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RADIATION EFFECT ON THE PERMEABILITY OF YEAST CELLS TO SODIUM AND POTASSIUM IONS

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Kwan Hsu

Thesis

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ABSTRACT

The movement of potassium ions from the cell to the outside medium where the potassium concentration is lower than that inside the cell, is often considered as a diffusion process, whereas the movement in the opposite direction is defined as active transport. If the process is pure diffusion then the amount of potassium leakage should be expected to be a linear function of the cell surface area, and if there is energy involved in the active transport then the amount of potassium uptake is expected to be a linear function of the cell volume.

Haploid, diploid, tetraploid, and hexaploid cells of a related polyploid series of yeast, Saccharomyces cerevisiae, were used in this study. The cells have characteristics surface areas and volumes, which increase with ploidy. The dry weight, the ash weight, the potassium and the sodium contents of the cells were determined. The effects of nitrogen, oxygen, and dextrose on the uptake and leakage of sodium and potassium ions were studied with and without radiation.

The potassium content of the cells was proportional to the dry weight, whereas the sodium content was proportional to the cell surface area with the exception of the haploid.

The potassium leakage from the cells into the distilled water medium was found to be largely due to a diffusion process. The amount and the time rate of K^+ loss were larger and higher in cells of larger ploidy and with higher dose. The K^+ leakage was proportional to the cell surface area in both control and irradiated cells.

In the presence of dextrose, active transport took place. The ratio of the potassium uptake by the hexaploid to that by the diploid was 3:1, the same as the ratio of their respective volumes. Oxygen also initiated the active transport process. Nitrogen did not have any obvious effect on the leakage and the uptake of potassium.

The order of the radiosensitivity for potassium retentivity of cells of different ploidies does not follow the same sequence as that of lethality. Radiation up to 107 kr did not seem to impair the active transport process.

In contrast to the behavior of potassium ions, loss of the sodium ions from the cells to the distilled water medium was not found to vary with dose. The outward movement of the sodium ions could not be explained as primarily due to a diffusion process. Nitrogen and dextrose did not have any obvious effect on the sodium leakage. Oxygen, however, was found to increase the rate of sodium leakage.

INTRODUCTION

Permeability phenomena of the membrane of living cells often attract the attention of biochemists and biophysicists, as well as physiologists. For growth and maintenance of life, the cells have to take in various substances from the bathing medium and to remove metabolites and waste products to the extracellular fluid.

The term "permeability," in its strict sense, is a description of the property of the cell membrane which allows molecules or ions to pass in and out. It is also a measure of the quantity that passes through the membrane. The permeability phenomena, therefore, are those related to molecular and ionic transports, including the electrical conductivity of the cell membrane and the membrane potential, etc. Much work has been done on the permeability and the permeability phenomena of the cell membrane. Review particles are found in many journals and books. Examples can be found in Annual Review of Biochemistry (1-4), Annual Review of Physiology (5-14), Annual Review of Plant Physiology (15-20). A few books and symposia are devoted to the same subject matter (21-27).

Permeability studies have been done on many organisms with various methods. For example, for permeability to water, Jacobs and Stewart observed the osmotic volume change (28), Hevesey, Hofer, and Krough used heavy water as tracer (29), and Pigon and Zeuthen used Cartesian Diver Balance (30). For permeability to monelectrolytes, Collander used a microchemical method (31); to weak base, Ayrapää used staining (32); to salts, Scarth used the plasmolytic method (33). Paulson, Sylven, and Hirsch used the interferometric method to determine the diffusion rate into cartilage and other mesenchyme tissue (34). For permeability to ions, one may use the conductivity as a measure of permeability (35). Cole determined the impedance (36), Hodgkin and Huxley measured the membrane potentials (37), Levi and Ussing used tracers (38), and Lehman and Krusen used ultrasound to study the ion transport through biological membranes (39). Besides experimental studies many people approached the problem mathematically (40-43) and theoretically (44-51). Many models and hypotheses have been suggested to explain the transport mechanisms. For example: Dean suggested a sodium (and potassium) pump (52); Franck and Mayer, a diffusion pump (53); and Conway, a redox pump (54 a, b). Danielli postulated activated diffusion (55); Osterhout, a membrane carrier (56); and Ling, fixed-charge hypothesis (57). Teorell formulated the "fixed charge" membrane theory (58) and Papenheimer, Renkin, and Borrero, pore theory (59). Patlak expounded the "Maxwell Demon," the gate-type noncarrier mechanism (60).

