tration than normal, the inhibitory effect was markedly reduced when the lower limbs were excluded from the action of the agent. On the basis of these findings, it is postulated that the inhibitory effect of $HN_2$ on the Shwartzman phenomenon is the result of the systemic rather than a local action of the agent.

Studies in progress on the peripheral blood and on the histologic changes in the bone marrow after the administration of $HN_2$, suggest that protection of the marrow may be a factor in the decreased inhibition seen after the aortic occlusion.

*Summary and conclusions.* 1. Inhibition of the Shwartzman phenomenon by $HN_2$ is confirmed. 2. Protection of the preparatory site from $HN_2$ fails to influence the inhibition. 3. Protection of the lower limbs from the action of $HN_2$ by aortic occlusion decreases or prevents the inhibition.

Received June 15, 1950. P.S.E.B.M., 1950, v74.

## Effects of X-Rays on Size of Yeast Cells.* (18037)

KIRKLAND C. BRACE.† (Introduced by R. E. Zirkle)

*From the Institute of Radiobiology and Biophysics, University of Chicago.*

Enlargement of cells after exposure to ionizing radiation has been observed in many types of biological material, although it is only infrequently mentioned in the literature (1-4). There are two general explanations for this enlargement. First, it may be due to swelling, which can be defined as enlargement resulting chiefly from selective increase of one of the cell constituents, namely water. Second, it may be due to nonselective and continued formation of protoplasm and other cell constituents with a simultaneous slowing of the division rate.

The purpose of this work has been to determine whether the increased size of x-irradiated yeast cells is selective or nonselective. To obtain evidence bearing on this question, irradiated and nonirradiated cells have been compared with respect to two properties. The first is their specific gravity. This quantity is a fair indication of the amount of nonaqueous materials in the cells, since most of these are heavier than water. If the irradiated cells have a significantly lower specific gravity than the nonirradiated ones, this indicates that enlargement is at least partly due to simple swelling. On the other hand, if there is no significant difference between the specific gravities, this indicates that enlargement is due to nonselective increase in amounts of aqueous and nonaqueous material. The second basis of comparison is total nitrogen content, which may be taken as a rough index of the amount of protoplasm in the cells. If the nitrogen content of the irradiated cells is significantly lower than that of the controls, this indicates selective enlargement whereas substantially identical nitrogen contents are evidence for nonselective enlargement.

The yeast was a single-cell strain of *Saccharomyces cerevisiae* (Schenley No. 52). The cells were grown at 28°C in an aqueous solution containing 2% glucose and 0.3%

* This work was done in the laboratory of Professor R. E. Zirkle as an adjunct to work being performed under Contract No. NR 116 256 with the Office of Naval Research in cooperation with the United States Atomic Energy Commission. The author thanks the United States Public Health Service for the opportunity to carry out this research and Professor W. L. Doyle, Department of Anatomy, University of Chicago, for instruction in microchemical technics.

† Present address: National Institutes of Health, Bethesda, Md.

1. Holweck, F., and Lacassagne, A., *C. R. soc. biol.*, 1930, v103, 60.

2. Robertson, M., *Brit. J. Radiol.*, 1932, v8, 502.

3. Lea, D. E., Haines, R. B., and Coulson, C. A., *Proc. Roy. Soc., B,* 1937, v123, 1.

4. Failla, G., *Am. J. Roentgenol.*, 1940, v44, 649.

yeast extract. The source of radiation was a General Electric Maximar X-ray machine operated at 230 kv and 15 ma. A yeast culture to be irradiated was placed in a 15 ml Carrel flask and the flask was placed close to the window of the x-ray tube. No filter was used except the tube wall and window and the wall of the flask. The dose rate was approximately 850 r/min as measured with a Victoreen condenser r-meter. No attempt was made to maintain constant temperature or to agitate the material during irradiation.

As a preliminary to the studies on specific gravity and nitrogen content, irradiated and nonirradiated cells were observed under the microscope. When cells were grown in liquid medium, irradiated in the nondividing condition, and transferred to fresh liquid medium, a very characteristic pattern of changes occurred. During the first few hours after irradiation, the cells appeared normal or nearly so, except for an occasional cell that was small and shrunken. A large fraction of the cells formed doublets with a peculiar dumbbell shape. This has been observed by Lacassagne(1) and many others. During the next few hours there was a gradual increase in cell size, most of the cells being paired or in clusters of four to eight. The cells that did not enlarge were nearly all single. The maximum cell size was reached 6 to 8 hours after the onset of growth; this represents a period of 3 or 4 normal division times. During the next 4 hours the swollen cells gradually disappeared and large numbers of normal cells appeared. After 20 hours the large cells were very rare. All of these changes could be stopped by low temperature or by exhaustion of food in the medium.

