One might suppose that, if zinc does complex cyanide with a stability sufficient to interfere with this method of determining cyanide, absorbance would decrease as zinc content increases, falling to zero with the addition of an amount of zinc proportional in some manner to the amount of cyanide present. However, Fig. 1 shows that, as the zinc content is increased, the absorbance increases to a flat maximum, then rapidly falls to zero with addition of a specific amount of zinc which is independent of the amount of cyanide present. The increases in absorbance are neither constant nor proportional to the absorbance in the absence of zinc.

Since the cyanide solutions are basic and the zinc solutions are acidic, the effect of *p*H was briefly investigated. Solutions of NaOH and HCl were made up which matched the zinc and cyanide stock solutions with respect to *p*H. A colored cyanide solution is decolorized by HCl and recolored by NaOH. After the initial addition of HCl, a single drop of base or acid is sufficient to change the color, as one would expect at the end point in an acid-base titration in which phenolphthalein is used as the indicator.

On the other hand, a cyanide solution which has been decolorized with zinc can be recolored by adding cyanide, but more than three times the amount of cyanide originally present is required, and the second color is weaker than the first. This second color can then be discharged with about one-tenth the amount of zinc first required. Subsequent recolorization and decolorization requires an excess of cyanide and only a small amount of zinc.

It is thus apparent that the phenolphthalin method for determining cyanide is subject to interference by zinc. Often when an ion interferes with an analytical method, the method can be adapted as a means of analyzing for that ion. The shape of the curves in Fig. 1 indicates that this cannot be done in the present case. However, by standardizing cyanide in the presence of, say, 6 parts per million of zinc, one would be operating in the flat portions of the curves, and then one could determine cyanide in the presence of zinc without error, as long as the zinc concentration did not vary beyond the limits of 3 to 10 parts per million.

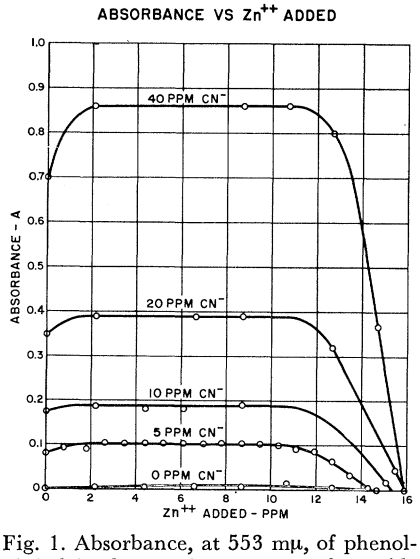

Fig. 1. Absorbance, at 553 mμ, of phenolphthalein due to the presence of cyanide versus amount of zinc added.

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Reactivation of Cytochrome Oxidase by Lipide

There are reports in the literature which indicate that lipides or lipide-soluble substances may be important for the normal activity of respiratory enzymes. For example, Nason and Lehman (*1*) have investigated the restoration by tocopherol of DPNH- and succinate-cytochrome *c* reductase activity of rat skeletal muscle following isooctane extraction and aging to reduce the activities. Martius and Nitz-Litzow (*2*) have proposed that vitamin K_1 is a necessary component of the DPNH-cytochrome *c* reductase system. They have based their hypothesis on (i) the inhibitory effect on oxidative phosphorylation of dicoumarols and related compounds, (ii) the reduced phosphorylation in mitochondria from the livers of vitamin K-deficient chicks (which is restored by vitamin K_1), and (iii) the identification of a DPN-dependent vitamin K_1 reductase in pig liver mitochondria.

