosphate groups from respiration in the cell is correct, then dinitrophenol can be used to determine whether phosphorylations are involved in the accumulation mechanism. It was realized that phosphorylations might be involved in two ways:

(*a*) directly or indirectly in the accumulation mechanism itself,

(*b*) indirectly in maintaining cell organization, in particular the resistance of the cytoplasm, which prevents the leakage of accumulated salt. The observed accumulation rate at any time must be the difference between a rate of uptake and a rate of leakage (Krogh 1946; Robertson and Wilkins 1948). Hence the experiments to be described deal not only with the effect of dinitrophenol on accumulation but also with the effect of dinitrophenol on leakage.

II. Materials and Methods

Xylem parenchyma from carrot root, *Daucus carota* L. was cut into discs and washed for at least 96 hours by the methods described in earlier papers of this series (Robertson and Turner 1945). In some experiments discs were weighed the day before the experiment, placed in distilled water in small flasks, and aerated overnight. They were then blotted with filter paper and transferred to the experimental vessels. This procedure, by eliminating weighing on the day of the experiment, minimized the handling and shortened the period for settling down.

For one series of experiments, sets of 80 discs were taken immediately after cutting and threaded on fine silver wire, each disc being separated from its neighbour by a small glass bead. These were then washed and aerated in the usual manner.

(*a*) *Respiration*

The respiration was measured by standard Warburg technique. The vessels were shaken at 100 oscillations per minute in a thermostat at 25 or 27°C.

One gram of tissue and a total of 5 ml. solution was used in the vessels. The reagent to be tested was placed in the side-arm. When this was tipped into the vessel, the same volume of distilled water was tipped into the controls. When potassium cyanide was to be added, the vessels were removed from the manometers and the required amount of potassium cyanide added to the solution in contact with the tissue. The potassium hydroxide in the centre tube of the vessel was replaced with potassium hydroxide containing the appropriate quantity of potassium cyanide (Krebs 1935). When carbon monoxide

was used, the technique was the same as that described by Weeks and Robertson (1950); nitrogen-oxygen mixtures with the same oxygen concentration as the carbon monoxide-oxygen mixtures were used on replicate sets of tissue to determine the effect of low oxygen on the respiration rate.

Respiration results are expressed as cu. mm. O_2/hr./g. fresh wt. Where dinitrophenol was added, the respiration rates were compared one and a half hours after the addition.

(*b*) *Salt Uptake*

Salt uptake was measured by following the changes either in conductivity or in chloride concentration, in the solution surrounding the tissue. In most experiments, the weighed discs were placed in the solution in 50 ml. florence flasks which were held in a thermostat on a carrier attached to the Warburg shaker. The tissue volume ratio was varied according to the requirements of the experiment; not less than 2 g. of tissue were used. In the series of experiments with discs threaded on silver wire, the threaded discs were placed in the solution in 100 ml. florence flasks in a thermostat and a continuous current of air bubbled through the solution. The air was passed through towers of water at the temperature of the thermostat before passing through the vessels to ensure that no water would be lost by evaporation.

In the conductivity experiments, a conductivity pipette was fitted in the cork of each flask and samples withdrawn periodically from the solution surrounding the discs, the conductivity measured, and the sample returned. Replication between sets of discs was good.

When changes in chloride concentration were to be determined, seven or more sets of discs were used for each treatment. The solution was poured off the first set after half an hour, off the second set after one hour, and so on. Samples of the solution were analysed for chloride by the indirect method with silver nitrate and thiocyanate.

Salt accumulation is expressed as g. mol. salt accumulated/hr./g. fresh wt. Where g. mol. salt could not be estimated (as in the very dilute solutions resulting from leakage), results are expressed in conductivity units, mhos/hr./g. fresh wt.

(*c*) *Leakage of Phosphate*

In some experiments, the external solution was analysed to determine how much phosphate leaked from the tissue in the presence of dinitrophenol. Phosphate was determined colorimetrically after the formation of phosphomolybdate.

III. Results

(*a*) *Respiration*

The results of a typical respiration experiment are given in Figure 1; dinitrophenol in four concentrations was added to tissue in distilled water and in 0.05M potassium chloride. The stimulation of respiration by dinitrophenol

was marked in all but the set of discs in water to which the lowest concentration of dinitrophenol was added. The stimulation of the respiration occurred within 30 minutes of the addition of the dinitrophenol.

