strated by their associated extinction contours. Their shape was further confirmed by taking stereoscopic pairs and examining them in a stereo viewer. Conclusive evidence as to the fiber formation mechanism in this alloy has not yet been obtained, but some of the replica electron micrographs obtained suggest that it may be the same as in the Au-Ni system.

Eventually fiber-reinforced alloys in a wide range of other alloys may be prepared with our technique. The straightforward approach is to prepare the fibers first, often by growth from the vapor phase, and then to permit an appropriate matrix material to solidify around them. This is a difficult and costly process, and it is therefore desirable to devise methods of growing the fibers *in situ*. During the past few years some progress in this direction has been made with unidirectional solidification of eutectic alloys (*7*), but this approach is of limited value because the volume fraction of fibers obtainable is fixed by the necessity of using alloys at or near eutectic compositions. Our technique involves only heat treatment in the solid state, and the volume fraction of fibers can be varied over a wide range with variation of the alloy composition.

A. FESOLOWICH
A. A. JOHNSON
J. L. BESTEL, K. MUKHERJEE
Department of Physical and Engineering Metallurgy, Polytechnic Institute of Brooklyn, Brooklyn, New York 11201

References and Notes

1. J. Ordonez, A. A. Johnson, K. Mukherjee, *Nature* **217**, 442 (1968).
2. A. A. Johnson, E. J. Hughes, P. W. Barton, *Int. Dent. J.* **18**, 655 (1968).
3. A. J. Ardell and R. B. Nicholson, *Acta Met.* **14**, 1295 (1966).
4. J. D. Eshelby, appendix to (*3*).
5. F. R. N. Nabarro, *Proc. Phys. Soc. London* **52**, 90 (1940); ———, *Proc. Roy. Soc. London Ser. A* **175**, 519 (1940).
6. The results on Al-Zn are from J. L. Bestel, thesis, Polytechnic Institute of Brooklyn (1970).
7. R. W. Hertzberg and R. W. Kraft, *Trans. Met. Soc. Amer. Inst. Met. Eng.* **227**, 58 (1963); R. W. Hertzberg, *Trans. Amer. Soc. Metals* **57**, 434 (1964).
8. Supported in part by NIH grant DE 02422-04 and NIDR training grant ITIDE 189-01. We thank R. W. Bauer for preparing some of the replica micrographs.

7 October 1969; revised 23 December 1969

Lead Effects on Corn Mitochondrial Respiration

Abstract. *Oxidation of exogenous nicotinamide-adenine dinucleotide and succinate by corn mitochondria was measured as a function of lead chloride concentration. Lead chloride (50 to 62 micromoles per liter) stimulated oxidation of exogenous reduced nicotinamide-adenine dinucleotide by 174 to 640 percent depending on the reaction mediums, whereas lead chloride (12.5 micromoles per liter) inhibited succinate oxidation by more than 80 percent. When inorganic phosphate was included in reaction mediums the subsequent addition of lead was without effect due to the low solubility of lead phosphate. If addition of lead was followed by addition of phosphate the inhibition of succinate oxidation by lead was released, but there was no reduction in the stimulation of oxidation of reduced nicotinamide-adenine dinucleotide by lead. The effects of lead on plant growth might be accentuated under conditions of phosphate deficiency.*

Each year motor vehicles in the United States liberate approximately 225×10^6 kg of particulate lead into the atmosphere. About 50 percent of this lead is deposited within 30 m of the roadways (*1*), and the remainder is scattered over large areas. Lead accumulation in soils near roads varies with traffic volume and decreases rapidly with distance from the road. Lead has been found at concentrations of 128 to 700 ppm in soil adjacent to 12 highways in the Minneapolis-St. Paul area (*1*). Grass collected near an intersection of two heavily traveled highways near Denver, Colorado, contained as much as 3000 ppm lead, and at Canandaigua, New York, 16 vegetable samples from gardens less than 50 feet (15.2 m) from roads averaged 115 ppm lead and ranged from $<$ 10 ppm to 700 ppm (*2*).

