alent formulations should release more DO than HDA in the field.

Fragments were seen at m/e 235 (M+1, 7%), 236 (M, 4%) and 237 (M+1, 3%) by methane chemical ionization mass spectrometry and were consistent with the desired diolefin.

The bioassay method for studying disruption of mating communication in the corn earworm via air permeation in the field has been described elsewhere[5]. The DO was evaporated from closed No.3 Beem® embedding capsules, each containing 5 mg of the material. Each capsule was attached to a wooden stake at a height about 15 cm above the plant canopy. A treatment consisted of 16 equally-spaced evaporators within a 100 m^2 plot. At the center of each plot was a cone-trap[5] baited with 3 virgin female *H. zea*. The experiment consisted of 6 trapping sites in a peanut field (2 rows of 3 traps each) with 100–180 m between traps. The treatments were applied to every other site and each time the traps were checked the treatment were moved to the adjacent site. The traps were inspected 9 times from July 11–23, 1980. Each time the treatment was moved to a previously untreated trap site, the number of males captured by that trap was reduced. Traps at untreated sites captured a mean of 12.4 ± 2.7 (SE) males/trap per trapping interval and traps in DO-permeated areas captured 1.9 ± 0.5 males/trap per trapping interval. These means are statistically different, ANOVA, at the 1% level of significance. The greatest reduction relative to the controls was 91.9% and the least reduction 75%.

Inhibition of the corn earworm was determined by placing 2 cone traps 50 m apart in a corn field for 6 nights. Each night each trap was baited with 3 virgin *H. zea* females. An embedding capsule containing 5 mg of DO was attached adjacent to the female's cage in 1 trap. The treatment was alternated nightly between the traps. The traps without DO captured a mean of 23.3 ± 1.2 (SE) males/trap per night while traps baited with DO and females captured a mean of 18.5 ± 0.9 (SE) males/trap per night. The means are statistically equivalent, ANOVA, at the 5% level.

Disruption of mating communication in the tobacco budworm was attempted in a tobacco field for 5 consecutive nights, August 1–6, 1980, using the same technique. Control traps captured a mean of 2.6 ± 0.5 (SE) males/trap per night while traps in the treatment area captured a mean of 3.8 ± 0.8 (SE) males/trap per night. The means are statistically equivalent, ANOVA, at the 5% level.

It appears that electron density around the position occupied by the oxygen atom in the natural pheromone, HDA, may be very important; thus, its substitution with a double-bonded carbon as in DO is possible with retention of activity. Interestingly, while the formate disrupted both *H. zea* and *H. virescens*, DO disrupted mating communication only in *H. zea* when released at the same single rate described here. This failure in *H. virescens* suggests that, while isosteres, analogs, enantiomers and cogeners of insect sex pheromones have variously demonstrated effects as attractants, inhibitors or disruptants, it is not yet possible to predict such effects.

Comparable reduction in catch of *H. zea* using HDA in the air permeation technique in a Hercon formulation were 90–100% over approximately the same period of field trapping as the present work, while reductions using the formate in Conrel hollow fibers were also 90–100%. Direct comparison of results with HDA and formate to the 75–92% reductions found here with DO are not appropriate since different formulations were used, but preliminary results are encouraging. Also, it is possible that a blend of DO with small amounts of aldehydes and/or formates may approximate the activity of the best aldehyde blend[6].

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6 Mention of a commercial or proprietary product does not constitute an endorsement by the U.S. Department of Agriculture.

Phosphate mediated regulation of some of the enzymes of carbohydrate metabolism in *Neurospora crassa*

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Summary. The levels of FDP aldolase, isocitrate lyase, isocitrate dehydrogenase, malate dehydrogenase and glucose-6-P dehydrogenase were found to be rugulated by inorganic phosphate in the growth medium. The possible regulatory aspects have been discussed.

It has been suggested that phosphate exerts a direct control on the selection of primary and secondary metabolism of the cell[1]. Though the regulation of secondary metabolism by inorganic phosphate is well documented[2–4], information on the role of inorganic phosphate in the regulation of enzymes is scarce. The present study is focused on the role of inorganic phosphate in the regulation of some of the enzymes of carbohydrate metabolism. These studies may be useful for understanding the regulation of secondary metabolism through control of the levels of enzymes involved either directly in a biosynthetic pathway or in providing intermediates.

Table 1. Changes in growth, glucose utilization and ketoacids in *N. crassa* grown under low and high phosphate conditions

Growth condition	Growth (dry wt, g/l)	Glucose uptake (g/l)	Ketoacids (mg/l)
Low phosphate (KH_2PO_4 0.01%)	4.8	25.5	302.1
High phosphate (KH_2PO_4 1.0%)	5.7	42.3	209.7

The carotenogenic strain of *N. crassa* (wild type) was maintained on Sabouraud's agar slants. The composition of the synthetic medium employed was the same as that described earlier[5]. The culture was grown in 100 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (180 rpm) at 30 °C for 6 days. The mycelia were harvested by filtration and stored at −5 °C before use. For growth measurement, the mycelia were dried at 50 °C to a constant weight. The growth was expressed as dry mat weight per l.