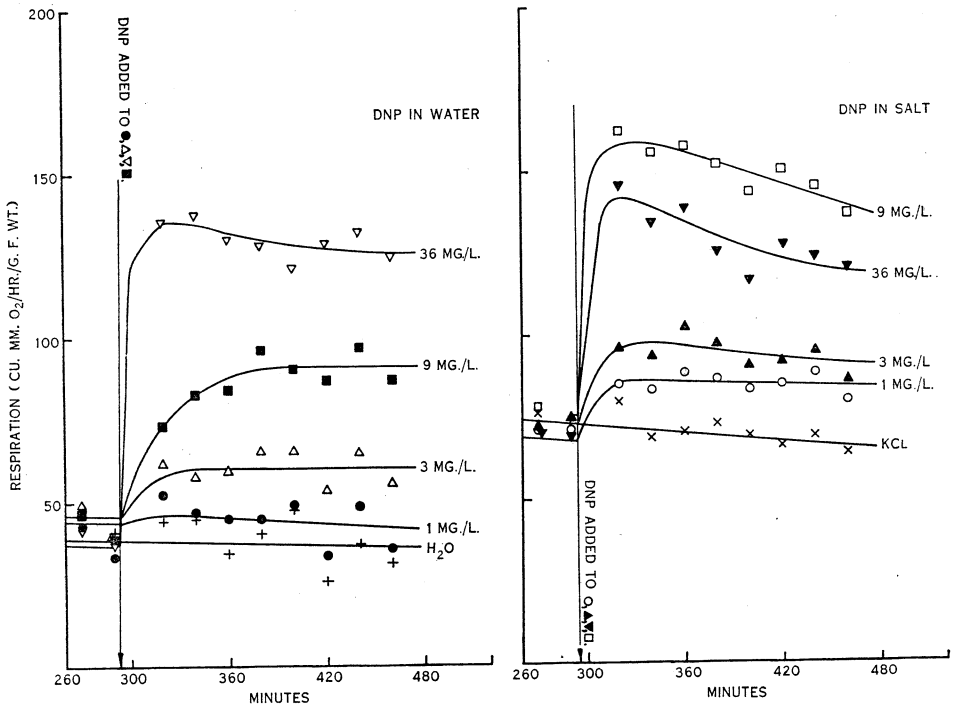

Fig. 1.—Effect of four different concentrations of dinitrophenol (DNP) on the respiration rate of carrot tissue in water and in 0.05M KCl solution. The respiration rates in water and in salt had been established before the dinitrophenol was added.

The effect of concentration of dinitrophenol in the experiment just described and in other experiments is summarized in Figure 3. Here the dinitrophenol respiration, i.e. the respiration rate in dinitrophenol over and above that in water or in salt 1½ hours after the addition of dinitrophenol, is plotted against concentration of dinitrophenol. At low concentrations of dinitrophenol, the magnitude of the dinitrophenol respiration is much the same in water and in salt. In higher concentrations, whereas the dinitrophenol respiration in water is still increasing with concentration at 36 mg./l. dinitrophenol, it reaches its maximum in salt at about 9 mg./l. and then begins to decrease as the concentration increases. The stimulatory effect seems to be specific to 2,4-dinitrophenol, as *o*-nitrophenol, *p*-nitrophenol, and picric acid (2,4,6-trinitrophenol) did not affect respiration rate in the same concentrations.

(*b*) *Accumulation*

A typical experiment on the effect of dinitrophenol on accumulation is shown in Figure 2. When the dinitrophenol was added to the accumulation

vessels, a small amount of concentrated potassium chloride was added to offset the dilution effect*. The discs used in this experiment were from the same batch as those used in the respiration experiment, but had been cut for a longer period. The dinitrophenol inhibited the uptake of ions as judged by the conductivity change in the external solution, the effect of dinitrophenol in each concentration becoming apparent immediately after the addition.

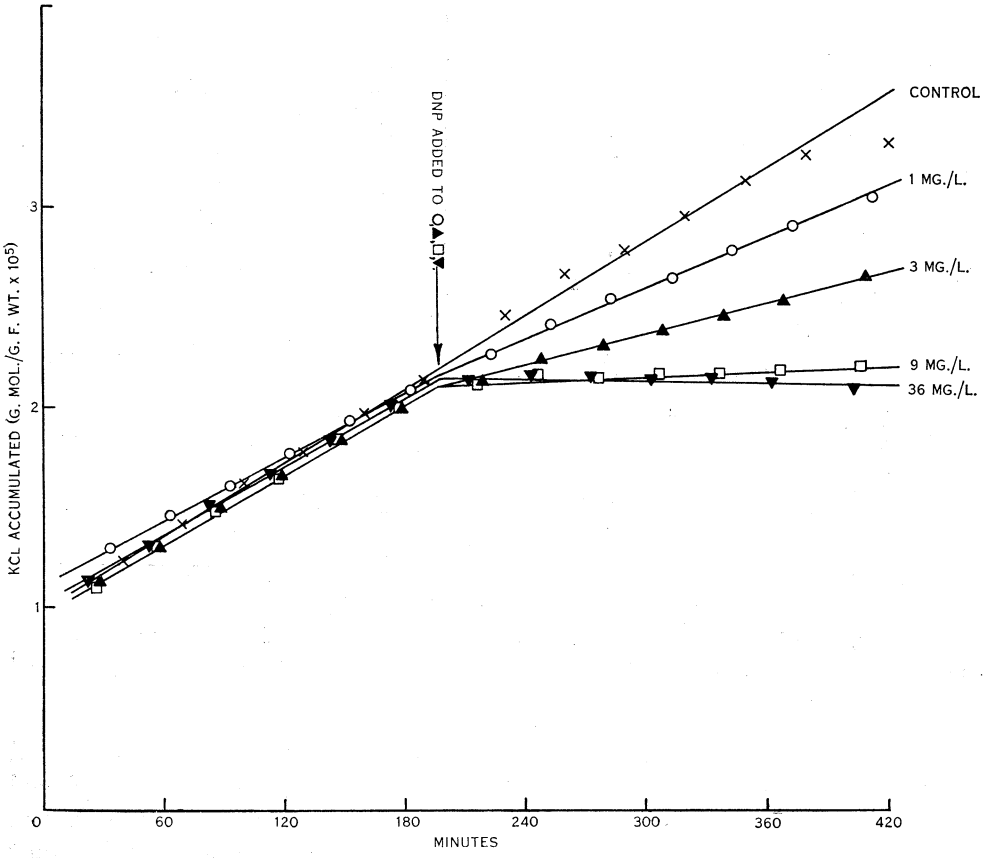

Fig. 2.—Effect of four different concentrations of dinitrophenol (DNP) on the amount of potassium chloride accumulated by carrot tissue in 0.05M KCl solution.

In all experiments, summarized in Figure 3, inhibition of accumulation increased sharply with increasing concentration of dinitrophenol; at concentrations above about 20 mg./l. there was a leakage of ions.

Since in these experiments the inhibition of accumulation had been determined from the conductivity change in the external solution, an experiment was carried out to determine whether the uptake of chloride itself was inhibited.

