Table 1. Pain threshold values in the 24 subjects.

Males	Threshold	Standard error	Females	Threshold	Standard error
E.L.	8.5	0.13	J.G.	6.4	0.06
R.E.H.	7.9	.05	P.C.	7.8	.10
J.R.	6.7	.05	D.Q.	7.1	.03
F.R.P.	7.4	.04	M.V.	8.0	.05
B.O.	7.5	.03	T.H.	6.6	.06
J.P.K.	8.6	.07	M.S.	7.3	.05
N.M.P.	8.0	.04	J.M.	8.1	.03
R.S.V.	8.1	.07	T.F.	7.4	.04
J.G.	7.2	.05	N.K.	8.3	.06
S.L.	8.0	.05	L.L.	8.9	.10
M.S.	7.8	.04	I.D.	7.8	.29
E.H.	7.8	.01	M.P.	7.7	.07

within the range of the standard deviation in every case. The mean and standard error for the male and female groups were 7.7 ± 0.16 and 7.6 ± 0.20, respectively.

With the method of algesimetry described, it was observed that the end-point is sharp eliciting of a spontaneous response. The range of the standard errors of the pain-threshold determinations made on the same individual suggests a highly significant degree of reproducibility. The variation in pain thresholds between different subjects was of a low order, and no significant sex difference was observed. The apparatus is compact, simple to use, inexpensive, and requires no special training on the part of the operator. Furthermore, reliable results can be obtained in untrained subjects.

The ear lobule as the site of stimulation appears to be advantageous, because it has a relatively uniform thickness in different individuals and is composed only of skin, areolar, and adipose tissues. The anatomy of the earlobe permits passage of the stimulating current through its entire thickness; the stimulus is therefore more uniform than in methods where surface stimulation is applied. This method is currently being employed to investigate the effects of Nisentil® on pain threshold in man.

Since the time that this paper was accepted for publication, it has come to our attention that a similar method, utilizing electric stimulation of the earlobe, has been employed by Hofmann and his coworkers (*9*) for the study of the changes in pain threshold caused by various combinations of analgesic drugs.

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Radiation-Induced Chlorophyll-less Mutants of *Chlorella*

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Although many physiologic investigations have been made of *Chlorophyceae,* particularly *Chlorella,* there are very few publications on genetic variation in this group. Reports of chlorophyll-less mutants have been confined to species of *Chlorella.*

Beijerinck (*1*) reported the natural occurrence of yellow and colorless colonies of *Chlorella variegata* on culture mediums. This observation was later confirmed by Meyer (*2*), who attributed the presence of yellow and colorless forms to variation within the normal green colony. Recently Granick (*3*) induced colorless, pale yellow, and light green mutant colonies in *C. vulgaris* with x-rays. The present paper (*4*) presents the preliminary results of exposing cells of *C. pyrenoidosa* to ultraviolet light and the characterization of certain resulting mutants.

An isolate of *C. pyrenoidosa* obtained in pure culture from a single cell was used in this study. The cells were grown in continuous light in broth containing beef extract, tryptose, glucose, and sodium nitrate. A suspension of cells was prepared from cultures 7 to 10 days old by decanting the broth and adding distilled water. Fifteen milliliters of this suspension was pipetted into a Petri dish and exposed, at a distance of 3 cm, 1 to 4 min to ultraviolet light from a Westinghouse type SB Sterilamp. Cells were then distributed on the surface of agar plates and incubated in diffuse daylight for 3 wk.

Mutations were observed for the following characters: rate of growth, topography, and color of the colony; starch synthesis, plastid formation, and cell size. This report deals especially with induced changes in cell pigmentation.

Mutant colonies from the parent (C-1) were of lighter tints of green. Green colonies with yellow sectors and dwarf colorless colonies appeared frequently, but cells from these nongreen areas did not grow when transferred to fresh mediums. C-1 was very stable in culture and rarely gave rise to color mutations, either naturally or through the use of a mutagen. On one occasion, however, two yellow colonies were obtained from an aging culture, but in subsequent analysis of cell populations from cultures up to 5 mo old no color mutations were found.