More recently Crane, Hatefi, Lester, and Widmer (*3*) have reported the isolation from beef heart mitochondria of a quinone capable of undergoing reversible oxidation and reduction. This compound, with an absorption maximum at 275 mμ (designated as Q-275), will reactivate the succinoxidase system of heptane-extracted mitochondria, and in the reduced state can be oxidized by ferricytochrome *c* in the presence of the electron-transport particle. Previous reports that implicate a lipide in the cytochrome *c* oxidase portion of the chain include those that demonstrate an inactivating effect of lecithinases (*4*). More recently, Witter, Morrison, and Shepardson (*5*) have found that lysolecithin uncouples phosphorylation from oxidation of ascorbate-cytochrome *c* by the cytochrome oxidase contained in rat liver mitochondria and thus enhances oxygen uptake by about 100 percent.

In the course of attempting to purify the cytochrome oxidase of beef heart mitochondrial fragments (*6*) by extracting with surface active agents, we found that, after treatment of the particles on a cellulose column with deoxycholate (1.9 percent) and with cholate (4.0 percent), the cytochrome oxidase that was finally solubilized with 3 percent deoxycholate (S3) was relatively inactive. The addition of the 4 percent cholate extract (S2), however, reactivated some of the enzyme (Table 1, experiment 1). In another experiment the 1.6-percent deoxycholate extract (S1) proved to be an even better activator (Table 1, experiment 2) of a slightly active 2.5-percent deoxycholate extract (S3).

The activating substance, free of surface-active agents, proved to be heat stable and extractable by butanol but not by ethyl ether or petroleum ether. A number of lipides and lipide-soluble substances were tested for their capacity to activate the enzyme. The following compounds were ineffective: oleic acid, vitamin K_1, cholesterol, DL-α-tocopherol phosphate, choline, and phosphoryl choline. In Table 1, experiment 3, are presented those compounds which proved to be effective activators. There are several possibilities to be considered for the activating effect by these phospholipides:

Table 1. Reactivation of cytochrome oxidase. Cytochrome oxidase activity (*9*) is expressed as the first-order velocity constant.

Fraction	Activity ($\times 10^{-3}$ sec^{-1})
Experiment 1	
S3	0.47
S2	0
S3 + S2	1.34
Experiment 2	
S3	0.69
S1	0.75
S3 + S1	8.54
Experiment 3	
S3	0.93
S3 + animal lecithin (pract.)	4.85
S3 + animal lecithin (purified by chromatography)	1.60
S3 + vegetable lecithin	2.45
S3 + cephalin (impure)	2.80
S3 + dimyristoyl lecithin	2.88

(i) that they are actually involved in electron transport; (ii) that they are facilitating an optimal rearrangement of the reacting components; (iii) that they are removing a surface-active agent which has become attached to the enzyme and has inhibited it. The last possibility is rendered unlikely by the result that removal of deoxycholate with resin from an S3 supernatant does not lead to reactivation.

These results explain a discrepancy observed in our laboratory in 1950. When studying the copper content of cytochrome oxidase (*7*), we noted that there was poor correlation in many instances between the oxygen uptake catalyzed by a fraction and the height of the *alpha*-absorption peak in the reduced state. The ratio, O_2 uptake : 601-mμ absorption, decreased as succeeding fractions were made by extracting heart muscle particles with 1 percent deoxycholate. It may now be suggested that the third and fourth fractions were probably deficient in the lipide or in the lipide-soluble substance being discussed here (*8*).

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Production of Gibberellin-like Substances by Plant Tissue Cultures

One of the most discussed as well as one of the most investigated problems today in the fields of plant physiology and plant agriculture generally is that of the gibberellins and their action on plant growth (*1, 2*). That this group of fungal metabolic products has many very dramatic effects on plants has been amply demonstrated (*2, 3*). How these chemicals act and exactly how valuable they will be to agriculture are two questions which still remain unanswered.

Recently, investigators have reported gibberellin-like activity in extracts from several plants (*4–6*). Most of this type of investigation has been carried out by Phinney and his colleagues. In surveying numerous plants and plant parts, a surprising number of active extracts was found. This led Phinney to suggest that gibberellin-like compounds are widespread and might be universal in their occurrence among flowering plants (*6*). The greatest activity was found in the green seeds, young fruit, and endosperm of several plant species.