* This was not considered necessary in the Warburg vessels, since the concentration of chloride used would be adequate to ensure the full salt respiration (Robertson and Wilkins 1948).

The results of this experiment are given in Figure 4. Uptake was measured both by the change in conductivity and the change in chloride concentration. Fourteen sets of discs were taken; seven sets were placed in 0.05M potassium chloride and the remaining seven sets were placed in 0.05M potassium chloride with dinitrophenol (9 mg./l.). Samples for conductivity and chloride determinations were taken from each replicate at the required time. The conductivity results showed a complete inhibition of accumulation by dinitrophenol, after the initial uptake due to the physical equilibration. The chloride results indicated that, apart from the initial uptake, a very small amount of chloride entered over a short period, but this may not be significant. These results show that inhibition judged from conductivity determinations is an inhibition of the uptake of chloride itself, and is not due to an appreciable increase in leakage of other ions into the external solution.

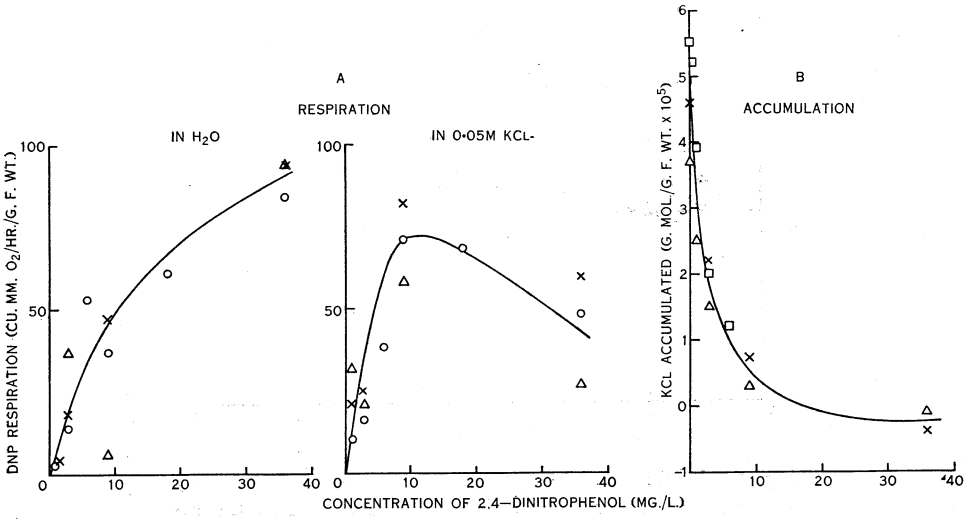

Fig. 3.—Relationship between concentration of dinitrophenol (DNP) and (*A*) the rate of the dinitrophenol respiration (i.e. the rate in DNP over and above that in water or in salt), and (*B*) amount of accumulation in carrot tissue; ×, O, and △, discs from one batch of carrots, respectively 144, 264, and 312 hr. from cutting; □ from another batch of carrots, 96 hr. from cutting.

(*c*) *Leakage*

In several experiments the change in conductivity produced by discs in water and in dinitrophenol was followed. This gave an estimate of the total loss of all electrolytes from the tissue. There was little or no leakage in water. Leakage in dinitrophenol was small but was always greater than that in water. If tissue that had been accumulating salt for some time was transferred to water or to dinitrophenol, the rate of leakage was greater than when the discs had had no pretreatment in salt. This can be seen from the results of the experiment given in Figure 5. Ten sets of discs were taken. Five sets were placed in water or dinitrophenol at four concentrations, 1, 3, 9, and 36 mg./l., and the conductivity changes followed. The remaining five sets

were placed in 0.05M potassium chloride and the conductivity changes followed for four hours. At the end of the four hours, the salt was drained off, the discs rinsed three times in distilled water, which was then drained off, and the required volume of water or dinitrophenol added. Other work has shown that this rinsing is sufficient to remove most of the salt from the intercellular spaces and cell surfaces. The rates of leakage with and without pretreatment in salt are plotted against concentration of dinitrophenol.

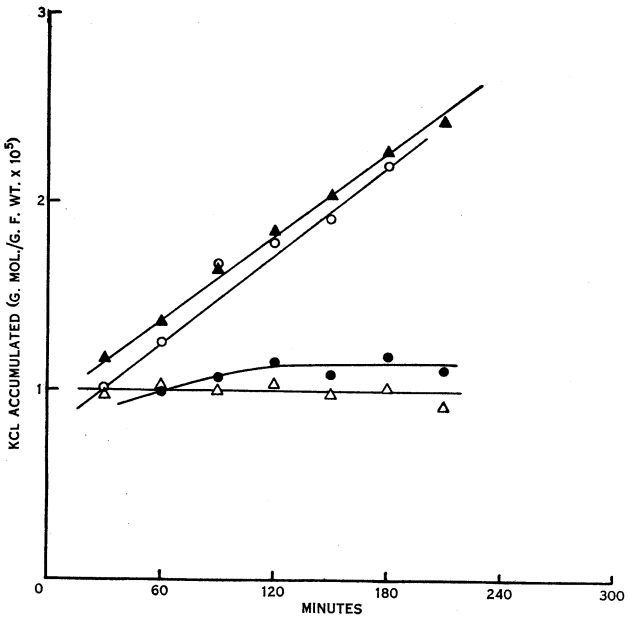

Fig. 4.—Inhibition of accumulation by DNP as measured by conductivity change and also by chloride change in the external solution (0.05M KCl). The upper curves show the amount of KCl accumulated from 0.05M KCl; ▲ from conductivity method; O from chloride method. The lower curves show the amount entering from 0.05M KCl in the presence of dinitrophenol (9 mg./l.); △ from conductivity method; ● from chloride method.

