bility is small and the response to red radiant energy is very reproducible. The material is easy to handle and manipulate. The assay is reliable between 10^{-3} $\mu w/cm^2$ and 10 $\mu w/cm^2$ of red (625 to 700 mμ) and the photoresponse is directly proportional to the logarithm of the irradiance.

The technic consists of excision of the hook from 6-day-old seedlings of Black Valentine bean and exposure to red energy (625 to 700 mμ). The angle of hook opening is a quantitative measure of the photomorphogenic effect. The presence of bud, leaves or cotyledons, particularly the latter, causes an effect opposing the photoreaction, as does also 3-indoleacetic acid.

The photoresponse increases with age of the seedling up to seven days, but at this age the hook has progressed up the stem close to the cotyledonary node; therefore, 6-day-old seedlings were found most suitable. In complete darkness no opening could be observed for 16 to 24 hours following excision; with red energy, the lag period was 4 hours and the subsequent opening was at a markedly increased rate as compared to the dark. The maximum opening in the red occurred at 25° C; in the dark at 30° C.

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SOME FACTORS AFFECTING ABSORPTION AND TRANSLOCATION OF ZINC IN CITRUS PLANTS[1,2]

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Zinc deficiency occurs almost universally in citrus plants. In its more severe stages this disorder seriously reduces tree growth and fruit production. Soil treatments have not been altogether successful in solving this problem. Foliar sprays of zinc are commonly used in the form of oxide, sulfate, hydroxide, or carbonate, materials which are frequently incorporated in sprays applied for pest control purposes. Variability of results raises a number of questions concerning factors affecting absorption and translocation of zinc. The experiments reported here served to identify some of these factors and suggest procedures for additional experiments. The variables studied were the following: 1) placement of material on leaves, 2) leaf age, 3) concentration of zinc used, 4) leaf vs. root application, and 5) amount and chemical form of zinc applied to soil.

Materials and Methods

In these studies the radioactive isotope zinc-65 was used as a tracer in following the movement of zinc in citrus plants. At the time of shipment the solution contained 3.78 mg Zn per mc, in the form of $ZnCl_2$. In this report, the symbol Zn* is used to denote a mixture of Zn^{65} with the naturally occurring isotopes. Concentrations of Zn^{65} are expressed in terms of activity at the time of shipment. The experiments covered a span of approximately one half-life (250 days).

Elapsed time between treatment and harvest is indicated in the description of each experiment.

Three types of plants were used: 1) 3-year-old navel orange trees growing out-of-doors in solution cultures; 2) Koethen sweet orange seedlings, 15 to 25 cm tall, grown in the greenhouse in solution cultures or in pots of soil; 3) rooted cuttings of Eureka lemon, 15 to 25 cm tall, grown in the greenhouse in solution cultures.

Except where otherwise noted, nutrient solutions were maintained in the pH range 4.0 to 4.5. Their composition was the same as that reported for previous studies (3). All plants were adequately supplied with zinc prior to the time of treatment.

Preparation of Plant Material for Analysis: In a preliminary study it was found, in agreement with Smith et al (1), that a solution of Dreft detergent acidified to 0.3 *N* with HCl was more effective in removing zinc applied to the leaf surface than was Ivory soap or a 10 % solution of sodium ethylene diamine tetra-acetate (NaEDTA) adjusted to pH 5.

[1] Received March 13, 1956.
[2] Paper No. 903, University of California Citrus Experiment Station, Riverside, California.
[3] This work was done while the second author was in the United States as a Fellow of the Institute of International Education. Present address: Agricultural Research Station, Rehovot, Israel.

Acid-detergent solution was therefore adopted as a standard washing material in these experiments.

Ashing was done in an electric muffle furnace at 500° C. The ash was dissolved in dilute HCl, dried, taken up with a few drops of HCl, diluted to volume, and filtered.

ASSAY FOR RADIOACTIVITY: Autoradiograms were made by pressing dried plant materials against Eastman Industrial (Type K) x-ray film. Duration of exposures was from 12 to 15 days.