For enzyme assays, a cell-free extract was prepared in 0.05 M Tris-HCl buffer of pH 7.5 containing 0.4 M sucrose, by grinding the frozen mycelia with a pestle in a chilled mortar with glass powder so as to get a 30% (wt/vol.) extract. The extract was centrifuged at 5000 × g for 15 min. The supernatant was recentrifuged at 15,000 × g for 30 min and the 2nd step supernatant was used for cytosolic enzyme assays. The 15,000 × g pellet obtained was sonicated 4 times for 30 sec, with intervals of 30 sec, in the cold, in the same buffer. The sonicated material was then centrifuged at 15,000 × g for 30 min and the resulting supernatant was used for the assay of mitochondrial malate dehydrogenase activity.

The assay methods used for FDP aldolase (fructose-1, 6-diphosphate D-glyceraldehyde-3 phosphate-lyase, EC 4.1.2.13), isocitrate dehydrogenase (threo-D-isocitrate: NADP oxidoreductase (decarboxylating), EC 1.1.1.42), malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37), glucose-6-P dehydrogenase (D-Glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49), and isocitrate lyase (threo-D-isocitrate glyoxylate lyase, EC 4.1.3.1) were the same as described by Jagannathan et al.[6], Ochoa[7], Ochoa[8], Kornberg and Horecker[9] and Dixon and Kornberg[10] respectively. The protein and keto acids were assayed according to the methods of Warburg and Christian[11] and Friedemann[12] respectively. The residual sugar (glucose) in the growth medium was determined by the method of Bernfeld[13]. The phosphate was added to the medium as KH_2PO_4. 'Low phosphate' indicates the addition of 0.01 g% KH_2PO_4 while 'high phosphate' means the addition of 1.0 g% KH_2PO_4 to a synthetic medium devoid of phosphate. These concentrations of phosphate were selected since they had been used earlier in this laboratory[14].

Neurospora crassa grown in high phosphate conditions showed higher growth and sugar utilization as compared to low phosphate medium (table 1). Earlier, Liras et al.[15] and Martin and Daniel[16] had shown that upon addition of 5 mM phosphate to cells, the rate of glucose utilization and of growth were increased. Similar observations were also made by Perlman and Wagman[17] and Di Macro[18] in *Streptomyces* sp.

Neurospora crassa showed higher levels of keto acids in cultures grown in low phosphate medium as compared to high phosphate (table 1). The decrease in keto acids in high phosphate has also been demonstrated by Toropova et al.[19] in *Proactinomyces vulgaris.* The reduction in the levels of keto acids suggests that carbohydrate metabolism might have been affected in the presence of high phosphate. The activity of the glycolytic enzyme FDP aldolase was found to be much lower in high phosphate medium as compared to low phosphate grown cultures (table 2). Similarly the activities of both cytosolic and mitochondrial malate dehydrogenase (tables 2 and 3) were also found to be affected. Cytosolic (NADP) isocitrate dehydrogenase was not detectable in high phosphate conditions. The reduced levels of these dehydrogenases and FDP aldolase may be partly responsible to some extent for low levels of keto acids in high phosphate grown cultures.

The glucose-6-P dehydrogenase was also found to be affected. The activity was not detectable in high phosphate conditions (table 2). Earlier, Hošťálek[20] and Harold and Hošťálek[21] also demonstrated that increased phosphate concentrations in the medium decreased the activity of the pentose phosphate pathway.

The activity of the glyoxylate bypass enzyme isocitrate lyase was also found to be reduced in high phosphate grown *N. crassa* as compared with that grown in low phosphate (table 2).

There are many possible reasons for low levels of enzymes in cultures grown in high phosphate medium, and they may vary for different enzymes. The high phosphate grown culture may contain a phosphorylated enzyme which is less active than the dephosphorylated form; e.g. Garnak and Reeves[22] found that the NADP isocitrate dehydrogenase of *E. coli* strain K becomes phosphorylated in vivo which results in a reduction of the enzyme activity. b) The high phosphate grown culture may produce higher levels of a repressor which may control enzyme synthesis at the transcriptional level; e.g. the synthesis of alkaline phosphatases has been shown to be derepressed in environments with a

Table 3. Mitochondrial malate dehydrogenase activity from *N. crassa* grown under low and high phosphate conditions

Growth conditions	Malate dehydrogenase (units/mg protein)
Low phosphate (KH_2PO_4 0.01%)	44
High phosphate (KH_2PO_4 1.0%)	9

Unit, Amount of enzyme which brings about a change of 0.01 OD per min at 30 °C.

