TABLE 1

CONTROL GROUP OF 20 IRRADIATED RATS*

No. of days of survival	11	12	13	14	15	16	17	18	19	20	21	22	23
No. of rats that succumbed	1	2	2	1	3	0	3	2	1	0	0	0	1

* These rats did not receive vitamin P.

sess very similar antihemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones'' (*3*, *4*).

In our investigation, 50 rats of British brown breed (obtained from Francis Carter Wood of St. Lukes Hospital, New York City) were submitted to x-ray irradiation. One group of 20 rats served as control, and a second group of rats was given vitamin P compound (CVP compound) isolated from citrus waste.[3] The average weight of the rats was 180 g, ranging from 160 to 205 g. The rats were kept on regular Purina Rat Ration. The radiation factors were 250 kv, 15 ma, with 0.5-mm Cu and 3.0-mm Bakelite filters. Target distance was 27.5 cm, and 210 r/min was the dose rate. All rats received 800 r total-body radiation in a single exposure.

Sixteen rats of the control group (80%) succumbed during the second and third weeks after the exposure (Table 1). All of them manifested gross hemorrhages of various gravity and pronounced pathological lesions in the adrenal glands. The zona fasciculata and zona reticularis were particularly affected, with argentaffin fibrils showing signs of degeneration. Four rats (20%) survived in spite of numerous petechial hemorrhages and generalized purpura.

TABLE 2

IRRADIATED RATS GIVEN 40 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24
No. of rats that succumbed	1	0	0	0	1	1	1

The treated animals were divided into two groups. Ten rats received orally 4 mg of vitamin P compound per day for 10 days, 3 days prior to radiation and 7 days after radiation. Twenty rats received 5 mg of vitamin P per day for 30 days, 7 days prior to radiation and 23 days after radiation.

In the group of animals (Table 2) which received a total amount of 40 mg of vitamin P compound, the mortality from irradiation was reduced to 40%. Moreover, those rats which did not succumb to the injurious effect of radiation lived longer. The petechial hemorrhages in the treated animals were considerably less pronounced, but some pathological changes in the adrenal cortex were observed, mostly in the zona reticularis (vacuolization).

In the group given a total of 150 mg of vitamin P compound in a period of 30 days, mortality from irridiation was reduced to 10% (Table 3). In this group, petechial hemorrhages were very slight and in some rats apparently absent.

[3] This compound, containing four identified factors naturally present in citrus fruit, was obtained from Vitamerican Company, Paterson, New Jersey.

TABLE 3

IRRADIATED RATS GIVEN 150 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24	25
No. of rats that succumbed	1	0	0	0	0	0	0	1

From these observations it appears that the vitamin P compound, which contains four flavonoids naturally present in citrus fruit, gives considerable protection to rats against a total-body, near-lethal dose of radiation.

In our previous publication (*5*), we stressed the importance of making a clear distinction between increased capillary permeability and capillary fragility. In radiation injury, there seems to be present a pronounced increase in capillary fragility which might be prevented by large doses of flavonoids naturally present in citrus fruit.

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Likelihood of Photorespiration or Light-inhibited Respiration in Green Plants[1]

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Generally speaking, there are two ways in which light could stimulate respiration: either directly by photosensitization or indirectly through photosynthesis, thereby increasing the amount of respirable metabolites. It is the direct effect of light on respiration (photorespiration), commencing upon exposure to light and stopping immediately upon the return to darkness, that has been a source of disturbance to those interested in measuring photosynthesis, as such a process escapes measurement. Whenever light has been reported to stimulate respiration, the stimulation has been of the persistent or indirect type and could be explained either on the basis of an accumulation of photosynthates, or on the basis of light absorption by the carotenoids, in which case respiration has been found to increase slowly in the light and persist for a short time in the dark. The possibility also

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exists that light inhibits respiration. It would follow that photosynthetic determinations above and also below the compensation point, where oxygen evolution is measured as a decrease in respiration, would become of doubtful significance.

McAlister (*8*) concluded from his experiments with wheat seedlings that light does not have a direct effect on respiration, since the rate of respiration after a period of illumination was equal to that before illumination and was independent of light intensity. Gaffron (*6*) arrived at a similar conclusion from an experiment in which cyanide inhibited the respiration of *Scenedesmus* by approximately 96% but had no effect on the calculated "true photosynthesis." Such a result can also be interpreted on the basis that cyanide does not affect photorespiration. The fact that Emerson and Lewis (*4*) did not find an effect of temperature on the calculated quantum yield also suggests that light does not have a direct effect on respiration. More recently, Burk *et al.* (*2*) and Warburg *et al.* (*10*) came to a similar conclusion. Kok (*7*) concluded, from experiments in which oxygen evolution was measured in the dark and in light of increasing intensity, that respiration is inhibited at high light intensities.