Though leakage in dinitrophenol was greater than in water, it did not account for the inhibition of accumulation observed. In several experiments the leakage in water and in dinitrophenol and the uptake in salt and in salt with dinitrophenol were determined in replicate sets of tissue. The results of one such experiment (discs threaded on wire) are given in Figure 6. The rate of leakage into water containing dinitrophenol was small compared with the difference between the rates of uptake from potassium chloride and from potassium chloride containing dinitrophenol. In some experiments, the leakage measured this way accounted for more of the difference between the normal and the inhibited accumulation, but greatest conductivity change in the external

solution (water containing dinitrophenol) due to loss of ions from the tissue accounted for only two-thirds of the difference and in most experiments for much less.

In four experiments, leakage from discs transferred to water from salt with dinitrophenol was not very different from that after transfer from salt alone, whether the dinitrophenol had caused partial inhibition of accumulation, complete inhibition of accumulation, or leakage. In the fifth experiment, the rate of leakage was greater after transfer from salt with dinitrophenol (36 mg./l.) than after transfer from salt alone. Even so, the rate of leakage into water accounted for only about 25 per cent. of the observed inhibition of accumulation in salt with dinitrophenol.

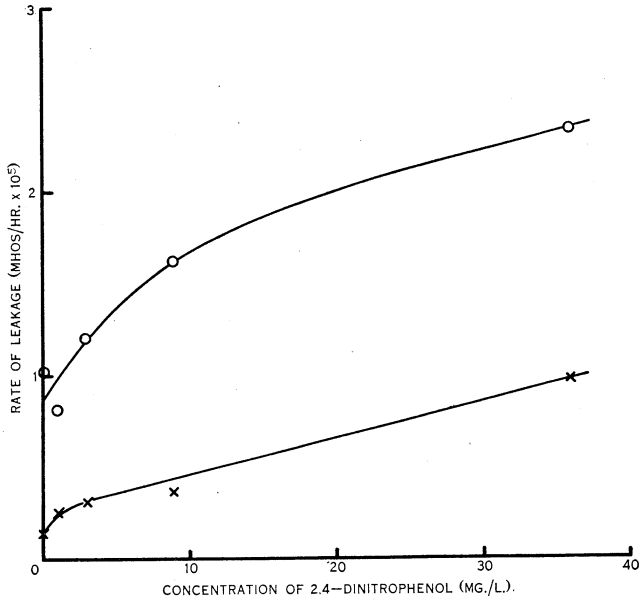

Fig. 5.—Rates of leakage from carrot tissue, measured by the increasing conductivity of the external solution, as a function of concentration of dinitrophenol; ×, no pretreatment; O, pretreatment in 0.05M KCl.

The effects on respiration of these transfers from salt to water and to dinitrophenol were examined. Respiration rates of tissue in water, in dinitrophenol, and in salt were determined; some sets of discs were then transferred from salt to water and to dinitrophenol and the changes in respiration rate followed. Respiration rates in water and in dinitrophenol were similar to those in Figure 1. The usual salt respiration was observed. Transfer from salt to water resulted in a decrease in respiration rate though the rate was still higher than that of the control in water. The respiration rates of sets of discs transferred from salt to dinitrophenol rose (at 36 and 9 mg./l.) or fell (at 3 and 1 mg./l.) to approximately the level of the respiration rates of their replicate sets of discs, which had had no pretreatment in salt.

(*d*) *Loss of Phosphate*

Since it has been shown by Teply (1949) that dinitrophenol has a specific effect in liberating inorganic phosphate from the cyclophorase system, the loss of phosphate from the tissue in the presence of dinitrophenol was examined in one experiment. It was found that tissue in water or potassium chloride solution lost little phosphate to the external solution over a period of five hours.

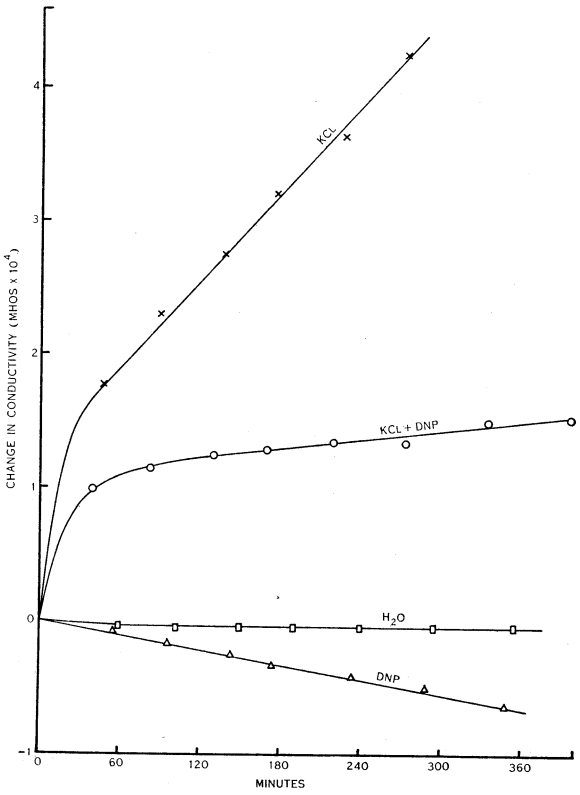

Fig. 6.—Uptake (positive) and loss (negative) of ions in carrot tissue in: 0.05M KCl, ×; 0.05M KCl with DNP (36 mg/l.), O; water, □; water with DNP (36 mg./l.), △. Results are expressed as conductivity change in the external solution.