Table 2. Isocitrate lyase, FDP aldolase, glucose-6-P dehydrogenase and cytosolic malate malate dehydrogenase and isocitrate dehydrogenase activities from *N. crassa* grown in low and high phosphate conditions

Growth conditions	Isocitrate lyase		FDP aldolase		Glucose-6-P dehydrogenase		(NADP) isocitrate dehydrogenase		Malate dehydrogenase	
	U/g dry wt	U/mg protein	U/g dry wt	U/mg protein	U/g dry wt	U/mg protein	U/g dry wt	U/mg protein	U/g dry wt	U/mg protein
Low phosphate (KH_2PO_4 0.01%)	2927	17	3476	20	9604	55	1646	9	6860	31
High phosphate (KH_2PO_4 1.0%)	2279	4	1641	3	ND	ND	ND	ND	1099	2

ND, Not detectable; U, Units. Units for glucose-6-P dehydrogenase, isocitrate dehydrogenase and malate dehydrogenase. Amount of enzyme which brings about a change of 0.01 OD at 340 nm/min at 30 °C. Units for isocitrate lyase and FDP aldolase: Amount of enzyme which brings about a change of 0.01 OD at 324 and 240 nm respectively per min at 30 °C.

low inorganic phosphate concentration[23]. Similarly an acid phosphatase[24], 2 extracellular nucleases[25] and ribonucleases[26] have been reported to be derepressed under orthophosphate limiting conditions. c) The high phosphate grown culture may produce an inhibitor of the enzyme activity. In our studies, high phosphate grown cultures were found to produce an inhibitor (unpublished results) which was found to be responsible for inhibition of the activities of glucose-6-P dehydrogenase and isocitrate lyase, but not of isocitrate dehydrogenase, since the addition of crude cell-free extract of a high phosphate grown culture to a low phosphate grown cell-free extract was found to inhibit the activities of these enzymes. The rate of inhibition was also proportional to the concentration of inhibitor (data not presented here).

These results indicate that phosphate plays an important role in the control of the activity of a variety of enzymes. This may help us in understanding the regulation of the production of secondary metabolites by inorganic phosphate, and hence these studies might help in increasing the production of secondary metabolites, through control of enzyme activities by control of inorganic phosphate levels.

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The increase of thiobarbituric acid reacting substances in rats with experimental chronic hypoxia

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Summary. Feeding under conditions of reduced oxygen supply prompted an increase in serum, arterial and brain tissue levels of thiobarbituric acid (TBA)-reacting substances. These observations indicated the possibility that hypoxia might be one of the factors predisposing to the accumulation of lipid peroxide.

It has been demonstrated in animal experiments and other studies that damage is caused to arterial tissue under conditions of oxygen insufficiency, such as hypoxia due to diminished lung function on diseases or smoking[1-3]. On the other hand, Glavind[4] has reported that the content of lipid peroxide in the arterial wall is elevated with increasing severity of arteriosclerosis. We focused our attention on lipid peroxidation as one of the important factors in hypoxia-induced damage, and an investigation was carried out in rats with experimental chronic hypoxia; the levels of TBA-reacting substances in the serum, artery, brain and liver were determined.

Materials and methods. Adult female Wistar strain rats weighing between 250 and 300 g were used in all experiments. In order to induce hypoxia, the rats were kept in airtight 60-l cages which were ventilated with a mixed gas containing 85% nitrogen and 15% oxygen at a rate of 0.5 l/min. After 2 or 4 weeks of feeding under these conditions, blood was drawn and liver, brain and abdominal aorta were removed. The tissues were perfused with cold saline solution to remove blood. The levels of TBA-reacting substances in the serum and tissue were determined by the method of Yagi et al.[5,6].

Results. At 2 weeks of feeding under conditions of reduced oxygen supply Po_2 decreased by about 16%, as compared to the level determined before the beginning of the experiment, and at 4 weeks Po_2 showed a tendency to decrease further. This observation indicates that hypoxia was induced in all the rats kept under this condition (table 1). When the animals were kept under conditions of reduced oxygen supply, TBA-reactants in the serum, in the abdominal arterial wall and in the brain tissue showed a significant increase at 2 weeks, whereas in the liver tissue

Table 1. Change of Po_2, PCo_2 and pH levels under hypoxic conditions

	0 (control) (n=8)	2 weeks (n=7)	4 weeks (n=7)
pH	7.43±0.03	7.47±0.04	7.41±0.03
PCo_2	35.9 ±3.5	32.1 ±4.9	34.8 ±4.6 mmHg
Po_2	90.2 ±4.5	76.4 ±3.2*	72.3 ±9.7 mmHg*

Results are expressed as mean value±SD. * $p < 0.001$ for difference from controls by Student's t-test.