WEAK LUMINESCENCE FROM THE YEAST SACCHAROMYCES CEREVISIAE AND THE EXISTENCE OF MITOGENETIC RADIATION

Terence I. Quickenden

School of Chemistry, University of Western Australia, Nedlands, 6009, W.A. Australia

Shane S. Que Hee

Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Canada, S7N OWO.

Received August 7,1974

SUMMARY: A weak luminescence has been detected from liquid cultures of the yeast Saccharomyces cerevisiae, during two different stages of its growth cycle. The most intense emission occurred during the latter portion of the logarithmic stage of growth and varied from culture to culture within the range 160-1540 counts/sec. A less intense emission of 290-1220 counts/sec was observed during the early stationary phase. The luminescences lasted for periods in excess of three hours, and were measured with a photon counter sensitive to wavelengths in the range 180-650 nm. The quantum efficiency of the log phase emission varied from 0.07 to 0.7 photons per cell division for the various cultures. These observations provide limited support for certain published claims relating to the existence of a so-called mitogenetic radiation from dividing cells.

Between 1920 and 1935, over 500 papers were published in the Russian and German literature on the subject of mitogenetic radiation. These studies originated with the work of A.G. Gurwitsch (1-4) who found that living cells emitted a radiation which could stimulate other cells to divide. Subsequent experiments indicated that it was ultraviolet light of intensity 10-1000 photons cm^{-2} sec^{-1}, and of wavelength 190-280 nm, or 320-350 nm. The radiation was observed (1-4) from a wide variety of plant and animal cells during mitosis, and also from a number of chemical reactions. It was usually detected by its ability to stimulate cell division in a variety of biological detectors such as yeast cultures, bacterial cultures, and plant meristems, although later workers introduced physical detectors such as the photographic plate and the u.v. sensitive Geiger tube.

Western attempts to reproduce the mitogenetic phenomena were singularly unsuccessful, despite a series of very careful investigations (5-7) during the 1930's. Work on mitogenetic radiation subsequently ceased in Western

countries, but is still carried on (2-3, 8-11) in the Soviet Union. Nevertheless, recent Western work by Metcalf and Quickenden (12), and by Doman (13), using modern photon counting equipment, has failed to detect luminescence from logarithmic cultures of yeast and bacteria, and from germinating seedlings. Vladimirov (14) has recently discussed in some detail, the contradictory status of mitogenetic radiation. Apart from an occasional news report (15), the subject is now infrequently mentioned in the Western literature. The aim of this present work was to examine yeast cultures for luminescence, over their entire life cycle, using sensitive photon counting equipment and optimal light collecting techniques.

EXPERIMENTAL: The luminescence was measured with an E.M.I. 6255B photomultiplier tube, sensitive in the region 180-650 nm. The tube was attached to a photon counter (16, 17) comprising a pulse amplifier, a pulse-height discriminator, and a ratemeter which gave a continuous chart record of the count rate of those pulses which surmounted the discriminator setting. This apparatus has been described elsewhere (18). The photomultiplier E.H.T. voltage was set at 1735 V, and the discriminator was set at 12.5 mV, to optimise (19) the ratio R^2/B, where R is the count rate due to the incident light, and B is the background count rate.

The cuvette which contained the yeast culture, was attached with a non-luminescing glue to the end-window of the photomultiplier tube, which formed the base of the cuvette. The remainder of the cuvette comprised a vitreosil tube of internal diameter 4.5 cm and of length 26.0 cm. The tube was coated externally with a reflecting layer of vacuum deposited aluminium, and was provided with a polished stainless steel lid. The lid contained holes to permit the entry of various stainless steel tubes which were used for withdrawing and inserting samples of yeast, and for oxygenating the suspension in the cuvette at a constant rate of 0.14 cm^3 sec^{-1} to ensure that growth occurred under conditions of oxygen saturation. The stirring produced by the bubbles also ensured that the suspension was homogeneous and at a uniform temperature. Although placement of the warm yeast suspension in direct contact with the end-window of the photomultiplier tube leads to a high photomultiplier background of ca. 300 counts sec^{-1}, and necessitates good temperature regulation to keep the background constant, it has the advantage of allowing very efficient collection of light from a large volume of suspension.

The temperature of the cuvette and the photomultiplier tube was kept at 306.2 ± 0.03 K by a constant stream of thermostatting water which passed through a surrounding, light-proof, brass jacket and through a stainless steel tube which passed through the suspension in the cuvette. The temperature of the suspension and the adjacent photomultiplier cathode was monitored continuously by a thermistor. Careful control and measurement of the temperature was necessary because the background count rate from the photomultiplier had a temperature coefficient of 41 counts sec^{-1} K^{-1}. The temperature of 306.2 K ensured optimal yeast growth, and the allowed variation of ± 0.03 K corresponded to changes of less than 0.1% in the growth rate.