In order to follow the history of single irradiated cells, cultures were grown in liquid medium diluted to 1% of the usual concentration of glucose and yeast extract, and the cells were transferred directly from the Carrel flask to the free surface of a level plate of potato-dextrose agar. A block of agar about 1 cm square was then transferred to a special ruled slide with the cells and the rulings on substantially the same plane. To prevent evaporation the square of agar was covered with a depression slide taped to the ruled slide. These agar cultures were incubated at 28°C, and several hundred individual cells and their descendants were followed until overgrowth occurred. Practically none of the cells disintegrated. Most of them progressed to the doublet stage and then started to enlarge without dividing. After a delay of several hours most of the cells were able to resume a normal division rate and returned to normal size. The large colonies derived from rapidly recovering cells soon outgrew the remainder of the colonies. The longer the delay before normal division returned, the more enlarged the cells became. A few cells were able to undergo only 3 or 4 divisions, and small colonies of giant cells resulted. Some of these colonies of giant cells gradually disappeared, presumably by lysis, while others persisted for long periods without change. The single cells which failed to enlarge were able to divide after a delay (10 hours or more).

In order to compare the specific gravities, the nitrogen contents and the mean volumes of irradiated and nonirradiated cells and their descendants, the yeast samples were cultured in liquid medium at 28°C and the following quantities were determined: 1. The specific gravity of the total suspension ($\varrho_s$) and of the medium alone ($\varrho_m$). These measurements were made with a kerosene-bromobenzine gradient tube, as described by Linderstrøm-Lang and Lanz(7) and modified for use with yeast by Atkins *et al.*(8). The standard solutions consisted of copper sulfate in distilled water and were kept under oil. 2. The fraction (F) of the culture volume occupied by the cells. This was determined by drawing a well-mixed sample of the culture into a calibrated Van Allen blood volume index tube and centrifuging until the packed cell volume became constant. 3. The amount of nitrogen per $mm^3$ of cell suspension ($Q_s$) and of medium alone ($Q_m$). These quantities were determined by the micro-Kjeldahl technic devel-