The loss of activity as seeds reach maturity, the location of active extracts in stem tips (*7*), and the general activity of immature tissues indicate that immature tissues—rapidly dividing meristematic areas—are the sites of synthesis of these gibberellin-like materials. If this is the case, plant-tissue cultures should be an excellent place to look for such activity. If it is found, this should lend much weight to the hypothesis of universal occurrence in the plant kingdom. Moreover, tissue-culture techniques should lend themselves to investigations of such problems as rate and site of synthesis, nutritional factors affecting synthesis, and control of synthesis.

This is a preliminary report of the results of our survey of plant tissue cultures as sources of gibberellin-like factors.

The tissues used in this study are well-established cultures which have been maintained in our laboratory for several years. The conditions under which they have been grown have been discussed in previous publications (*8*). Extractions were made, in each case, of 20 g (wet weight) of tissue about 4 weeks after its latest subculture. The method of extraction was patterned after that used by Phinney and his co-workers (*5*) and consisted of (i) extraction of diced tissue with acetone-water (1:1) on a shaker for 24 hours; (ii) filtration of the solvent, and (iii) reduction in volume over a steam bath to 5 ml. This 5-ml sample was sprayed with a deVilbiss atomizer on five dwarf pea plants (*9*) (1 ml per plant) which had been planted in sand 1 week previously. Growth of the plants was measured 3, 5, and 7 days after application, and the results were compared with measurements of control plants and of plants sprayed with standard gibberellin solutions.

Table 1. Plant tissue-culture extracts which have gibberellin-like effects on dwarf pea seedling growth. (LP) Nickell low-phosphate medium (*11*); (24) Burkholder and Nickell high-phosphate medium (*12*); (CM) coconut milk (18 percent by volume); (YE) Mead Johnson yeast extract (5 g/l); (2,4-D) 2,4-dichlorophenoxyacetic acid (0.6 part per million); (*p*Cl) *p*-chlorophenoxyacetic acid (0.6 part per million). Effects listed below are related to untreated plants (O) and gibberellin-treated (0.5 part per million) plants (++++).

Plant source	Common name	Plant part	Type tissue	Medium used	Relative effect
Vinca rosea	Periwinkle	Stem	Crown gall	LP	++
Helianthus annuus	Sunflower	Petiole	Crown gall	LP	+++
Melilotus officinalis	Sweet clover	Stem	Crown gall	LP-YE-*p*Cl	+
Melilotus officinalis	Sweet clover	Root	Virus tumor	24-YE-*p*Cl	+
Melilotus officinalis	Sweet clover	Stem	Virus tumor	24-YE-*p*Cl	+
Melilotus officinalis	Sweet clover	Root	Callus	LP	+
Agave toumeyana	Century plant	Leaf	Callus	White-2,4-D-CM	++
Ilex aquifolium	Holly	Stem	Callus	White-2,4-D-CM	+
Phaseolus vulgaris	Pinto bean	Cotyledon	Callus	White-2,4-D-CM	+

It should be noted that positive growth effects were obtained with extracts of both monocots and dicots, as well as with both leguminous and nonleguminous dicots. Activity is not limited by the type of tissue or by the plant part from which it originated, for stems, roots, leaves, and cotyledons are all represented, as well as nonpathological callus tissues and tissues of virus tumor and crown gall origin. This represents a wide distribution of plant material from many points of view: taxonomical, physiological, morphological, and pathological.

Responses of the magnitude obtained with the standard gibberellin solutions and with the plant extracts were not obtained with any other solutions of specific chemicals tested. These included several common auxins, purines, numerous antibiotics, organic acids, amino acids, and various types of chelating agents. Nor did the media on which the tissues were grown cause this response.

The production of substances which elicit a response in test plants similar to that elicited by the fungal metabolic products, the gibberellins, has been demonstrated for tissue cultures of higher plants—in many cases tissues which have been maintained for years on a synthetic medium. The wide variety of plants represented by these cultures lends strong support to the hypothesis that the