

TABLE I.

No. of exps.	Cell	Element	pH	Activity (mC/liter)	Duration of exp. hr	Effect
2	Nitella	KCl	7.3	4	24	None
6	"	NaCl	8.2	20	24	"
6	"	"	7.3	5	4	"
4	"	"	8.2	2.2	24	Slight increase
6	Elodea	"	7.2	13		None
6	Sheep r.b.c.	"	7.3	—	4	"
6	Chicken r.b.c.	"	7.3	5	4	"

according to the usual method. The pH of the NaCl and KCl solutions was 7.2. In certain cases with pond water it was 8.2. Controls with non-radioactive salts were done in all cases. Table I gives the results.

In most cases there was no observable effect on O₂ consumption as indicated by the Warburg method. The exception is that of *Nitella* when a low activity of NaCl (2.2 mC/liter) was used, and a slight acceleration was noted. There was, however, no immediate rise in oxygen consumption within a few minutes corresponding to the rapid ion exchange as shown in the induced absorption or first phase of ion exchange as illustrated by S. C. Brooks.¹ More refined methods may show other results.

It is concluded that there is no effect upon oxygen consumption because of the radioactivity of these ions.

10975

Ultraviolet Absorption Spectra of Active and Inactive Yeast.

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The ultraviolet inactivation spectrum of yeast has been investigated by several workers, notably Wyckoff and Luyet,¹ Ehrismann and Noethling,² Oster,³ and Schreiber.⁴ These workers are in substantial agreement on the most effective wave-length region and on the

¹ Brooks, S. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 856.

¹ Wyckoff, R. W. G., and Luyet, B. J., *Radiology*, 1931, **17**, 1171.

² Ehrismann, O., and Noethling, W., *Z. Hygiene Infektionskr.*, 1932, **113**, 597.

³ Oster, R. H., *J. Gen. Physiol.*, 1934, **18**, 251.

⁴ Schreiber, H., *Strahlentherapie*, 1934, **49**, 541.

absolute energies required to produce a given degree of inactivation. It turns out that there is a maximum effect around 2600 Å, some 500 ergs/mm² of incident energy being sufficient to produce 50% inactivation.

It has been generally assumed that the effect of the radiation is on the known content of yeast nucleic acid or its component radicals. The wave-length characteristics of the inactivation curves support this assumption.³ Nevertheless, all previous attempts to secure direct quantitative measurements of ultraviolet absorption by yeast cells were either incomplete or failures. Although an absorption curve for yeast extract has been recently published,⁵ it does not necessarily correspond to the values for the intact cells. Using the method of Gates,⁶ Oster³ pressed the cells between quartz plates in order to obtain a thin layer, but without success. Lucas,⁷ Köhler,⁸ and Wyckoff⁹ published a few photomicrographs of individual yeast cells taken with monochromatic radiation at 2750 Å but no quantitative data. The transmission curve of Ehrismann and Noethling² for an unmentioned species of *Saccharomyces* cells in suspension in a mixture of glycerine and water seems in serious error and obviously does not represent the absorption by the individual organisms. On the other hand, the data of Caspersson¹⁰ on selected individual cells of a brewers' yeast by the quartz microscope method are sufficiently quantitative, save possibly for the omission of the thickness of the cells, but are for only four wave-lengths. Caspersson determined absorption as a function of age or activity of cells and found older cultures to have a lessened absorption.

The present paper reports more complete absorption data, taken at 16 wave-lengths ranging from 2300 to 3650 Å, and on both an active 2-day and an inactive 16-day culture.

The extinction coefficients for an 8-micron layer of pressed cells of *Saccharomyces ellipsoideus* were determined photoelectrically. A CsO-on-Ag photocell in a quartz envelope was employed in connection with a direct-current amplifier. Galvanometer deflections corresponding to the ultraviolet transmission through the absorbing layer or through the control layer, were read alternately.

The radiation was rendered singly monochromatic by a Hilger medium quartz spectrograph. Mercury emission lines from a small

⁵ Loofbourow, J. R., Cook, E. S., and Stimson, M. M., *Nature*, 1938, **142**, 573.

⁶ Gates, F. L., *J. Gen. Physiol.*, 1930, **14**, 31.

⁷ Lucas, F. F., *J. Franklin Inst.*, 1934, **217**, 661.

⁸ Köhler, A., *Z. wiss. Mikr.*, 1904, **21**, 273.

⁹ Wyckoff, R. W. G., *Cold Spring Harbor Symposia*, 1934, **2**, 39.

¹⁰ Caspersson, T., *Skand. Arch. Physiol.*, 1936, **73**, suppl. 8.

water-cooled arc furnished the source. The exit slit of the monochromator was reduced to a circular opening 0.5 mm in diameter against which the quartz slide with the sample was placed. No appreciable change in transmission by the yeast occurred during the period of the measurements at the radiation intensities used.