7. Linderstrom-Lang, K., and Lanz, H., Jr., *C. r. trav. lab. Carlsburg, Ser. chim.*, 1938, v21, 315.

8. Atkins, L., Feinstein, M., and Gray, P. P., *Wallerstein Lab. Communications*, 1948, v11, 289.

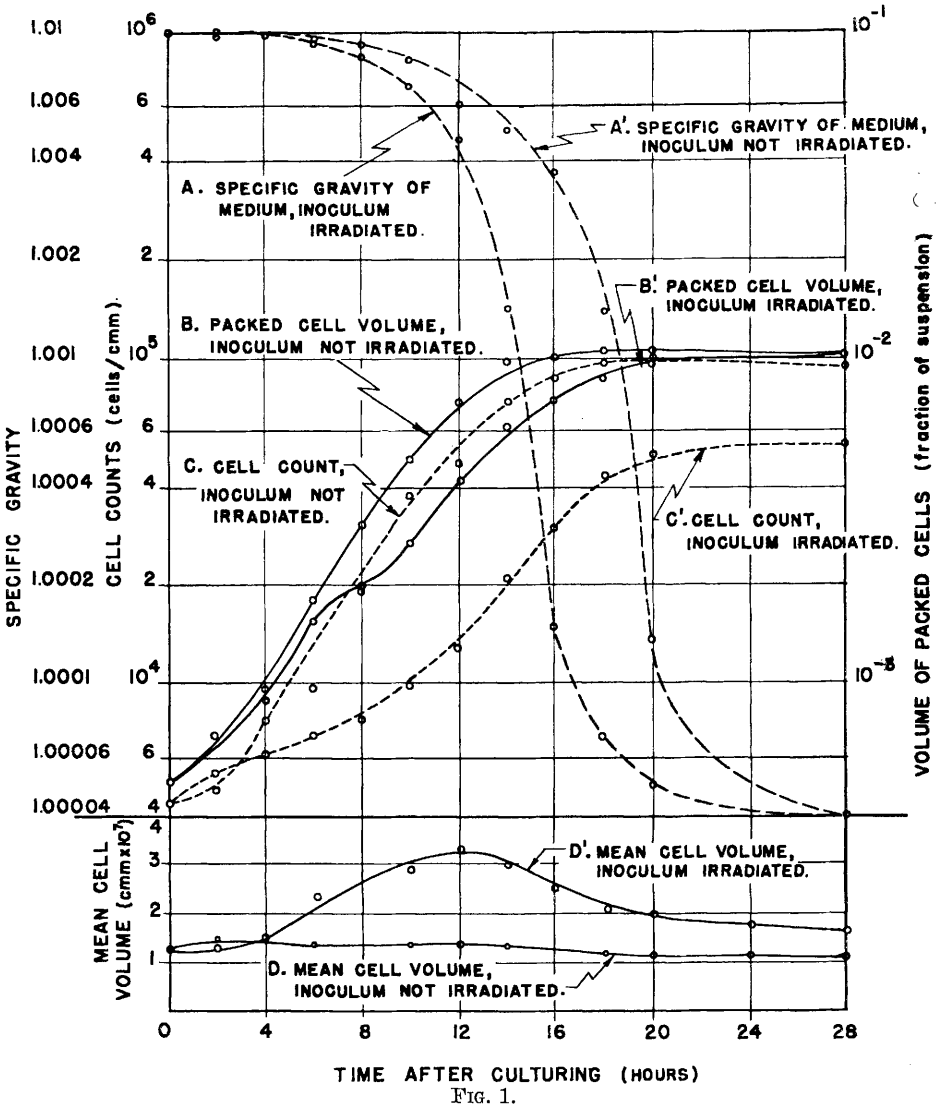


FIG. 1.

Time courses of changes in properties of yeast suspensions grown from x-rayed and non-irradiated cells.

oped by Linderstrom-Lang and coworkers and modified by Doyle and Omoto(6). 4. The number N of cells per $mm^3$ of suspension. This figure was obtained by hemocytometer count of an undiluted sample of cell suspension.

From these data the following quantities were calculated: 5. The specific gravity of the cells ($\rho_c$) was derived from the relationship

$$F\rho_c = \rho_s - (1 - F)\rho_m \qquad (1)$$

6. The nitrogen content of the cells in micrograms per cmm, was

$$Q_c = \frac{Q_s - Q_m}{F} \qquad (2)$$

7. The mean cell volume in $mm^3$ was equal to F/N.

In preliminary experiments, in which graded x-ray doses were used, it was found that maximal enlargement of cells could be produced by 20,000 r. Accordingly this dose was used in all subsequent experiments.

Further preliminary experiments were performed to determine the time course of cer-

tain changes which occur when normal and irradiated cells grow and multiply in liquid medium. A stock culture of cells was allowed to grow until the glucose in the liquid medium was exhausted. A sample was irradiated in a sterile Carrel flask, and a control sample was handled identically except for irradiation. From each of these 2 samples, 1 ml of cell suspension was immediately transferred to 25 ml of fresh sterile liquid medium in a 125 ml Erlenmeyer flask and incubated at 28°C. For 20 hours thereafter, samples were taken at intervals from both of these cultures for determination of cell count, packed cell volume and specific gravity of the medium. The results are plotted in Fig. 1. The time courses of decrease in specific gravity of the cell media are shown by the dashed curves A and A'. The chief conclusion to be drawn from these curves is that the irradiated cells and their progeny (curve A') lag about 4 hours behind the controls in their removal of glucose from the medium.‡ The packed cell volumes (solid curves B and B') increase at substantially the same rate, except that, during a 4-hour period starting about six hours after culturing, the irradiated cells and their descendants increase much more slowly than the controls (note kink in curve B'). This temporary lag may be due to lysis of some of the swollen cells. The cell counts (curves C and C') show that during the first two hours the irradiated cells divide slightly more rapidly than the controls (this was consistently observed) but that thereafter they divide more slowly. From the packed cell volumes and the cell counts, the mean cell volumes can be calculated. It will be noted that the cells derived from nonirradiated cells (curve D') remain substantially constant in mean volume but that the irradiated cells and their progeny (curve D) are maximal in mean volume about 12 hours after culturing and, with the exception of the first 4 hours, are always larger than the controls.

In each of the final experiments several different measurements had to be made on the culture derived from irradiated cells and on its control. Since the cultures changed so rapidly during the logarithmic growth phase, good comparative measurements would have been very difficult to obtain during this period. This difficulty was overcome by so adjusting the amount of irradiated (or control) suspension transferred to fresh medium that the glucose became exhausted and growth stopped when the mean cell volume was approximately maximal. In this way samples of large cells could regularly be obtained at the time when the difference between the specific gravity of the suspension and that of the medium was maximal. The cell counts and the packed cell volumes were first determined. If the mean cell volume of the irradiated cells and their progeny was at least twice that of the controls, each culture was divided into 2 portions, one of which was centrifuged to obtain a sample of cell-free medium. The specific gravity and the nitrogen content of each sample were then determined, each measurement being performed in triplicate.