Yeast is one of the many organisms of which the membrane permeability has been studied for quite a long time. One of the earliest investigations was done by Paine (61), who in 1911 studied the differential permeability of yeast cells to various sodium salts. In recent years, the permeability studies on yeast have been concentrated on the permeability to cations, especially to potassium and sodium ions. These two ions are of interest because most cells contain higher concentration of potassium and lower concentration of sodium than the extracellular fluids. Extensive work in this field has been done in this country by Rothstein and his associates (62-69), and in Ireland by Conway and his associates (70-82). Both Rothstein (62) and Conway (71) found that during fermentation potassium ions moved into the cell in exchange for hydrogens ions. As a consequence, the pH of the unbuffered external medium reached a value of 1.78 and even lower with prior oxygenation (83). The source of the H^+ ions was found to be the succinic acid (72). Both investigators believe that there is a carrier system that actively transports the potassium ions into the cell. In the absence of potassium ions in the external fluid, other monovalent cations can also be transported into the cell by the same carrier in exchange for hydrogen ions (64). Conway determined the relative affinities of the potassium carrier for various cations. In descending order, they are K^+, Rb^+, Cs^+, Na^+, Li^+, and Mg^{++} (80); the affinities of K^+ and Na^+ are in the ratio of approximately 25/1. Although the K^+ carrier actively transports Na^+ into the cell, Conway demonstrated that there is another carrier responsible for the Na^+ extrusion (77). He was able to explain the transport mechanism of the two carriers by his redox-pump theory (83-84). From the permeability studies on yeast cell, Rothstein concluded that there are three carrier system: one for the inward transport of K^+, one for the outward transport of Na^+, and a third for the uptake of phosphate ions. He pictured the cell wall as composed of two compartments: the outer one contains the glycolytic machinery of the cell and the inner contains the three carriers or pumps (64, 65, 68).

The effect of radiation on the permeability of living cells has attracted many cell physiologists. Earlier work on this subject was reviewed by Heilbrun and Mazia (85). The early work on the radiation effect on the permeability of yeast cell was done by Hevesey and Zerahn (87). An LD_{90} X-ray dose of 30,000 r caused free P^{32} and K^{42} to migrate from the cell into the solution. With a partial lethal dose of uv, free P^{32} and organic-acid-soluble P^{32} were found to migrate from the cell into the solution, but there was no change in the migration of K^{42}. More recently, studies of the permeability of yeast to sodium and potassium ions have been extended to the effect caused by radiation; see, for example, Sanders (87) and Bruce (88). Sanders found that yeast cells that had been starved in 0.05 M of NaH_2PO_4 and irradiated in NaCl suspension with uv consisting 85% of 2537 A, accumulated Na^+ ions but lost K^+ ions to the medium. The permeability constant of Na^+ ions in the control cells was found to be 2.6×10^{-10} cm/sec, while that of irradiated cell was 1.2×10^{-8} cm/sec. The value for the irradiated cell is almost 50 times that for the control cell. Various studies indicated that the observed effect could be explained by the existing membrane theories. Bruce (88) found that X-rays increased the potassium leakage and decreased the potassium uptake. He studied the effects of various X-ray doses, inonic environments, substrates, and temperatures upon the potassium uptake and leakage. His results can be found in various reports (89-93) and were reviewed by Rothstein (94). Among the interesting results are those on the effect of temperature on K^+ transfer. He calculated the values for the Arrhenius constants for the uptake and the leakage of potassium in the irradiated and control cells. The value for potassium uptake was found to be higher in the irradiated cells, and that for leakage was lower. He concluded that the higher value for potassium uptake indicates that more energy is required for uptake of potassium after irradiation, and the lower value for leakage indicates an association with a diffusion process (88, 94).