A glass-walled dipping counter was used for radioactivity counts. Time required for 3200 counts was recorded, and this value was referred to a similar count on a reference solution of Zn^{65} to correct for decay.

MEASUREMENT OF ZINC ABSORPTION: Absorption of zinc resulting from applications to leaf surfaces was measured in two ways, each with its uncertainty. 1) Leaves were washed with acidified detergent to remove surface residue; the zinc that remained was assumed to have been absorbed. This assumption is in error to the extent that zinc was retained by wax, cuticle, or cell walls, all unknown quantities. Furthermore, removal of part of the applied material made it difficult to measure the total amount applied. 2) Where single-drop applications on leaves were made, the surface residue was eliminated in some cases by removing, with a punch, a circular disc of 1 cm^2 area which included the spot to which zinc solution had been applied. Thus, only zinc which was translocated from the point of application was considered to have been absorbed. Although the method is in error by the amount of zinc that was absorbed but did not move beyond the 1 cm^2 area, it provides assurance that all zinc measured had been absorbed. This method also served to prevent the halo effect in auto-

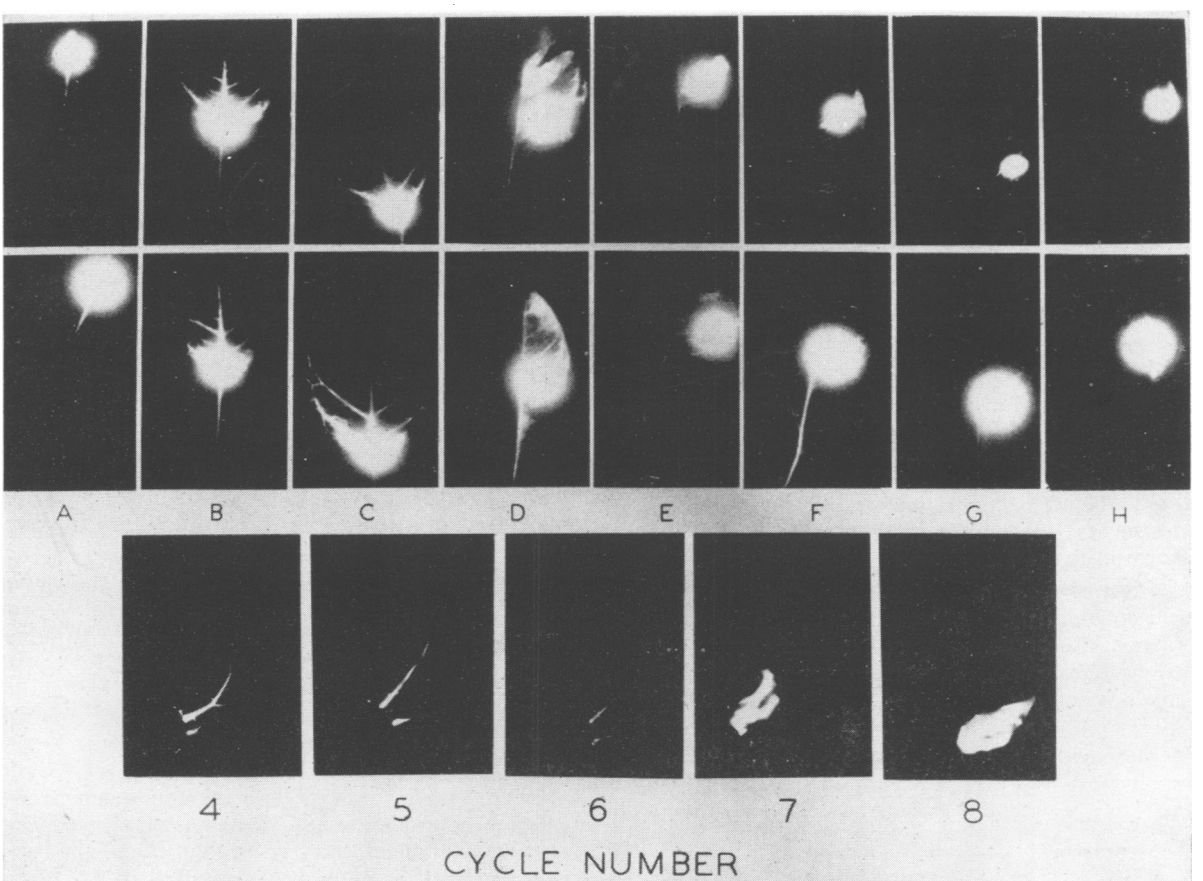

FIG. 1 (*above*). Autoradiograms of orange leaves to which drops of Zn* solution were applied at various locations on the upper surface: A, midrib near tip; B, midrib center; C, midrib near base; D, lateral vein; E, Mesophyll near tip; F, mesophyll center; G, mesophyll near base; H, near leaf margin (1/3 natural size). Leaves were harvested at 3 days. Those in top row were washed in acidified detergent; those in second row in a 10 % solution of Na_4EDTA (Expt 2).

FIG. 2 (*below*). Autoradiograms of lemon leaves in successive growth cycles,[4] after single-drop applications of Zn* (2/3 natural size). Leaves were harvested at 3 days. Halo was eliminated by removing part of leaf to which Zn* had been applied. Exposures of leaves from cycles 1 to 3 were blank, indicating no translocation from point of application (Expt 4).

radiograms that results from local high activity. Total activity applied can be closely estimated from the sum of counts on the leaf and on the disc that was removed from it.

Experimental Procedures and Results