The yeast studied was diploid Saccharomyces cerevisiae, strain Y1, from the microbiological collection at the University of Queensland. The yeast was grown in a fully defined, sterile, nutrient medium similar to Wickerham's diagnostic morphology medium (20), except that the concentrations of the vitamins, amino acids, and glucose, were twice those specified by Wickerham. The nutrient medium was stored in blackened bottles under carbon dioxide at a temperature of 277 K, to avoid photochemical and thermal decomposition. The yeast was pre-trained to minimise any effects due to its immediate past history, by sub-culturing 0.1 ml samples of the yeast into 50 ml of nutrient medium, in darkness, under anaerobic conditions, at 306.2 K, daily, for one week.

Asynchronous cultures were grown either from logarithmic or stationary phase inocula. Logarithmic inocula were prepared by placing a 0.1 ml sample of the pre-trained yeast into 50 ml of nutrient medium at 306.2 K. When the culture had reached an optical density of 1.20 (measured at 540 nm in a 1 cm cell) 9 ml of the suspension was injected into 350 ml of nutrient medium in the yeast cuvette, prior to commencing luminescence measurements. Stationary phase inocula were prepared by placing 0.1 ml of the pre-trained yeast into 50 ml of nutrient medium. The medium was then incubated with continuous agitation at 306.2 K for ten days, before dilution to an optical density of 1.20, followed by injection of a 9 ml sample into the yeast cuvette. In the case of synchronous cultures, the cells were brought to a uniform stage of growth by the method of Williamson and Scopes (21).

Before luminescence measurements were commenced, the photomultiplier tube was dark adapted until a constant background count rate was obtained. This took up to 48 hours if the tube had recently been exposed to a bright light. 350 ml of sterile nutrient medium which had previously been passed through a filter of pore size 0.45 microns, was then injected into the sterile, darkened cuvette through a stainless steel tube which did not permit the entry of light. The oxygen supply was turned on, and the temperature and the count rate were allowed to stabilise. This usually took several hours, and checks for contaminant growth were carried out on samples of the nutrient medium which were withdrawn during this period.

When the count rate had reached a stable level, a 9 ml inoculum of yeast, which had been diluted with nutrient medium to an absorbance of 1.20, was injected into the cuvette to initiate yeast growth. Yeast growth was monitored throughout the subsequent luminescence measurements by determining the number of cells in 0.5 ml samples, which were withdrawn periodically from the cuvette, and analysed with a Coulter counter (model B). The threshold levels on the Coulter counter were set so that particles with a radius exceeding 2.99 microns were counted. An entire run, from the injection of nutrient medium to the end of the stationary phase, usually took 30-35 hours. If the nutrient medium was maintained in the cuvette for a similar period of time, without inoculation of the yeast, the photomultiplier background plus the luminescence from the nutrient medium remained constant to within 0.5%, providing sterility was maintained. At the conclusion of each run, the cuvette was sterilised with formalin, and thoroughly cleaned before further use.

RESULTS AND DISCUSSION: Figures 1-4 show the luminescence intensity and number of cells as a function of time, for synchronous cultures and for asynchronous cultures grown from stationary phase inocula and log phase inocula. The maximum emission from log phase cultures varied from culture to