In the presence of dinitrophenol, however, a measurable amount of phosphate was lost and this increased with time. The results are shown in Table 1. The conductivity change in the dinitrophenol solution was examined simultaneously; if the phosphate leaking were regarded as having the conductivity of sodium dihydrogen phosphate, it would account for only about 4.5 per cent. of the total conductivity change due to leakage in dinitrophenol. Hence although some phosphate ions were lost in the presence of dinitrophenol, they did not account for more than a small fraction of the total leakage.

(e) Comparison of the Effects of Dinitrophenol and Potassium Cyanide

The results of accumulation experiments in which dinitrophenol and potassium cyanide were added both separately and simultaneously are given in Table 2. It can be seen that:

(1) Dinitrophenol either partly inhibits accumulation or completely inhibits accumulation and some ions may leak from the tissue,

(2) Cyanide either partly inhibits accumulation or completely inhibits accumulation and ions do not leak from the tissue,

(3) Dinitrophenol and cyanide together inhibit accumulation and ions may leak slowly from the tissue.

TABLE 1

LOSS OF PHOSPHATE FROM TISSUES IN DIFFERENT SOLUTIONS COMPARED WITH CHANGES IN THE SPECIFIC CONDUCTIVITY OF THE EXTERNAL SOLUTIONS

	External Solution							
	H_2O		DNP		KCl		KCl + DNP	
Time (hr.)	△ Sp. Cond. (mhos × 10^5)	P (mg./ml. × 10^4)	△ Sp. Cond. (mhos × 10^5)	P (mg./ml. × 10^4)	△ Sp. Cond. (mhos × 10^5)	P (mg./ml. × 10^4)	△ Sp. Cond. (mhos × 10^5)	P (mg./ml. × 10^4)
0	0	0	0	0	0	0	0	0
1	3.2	1.2	2.9	3.0	−22.4	0.6	−14.4	3.6
2	4.2	1.0	5.6	4.4	−25.3	0.0	−14.8	6.2
4	6.2	1.0	10.1	7.4	−35.4	0.6	−12.6	11.4
5	8.0	1.0	12.5	—	−37.6	0.0	−13.0	16.2

When cyanide (0.001M) was added to tissue in dinitrophenol or potassium chloride with dinitrophenol, the respiration was rapidly reduced to the same level as that of tissue in water with cyanide and salt with cyanide. This level varied in tissue from different carrots. The respiration rate in water might be unaffected, partly inhibited, or slightly stimulated by cyanide.

Leakage of ions from tissue in water, cyanide (0.001M), and dinitrophenol (36 mg./l.) was compared in one experiment. The results are given in Figure 7. There was only a slight leakage in water and a slightly greater leakage in cyanide, which went on for more than five hours, after which the rate of leakage in cyanide began to increase; the rate had become quite rapid by the end of the experiment. The rate of leakage in dinitrophenol was about four times that in water. Cyanide was added to one of the two sets of tissue in dinitrophenol after four hours. The rate of leakage decreased considerably though it was still greater than the rate of leakage in water.

In one experiment, tissue was transferred from potassium chloride (0.05M) to water, cyanide (0.001M), dinitrophenol (36 mg./l.), and cyanide with dinitrophenol. After the transfer four readings were taken and then the vessels

were left shaking overnight and further readings taken in the morning. The results are shown in Figure 8. There was a slow leakage in water. In cyanide and cyanide with dinitrophenol there was at first a small uptake (initial uptake) and after this a slow leakage. Overnight, however, the leakage from tissue in cyanide ceased whereas the rate of leakage in cyanide with dinitrophenol increased. In dinitrophenol the rate of leakage was fairly high and continued at the same rate overnight.

TABLE 2

EFFECTS OF CYANIDE AND DINITROPHENOL ON THE ACCUMULATION RATE OF POTASSIUM CHLORIDE IN CARROT TISSUE

Experiment	Time from Cutting (hr.)	Replicate Number	Accumulation Rate (g. mol./g./hr. × 10^5)			
			KCl*	KCl* + DNP†	KCl* + KCN‡	KCl* + KCN‡ + DNP†
NL1	144	1	0.36			
		2	0.38		0.25	
		3		0.21		
		4		0.21		0.03
		5	0.32	0.18		
		6	0.38	0.17		0.03
NL2	168	1	0.38			
		2	0.41		0.14	
		3	0.38	0.15		
		4	0.40	0.17		0.00
		5		0.16		
		6		0.20		0.03
BE1	96	1	0.38			
		2	0.52	—0.12		
		3	0.47			0.00
		4			0.07	— 0.05
		5	0.65		0.04	
		6		—0.06		— 0.02

* 0.05M. † In NL1 and NL2, 3 mg./l; in BE1, 36 mg./l. ‡ 0.001M.

In the same experiment the rates of leakage in water, after pretreatment in salt (0.05M potassium chloride), salt with cyanide, salt with dinitrophenol, and salt with cyanide and dinitrophenol, were measured. Pretreatment in salt plus cyanide resulted in a slow leakage; the rate of leakage in water after pretreatment in salt with cyanide and dinitrophenol was greater, but not as great as that after pretreatment in salt with dinitrophenol alone.

