The optical density of a TMV suspension is proportional to its concentration (*6*). To determine whether the optical density of the assay fraction, minus that of the healthy control, is proportional to the concentration of virus in the homogenate, dilutions of homogenates from infected tissue were prepared by adding various volumes of homogenates from uninfected tissue, and the assay was completed (Fig. 1).

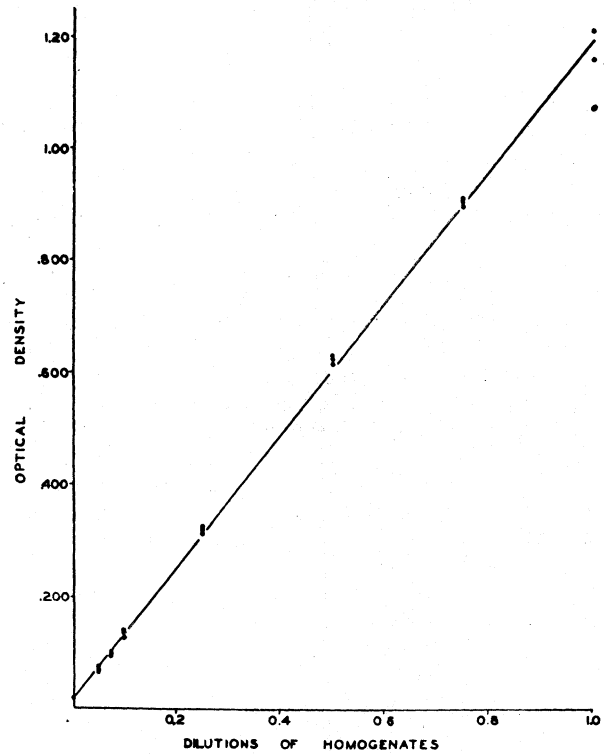

FIG. 1. Linear relationship between known dilutions of TMV in homogenates and the optical density obtained (circles) by use of the chloroform emulsion method.

It was found that a straight line could be drawn which falls very close to the mean of each dilution, including the mean of the control. The average deviation of all samples from this straight line is 4.5%, with a maximum mean deviation of 8.1% for any dilution. It can be seen that in the twentyfold dilution range tested (Fig. 1), the optical density (at 260 mμ) of the assay fraction of each dilution, minus that of the healthy check, is inversely proportional to the dilution of the homogenate, or directly proportional to the virus concentration in the homogenate.

To estimate percentage recovery, 0.5 ml aliquots of various dilutions of purified virus having a known optical density were added to incompletely homogenized healthy tissue. The homogenizing was completed, and the samples were assayed. For three experiments, the average recovery was 89%, with a maximum mean deviation of 9% for any dilution. Within a single experiment, the average recovery for any virus dilution did not differ from that of any other dilution by more than 4%. Takahashi has shown that not all the virus present in the tissue is extracted by homogenizing, and that additional virus may be recovered from the residue of the first slow-speed centrifugation (*7*). Therefore, the percentage of virus recovered by this assay method is based on the free virus, and not on the total amount in the infected tissue.

The results obtained show that the chloroform emulsion technique provides a rapid and relatively accurate assay method for TMV. Even with young leaf tissue, where it is difficult to separate virus from chlorophyll, the chloroform method is very effective. It is possible that the technique may also be useful in the purification or assay of animal viruses or other plant viruses.

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The Disruption of Mitochondria of Saccharomyces by Ultraviolet Irradiation[1]

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Coagulation of cellular protein has been the principal cytoplasmic change attributed to ultraviolet irradiation (*1, 2*). We report here a characteristic disruption of mitochondria which becomes microscopically detectable prior to the onset of cytoplasmic precipitation. The effect is first observed with ultraviolet doses sufficient to reduce irradiated yeast suspensions to approximately 1% survival, and becomes more pronounced as dosage increases.