A light green mutant, CM-1, obtained from the parent produced mutants when exposed to ultraviolet

Table 1. Characteristics of parent and mutant lines in diffuse daylight on meat extract-tryptose medium.

Parent and mutants	Colony color	Range in cell size (μ)	Typical autospore formation	Plastid formation	Starch synthesis
Parent					
C-1	green	2.0– 3.5	+*	+†	Normal
Mutants					
CM- 5	yellow	2.0– 3.5	+	+	Normal
CM-24	white	3.0–11.0	+	+	> Normal
CM-29	white	3.0– 7.0	+	–	Normal
CM-30	white	3.0– 7.0	+	+	None

* + indicates presence; – indicates absence of character.
† Cells stained with crystal violet and examined at approximately 1000 magnification.

light similar to those produced by C-1. Neither yellow nor white colonies were obtained from this line. It did, however, give rise to CM-3, a light olive-green mutant, which in turn produced several yellow colonies after treatment. One such yellow mutant, CM-5, yielded white, red, and green colonies after exposure to ultraviolet light. A white mutant, CM-24 (Table 1), gave rise spontaneously to a pale yellow mutant, CM-35. This yellow line mutated to light green after exposure to ultraviolet light. Thus it was possible to induce white mutants, apparently without chlorophyll, stepwise from the green parent. It was also possible to obtain green colonies similar to the parent stepwise from the white lines.

White mutants CM-29 and CM-30 were stable in culture, but this was not true for most of the yellow and light green mutants. When maintained on beef extract-glucose agar, particularly in light, cultures tended to revert to the color of the line from which they were immediately derived. This difficulty of maintaining pure stocks was overcome by growing variable lines on a medium composed only of inorganic salts and glucose.

Like the parent, C-1, colorless mutants reproduce by autospores. In this process the cell protoplasm divides to produce a tetrad of cells. Although the tetrad is transitory, two cells of different size may adhere in such a way to make it appear that cell multiplication is by budding or fission. The protoplasm frequently divides to produce only two globose spores that lie free within the wall of the parent cell.

The white mutants occasionally formed filamentous cell protrusions similar to the fusion tubes of certain yeasts. These tubes were 1 to 2 μ in diameter and approximately 2 to 4 μ long. Other morphological characters of selected mutants are summarized in Table 1.

Chlorophyll-less mutants were not deficient for vitamins or other growth factors. All the lines grew rapidly in a medium comprising inorganic salts and glucose. In the dark, the parent and mutants (Table 1) behaved similarly in sugar assimilation tests. Glucose and galactose were strongly assimilated, lactose and maltose weakly, and sucrose was not utilized. Fermentation tests with these sugars were negative. Nitrate and nitrite nitrogen were utilized by C-1 and by the white lines, but growth was sparse when cultures were supplied with either asparagine or ammonium chloride.

Permanently colorless forms analogous to the colorless mutants of *C. pyrenoidosa* have been reported from nature. These forms have been referred to the genus *Prototheca,* which was created in 1894 by Krüger (*5*) to accommodate two unicellular organisms that he considered fungi. Krüger (*5*) pointed out the close resemblance of *Prototheca* spp. in form and development to certain lower green algae, particularly to such forms as *C. vulgaris* Beijerinck. A comparison of the white mutants (Table 1) with *P. chlorelloides* obtained from the University of Indiana culture collection confirmed the similarities in ontogeny and morphology of the two genera. Since the species of *Prototheca* are alga-like in their reproduction and show no direct connection with existing groups of fungi, they have been regarded by some phycologists as colorless algae.

It may be inferred from the results presented in this paper that the colorless forms of the genus *Prototheca* could have arisen by mutation from the *Chlorophyceae.* Indeed Beijerinck (*1*) assigned a colorless mutant of *C. variegata* to a new species *P. krugeri.* In his opinion this was a transition from an alga to a fungus.

It has not been possible to study the range of potential characters that may be produced in the colorless mutants. Studies are therefore being continued to determine the limits of genic variation and phenotypic variability.

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