(*f*) *Effects of Dinitrophenol and Carbon Monoxide*

The cyanide inhibition of the respiration stimulated by dinitrophenol suggests that this respiration, like the salt respiration, proceeds via the cytochrome-

cytochrome oxidase system. To establish whether the cytochrome oxidase system was involved, carbon monoxide, a specific inhibitor of this oxidase, was used in both light and darkness. A typical experiment is illustrated in Figure 9. A large increase in respiration rate was observed after the addition of the dinitrophenol (6 mg./l.). When the steady respiratory drift had been established, the air in some Warburg flasks was replaced by 93.5 per cent. CO-6.5 per cent. O_2, and respiration rates were then measured in both light and darkness. Carbon monoxide strongly inhibited the dinitrophenol respiration in

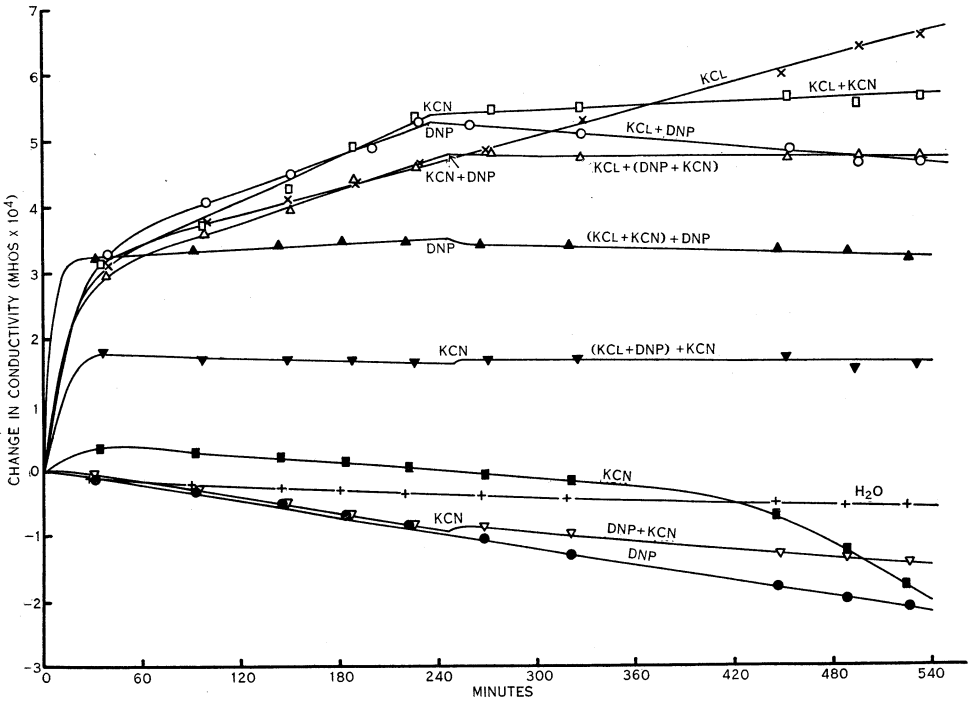

Fig. 7.—Effects of dinitrophenol (32 mg./l.) and of potassium cyanide (0.001M) on leakage in water and in potassium chloride solution (0.05M) with time. Results for uptake (positive) and leakage (negative) are expressed as change in conductivity of the external solution.

darkness, and bright light almost completely reversed this inhibition if allowance is made for the drift due to the low oxygen concentration. The effects of the gas mixtures on the respiration in water were also investigated but the results are not shown here; the respiration in water was depressed by CO in darkness to almost the same level as that in dinitrophenol, and was almost unaffected by low oxygen concentration. The small carbon monoxide inhibition was completely reversed by light. These results show conclusively that cytochrome oxidase is responsible for part of the respiration in these carrots, both in dinitrophenol solutions and in water, and that all the dinitrophenol respiration is mediated by cytochrome oxidase.

IV. Discussion

From the above results it may be concluded that:

(1) Dinitrophenol inhibits salt accumulation and at high concentrations may even cause a leakage of ions from tissue in salt.

(2) Dinitrophenol increases the leakage of ions from tissue in water and from tissue transferred from salt to water; even if dinitrophenol caused the same amount of leakage from tissue in salt (though this leakage would tend to be less because the concentration gradient would be less), it would not account for the inhibition of accumulation as determined by the conductivity method.

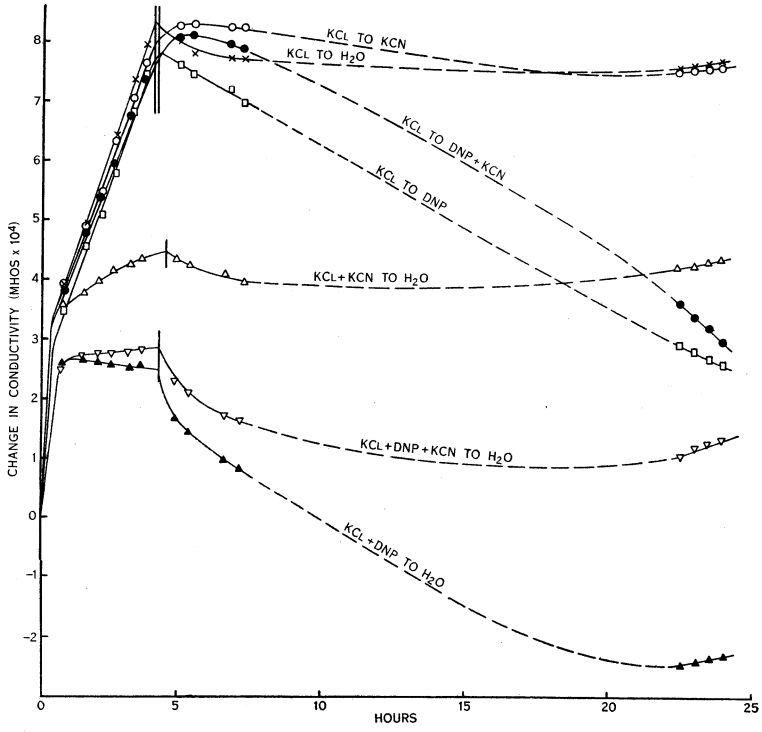

Fig. 8.—Effects of dinitrophenol (32 mg./l.) and potassium cyanide (0.001M) on leakage over long periods after pretreatment with 0.05M KCl, with KCl and KCN, with KCl and DNP, and with KCl, KCN and DNP. Results are expressed as change of conductivity of the external solution.