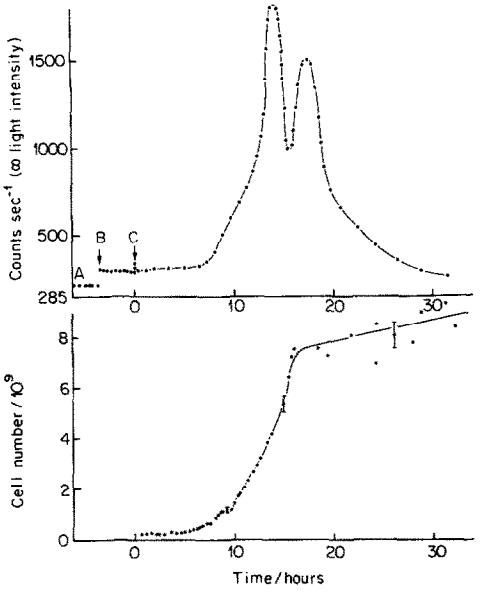

FIG. 1. Inoculum subjected to 4 cycles of synchronisation

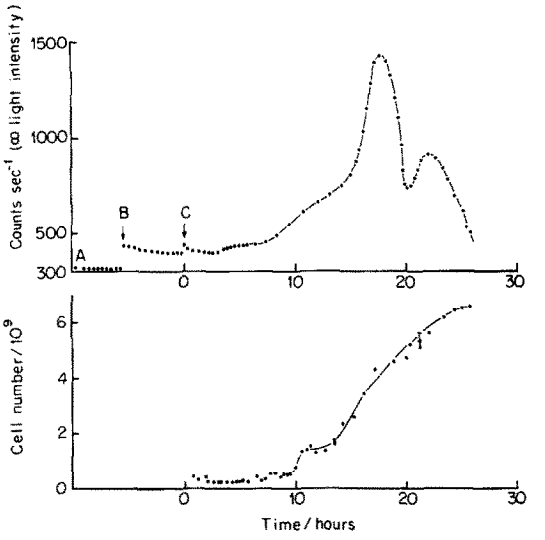

FIG. 2. Inoculum subjected to 6 cycles of synchronisation

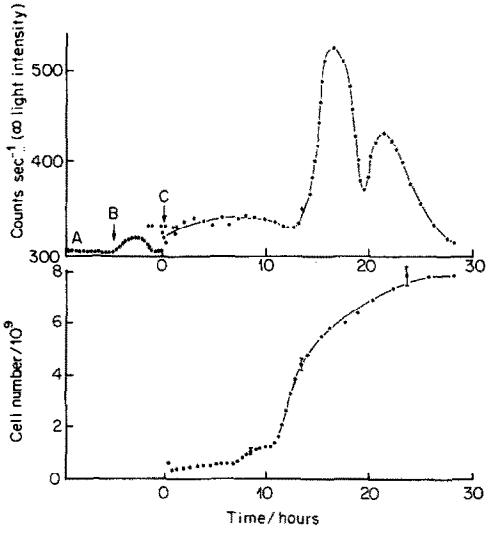

FIG. 3. Log phase inoculum

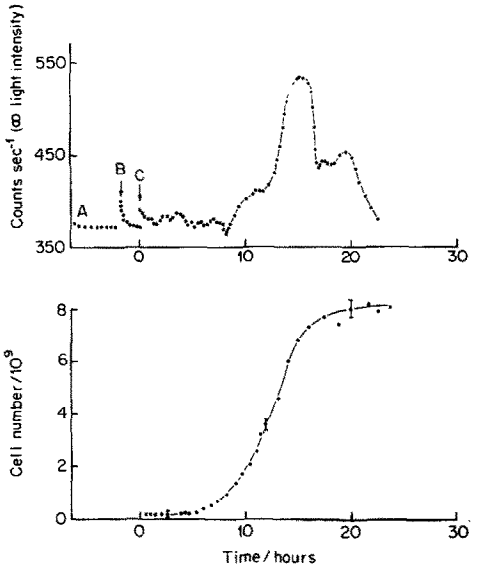

FIG. 4. Stationary phase inoculum (10 days old)

Figs. 1-4. Luminescence and growth curves of the yeast Saccharomyces cerevisiae after inoculation into a nutrient medium. A-B: photomultiplier background; B: introduction of nutrient medium; C: inoculation of yeast.

culture within the range 160-1540 counts sec^{-1}, and the corresponding range for stationary phase emission was 290-1220 counts sec^{-1}. These intensities represented a two-fold to five-fold increase in background count rate from the photon counter, and such increases were maintained for periods in excess of three hours. The background count rate included photomultiplier background and a weak luminescence from the nutrient medium, due to a previously reported (18) excitation by ambient ionising radiation. The total background count varied by less than 0.5% over periods of time similar to the duration of a run. There is accordingly little doubt as to the existence of the two major luminescence peaks which were observed from all the cultures examined.

Calculations on the maximum log phase emission indicated quantum efficiencies ranging from 0.07-0.7 photons per cell division for the various cultures. These figures were obtained on the basis of a mean photomultiplier cathode efficiency of 0.16 photoelectrons per photon, and an estimated light collection efficiency of 10%.

The experiments reported in this paper do not determine the ability of the yeast luminescence to stimulate cell division, nor do they establish the wavelength of the radiation, other than to localise it in the wavelength range 180-650 nm. Nevertheless, the log phase luminescence does possess two characteristics ascribed to mitogenetic radiation. In all cases, the radiation was observed to be at a maximum when the division rate was at a maximum, i.e. during the latter portion of the log phase. Secondly, the quantum yield of the log phase luminescence is surprisingly close to unity, and although approximate, is unlikely to be in error by more than one order of magnitude. A quantum yield of one photon per cell division suggests that the emission of a photon may be closely connected with the act of cell division, rather than being an incidental chemiluminescence from metabolic and other biochemical reactions in the cell. The observed correlation between cell division and luminescence, distinguishes the present observations from those of Stauff (22, 23) who has observed a weak yeast luminescence, which was not related to growth.