All such experiments, however, are of an indirect nature and have, therefore, the limitations inherent in indirect experimentation. Although results obtained thereby are frequently correct, they should, if at all possible, be tested with a direct method. Heretofore, the separation of photosynthesis from respiration in light was only possible by the use of poisons, which in many instances are undesirable because of their known or unknown effects on processes other than those to be specifically inhibited. A technique is now available by which these difficulties can be eliminated. In artificially induced mutant strains which are unable to photosynthesize or evolve oxygen in light, but yet are green, respire, and grow when supplied with a suitable carbon source, photosynthesis is completely divorced from respiration, and the problem of whether or not light directly affects respiration is rendered amenable to direct experimentation. Such mutant strains of *Chlorella* have been obtained as the result of ultraviolet irradiation and have been the object of photosynthetic studies (*3*). Strains 322 and 349 which possess these characteristics afforded an excellent opportunity for the study of the influence of light on respiration. If a process such as photorespiration, or light-inhibited respiration, exists, it would be extremely likely that it could be demonstrated by determining the respiration rates of these strains in the light and in the dark.

Oxygen-uptake measurements were made with the cells suspended in 0.1M Emerson and Lewis carbonate bicarbonate buffer solution (*5*), as these experiments were performed in conjunction with oxygen liberation studies. The cells were grown on solid stock medium slants which contained the following, per liter of nutrient solution: KNO_3, 1.21 g; $MgSO_4$, 1.20 g; KH_2PO_4, 1.22 g; 1,000 × Pratt's iron solution (*9*), 1 ml; Arnon's A4 trace element solution (*1*), 1 ml; tryptone, 4.5 g; dextrose, 10 g; yeast extract, 0.36 g; agar, 10 g; distilled water, to make 1 liter. Cultures were grown under continuous light for 11 days, after which the cells were harvested, washed in distilled water, and suspended in 0.1M carbonate bicarbonate buffer solution. Two-milliliter portions of the suspensions were added to the main chambers of Warburg reaction vessels. The vessels were attached to manometers, immersed in a constant temperature water bath (27° C), and shaken. After 15 min for equilibration, readings were taken during 30-min light periods and 30-min dark periods. The light intensity at the level of the vessels was about 500 ft-c. Wet-packed cell volumes were determined from aliquots of the suspensions.

TABLE 1

RESPIRATION RATES OF MUTANT STRAINS OF *Chlorella* IN LIGHT AND IN THE DARK

Strain	Q_{O_2} (cu mm of O_2/hr/cu mm of cells)	
	In dark	In light
No. 322	−1.64	−1.77
	−1.76	−1.70
	−1.70	−1.39
	−1.52	−1.20
	−1.64	−1.26
	−1.26	−0.95
	−2.13	−1.73
	−1.79	−0.98
	−1.90	−1.84
	−1.33	−1.44
	Average = −1.67	Average = −1.43
No. 349	−3.98	−3.85
	−3.79	−4.05
	−3.66	−3.92
	−3.34	−3.34
	−3.39	−3.51
	−3.62	−3.34
	−3.45	−3.63
	−3.82	−4.01
	−3.95	−3.95
	Average = −3.67	Average = −3.73

Respiration rates (cu mm of oxygen taken up per hr per cu mm of cells), calculated from the amounts of oxygen taken up during the 30-min light and dark periods, were determined for several cultures of each strain. The results indicate that light had no effect on the respiration of strains 322 and 349, as their respiration rates were approximately equal in the light and in the dark (Table 1). It is noted that positive pressure changes were not observed upon exposing these strains to light, as is normal and as is found for the parental type. The argument could be presented that the gene mutations which resulted in the inability of these strains to photosynthesize also resulted in their inability to carry on photorespiration. This is unlikely, however, as there is considerable evidence that indicates they are blocked in different reactions, and neither exhibited a significant increase in respiration in the light.

This experiment presented an excellent chance for photorespiration to be exhibited if it existed. However, it was not observed. Also, since the rates of respiration were approximately the same in the light and in the dark, it is extremely unlikely that light inhibits respiration. It seems certain, therefore, that light does not have a direct effect on respiration.