From the above studies, it is apparent that the movements of sodium and potassium ions are influenced by physiological conditions and are related to membrane phenomena. Whatever the process is diffusion or active transport, it is of interest to study the movements of the potassium and sodium ions for cells of different ploidies under similar conditions, and the effect of radiation on the movements. Cells of different ploidies have characteristic surface areas and volumes, which increase with higher ploidy. If the process is strictly diffusion then the amount of leakage, or the permeability constant, should be a function of the surface area; whereas if there is any energy involved, as in active transport (or leakage), the uptake might be expected to be a function of the volume. The study of the dose effects on lethality and potassium leakage may indicate whether there is any correlation between the two.

MATERIAL AND METHODS

YEAST CULTURE

The cultures used were from a related polyploid series of Saccharomyces cerevisiae. They were S163A (haploid), X320 (diploid), X323 (tetraploid), and X362 (hexaploid) developed by Mortimer (95). These cells can reproduce vegetatively by budding. In this investigation, they were grown in liquid medium and harvested at the stationary-phase state of growth. To test the ability of colony formation, they were plated on agar medium in the conventional method.

GROWTH MEDIUM

1. Liquid Medium (PDB)

Difco Potato Dextrose Broth	4%
KH_2PO_4	0.9%

They were autoclaved separately for 15 minutes under 15 lb pressure.

2. Solid Medium (YEAPD) for slants and plates

Yeast extract	1%
Peptone	1%
Dextrose	2%
Agar	2%

Adenine 20 mg/1000 ml of final solution.

PREPARATION OF THE YEAST

The yeast was grown in the PDB liquid medium at 30°C. Cells grown in this medium were found to have very few budding cells in the stationary phase of the growth cycle (96). For inoculation, a distilled water suspension was made from a 24-hour culture on YEAPD slant that had been incubated at 30°C. The inoculum was adjusted to approximately 10^5 cells/ml of the growth medium. For each experiment 1000 ml of the medium was used, and divided among five 1-liter Erlenmeyer flasks, which were fitted on a rotary shaker. The cells were harvested 36 hours after inoculation. They were centrifuged to separate the cells from the growth medium. The cells were then washed twice with sterile distilled water. Each washing was done by suspending the cells in 600 ml of water and recentrifuging. In order to keep the cells under similar conditions for different experiments, each centrifugation was done at 1500 rpm for 10 minutes. In some experiments, the growth medium and the supernatant fluid from the washing were kept for Na and K determinations. After washing, the cells were again suspended in sterile distilled water. An aliquot of the suspension was used to determine the cell concentration, the viability, the dry weight, and the ash weight. Cell concentration was determined by counting the cells in a haemocytometer. Viability was expressed in percentage as the ratio of the colonies formed from a plating of a serial dilution and the cell count. The remainder of the suspension was kept in the refrigerator (5°C) until it was ready for irradiation. The experimental procedure is shown diagramatically in Fig. 1.

DRY WEIGHT AND ASH WEIGHT DETERMINATION

An aliquot was pipetted into a platinum dish, which was placed inside a glass container or beaker. A large watch glass was put on top of the beaker in such a manner as to prevent foreign particles from falling in but to allow the vapor to escape. The beaker with its contents and the watch glass was put in a drying oven set at 65°C. When the water had all evaporated and the cells dried, the dish was transferred to a desicator until it was cooled. The difference in the weights of the dish with and without the cells gave the dry weight. The platinum dish was then ashed in a muffle furnace at 550°C. The ash weight was determined when the dish was cool. The ash content of the cells was collected by washing the dish several times with 0.1 N HCl. The solution was transferred directly into a volumetric flask and diluted to 10 ml. It was set aside for the determination of the Na and K contents of the cells.