The stationary phase emission in figures 1-4 is clearly separated from the log phase emission, and is reminiscent of Lepeschkin's (24) ultraviolet "necrobiotic radiation" from dying cells. Unfortunately, the Coulter counter does not distinguish between viable and non-viable cells so it was not practicable to search for a correlation between the rate of cell death and the intensity of the stationary phase emission. An alternative source of the stationary phase emission, is the weak luminescence at ca. 530 nm observed by Vladimirov et al. (14) from lipid oxidation. It is well known that lipids accumulate considerably in yeast during the stationary phase.

Synchronisation of several yeast cultures (figures 1-2) did not produce any identifiable systematic changes in light emission, although the stepwise increases in cell number were clearly apparent in the growth curves during the early portion of the log phase. The luminescence intensity showed more tendency to fluctuate during the period of the synchronous divisions, but the cell number was not sufficiently large at this stage, to produce a luminescence strong enough to resolve into the individual fluctuations found by Konev (8) to be associated with each synchronous division.

The present results indicate that a case exists for the reinvestigation of some of the mitogenetic phenomena, using modern photon and cell counting equipment, and taking due note of the current East European work in the field. It is clearly important to distinguish between luminescences which are significantly related to the act of cell division, and those which are incidental by-products of biological reactions which happen to be chemiluminescent. The authors are currently attempting to determine the spectral distribution of the yeast luminescence.

We are grateful to Professor L.E. Lyons for providing facilities in the Department of Chemistry at the University of Queensland where this work was carried out. We are also grateful to Dr. H.W. Doelle of the Department of Microbiology for advice on microbiological matters, and to Dr. E.H. White of the Department of Chemical Engineering for the use of his Coulter counter.

REFERENCES:

1. Gurwitsch, A.G., and Gurwitsch, L.D., "Die mitogenetische Strahlung", Gustav Fischer Verlag, Jena (1959).
2. Gurwitsch, A.A., Eremeyev, V.F., Karabchievsky, Yu.A., Dokl. Akad. Nauk SSSR, 178, 1432 (1968).
3. Gurwitsch, A.A., "Problems of Mitogenetic Radiation as an Aspect of Molecular Biology", Meditsina, Leningrad (1968).
4. Rahn, O., "Invisible Radiations of Organisms", Börntraeger, Berlin (1936).
5. Gray, J., and Oullet, C., Proc. Roy. Soc. B, 114, 1 (1933).
6. Lorenz, E., J. Gen. Physiol., 17, 843 (1934).
7. Hollaender, A., and Claus, D.W., Bull. Natn. Res. Coun. Wash., No. 100 (1937).
8. Konev, S.V., "Fluorescence and Phosphorescence of Proteins and Nucleic Acids", (English transl.), Plenum Press, New York (1967).
9. Konev, S.V., Lyskova, T.I., and Nisenbaum, G.D., Biophysics (USSR) (English transl.), 11, 410 (1966).
10. Barenboim, G.M., Domanskii, A.N., and Turoverov, K.K., "Luminescence of Biopolymers and Cells", (English transl.), Plenum Press, New York (1969).
11. Veselovskii, V.A., Sekamova, Ye.N., and Tarusov, V.N., Biophysics (USSR) (English transl.), 8, 147 (1963).
12. Metcalf, W.S., and Quickenden, T.I., Nature, 216, 169 (1967).
13. Doman, M.J., "A Study of Luminescent Processes in Aqueous Media with Reference to Bioluminescence", Doctoral Dissertation, University of New York at Buffalo (1968).
14. Vladimirov, Yu.A., "Ultraweak Luminescence Accompanying Biochemical Reactions", English transl., NASA, C.F.S.T.I., Springfield, Vermont (1966).
15. New Scientist, 57, 172 (1973).
16. Morton, G.A., Appl. Opt., 7, 1 (1968).
17. Malmstadt, H.V., Franklin, M.L., and Horlick, G., Anal. Chem., 44, 63A (1972).
18. Quickenden, T.I., and Que Hee, S.S., Radiat. Res., 46, 28 (1971).
19. Loevinger, R., and Berman, M., Nucleonics, 9, No. 1, 26 (1951).
20. Wickerham, L.J., Tech. Bull. U.S. Dep. Agric. Washington (1951) No. 1029.
21. E. Zeuthen (ed.), "Synchrony in Cell Division and Growth", Interscience, New York (1964) p. 589.
22. Stauff, J., and Reske, G., Naturwissenschaften, 51, 39 (1964).
23. Esser, A., and Stauff, J., Z. Naturforschung, 23b, 1554 (1968).
24. Lepeschkin, W.W., Protoplasma, 20, 232 (1933).