(3) Dinitrophenol stimulates the respiration of tissue in water and of tissue in salt; this stimulated respiration is sensitive to cyanide and is light-reversibly inhibited by carbon monoxide.

(4) Cyanide does not cause any appreciable leakage of ions from tissue in water at least for several hours, and apparently retards leakage from tissue in dinitrophenol.

(5) Cyanide inhibits salt accumulation and during the period of an experiment causes no leakage of ions from tissue in salt.

Thus dinitrophenol, like salt, allows more active functioning of the cytochrome system and its effect is additive to the salt effect; in spite of this, accumulation is inhibited. It has been shown both by the measurement of leakage rates and by the measurement of chloride uptake that the inhibition is not accounted for by a leakage of electrolytes that have previously been accumulated.

Hence it must be concluded that dinitrophenol either inhibits the transport mechanism itself or increases leakage only in the critical region across which the transport mechanism operates, so that ions being moved into the cell leak back at such a rate that there is no net accumulation.

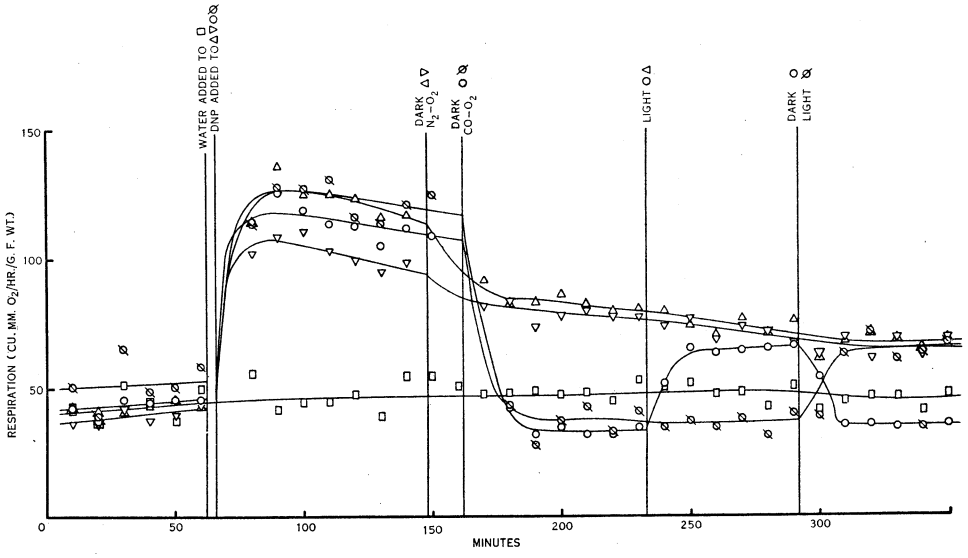

Fig. 9.—Effect of carbon monoxide on the respiration of carrot tissue in KCl solution (0.05M) with and without dinitrophenol (6 mg./l.); gases contained 6.5 per cent. O_2.

(*a*) *Effect on Accumulation*

Whatever the mechanism of the action of dinitrophenol, there is ample evidence (Loomis and Lipmann 1948; Cross *et al.* 1949; Teply 1949) that it interferes with the transfer of energy-rich phosphate from respiratory reactions to other processes. There is as yet insufficient evidence to decide how an inhibition of energy-rich phosphate transfers might interfere with the ion transport mechanism.

It may be that, in the presence of dinitrophenol, the cytochrome system, though operating at a greater rate in the presence of dinitrophenol, is no longer in a position to transport anions. There is increasing evidence, particularly that of Green, Loomis, and Auerbach (1948) and of Hogeboom,

Schneider, and Pallade (1948), that the succinoxidase system is associated with mitochondria. Similar conclusions about plant succinoxidase have been reached by duBuy, Woods, and Lackey (1950). Further, interference with the normal energy-rich phosphate transfer system brings about some loss of function and integrity in the mitochondrial particle (Teply 1949; Harman 1950*a*). Hence it may be that the accumulation occurs only if the cytochrome oxidase system is organized in the intact mitochondrion and that the dinitrophenol interferes with this organization. Though Harman (1950*b*) did not find any accumulation of sodium or potassium in the mitochondria, Mullins (1940) found that both phosphate and potassium accumulated in the protoplasmic granules of *Nitella.*

Alternately, the transport by the cytochrome system may be only one stage in a mechanism consisting of several stages in series, at least one of which requires a phosphorylation. In this hypothesis some of the energy for the transport of ions would be derived from unstable phosphate esters, as suggested by Davies and Ogston (1950) in explaining the secretion of hydrochloric acid by the gastric mucosa. Finally it is possible that the cytochrome system plays no direct part in the accumulation mechanism; if this were so a new explanation of the quantitative relation between salt accumulation and salt respiration (Robertson and Wilkins 1948) would have to be sought.

(*b*) *Effect on Respiration*

It is not yet clear how dinitrophenol increases the respiration rate, though the suggestion that it liberates free phosphate which has been limiting the rate of respiration is plausible (Teply 1949). It seems likely that it would have an effect in carrot tissue similar to that in *Avena* coleoptile (Bonner 1949*b*); both tissues show respiration stimulated by adenylic acid.