Two complete experiments were carried out as just described, and the experimental measurements are presented in Table I along with the quantities which can be calculated from them—namely the specific gravity of the cells alone and the nitrogen content of the cells. It will be noticed that in both experiments the specific gravity and the nitrogen content of the irradiated cells and their progeny are slightly but consistently less than the controls. The individual differences are probably within the experimental error of the measurements of specific gravity differences and of nitrogen content, the triplicate determinations of which deviate as much as 5 per cent from the mean. The consistency of direction of the differences is probably due to the tendency of the enlarged irradiated cells to clump and thus fail to pack as well as the controls. The corresponding values of packed cell fraction F would thus tend to be high, and the calculated values of nitrogen content ($Q_c$) and specific gravity ($\varrho_c$) would therefore tend to be low, as observed. However, these discrepancies, compared to the large differences in cell volume, are small, and

‡ The decrease in specific gravity is a fairly good index of glucose depletion despite the complication of alcohol formation.

TABLE I.
Comparison of Cells Derived from Normal and Irradiated Yeast Cells.

| | Exp. 1 | | Exp. 2 | |
|---|---|---|---|---|
| | Normal | Irradiated | Normal | Irradiated |
| Cells per cmm of culture (N) | 101,300 | 46,400 | 153,000 | 69,000 |
| Fraction of culture volume occupied by packed cells (F) | 0.0108 | 0.0115 | 0.0147 | 0.0134 |
| *Mean cell volume* in cmm × $10^7$ (F/N) | 1.07 | 2.48 | 0.96 | 1.94 |
| Nitrogen content of cell suspension in $\mu$g/cmm ($Q_s$) | 0.318 | 0.323 | 0.382 | 0.384 |
| Nitrogen content of medium in $\mu$g/cmm ($Q_m$) | 0.094 | 0.109 | 0.091 | 0.135 |
| *Nitrogen content of packed cells* in $\mu$g/cmm ($Q_c$) | 20.7 | 18.6 | 19.8 | 18.6 |
| Specific gravity of suspension ($\rho_s$) | 1.000674 | 1.000632 | 1.000787 | 1.000648 |
| " " " medium ($\rho_m$) | 1.000010 | 1.000016 | 0.999886 | 0.999861 |
| *Specific gravity of cells* ($\rho_c$) | 1.061 | 1.054 | 1.061 | 1.060 |

one may regard the data for the irradiated and control cultures to be substantially equal.

Accordingly it is to be concluded that, in the case of the yeast cell, the enlargement observed after irradiation is chiefly if not entirely due to a nonselective increase in water and nonaqueous constituents. This nonselective increase probably proceeds through normal mechanisms which are much more resistant to irradiation than is the mechanism of cell division.

So far as comparisons can be made, a similar state of affairs was produced in the protozoan *Bodo caudatus* by Robertson(2). However, the enlargement of certain types of cells, *e.g.* erythrocytes(5), after irradiation seems to be due almost entirely to uptake of water (swelling). Failla(4) has discussed this phenomenon and has offered a theory to explain it.

*Summary.* When yeast cells were exposed to 20,000 r of x-rays and cultured in liquid medium, they and their descendants attained a mean cell volume more than twice that of cells derived from nonirradiated controls. On the other hand, there were no corresponding differences in specific gravity and nitrogen content per unit of cell volume. It is accordingly concluded that enlargement of yeast cells after irradiation is largely a nonselective increase in cell constituents rather than a swelling due mostly to uptake of water.

5. Ting, T. P., and Zirkle, R. E., *J. Cell. and Comp. Physiol.*, 1940, v16, 189.

6. Doyle, W. L., and Omoto, J. H., *Analytical Chem.*, 1950, v22, 603.

Received June 19, 1950. P.S.E.B.M., 1950, v74.

## Survival of *Trichomonas vaginalis* in Vaginal Discharge.* (18038)

JOHN F. KESSEL AND CONSTANCE F. THOMPSON

*From the Department of Microbiology, School of Medicine, University of Southern California and the Pathology Laboratory, Los Angeles County Hospital.*

The transmission of *Trichomonas vaginalis* is partially dependent upon the ability of the flagellates to survive in natural discharges. Vazquez-Colet and Tubanqui(1) reported that *Trichomonas vaginalis* would survive in a semi-dry state for 6 hours and Swift(2) stated that the trichomonads could be cultured from vaginal discharge that had been dried for 3 hours. Kirby(3) reported that most trichomonads in vaginal discharge under paraffin-sealed cover-slip preparations kept at room temperature survived for 24 hours

* Aided by a grant to the Microbiology Research Fund, by Protecto Products Co.

1. Vazquez-Colet, A., and Tubanqui, *Med. J. Philippine Islands, M. A.*, 1936, v16, 231.

2. Swift, B. H., *Med. J. Australia*, 1937, v1, 123.

3. Kirby, H. J., *J. Parasit.*, 1943, v29, 422.