Vegetation near roads might be in danger if the present trend continues. Inhibitions of various animal enzyme systems is attributable to lead (*3*), but little is known of the effect of lead on plant enzyme systems. Hammett (*4*) showed a decrease in root tip mitosis at very low concentrations of lead. This study was therefore initiated to test the effect of lead on substrate oxidation in mitochondria of corn.

Mitochondria were isolated from 3-day-old etiolated corn shoots (*Zea mays* L., WF9 $\times$ M14) (*5*). Experiments were performed with the addition of mitochondrial suspensions (approximately 1 mg of protein) and $PbCl_2$ in known concentrations to a completely filled 4-ml glass reaction cell that was temperature controlled ($27° \pm 0.2°C$) and equipped with a Clark oxygen electrode (Yellow Springs). The reaction mediums contained 200 m*M* KCl or 300 m*M* sucrose, 20 m*M* tris (*p*H 7.5), and 1 mg bovine serum albumin (BSA) per milliliter under most conditions. Reduced nicotinamide-adenine dinucleotide (NADH) or succinate was added 4 minutes after addition of mitochondria to the medium, and rates of respiration were determined as a function of concentration of $PbCl_2$.

Micromolar concentrations of $PbCl_2$ influenced oxidation of both exogenous NADH and succinate in either KCl- or sucrose-containing mediums. The effect of lead was not the same on the two substrates tested, even though both are donors in electron transport. In a KCl medium, succinate oxidation was inhibited 37 percent by 6 μM $PbCl_2$, and 100 percent by concentrations of 25 μM or greater (Table 1). Oxidation of exogenous NADH by mitochondria in a KCl medium was stimulated 174 percent by 62 μM $PbCl_2$, with some stimulation (28 percent) at $PbCl_2$ concentrations as low as 6 μM (Table 2). Even greater stimulations of oxidation of exogenous NADH were found with mitochondria suspended in a sucrose medium (Table 2). Oxidation of succinate was inhibited in sucrose as it had been in KCl and was independent of the presence of BSA (Table 1).

Studies measuring oxidation of exogenous NADH and succinate in a diaphorase reaction with 2,6-dichlorophenolindophenol as the electron acceptor, and 1 m*M* KCN as the regular blocker of electron transport, gave results similar to those obtained by measuring oxygen uptake. In whole mitochondria oxidation of exogenous NADH was stimulated, but oxidation of succinate was inhibited. Even when mitochondria were disrupted by high-frequency sound for 2 minutes and centrifuged at 46,000*g* for 30 minutes, the supernatant maintained activity of NADH diaphorase that was stimulated 12 to 25 percent by 62 μM $PbCl_2$.

The diaphorase reaction is a general reaction that is catalyzed by flavoprotein and involves, among others, flavoproteins from the first part of the electron-transport chain. Since these flavoproteins are utilized solely in the diaphorase reaction, and are a part of the

Table 1. Rate of oxidation of succinate by corn mitochondria in relation to concentration of $PbCl_2$. Succinate (10 mmole/liter) was added after 4 minutes to mitochondria (approximately 1 mg of protein) that were suspended in 4-ml reaction vessels that contained 300 m*M* sucrose or 200 m*M* KCl and 20 m*M* tris (*p*H 7.5). Bovine serum albumin (BSA, 1 mg/ml) was included in the reaction mediums as given below. Figures obtained from experiments performed in duplicate were averaged and represent oxygen uptake (nmole/min) with the percent of the control in parentheses.

$PbCl_2$ (μmole/liter)	Respiration rate		
	Sucrose		KCl + BSA
	No BSA	BSA	
0	50 (100)	62 (100)	60 (100)
1.5	50 (100)	62 (100)	60 (100)
3	11 (22)	45 (73)	50 (83)
6	2 (4)	14 (23)	38 (63)
12.5	0 (0)	8 (13)	12 (20)
25	0 (0)	0 (0)	0 (0)
50	0 (0)	0 (0)	0 (0)