The respiration of carrot tissue, both in water and in salt, was stimulated by even the highest concentration of dinitrophenol used (36 mg./l.), while Bonner (1949*b*) found that the respiration of *Avena* coleoptiles, though stimulated at low concentrations (below 6-7 mg./l.), was almost completely suppressed at higher concentrations (10 mg./l.). While this difference may be inherent in the tissue, it may be only apparent, since Bonner was using solutions buffered at pH 4.5. In most of our experiments no buffer solution was added but the pH of the external solution changed only from pH 5.0 to 5.5. An experiment with carrot tissue in dinitrophenol (9 mg./l.) buffered at pH 3.5, 5.5, and 7.5 with citrate-phosphate showed that the effect of dinitrophenol was influenced by the pH level. At pH 3.5 respiration was almost completely suppressed, whereas at pH 5.5 and 7.5 the respiration was stimulated. At low pH values, owing to the suppression of ionization of both the dinitrophenol and the weak electrolytes of the cytoplasm, the concentration of dinitrophenol in the cell would probably be greater than at higher pH values.

Bonner found that the inhibitory effect of dinitrophenol on respiration was not shown if pyruvate was supplied as a respiratory substrate. He suggested that, at high concentrations, dinitrophenol interferes with the normal

production of pyruvate from hexose, a process known to require adenosine-triphosphate, formation of which may be inhibited by dinitrophenol. Some similar effect of dinitrophenol may account for the submaximal effect of high concentrations (above about 10 mg./l.) on the respiration of tissue in salt. The effect of inhibition of the production of pyruvate would be expected to be more marked in tissue in salt than in tissue in water, since presumably the rate of utilization of pyruvate is greater in salt than in water.

(*c*) *Effect on Leakage*

Leakage of ions from carrot tissue in water is very small. When dinitrophenol is applied to the tissue, the leakage is increased. The reason for this increase is not yet known. If the resistance in the cell to ion diffusion were dependent for maintenance wholly on phosphorylations, then a greater increase in leakage in dinitrophenol would be expected. The fact that the resistance does not decrease markedly in the presence of dinitrophenol may mean that a continuous supply of energy-rich phosphate is not necessary for its maintenance or that sufficient energy-rich phosphates are still available.

The ions appearing in the external solution have not yet been fully identified and may be ions liberated from the cytoplasm owing to some effect of the dinitrophenol. Phosphate, which may correspond to the "gel phosphate" liberated by dinitrophenol from the cyclophorase system (Green *et al.* 1949; Teply 1949), is apparently liberated, but accounts for only a small fraction of the total ions lost to the external solution.

Another possible explanation of the leakage is that the dinitrophenol, without having much effect on the overall cytoplasmic resistance, inhibits the mechanism that normally operates to transport back to the cell interior the small quantity of ions that escape through the high-resistance region.

Dinitrophenol alone increases leakage more than dinitrophenol with cyanide. Leakage is also greater in water after transfer from salt with dinitrophenol than after transfer from salt with dinitrophenol and cyanide. This may be a real difference due to some antagonism between the dinitrophenol and the cyanide and might, for instance, be connected with the effects of these substances on the mitochondria. The problem of leakage obviously requires more thorough investigation.

It must be concluded that the dinitrophenol inhibits some process intimately connected with accumulation; it does not inhibit the cytochrome-cytochrome oxidase system; hypotheses of salt accumulation must allow for this conclusion.

V. Acknowledgments

The authors wish to express their gratitude to Professor J. S. Turner, University of Melbourne, and to Dr. J. R. Vickery, Chief of the Division of Food Preservation and Transport, C.S.I.R.O., for their helpful criticism of the manuscript.

VI. References

BONNER, J. (1949*a*).—*Amer. J. Bot.* **36**: 323.
BONNER, J. (1949*b*).—*Amer. J. Bot.* **36**: 429.
CROSS, R. J., TAGGART, J. V., COVO, C. A., and GREEN, D. E. (1949).—*J. Biol. Chem.* **177**: 655.
DAVIES, R. E., and OGSTON, A. G. (1950).—*Biochem. J.* **46**: 324.
DUBUY, H. G., WOODS, M. V., and LACKEY, M. D. (1950).—*Science* **111**: 572.
GREEN, D. E., ATCHLEY, W. A., NORDMANN, J., and TEPLY, L. J. (1949).—*Arch. Biochem.* **24**: 359.
GREEN, D. E., LOOMIS, W. F., and AUERBACH, V. H. (1948).—*J. Biol. Chem.* **172**: 389.
HARMAN, J. W. (1950*a*).—*Exp. Cell Res.* **1**: 382.
HARMAN, J. W. (1950*b*).—*Exp. Cell Res.* **1**: 394.
HOAGLAND, D. R. (1944).—"Lectures on the Inorganic Nutrition of Plants." (New York.)
HOGEBOOM, G. H., SCHNEIDER, W. C., and PALLADE, G. E. (1948).—*J. Biol. Chem.* **172**: 619.
KREBS, H. A. (1935).—*Biochem. J.* **29**: 1620.
KROGH, A. (1946).—*Proc. Roy. Soc.* B **133**: 140.
LOOMIS, W. F., and LIPMANN, F. (1948).—*J. Biol. Chem.* **173**: 807.
LUNDEGARDH, H. (1945).—*Ark. Bot.* **32**A (12): 1.
MULLINS, L. J. (1940).—*Proc. Soc. Exp. Biol. N.Y.* **45**: 856.
NANCE, J. F. (1949).—*Science* **109**: 174.
NEWCOMB, E. H. (1950).—*Amer. J. Bot.* **37**: 264.
ROBERTSON, R. N., and TURNER, J. S. (1945).—*Aust. J. Exp. Biol. Med. Sci.* **23**: 63.
ROBERTSON, R. N., and WILKINS, MARJORIE J. (1948).—*Aust. J. Sci. Res.* B **1**: 17.
TEPLY, L. J. (1949).—*Arch. Biochem.* **24**: 383.
WEEKS, D. C., and ROBERTSON, R. N. (1950).—*Aust. J. Sci. Res.* B **3**: 487.