For this study we have employed a procedure which consistently leads to optimal production of mitochondria in baker's yeast. Actively growing cells were harvested, washed once with distilled water, and transferred to a solution of 3% monopotassium phosphate and 3% dextrose, the final dry weight of yeast being about 1%. Two hundred and fifty ml of this suspension was shaken in a 500 ml flask, 8–12 hr at 30° C, until the sugar was completely exhausted. The yeast cells from such a preparation, with their large mitochondria, are shown in Fig. 1. In general, an inverse relation exists between the size of the mitochondria and the metabolic activity of the cell (*8*). Large mitochondria may be considered to consist of an enzyme protein core essentially isolated from the cytoplasm by a lipoid cortex (*9*). As cellular activity declines, mitochondria increase in size.

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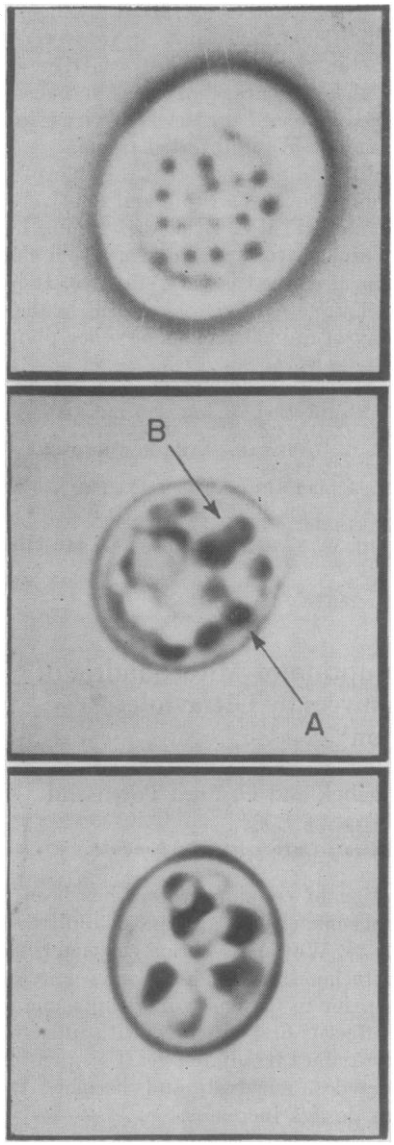

FIGS. 1–3. All illustrations represent unstained cells.

1—A yeast cell showing characteristic arrangement of large mitochondria. The structures depicted have been described as lipoidal granules (*3*), metachromatic granules (*4*), and volutin granules (*5*), as well as mitochondria (*6*). Recently, however, B. D. Mundkur (*7*) has shown them to be centers of strong cytochrome oxidase activity and has conclusively established their identity as mitochondria.

2—A cell revealing an early stage of mitochondrial disruption effected by moderate doses of ultraviolet radiation. *A*, indicates the swelling mitochondria; *B*, incipient coalescence of adjacent swollen mitochondria.

3—A cell with completely disrupted mitochondria after 10 min ultraviolet irradiation. The cell is noticeably shrunken and the vacuole unobservable.

The yeast cells containing large mitochondria were harvested, washed free of the potassium phosphate with distilled water, and suspended in distilled water to a cell concentration of approximately 2×10^4 cells/ml. Fifteen ml of the suspension was irradiated in an open Petri dish without agitation at a distance of 60 cm from a Hanovia quartz envelope ultraviolet lamp delivering 95% of its ultraviolet energy at a wavelength of 2537 A. During the course of these studies the temperature of the suspensions during irradiation ranged from 26° to 28° C. Photographs were made after 2 min (approximately 1% survival as determined by plate counts) and after 10 min exposure (no survival detected by plating).

Following irradiation of cells containing an abundance of large mitochondria, slight swelling of the mitochondria occurs after 2 min, and complete disruption and obvious coalescence, producing in some cells a single large mass, occur after 10 min exposure (Figs. 2, 3). The fused amorphous masses are readily stained with Sudan black. Although there is considerable cellular shrinkage and the nuclear vacuole is usually obscured, there is no apparent precipitation of the cytoplasm. Unirradiated controls showed slight disruption of mitochondria but no cellular shrinkage after 4–5 hr at 28° C.

In contrast to the results obtained in the treatment of cells with large mitochondria, a different result is observed when cells containing smaller mitochondria are irradiated. These cells were obtained by centrifuging and washing cells from the flask containing yeast, phosphate, and glucose after 2 hr shaking and before the dextrose was exhausted. Presumably these organisms differ from those containing large mitochondria in possessing lesser amounts of lipid in the external cortex and less extensive aggregation of enzyme at the mitochondrial core. In such cells disorganization of the mitochondria was not detectable after 10 min irradiation, although definite precipitation of the cytoplasm and cellular shrinkage were already apparent.

