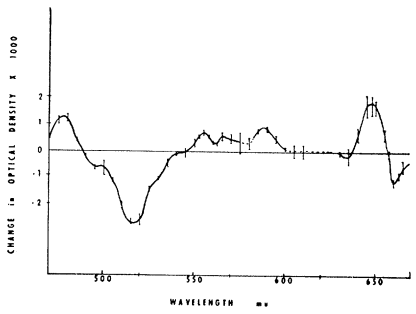

Fig. 2. Absorption spectrum changes in *Chlorella* produced by prior illumination. Spectrum measured with a difference spectrophotometer about 1.5 sec after illumination of 30-sec duration.

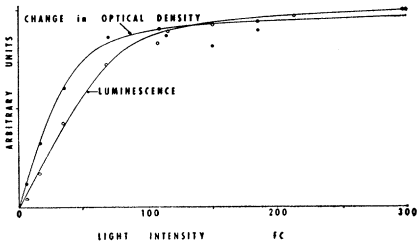

Fig. 3. Luminescence and 525-mμ absorption measured concurrently as a function of illumination intensity. Optical density and luminescence measured 1.5 sec after illumination for about 0.1 sec in a flow system.

to be reported fully elsewhere strongly suggest that chlorophyll itself participates directly both in the photochemical and later steps in photosynthesis as a chemical reagent rather than simply as a light-trapping agent (*10*).

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10. This work was supported in part by a grant from the U.S. Atomic Energy Commission. Thanks are due to L. R. Kruger for expert assistance in the construction of the apparatus, and to C. S. French and J. H. C. Smith for many helpful suggestions and discussions of the experimental techniques and results.

8 November 1955

Carbon Dioxide Sorption by Yeast

The sorption of carbon dioxide by some relatively dry food products, such as milk powder (*1*) and the nut meats (*2*) has been reported. A similar phenomenon occurs during the exposure of granular, dry yeast. We have noted that the uptake of carbon dioxide is equally as great with fresh preparations of active dry yeast as it is with yeast in which fermentative activity has been destroyed by heat treatment.

When dry yeast is sealed into an ordinary can in an atmosphere of carbon dioxide, the degree of sorption is high enough to cause deformation and collapse of the can. Pressure measurements in a comparable closed system using relatively large amounts of yeast in proportion to the volume of carbon dioxide have shown a minimum sorption capacity of about 0.3 ml/g of yeast after 5 days of contact. Indications have been obtained, however, that this value may increase considerably in a system in which the sorption mechanism is not required to operate against a self-induced reduced pressure.

For the study reviewed in this report, measurements were made in a Warburg microrespirometer in which the reduced pressure could be equalized by venting the apparatus. These experiments demonstrated a continuous but progressively decreasing rate of uptake of carbon dioxide by dry yeast during a period of 10 days. At this time the volume of carbon dioxide that had been taken up by 0.5 g of yeast in an 18-ml test system was at least 3.0 ml.

The afore-mentioned experiment was repeated with radioactive carbon dioxide (*3*) in an attempt to determine the nature of the sorption process. Fractionation of the yeast after prolonged exposure to the labeled carbon dioxide was attempted to detect the yeast components responsible for the uptake of the gas.

This approach failed in its original purpose. It was found that in each of the two trials made, the degree of radioactivity of the yeast after removal from the test system was very much lower than would be expected on the basis of the measurements of uptake volume. Less than 1 percent of the expected radioactivity was found in the yeast following a 15-minute air sweeping that was given the yeast immediately preceding the first count-rate determination. Apparently this flushing of the excess carbon dioxide also causes an extremely rapid loss of sorbed gas from the yeast. Failure to remove the interstitial carbon dioxide would yield count rates that would obviously not be a measure of the sorbed gas.

Table 1. Loss of labeled carbon dioxide from dry yeast on exposure to ordinary atmosphere.

Time after first count-rate determination (hr)	Proportion of original activity remaining in yeast (%)
1	85
2	76
3	69
4	62
5	58
24	48
72	6
96	3
240	2

The further observation that the radioactivity of the yeast after removal from the test system was decreasing rapidly precluded consideration of fractionation of the yeast. As shown in Table 1, the steady diffusion of labeled carbon dioxide from the yeast on exposure to the ordinary atmosphere suggests that carbon dioxide forms no stable addition or reaction complex with any constituent of yeast.

These findings suggest that only a transient association exists between the yeast and the carbon dioxide. The extent of this association is governed by the relative proportion of yeast to gas in the test system and by the time of contact or exposure.

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22 September 1955

Pseudotuberculosis in Experimental Animals

In our experience 3 years ago, about 2 percent of the mice purchased from a commercial breeder succumbed to pseudotuberculosis (*1*) while in quarantine prior to use. *Corynebacterium pseudotuberculosis murium* was readily isolated from the lesions. When subjected to sublethal total body x-radiation (350 r) 65 to 75 percent of the animals with latent infection died with active pseudotuberculosis (*2*). Recently it has been found that

(latent?) pseudotuberculosis was present in mice obtained from another institution that supplies animals to many laboratories throughout the country. "Activation" of this infection by radiation and other stressing agents (*3*) may invalidate experimental results. It is, therefore, advisable to reemphasize the importance of post-mortem examination of animals that die without any apparent cause, as well as of systematic examination of all dead experimental animals. In our experience, latent pseudotuberculosis in a resident animal colony presents a very serious problem. Eradication of infection in such a herd is a difficult task with uncertain results.