Experiment 1: Several leaves of uniform age and size were selected for treatment on a single 3-year-old navel orange tree. Treatments consisted (a) of single-drop applications of Zn* solution (approx. 0.04 ml), and (b) of spreading Zn* solution over one lateral half of the leaf. Both types of treatment were tested on both upper and lower sides of the leaves. Each leaf received approximately 3 μgm of zinc having activity of 0.8 μc. The leaves were harvested 3 days after treatment. Amounts of Zn* absorbed were calculated from the counts.

Results, which are summarized in table I, indicate that the zinc was absorbed equally well through the upper and lower surfaces of the leaves. This opposes the hypothesis that zinc enters primarily through the stomata, which occur on the lower side only. Turrell (2) has already suggested that aqueous solutions cannot penetrate citrus leaves by way of the stomata, and has discussed the possibility of a pathway through wax canals in the cuticle.

The data also show that absorption was less effective when the applied zinc was spread over a considerable area of leaf surface. This suggests that penetration and movement of zinc in leaves is a function of the concentration applied per unit area.

Experiment 2: Single-drop applications of Zn* solution were made at various positions on the upper surfaces of leaves. Duplicate leaves were used for each treatment but they were washed by different methods, one with acid detergent and the other with NaEDTA. The leaves were harvested 3 days after treatment. The autoradiograms of the leaves are shown in figure 1.

Most of the autoradiograms of leaves washed with acid detergent have a smaller halo than those washed with chelating agent; this in in agreement with previous tests on efficacy of washing procedure.

Table I

Zinc Absorbed through Leaf Surfaces (Expt 1)

Method of application	Zn* remaining after acid-detergent wash		
	Leaf surface treated		
	Upper	Lower	Av.
	μgm	μgm	μgm
Single drop	1.77	1.60	1.69
Spread over leaf surface	0.39	0.18	0.28

Results of F test: Upper vs lower surfaces—not significant. Drop application vs spreading—Significant at 1 % level.

Table II

Distribution of Zn* in Orange Plants at Two Periods after Application of Zn^*Cl_2 Solution to Young and Mature Leaves of Equal Sizes (Expt 3)

Age of treated leaf	Days between treatment and harvest	Zn* in plant parts at harvest †					
		Leaves		Stem		Roots	Treated leaf
		Young	Old	Young	Old		
		ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Young	5	0.007	0.001	0.002	0.02	0.02	24
	54	0.1	0.04	0.06	0.04	0.03	18
Old	5	0.00	0.004	0.00	0.02	0.009	47
	54	0.03	0.02	0.04	0.05	0.04	28

† Each figure is the mean value for 4 plants. Calculated from counts following application of known specific activities.