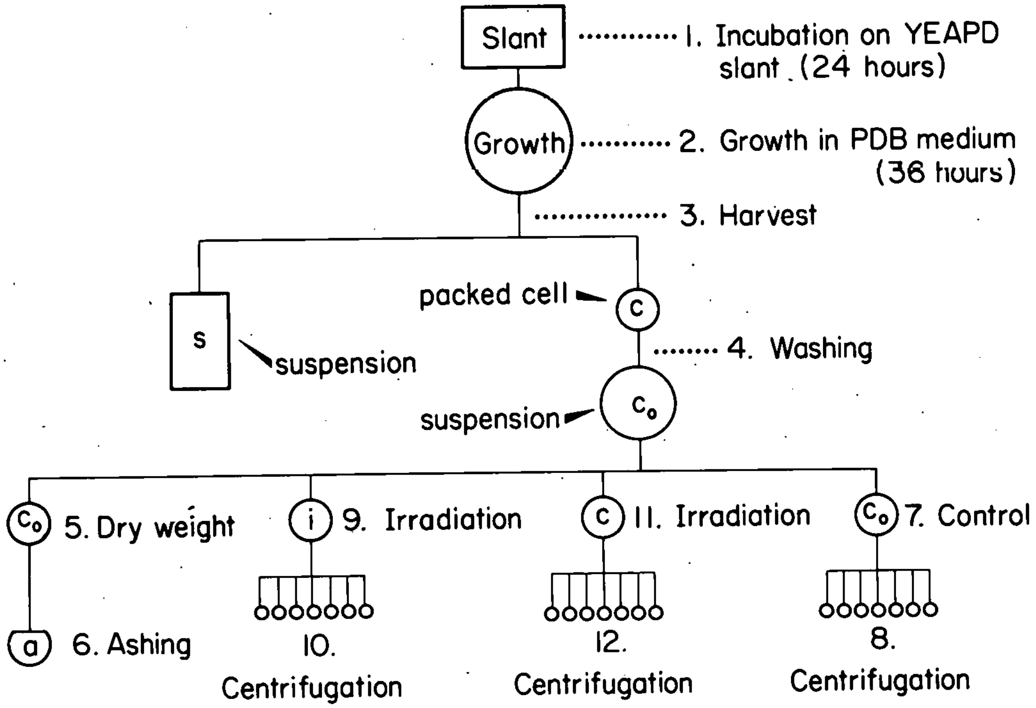

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Fig. 1. Schematic diagram of the experimental procedure.

Both the dry weight and the ash weight were expressed in micrograms per million cells ($\mu g/10^6$ cells), while the Na and K contents were expressed in $\mu g/10^8$ cells.

IRRADIATION

Irradiation was carried with a Philips 250-kv dc X-ray machine, operated at 250 kv at 15 ma with an external filter of 0.5 mm of Al. The inherent filtration of the machine was equivalent to 2mm of Al. The cell suspension was pipetted into two identical glass vials 4-1/8 in. in length and 1 in. in diameter. Each sample contained 14.5 ml of the suspension. The sample to be irradiated was placed 1 in. from the face of the filter holder, which is 19 cm from the focal spot. The control sample was put 2 meters away from the X-ray tube and was shielded with 1 in. of lead. Each glass vial was provided with a steel cap through which a glass tube was introduced for aeration. To maximize the effect of radiation, oxygen was bubbled through the suspension. The rate of flow was measured with a Fischer and Proctor's TRI-FLAT Flow Meter (02F-1/8-12-5) and adjusted to 500 ml per minute. Dose-rate determinations were made with various Victoreen thimble chambers of 250 r and 500 r range. The chamber was inserted in the middle of an empty glass vial identical with the one used for irradiation. The thimble was positioned at exactly the center of the volume occupied by the suspension. The average dose rate was found to be 1.34 ± 0.01 kr per minute.

Immediately after irradiation, an aliquot was taken out from each sample to determine the cell concentration and viability. A new determination of the cell concentration was necessary because of the possibility of evaporation. The survival of the irradiated cells was expressed in percentage as the ratio of the viabilities of the irradiated cells and the control cells. As soon as an aliquot was taken out from each sample the remainder was pipetted into four to seven centrifuge tubes in equal portions. In the later experiments, seven tubes were used. Each tube contained 2 ml of the cell suspension. At certain predetermined times one tube from each sample was centrifuged for 10 minutes at 2300 rpm. The supernatant fluids were transferred into different tubes for the determination of Na and K "leakage." To reduce sodium contamination from glassware, plastic centrifuge tubes were used in later experiments.

DETERMINATION OF THE SODIUM AND POTASSIUM CONTENTS

The sodium and potassium contents of the cells and of the supernatant fluids were determined by (1) neutron-activation analysis and (2) flame photometry.