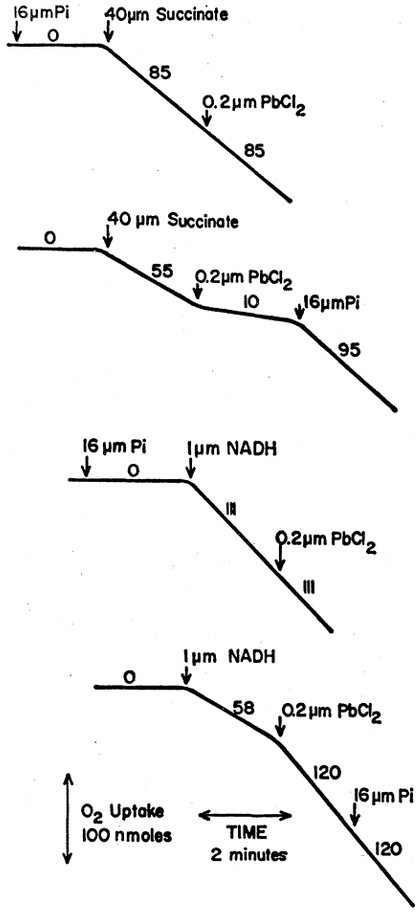

Fig. 1. Oxygen uptake by corn mitochondria (approximately 1 mg of protein) suspended in a 4-ml reaction vessel that contained 200 m*M* KCl, 20 m*M* tris (*p*H 7.5), and bovine serum albumin (BSA, 1 mg/ml). Additions made as indicated. Figures represent O_2 uptake (nmole/min) and were obtained from experiments performed in duplicate and averaged.

general series of reactions in electron transport, it is possible that the stimulation of oxidation of NADH by $PbCl_2$ as measured by oxygen uptake (and therefore involving the entire electron-transport chain) is at least partially associated with the first step of electron transport that is catalyzed by flavoprotein. For the same reasons the repression of oxidation of succinate may be the result of repression of activity of succinate dehydrogenase, a flavoprotein, by $PbCl_2$.

Although corn mitochondria typically contract (measured by changes in O.D.) when they respire in reaction medium that contains 200 m*M* KCl, those respiring in 62 μ*M* $PbCl_2$ showed no sign of contraction and might have been uncoupled. But, under respiratory control conditions lead did not reduce the respiratory control or ratios of adenosine diphosphate to oxygen atoms (ADP/O ratios) with either succinate or NADH as substrate. In experiments when inorganic phosphate (P_i) was present before the addition of lead, no inhibition of succinate oxidation or stimulation of NADH oxidation was observed (Fig. 1). When P_i was added after the lead-induced stimulation of NADH oxidation, no reduction in oxygen uptake was observed (Fig. 1). But addition of P_i, after the inhibition of succinate oxidation by lead, resulted in an oxygen uptake greater than that observed before the addition of lead (Fig. 1).

Large accumulations of lead in plants may influence growth if only a small amount of the lead is rendered soluble in a cytoplasmic solution that is deficient in P_i. Only 1 or 2 ppm of lead in the sucrose reaction mediums (without BSA) affected a decrease in the oxidation of succinate of greater than 75 percent, and about 5 ppm of lead caused nearly 100 percent inhibition of succinate oxidation when P_i was absent. Since succinate is a necessary link in Krebs cycle oxidations, the inhibition of one enzyme in the cycle could cause considerable inhibition of energy transfer to high-energy phosphate bonds, and, thus, of growth. Similarly, stimulation of oxidation of exogenous NADH by lead could have considerable influence on growth. The possibility of lead producing effects in vivo similar to those observed in our system is small if cellular P_i is present. This amount of P_i is likely sufficient to precipitate most of the lead as lead phosphate. It is also for this reason that we observed

Table 2. Rate of oxidation of exogenous NADH by corn mitochondria in relation to concentration of $PbCl_2$. After 4 minutes, 1 μmole of NADH was added to mitochondria (approximately 1 mg of protein) suspended in 4-ml reaction vessels that contained 300 m*M* sucrose and 20 m*M* tris (*p*H 7.5), or 200 m*M* KCl, 20 m*M* tris (*p*H 7.5), and bovine serum albumin (BSA, 1 mg/ml). Figures, obtained from experiments performed in duplicate, were averaged with the percentage of the control in parentheses.

