hypothesis, although other effects of the hemolytic agent on the structure of the cell cannot be ruled out. Inhibition of saponin or bile salt hemolysis by various sugars in isosmotic solutions has been described and the possible influence of reduced ionic strength discussed (*2*, p. 274). The sucrose inhibition of the present experiments is effective even with the ionic strength maintained constant. An increased internal osmotic pressure produced by a hemolytic agent might be due to (i) an increase of ions in the interior of the cell consequent to the loss of differential membrane permeability, or (ii) dissolving normally insoluble or structurally bound internal macromolecules. Experimental support of the first factor has been advanced by several investigators (*5, 6, 7*). However, a low concentration of resorcinol will allow marked cation shifts with only a little swelling (*2*, p. 244). The second possibility has apparently not yet been investigated, but it is noteworthy that the excess osmotic pressure, 0.1 isotonic, of the lowest sucrose concentration completely inhibiting resorcinol hemolysis in these experiments corresponds exactly to the osmotic pressure calculated for hemoglobin in red cells by Wilbrandt (*7*). Further work is being done to elucidate these mechanisms with quantitative volume and percentage hemolysis determinations.

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An Earlobe Algesimeter: A Simple Method of Determining Pain Threshold in Man

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A dependable and reproducible method for determining pain threshold is essential in the quantitative evaluation of analgesic drugs. Thermal radiation and electric stimulation of the tooth pulp, two commonly used methods, have recently been criticized (*1–3*), because the results obtained have not been uniformly reproducible even in trained subjects. Faradic stimulation, for the determination of pain threshold, was first clinically investigated by Martin *et al.* in 1913 (*4–7*). Macht and his associates (*8*) in 1915 used an inductorium to study quantitatively the analgesic action of opium alkaloids in trained subjects. It is the purpose of this paper to describe the use of the inductorium for the determination of pain thresholds in untrained subjects and to introduce the earlobe as the site of stimulation.

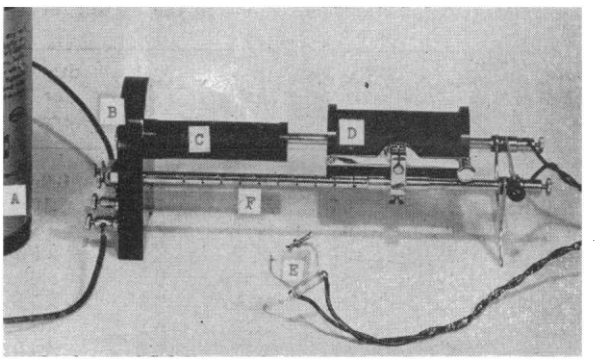

Fig. 1. Inductorium for determination of pain thresholds: *A*, battery; *B*, circuit breaker; *C*, primary coil; *D*, secondary coil; *E*, earpiece; *F*, calibrated scale.

The apparatus (Fig. 1) consists of a standard inductorium connected to a 1½-v dry cell battery. The primary and secondary coils are identically wired. A simple key is interposed between the battery and inductorium. Direct interrupted current is obtained through an electromagnetic circuit breaker. With the help of an electrocardiograph, the circuit breaker was regulated to produce faradic current of 60-cy/sec frequency. An adjustable earpiece is connected to the secondary coil of the inductorium.

The investigation was carried out on 12 male and 12 female untrained volunteers whose ages ranged from 21 to 36 yr. The subject lay on a bed in a quiet room and was allowed to rest for 20 min before the testing began. The earpiece was then applied to one of the earlobes and the screw was adjusted until good contact without discomfort was obtained. The secondary coil was then moved at a uniform slow speed toward the primary coil, and the subject was familiarized with the sensations of vibration, prickling, and sudden sharp pain (the end-point), which occur successively as the secondary coil is advanced. The volunteer was instructed to signify immediately the onset of the sharp pain. After this preliminary trial, 10 tests were carried out at 3-min intervals on each subject. The position of the secondary coil at the moment of response was read from the calibrated scale; this figure was taken to represent the pain threshold. After the 10 tests, one similar test was carried out on the other earlobe. During the experiments, the subjects could not see the markings on the scale, nor were the results discussed with them. All the tests were performed by the same investigator (M.S.).

Table 1 shows the means and standard errors of pain thresholds obtained in the male and female groups. The single test on the contralateral ear fell

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