The autoradiograms also show that the amount of absorption and translocation is related to the locus of application. Maximum translocation of zinc occurred when it was applied near the middle of the leaf. Least translocation resulted from application at the leaf margin.

Experiment 3: Sixteen Koethen orange seedlings growing in solution cultures were selected for uniformity. One leaf on each plant was dipped into a solution of Zn^*Cl_2 (having activity of 20 μc/ml) to which Vatsol spreader had been added at the rate of 0.02 %. All the treated leaves were of similar size, but half of them were young leaves and the other half were mature (old) leaves. Half of the plants in each group were harvested at 5 days and the remaining plants at 54 days. These samples were not washed. From the activity counts the amounts of zinc applied and translocated were calculated. Results are given in table II.

It is apparent that less zinc adhered to the young leaves than to the old leaves but that a higher percentage of it was absorbed and translocated to other parts of the plant from the young leaves (17 %) than from the old leaves (10 %). Initial translocation (5 days) from mature leaves was predominantly toward older leaves, whereas translocation from young leaves resulted in distribution throughout the plant. Over a longer period, however, the distribution was about the same in both cases and showed a strong tendency for accumulation of the zinc in young leaves. Substantial quantities were also transferred to the roots.

Experiment 4: One leaf in each growth cycle [4] on a rooted cutting of Eureka lemon received a single-drop application of Zn^*Cl_2 solution at 38 μgm Zn and

[4] Citrus trees in the field commonly complete 2 or 3 cycles of shoot elongation in a year; under greenhouse conditions as many as 6 or 7 cycles may occur. The term "growth cycle" is a colloquialism which refers to the portion of a shoot produced during one cycle. It is used here for want of a better term.

10 μc per ml. In this case the size of the drops was reduced to approximately 0.01 ml in order to reduce the tendency toward spreading or running. It was applied to the mesophyll about midway between the midrib and margin. Three days later the leaves were harvested, and the treated areas removed with a punch.

The autoradiograms (fig 2) show a clear tendency for greater spread of zinc into the mesophyll when applied to younger leaves.

EXPERIMENT 5: To compare distribution of zinc applied to leaves with that applied to roots, four 2-liter solution cultures were set up, each with 3 Koethen orange seedlings. To each of two of the cultures, 0.1 mc of Zn^*Cl_2 was added to the solution. Plants in the other two cultures received leaf applications as follows: five leaves about midway between root and tip of each plant were painted on the upper surface with a solution of Zn^*Cl_2, each leaf receiving about 0.1 ml of a solution containing 20 μc/ml. Plants were harvested 5, 10, and 119 days after treatment. Table III gives the amounts of zinc translocated, based on radioactivity found in the various plant parts. Figure 3 shows the distribution of radioactive zinc in leaves of various ages, following 10-day absorption of zinc through the roots.

In spite of wide differences in behavior among individual plants, two observations seem noteworthy, based on data in table III: 1) the distribution of Zn* in the plant tops after 119 days was about the same whether it was absorbed by leaves or by roots; 2) the rate of absorption and translocation was relatively constant.

TABLE III

DISTRIBUTION OF Zn* IN ORANGE PLANTS AT VARIOUS PERIODS AFTER APPLICATION TO LEAVES AND ROOTS (EXPT 5)

DUPLICATE PLANTS	MICROGRAMS Zn* IN PLANT PARTS †					
	DAYS AFTER APPLICATION TO LEAVES			DAYS AFTER APPLICATION TO ROOTS		
	5	10	119	5	10	119
Leaves (not including treated leaves)						
1	0.00	0.08	1.2	1.1	1.5	44
2	0.05	0.02	0.9	0.05	2.2	32
Stems						
1	0.07	0.11	0.8	1.2	4.4	16
2	0.09	0.08	0.6	0.4	3.8	19
Roots						
1	††	††	1.5	††	††	††
2	††	††	1.2	††	††	††
Daily average absorption by aerial portions of plants						
1	0.04	0.02	0.02	0.5	0.6	0.5
2	0.03	0.01	0.01	0.09	0.6	0.4

† Calculated from counts following application of known specific activities.

†† Not determined.