$PbCl_2$ (μmole/liter)	O_2 uptake (nmole/min)
200 mM KCl	
0	54 (100)
6	69 (128)
20	75 (139)
31	116 (215)
62	148 (274)
125	150 (277)
250	108 (200)
500	106 (197)
750	81 (151)
1000	71 (131)
300 mM sucrose	
0	30 (100)
6	52 (173)
12.5	80 (267)
25	176 (620)
50	222 (740)
100	218 (727)
250	218 (727)
500	192 (640)

no lessening of respiratory control or ADP/O ratios in the presence of 50 μ*M* $PbCl_2$ (these experiments are of necessity carried out in the presence of 4 m*M* P_i). Under normal growth conditions (with available phosphate) there have been no reports, to our knowledge, that indicate a significant effect of lead on the growth of the whole plant, even though Tso and Fisenne (*6*) reported that lead taken up through tobacco roots is concentrated in the younger leaves, a region of high mitochondrial activity.

From this data of effects of lead on enzymatic reactions in corn mitochondria, we conclude that plants that contain high concentrations of lead are unlikely to be affected by these concentrations due to the precipitation of the lead if sufficient phosphate is present. It is significant that the effects of lead seen in this study were observed at very low concentrations and that there was a sharp increase in the magnitude of influence within a small range of concentration. When effective concentrations of lead are reached in our environment, there could be a quick and dramatic reduction in plant growth under conditions of phosphate deficiency.

DAVID E. KOEPPE
RAYMOND J. MILLER
Department of Agronomy, University of Illinois, Urbana 61801

References and Notes

1. M. Singer and L. Hanson, *Soil Sci. Soc. Amer. Proc.* **33**, 152 (1969).
2. H. Cannon and J. Bowles, *Science* **137**, 765 (1962).
3. R. Groyer, A. Kroll, J. Kimball, *Lab. Invest.* **19**, 78 (1968); L. Tice, *J. Histochem. Cytochem.* **17**, 85 (1969); H. Moses and A. Rosenthal, *ibid.* **16**, 530 (1968).
4. F. Hammett, *Protoplasma* **5**, 135 (1929).
5. D. Kenefick and J. Hanson, *Plant Physiol.* **41**, 1601 (1966).
6. T. Tso and I. Fisenne, *Radiat. Bot.* **8**, 457 (1968).

7. We thank J. B. Hanson for helpful suggestions and C. Pinkham for technical assistance. Supported in part by a grant from the Office of Saline Water, Department of the Interior (14-01-0001-2153).

19 September 1969; revised 5 December 1969

δ-Aminolevulinic Acid Synthetase: Induction in Embryonic Chick Liver by Glucagon

Abstract. *Glucagon elicits a twofold increase in δ-aminolevulinic acid synthetase activity in the livers of 18-day-old chick embryos. This rise occurs when RNA synthesis is inhibited, but is prevented when protein synthesis is blocked. Cyclic adenosine monophosphate appears not to be involved.*

The first step in heme biosynthesis is the condensation of glycine and succinyl coenzyme A to form δ-aminolevulinic acid (ALA). This reaction is mediated by ALA synthetase, the rate-limiting enzyme in the pathway (*1*). This enzyme can be induced in embryonic chick liver by barbiturates, collidines, steroids, and certain other substances (*2*).

Acute intermittent porphyria is an inborn error of metabolism. Biochemically it is characterized by an excessive production of ALA and porphobilinogen. The primary lesion is an elevation in hepatic ALA synthetase (*3*). A high carbohydrate diet leads to a reduction in the formation of porphyrin precursors in patients with acute intermittent porphyria (*4*). Moreover, the induction of ALA synthetase in rat liver is blocked by glucose (*5*). To further our understanding of the relationship between carbohydrate metabolism and the regulation of heme synthesis, I investigated the effect of glucagon on the activity of ALA synthetase in embryonic chick liver.

Fertilized White Rock eggs were obtained from a local hatchery and kept at 38°C in a forced draft incubator. The samples under investigation were glucagon, cycloheximide, adenosine 3′,5′-monophosphate (cyclic AMP), and N^6,$O^{2'}$-dibutyryladenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) (Sigma Chemical Co.), and dactinomycin (Merck Sharp and Dohme).