Cultures of *Saccharomyces ellipsoideus*, kindly furnished by Professor W. E. Maneval, were grown on potato-dextrose agar at a temperature of 25°C. At the end of 48 hours, 5 samples were removed for absorption measurements. The same culture was retained an additional 14 days, at which time a further series of 4 samples was taken. Hence all determinations were made on samples

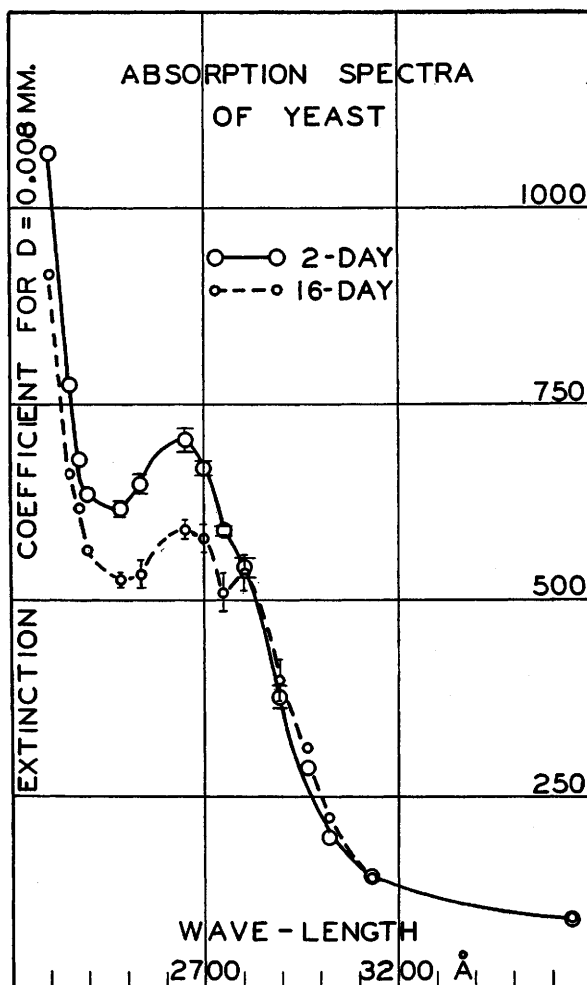


FIG. 1.

from the identical culture tube, but at a time interval of 2 weeks, in order to avoid possible variation among different cultures.

Yeast cells, obtained free from the agar substrate on a wire loop, were pressed between 2 quartz slides held apart by aluminum foil spacers. In this way a large relatively homogeneous area of uniform thickness was secured. The thickness of the layer was always 0.008 mm. A control layer of the same thickness was furnished by placing a small droplet of a 50% mixture of glycerine-water near the yeast sample on the same slide.

Results. The extinction curves determined on the 8-micron layers of the 2-day- and 16-day-old yeast cultures are shown in Fig. 1. The extinction coefficient, k , is plotted against the wave-length in Angstroms, where k is given by the expression, $k = (1/d)\log(I_0/I)$. Here I_0 is the incident flux and I the flux transmitted through the absorbing layer of thickness d in cm; decadic logarithms are used.

For the 2-day cultures, each point on the curve is an average of independent determinations on 5 samples from the same culture. Similarly, the 16-day curve represents the average of measurements on 4 different samples. Probable errors are indicated by bars and vertical lines for critical points of the curves.

The extinction coefficients for the 2-day-old yeast cultures are significantly higher than for the 16-day-old cultures in the region from 2800 Å to 2300 Å. Further, the older culture exhibits a more marked absorption band at 2800 Å.

The shape of the extinction curves obtained is similar to the inactivation curve of Oster⁸ for *Saccharomyces cerevisiae* and gives some justification to Oster's assumption that the extinction curve may be derived from the inactivation curve. The quantitative agreement, however, is far less satisfactory than would appear upon casual comparison. The discrepancy is particularly noticeable for the short wave-lengths. This means presumably that other substances than the one effective in inactivation are responsible in part for the ultraviolet absorption.

Oster's¹¹ finding that yeast cells from a 24-hour culture are more sensitive to ultraviolet radiation than a 15-day culture would seem to be accounted for, at least in part, by the greater absorption of radiation at the earlier stage of development. This dependence of yeast cell absorption on physiological activity is in harmony with the hypothesis of Caspersson¹⁰ regarding the increased nucleic acid metabolism of dividing cells. Caspersson's data on the extinction of selected individual yeast cells harmonize with his theory and are

¹¹ Oster, R. H., *J. Gen. Physiol.*, 1934, **18**, 243.

likewise in substantial agreement with the data presented here. His measurements having been taken at only 4 wave-lengths with the longest at 2750 Å, any increase in absorption with age at 2800 Å would not have been detected in his experiments.

Later data of Caspersson and Schultz¹² on the cytoplasm of *Drosophila* gut cells and on the root tips of *Allium sp.* do reveal an absorption band at 2800 Å for mature cells, while immature or actively dividing cells have their absorption maximum at 2600 Å. Characteristic of the latter type of absorption spectra are the recently obtained curves for the sporidia of *Ustilago zea*,¹³ the cytoplasm of *Drosophila* eggs,¹⁴ and the contents of *Zea mays* pollen grains.¹⁵

Summary. The ultraviolet absorption spectrum of the yeast, *Saccharomyces ellipsoideus*, exhibits a maximum of 2650 Å. The extinction coefficient for the maximum decreases for inactive cells, thus revealing a secondary maximum at 2800 Å. Agreement with the theory of Caspersson on nucleic acid metabolism as a function of division rate is obtained.

10976 P

Action of Tincture of Iodine when Injected into the Pericardial Sac.

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Since the appearance of the work of Musser and Herrmann¹ on the injection of tincture of iodine into the pericardial sac, no work has appeared on this subject. It was considered worth while to study this problem as an introduction to a general study of drugs administered by this route.

Eight healthy dogs of average size anesthetized with pentobarbital sodium were used in this series. Six dogs anesthetized with ether

¹² Caspersson, T., and Schultz, J., *Nature*, 1939, **143**, 602.

¹³ Landen, E. W., *J. Cell. Comp. Physiol.*, 1939, **14**, 217.

¹⁴ Caspersson, T., and Schultz, J., *Nature*, 1938, **142**, 294.

¹⁵ Uber, F. M., *Am. J. Bot.*, 1939, **26**, in press.

¹ Musser, J. H., and Herrmann, George R., *Proc. Soc. Exp. Biol. and Med.*, 1925, **23**, 212.