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28 December 1955

Artemia salina as a Test Organism for Bioassay

Bioassay methods for the detection of insecticide residues have a strong appeal because of the broad range of compounds to which they can be applied and because of their high sensitivity. Many bioassay methods have been devised using numerous test organisms (*1–5*). These procedures, with the exception of one or two (*6*), are based on the same fundamental technique.

For most of these bioassay methods it is necessary to maintain a culture of the test organism at all times. Many difficulties are encountered in breeding and rearing these organisms in the laboratory. In an effort to resolve this problem, we instituted a screening of test organisms. One organism, *Artemia salina,* a crustacean, commonly known as the brine shrimp, was superior to all others for this purpose. It not only demonstrated high sensitivity against a broad range of compounds, but it has one other outstanding characteristic—the eggs will remain viable for several years when they are stored in a dry condition. The necessity for maintaining cultures of the organism is eliminated; hatching can be obtained in less than 24 hours. *Artemia salina* has been the subject of physiological studies by zoologists for many years (*7*). More popularly, this organism has found wide use as food for tropical fish, and the dry eggs can be obtained readily at most tropical fish stores under the name "brine shrimp eggs."

Table 1. Composition of the salt solution used for hatching and rearing *Artemia* (in 1000 ml of distilled water and adjusted to a *p*H of 10.0 with sodium hydroxide).

Compound	Wt. (g)
Sodium chloride	30
Calcium sulfate	2
Magnesium sulfate	3
Magnesium chloride	8.5
Potassium chloride	0.8
Magnesium bromide	0.1

The eggs used in these experiments were hatched in a shallow glass tray approximately 16 in. long by 12 in. wide. A wooden divider extending across the tray and a short distance down into the solution was placed about 5 in. from one end to prevent the eggs that floated on the solution on the narrow side from drifting to the other side. The newly hatched nauplii swam under the divider and were drawn to the end of the tray by positive phototaxis. Here they were readily collected, free of eggs, by siphoning. The composition of the salt solution used for hatching and rearing *Artemia* is shown in Table 1.

Ordinary yeast made an excellent and convenient food. The yeast was suspended in water, and a few drops were floated on the salt solution. It was added in small quantities every second day. The optimum temperature for *Artemia* was found to be about 30°C. These organisms were reared in concentrations of salt ranging from 4 percent to near saturation. They required at least 3 weeks to reach the adult stage.

When *Artemia salina* was used as a test organism, a sensitivity to certain insecticides of 0.01 parts per million was demonstrated. Acetone solutions of insecticides were suspended in various concentrations in water or in salt solution of the same composition as the hatching or rearing salt solution. Toxicity was measured by placing the brine shrimp directly in the suspension of the test chemical. All age groups of *Artemia* from 24-hour-old nauplii to adults were tested and showed sensitivity to the presence of insecticides in microamounts.

A number of tests were made using adult brine shrimp as test organisms by placing them in measured amounts of water suspensions of insecticides in medicine droppers having an inside diameter of 5 mm.

Table 2. Time required to obtain fall of adult *Artemia* in varying concentrations of insecticides.

Insecticide	Concn.		
	1 ppm	0.1 ppm (min)	0.01 ppm
Chlordane	60–120 min	120–135	120–180 min
Methoxychlor	45– 60 min	45– 60	45– 60 min
Lindane	45– 60 min	60–120	60–120 min
Toxaphene	45– 60 min	90–120	18 hr
DDT	60 min	60	60–120 min
Acetone control (1:100)	24– 48 hr		
H_2O control	26– 50 hr		

The shrimp move freely in the columns of liquid and usually take a position at the top air-liquid interface. When the toxic material affects a shrimp, its swimming movements are rapidly curtailed, so that it is unable to remain at the top of the column and sinks to the bottom. Use of this fall as a criterion for the test yielded more rapid results than a mortality test. With the insecticides tested and concentrations used, readings were usually obtained in 45 minutes to 2 hours. Survival of the controls was in excess of 8 hours. Results obtained with lindane, methoxychlor, chlordane, DDT, and toxaphene are shown in Table 2.

Artemia salina exhibits a pronounced phototaxis, which, in view of the work of certain investigators (*6*), might also be used as a physiological criterion for a bioassay test. Initial tests using this characteristic have been promising.

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13 September 1955

Correction

One of the series of "Yale natural radiocarbon measurements, II" [*Science* 122, 954 (1955)], by Richard S. Preston, Elaine Person, and E. S. Deevey, was incorrectly reported through a technical error. The date for sample Y-293A (South Haven, Mich.: peat) was 10,790 ± 200 yr, not 9500 ± 250 yr as reported on page 958.

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16 February 1956