1. Neutron-Activation Analysis

The method consists in exposing the sample to thermal neutrons, which by nuclear (n, γ) reaction makes many elements radioactive. From the characteristics of the radioisotopes thus induced, the parent elements can be quantitatively determined. The number of atoms of one particular element originally present in the sample can be determined, from the equation:

$$N = \frac{\text{Disintegration rate per second}}{f\,\sigma_{act}\,(1-e^{-\lambda t})}, \qquad (1)$$

where

f = neutron flux in neutrons/cm^2-sec,
σ_{act} = activation cross section in cm^2,
λ = decay constant, fraction disintegration in reciprocal time,
t = irradiation time,

and the disintegration rate is that value determined immediately after the irradiation and corrected for the efficiency of the counter. For many elements this method is much more sensitive than other microchemical methods (97).

The reactor used was the Livermore Water Boiler Reactor. It is a thermal homogeneous light-water research reactor. It was operated at 500 watts and the maximal flux at the center of the spherical core (glory hole) was given as 2.4×10^{10} neutrons/cm^2-sec. (98). The flux distribution in the core could be determined from the equation

$$\phi = \phi_0 \frac{\sin k \cdot r}{k \cdot r} \qquad (2)$$

where ϕ_0 is the flux at the center, r is the distance from the center of the core, and k is a constant with a value of 0.31 (98).

Each sample was packed in a small polyethylene vial 1 in. long and 9/16 in. in diameter (Celluplastic, Newark, N. J.). Six samples could be irradiated at the same time. Six vials were in turn packed in a large polyethylene tube provided with a screw cap. The tube was 1 in. in diameter and 7 in. in length. It was graduated in tenths of an inch so that the exact location of each sample could be noted.

The samples were irradiated for 2 hours. Aliquots from each sample were taken out for counting with a G–M counter (Tracerlab., TGC 2/1B84) or in a well-type scintillation counter (Harshaw, Type 7F8), or both. Gamma-ray spectra of each sample were taken with a Nuclear Chicago Recording Spectrometer (Model 1820).

From the (n, γ) reaction, the radioisotopes that can be produced from sodium and potassium are Na^{24}, K^{40}, and K^{42}. Sodium found in nature consists of 100% Na^{23}, and potassium consists of 93.08% K^{39}, 0.012% K^{40} and 6.91% K^{41} (99). For a 2-hour bombardment the activity of K^{40} is practically nil because K^{40} has a half life of 1.3×10^9 years. Therefore, from the activity of Na^{24} and K^{42} the original amount of sodium and potassium in the sample can be calculated.

When a sample consists of only two isotopes one can in general determine the activity of each from the composite decay curve. The values of the half lives of Na^{24} and K^{42}, however, are 15.0 and 12.4

hours respectively. Their difference is so small that one cannot determine their activities very accurately by analyzing the decay curve.

Since both isotopes are β emitters and go through gamma transitions to the ground states, one can determine the activity of each by analyzing the β-ray absorption curve and the γ-ray spectrum of the sample. Na^{24} emits β rays of 1.39 Mev and K^{42} emits β rays of 3.55 Mev and 2.04 Mev (100). If one uses an absorber thick enough to "completely" absorb the 1.39-Mev β ray emitted by Na^{24} and if one knows what percentage of the activity due to K^{42} is absorbed, one can calculate the activity of each isotope in the sample. Since Na^{24} has two γ rays of 1.38 Mev and 2.75 Mev, while K^{42} has also two γ rays of 1.53 Mev and 0.32 Mev, it is impossible to choose an absorber that will "completely" absorb the activity due to Na^{24}. In general, if A_1 and A_2 are the activities of the two isotopes detected in the G-M counter without the absorber (except the inherent absorber consisting of the window and the air path), while $p_1 A_1$ and $p_2 A_2$ are those obtained with the absorber, then the values of A_1 and A_2 can be determined by solving the simultaneous equations

$$A_1 + A_2 = C_1 \qquad (3\text{-a})$$

$$p_1 A_1 + p_2 A_2 = C_2 \qquad (3\text{-b})$$

where C_1 and C_2 are the counting rates obtained with and without the absorber and corrected for the efficiency of the counter; p_1 and p_2 are the fractions of the activities remaining after absorption. The p_1 and p_2 values could be obtained experimentally by using pure Na^{24} and K^{42}. Results using the above method to determine known amounts of Na^{24} and K^{42} showed an error of more than 10%.