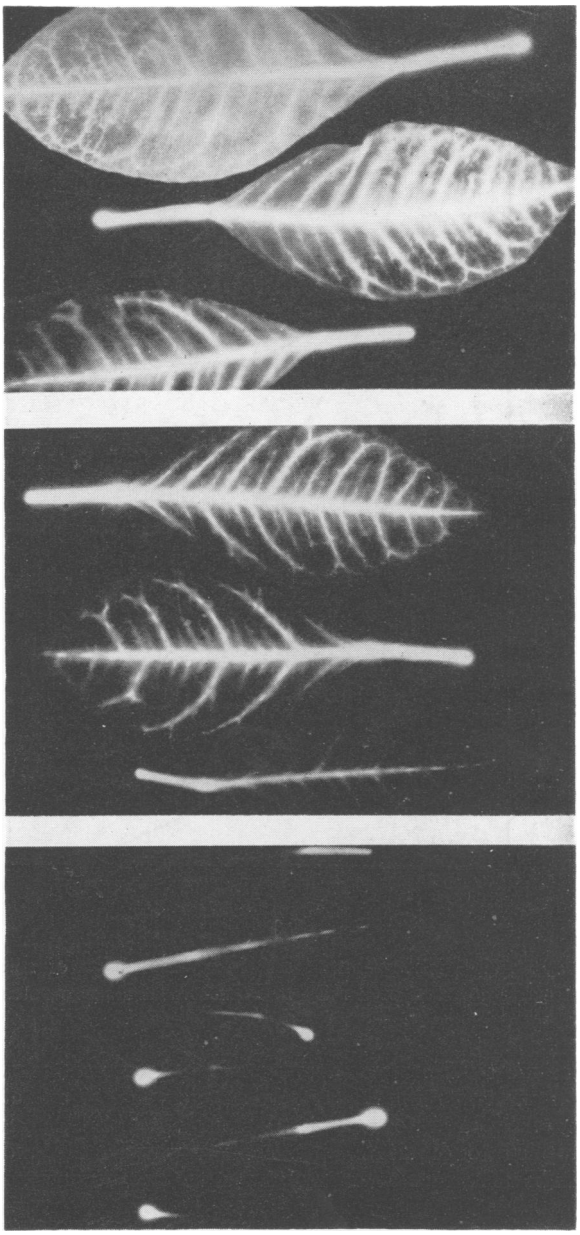

FIG. 3. Autoradiograms of orange leaves showing distribution of Zn* following absorption for 10 days from nutrient solution (2/3 natural size). Leaves shown are from 3 age groups with the youngest at the top (Expt 5).

EXPERIMENT 6: As a preliminary test of availability of zinc applied to soil, two zinc concentrations were compared in the forms of chloride and chelate (EDTA). The soil was a potting mixture made up of peat moss and a residual hillside soil of granite origin. This mixture was put into twelve 3-gallon glazed earthenware pots, in which Koethen orange seedlings were planted on October 21, 1953, one plant per pot. They were kept in the greenhouse thereafter. On March 5, 1954, eight of the pots containing