Yeast cells containing either large or small mitochondria showed no morphological alterations when held at 28° C for 3 hr in distilled water which had been previously irradiated for 30 min. It appears, therefore, that the changes we describe are due to direct ultraviolet absorption by the mitochondria and cytoplasmic matrix. The disruption of large mitochondria can be attributed to the high absorption of radiant energy of wavelength 2537 A by the sterols and unsaturated fatty acids of the lipid surface. It has been demonstrated that such absorption can effect decarboxylation of fatty acids (*10*). If loss of lipid polar groups occurs, a weakening of attraction between the surface lipid and proteinaceous mitochondrion core might be expected, resulting in the mutual attraction and coalescence of the lipid substances. Smaller mitochondria may resist disruption by ultraviolet irradiation as a result of the paucity of the absorbent material. The coagulation of the cytoplasm in cells containing such mitochondria may be attributable to the effects of irradiation on the large amounts of protein material free in the cytoplasm.

It must be pointed out that differences in the size and structural integrity of yeast cell mitochondria may depend upon osmotic and other physical factors, as well as metabolic states. Radiation-induced mito-

chondrial alterations, for example, are not detectable when cells are irradiated in 0.15 *M* glucose, 0.15 *M* monopotassium phosphate, or 0.15 *M* potassium chloride solutions. The precise roles of such extrinsic factors in the expression of radiation damage remain to be determined.

It is not possible at present to ascribe a fundamental relation to the disorganization of mitochondria and ultraviolet-effected cellular inactivation, since the changes reported here are brought about only under specific conditions and at relatively high doses. However, these striking phenomena, together with the demonstrated increase in radiation resistance with decrease in cytochrome activity (*11*), implicate the cytochrome-oxidase-rich mitochondria as critical ultraviolet-absorbing sites and suggest that mitochondrial damage may serve a characteristic function in cytoplasmically mediated recovery processes.

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Visible Intra-Epithelial Iron in the Mammary Glands of Various Species[1]

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The occurrence of stainable iron in the mammary epithelium of mice has been noted (*1–3*). Schultz also found it in the mammary glands of rats, both male and female, and in one case in a female guinea pig. Further investigation has shown the phenomenon to be so widespread that its significance should be assessed by workers in various fields.

The technique for demonstrating the iron is simple (*4*). The tissue is fixed in 10% formalin, preferably buffered (*5*), and stained by the method of Gömöri (*6*), using equal parts of 20% hydrochloric acid and 10% potassium ferrocyanide for about ½ hr. It is necessary to wash out the stain as completely as possible with distilled water. Clearing is done through 95% and absolute alcohol to xylol. If the pieces of tissue are large and are to be used as whole mounts, a bath of oil of origanum after the absolute alcohol aids in clearing. The extent of the iron deposition in whole mounts is best seen under a dissecting microscope with low or moderate magnification. Usually the iron is present to such a degree that the gland tree is clearly outlined (Fig. 1). The procedure can be carried out while the glands are still on the hides with small animals such as the mouse, or even the rat, and the gland viewed by reflected light, but staining is more satisfactory if, after fixation, the gland tissue is scraped off the hide. Care should be taken to avoid iron contamination as much as possible. With bigger pieces of tissue from the more extensive glands of larger animals, the material should be fixed and stained in blocks about 0.5 cm thick. Such pieces often show a marked staining reaction visible to the naked eye within a few seconds of immersion in the staining fluid.

FIG. 1. Area from a whole mount of a mammary gland of a rat about 9 months of age that had had a litter some time previously; stained by hydrochloric acid and potassium ferrocyanide. Stained iron appears black and outlines gland tree. × 35.

A useful procedure for large pieces of tissue is to run them up in thin but extensive slabs into celloidin and make sections 100–200 μ thick. These can be gathered alternately into two groups, one of which can be stained with hematoxylin alone and the other by the Gömöri technique. By examining these in xylol under low magnification a quick but thorough survey of

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