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Enzymic Conversion of Maltose into Unfermentable Carbohydrate

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Pigman (*6*) and Stark (*9*) reported that amylase preparations from molds, pancreas, and saliva are capable of synthesizing unfermentable carbohydrate or carbohydrates from maltose. However, the work of these authors is incomplete, since they did not separate or characterize the synthesized product.

While studying the enzymic hydrolysis of starch in distillery corn mash, by the use of submerged fungal cultures, we found that the secondary conversion of dextrins[1] into fermentable sugars was markedly inhibited by maltose.[2] The question was therefore raised as to whether the observed reduction in the rate of dextrin hydrolysis was actually a result of maltose inhibition or of the conversion of maltose into unfermentable carbohydrate. Conclusive data have since been obtained to show that such an enzymic conversion actually occurs.

The submerged fungal culture was prepared with *Aspergillus niger* NRRL 337 in a distillers' dried solubles-corn meal medium (*1*). One volume of the culture filtrate was mixed with two volumes of a 2.2% maltose[3] solution in 0.3 M acetate buffer (pH 4.4) and incubated at 30° C. Samples were taken periodically and analyzed for glucose, maltose, and unfermentable carbohydrate (dextrins) according to the tripartite method[4] of Stark and Somogyi (*10*). The data given in Fig. 1 show that, while a part of the maltose was hydrolyzed to glucose through maltase activity, another part was simultaneously converted into unfermentable carbohydrate. The presence of unfermentable material at zero hour was ascribed to dextrins contained in the fungal culture and in the maltose. During the reaction period the amount of unfermentable substance increased considerably, reaching a maximum at 8 hr, when 17.6% of the original maltose had been converted to unfermentable carbohydrate. A decrease in unfermentable material was observed after the maltose had been completely utilized (10–12 hr). The amount remaining accounted for only 10.2% of the maltose at the end of 24 hr. It is therefore concluded that the fungal culture must contain some enzyme or enzymes which catalyze the conversion of maltose into unfermentable carbohydrate. In the absence of maltose, this carbohydrate can be converted into glucose.

A similar experiment, in which glucose was used in place of maltose, showed no formation of unfermentable substance; the amount of glucose remained unchanged during the 24-hr reaction period. Apparently the enzyme or enzymes were inactive toward glucose.

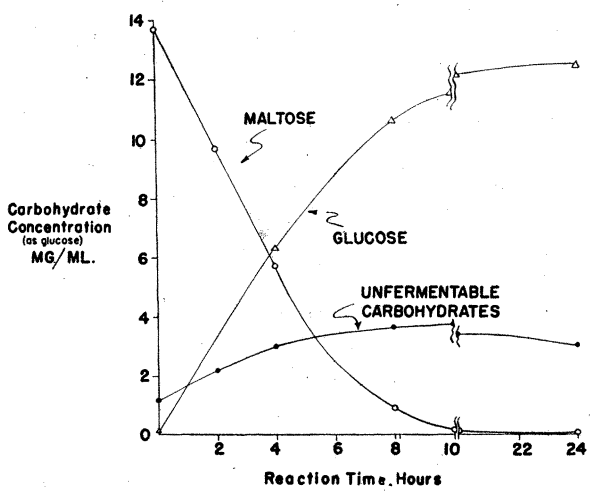

FIG. 1. Action of fungal enzymes upon maltose.

As a further confirmation of the formation of unfermentable substances, a similar experiment was conducted using a 10% maltose solution. Instead of using buffer, the filtrate of fungal culture was adjusted to pH 4.4 before mixing with the maltose solution. Ammonium sulfate, at a concentration of 1.5 g/l, was added as nutrient for subsequent yeast fermentation. After the fungal enzymes had been in contact with the maltose for 24 hr at 30° C, the mixture was sterilized and inoculated with an actively growing culture of distiller's yeast which had been grown in a yeast extract-glucose medium. At the end of fermentation (3 days), the medium was found to contain 40.7 mg of unfermentable carbohydrate per ml, equivalent to 41.5% of the maltose used (Table 1). The same table shows that the reaction mixture, which was sterilized immediately after mixing the maltose solution and the filtrate of the fungal culture, con-

[1] Like barley malt, the fungal culture enzymes hydrolyze starch primarily into fermentable sugars and dextrins.

[2] A report including this finding has been prepared for publication.

[3] Maltose (cp Fisher) recrystallized in the laboratory.

[4] Carbohydrate contents of samples either before or after treatment with baker's yeast were all determined as glucose by Somogyi's method (*8*) after complete acid hydrolysis in 0.69 *N* HCl solution for 2.5 hr in boiling water.