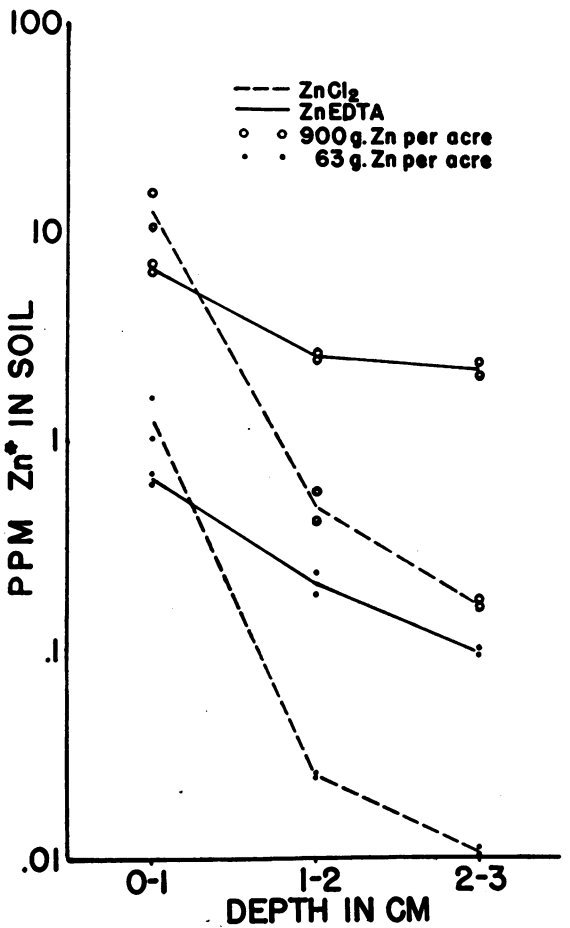

FIG. 4. Distribution of Zn* in surface 3 cm of soil after application of Zn^*Cl_2 and Zn*EDTA at two rates (Expt 6).

the most uniform plants were selected for treatment. All materials were added in solution in 200 ml of water applied to each pot. Four treatments, each applied to duplicate pots, consisted of Zn^*Cl_2 and Zn*EDTA, each at rates of 0.7 and 10 mg of zinc per pot. Activity in each case was the same, approximately 0.2 mc.

After 10 days all plants were harvested by cutting the stems at the soil surface. Leaves were washed, dried, and autoradiographed as previously described. Weak autoradiograms were obtained from plants that had received Zn*EDTA, but none from those receiving Zn^*Cl_2. Activity was observed principally in the petioles and midribs, except in the case of the youngest leaves, which had relatively uniform distribution of the applied zinc.

Soil samples representing depth increments of 1 cm were taken by boring with a piece of aluminum tubing (12 mm diameter). Each sample was a composite of four borings per pot. Samples were placed in tared 50-ml beakers and allowed to air-dry; they were then weighed and extracted overnight with 25 ml of normal HCl. Activities in the filtrates, calculated to parts per million of zinc, are plotted in figure 4.

Of the two zinc compounds applied, the chelated form moved more freely in the soil and probably more of it reached the root zone. This probably explains the presence of measurable activity in the plants treated with Zn*EDTA as contrasted with those treated with Zn^*Cl_2.

DISCUSSION

The experiments that have been described indicate some variables that must be controlled in studies of zinc absorption by citrus plants. Zinc may be absorbed and translocated more readily when applied 1) to young leaves than when applied to older ones; 2) at higher concentrations of zinc per unit area of leaf; 3) near the center of the leaf.

When solutions of zinc salts were applied to leaf surfaces, strong fixation occurred. Attempts to remove the untranslocated portion of zinc by various washing procedures were not completely successful. For this reason the technique of applying the zinc solution in single drops and then removing the treated area with a punch appears to have considerable merit. It should be useful in studies of the influence of environmental factors on zinc absorption. Most consistent results occur when the drops are kept small enough to avoid spreading and thereby maintain a uniform concentration of zinc per unit area in all treatments.

Since no differences in absorption and translocation were observed when comparing upper and lower leaf surfaces, it seems clear that absorption through stomata is unimportant if it occurs at all.

In the present experiments the ultimate distribution of applied zinc in the plant appeared to be independent of whether it was absorbed by the roots or by the leaves. It should be emphasized, however, that quite different results might be obtained if zinc-deficient plants were used.

The effect of chelation of zinc applied in solution to the soil, as shown in figure 4, is of considerable interest. These data for the surface 3 cm of soil suggest that the chelated form is less rapidly adsorbed by the soil than are the inorganic salts, and hence may be useful as a soil amendment for treating zinc deficiency.

SUMMARY

The radioactive isotope Zn^{65} was used in experiments for studying variables that affect absorption and translocation of zinc in citrus trees, particularly as a result of foliar application. It was found that absorption and translocation of zinc 1) occurred more rapidly in young leaves than in old leaves, 2) varied with concentration of zinc per unit area of leaf, and 3) was more rapid when zinc was applied near the center of the leaf than when applied near the margin. The fact that no differences in absorption and translocation were observed when comparing upper and lower surfaces of leaves leads to the conclusion that absorption through stomata is unimportant, if it occurs at all.