Each drug, dissolved in 0.5 ml of saline, was injected into the air space through a hole made in the shell of 18-day-old eggs. Glucagon was administered as a suspension. The hole was then closed with cellophane tape. Six hours later the ALA synthetase activity in individual livers was determined (*6*). The ALA synthetase activity in the liver of an 18-day-old chick embryo was around 8 nmole of ALA formed per gram of liver per hour and was relatively uniform from one individual to another (Table 1). The administration of glucagon resulted in an approximately twofold increase in the activity of the enzyme in 6 hours. The response to the hormone varied with only about half of the individuals showing an increase above normal. The increase induced by glucagon was not prevented by dactinomycin while dactinomycin alone was without effect. However, in the presence of cycloheximide this increase was inhibited.

To determine whether glucagon acts by way of cyclic AMP, cyclic AMP was injected into eggs and no effect was seen (Table 1). In the induction of tyrosine aminotransferase by cyclic AMP in fetal rat liver, more pronounced effects are achieved with its analog, dibutyryl cyclic AMP (*7*). However, dibutyryl cyclic AMP, when injected into eggs, was also ineffective in increasing the activity of ALA synthetase.

The inclusion of cyclic AMP in the assay medium at concentrations from 10^{-5} to $10^{-3}M$ had no effect on the activity. Similarly, glucagon ($10^{-7}M$) in the assay medium was without effect.

The induction of ALA synthetase in avian and mammalian liver by a variety of compounds led to a 10- to 40-fold increase in the activity of the enzyme (*5, 8, 9*), whereas the increase induced by glucagon was only twofold. However, this small increase is the result of protein synthesis as implied by its inhibition by cycloheximide.

The massive inductions of ALA synthetase in both chick and rat liver are subject to genetic regulatory mechanisms (*2, 9*). Because the increase induced by glucagon occurs in the presence of dactinomycin, an inhibitor of RNA synthesis, the regulatory mechanism is nongenetic.

Stimulation of glycogenolysis by glucagon is mediated by cyclic AMP (*10*). Glucagon activates adenyl cyclase, thus enhancing the production of cyclic AMP, which in turn activates phosphorylase. Because cyclic AMP has no effect on ALA synthetase activity when supplied to the embryo or when added to the homogenate, it is concluded that it is not involved in the induction.

The activity of ALA synthetase in fasted rats ranges from 8 to 24 nmole of ALA per gram per hour while it varies only from 4 to 15 nmole of ALA per gram per hour in fed rats (*6*). Fasting stimulates the release of glucagon by the pancreas. Thus the higher concentration of glucagon after fasting probably induces ALA synthetase in the rat. However, in their study of the induction of ALA synthetase in mature rat liver, Marver *et al.* (*1*) found that glucagon was not an inducer. The difference between their findings and mine may be developmental in origin. Greengard (*7*) has shown that glucagon is an inducer of tyrosine aminotransferase in fetal rat liver but that responsiveness to glucagon in the liver of the adult is lost. Alternatively, the lack of similarity between the observations could arise from a peculiarity of avian liver in view of the demonstration (*9*) that certain steroids are potent inducers of ALA synthetase in embryonic chick liver whereas they are ineffective in mammals.

Table 1. Effect of various compounds on ALA synthetase activity (nanomoles of ALA formed per gram per hour).

Compound	Embryos (No.)	Activity
None	6	8 ± 3
Glucagon (0.25 mg)	12	15 ± 10 ($P < .025$)*
Glucagon (0.25 mg) + dactinomycin (0.25 mg)	8	16 ± 7 ($P = .015$)*
Dactinomycin (0.25 mg)	8	6 ± 4
Glucagon (0.25 mg) + cycloheximide (5 μg)	8	5 ± 5
Cyclic AMP (0.5 mg)	8	6 ± 6
Dibutyryl cyclic AMP (0.5 mg)	4	10 ± 3

* Significantly different from untreated embryos with P calculated according to the Mann-Whitney test.