The single-drop technique that was used for applying solutions to leaf surfaces is a useful experimental method.

Ultimate distribution of applied zinc in the plant was found to be independent of whether it was absorbed by the roots or by the leaves, though this might not be the case in zinc-deficient plants.

An experiment to test movements of zinc through soil indicated better penetration of the chelated form than of the inorganic salts.

We are indebted to Professor H. D. Chapman for his counsel and support throughout the planning and conduct of this work.

The Zn^{65} which we used was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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THE METABOLISM OF GLUCOSE BY THE ALGA OCHROMONAS MALHAMENSIS[1,2]

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It has been demonstrated by many investigators that the growth of algae under autotrophic conditions generally is not increased in the presence of potential organic metabolites (12). Recently, however, Pringsheim (17) isolated a golden brown alga, *Ochromonas malhamensis*, which is an exception to this general rule, i.e., growth of this organism under optimal autotrophic conditions could be increased by the addition of a carbon source. These observations have stimulated further investigations to uncover the cause of this anomaly.

An investigation of the photosynthetic mechanism of Ochromonas showed that the chromatophores contained less chlorophyll a (13, 20) than other algae. Furthermore, it was noted that the rate of photosynthesis of Ochromonas (13) was much less than that of Chlorella and Euglena. From these observations it was postulated (13) that due to a low content of chlorophyll, Ochromonas could not reduce sufficient CO_2 by photosynthesis to supply its requirements for growth. For this reason, in order to obtain maximum growth, an organic substrate had to be added. Since Ochromonas[3] has been shown capable of photo-oxidizing isopropyl alcohol to acetone, the photosynthetic mechanism of Ochromonas may be more closely related to the system of purple bacteria than that of the other algae studied. Therefore, chlorophyll content may not be the only factor that causes the photo-organotrophic properties of Ochromonas.

The present work is a study of the glucose metabolism of Ochromonas in the dark under anaerobic and aerobic conditions and shows that the Embden-Meyerhof-Parnas pathway is the major route by which Ochromonas dissimilates glucose.

[1] Received March 20, 1956.

[2] This research was carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

[3] Vishniac, unpublished data.

Materials and Methods

Bacteria-free stocks of Ochromonas were cultured for one week in the light, using Hutner's (11) growth medium. The light was supplied by daylight-type fluorescent lamps at an intensity of 750 fc. The cells were transferred into 150-ml Florence flasks containing 30 ml of medium. The composition of this medium is shown in table I. The cells were grown on a New Brunswick shaker at 25° C. No special arrangements were used to illuminate the cells. Cells grown for one day were centrifuged from the growth medium with a force of $150 \times g$, washed and resuspended in 0.01 M potassium phosphate buffer, pH 5.

Five ml of suspension, which contained approximately 25 mg dry weight of cells, were added to the main compartment of a double side arm 50-ml Warburg vessel. One side arm contained the substrate and the other contained 0.1 ml of $4N$ H_2SO_4. In

Table I

Growth Medium for Ochromonas

Material	Gms/l
KH_2PO_4	0.3
$MgSO_4 \cdot 7\,H_2O$	0.6
$CaCl_2$	0.17
$(NH_4)_2SO_4$	2.5
Ethylenediamine tetraacetic acid	0.5
$(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$	0.020
Thiamine	0.002
Glucose	20
Biotin	4×10^{-6}
Vitamin B_{12}	5×10^{-6}
Trace metal mix *	12 ml

* Trace metal mix had the following composition (per liter): ethylenediamine tetraacetic acd, 0.5 gm; $MnSO_4 \cdot H_2O$, 6.15 gm; $ZnSO_4 \cdot 7\,H_2O$, 11.0 gm; $FeSO_4(NH_4)_2SO_4 \cdot 6\,H_2O$, 1.75 gm; $CoSO_4 \cdot 7\,H_2O$, 0.286 gm; $CuSO_4 \cdot 5\,H_2O$, 0.039 gm; H_3BO_3, 0.0571 gm; KI, 0.001 gm.