Another way to determine the activities of the two isotopes is by analyzing the γ-ray spectrum of the sample, provided the machine is able to resolve the two peaks, at 1.38 Mev for Na^{24} and at 1.53 Mev for K^{42}. Resolution of the two peaks depends not only on the inherent resolving power of the machine but also on the shapes of the individual spectra and the relative counting rates of the two isotopes. For Na^{24} and K^{42}, the Compton edge of the 1.53-Mev γ ray falls in the neighborhood of the 1.38-Mev photopeak of Na^{24}.

The relative counting rates of the two isotopes produced from equal molarity of sodium and potassium are

$$\frac{C_{Na}}{C_K} = \frac{\sigma_{act}^{Na^{23}} \; N_{Na} \; (1 - e^{-\lambda_{Na} t}) \epsilon_{Na}}{\sigma_{act}^{K^{41}} \; N_K \; (1 - e^{-\lambda_K t}) \; \epsilon_K}$$

where N_{Na} and N_K are the values of the natural abundance of Na^{23} and K^{41} respectively and ϵ_{Na} and ϵ_K are the efficiencies of the counter for the two isotopes. For a 2-hour bombardment the ratio becomes

$$\frac{C_{Na}}{C_K} = \frac{0.56 \times 100 \times 0.0879 \; \epsilon_{Na}}{1 \times 6.91 \times 0.1058 \; \epsilon_K} = 6.74 \; \epsilon_{Na}/\epsilon_K \quad .$$

In the yeast cells, however, the potassium content is much higher than that of the sodium, so that the ratio becomes less. With the Compton edge of the γ-ray from K^{42} overlapping this photopeak of Na^{24}, it becomes more difficult to determine the two activities.

The above methods are tedious and their accuracy depends upon the reproducibility of the calibration. In the presence of other radioisotopes that might be induced in the sample, the analysis becomes more cumbersome.

Another method of analysis made use of the chemical separation of Na^{24} and K^{42} by ion-exchange resin. Dowex 1-X4 (EDTA form) cation exchange resin (Bio-Rad Laboratories) was used. It was mixed with 2.5×10^{-3} M of ethylenediaminetetraacetic acid (EDTA) and adjusted to pH 11. The resin was packed in a column 1.6 cm in diameter and 18 cm long to a height of 10 to 12 cm. A solution containing Na^{24} and K^{42} was placed on the top of the column and 2.5×10^{-3} M of EDTA solution at pH 11 was used as the eluting agent. The eluant was fed into the column by a band of eight 100-ml syringes connected in parallel to an automatic syringe feeder which was run by a motor at 1 rpm. The eluate was allowed to flow through a polyethylene tube 1.5 mm in diameter, which was coiled in the middle around a spool. The spool was snugly fitted inside the well of the scintillation counter. The counter was connected to a scaling unit and a recorder (Leeds and Northrup Speedomax). From the chart speed of the recorder and the tracing of the activities detected by the counter as each isotope flowed through the coil, one could determine when to collect the portions that contained the two activities.

For calibrating the column a 2-hour bombardment of NaCl and KCl solutions were prepared in a concentration of 0.1000 g/ml each and then diluted after the bombardment so that the sodium contamination in the distilled water could be reduced. In one particular experiment, 10^{-4} g of NaCl in 200 λ (0.200 ml) and 10^{-3} g of KCl in 200 λ were used for the calibration. For a column 9 cm in height and a flow rate of 0.67 ml/min., K^{42} activity appeared in 15 minutes and flowed through the coil in 10 minutes, while Na^{24} activity went through the coil between 32 minutes and 68 minutes from the beginning of the elution. Recovery for both isotopes was 95.9%.

To determine the amount of Na^{24} and K^{42} in a sample, the unknown solution was put on top of the column and the portions of the total eluate that contained the activities were collected and counted. By comparing the activities of the unknown and the "standard" of the corresponding portions, the amount of the Na ions (or the K ions) could be determined.

2. Flame Photometry

The method consists in comparing the intensities of the emission line of one ion in two samples. If one sample contains a known concentration of the particular element then the concentration of the same element in the other sample can be calculated. Precision and accuracy of this method depend upon the operation of the instrument, such as the operation of the atomizer, the burner, the optical system, and the photodetector system. Spectroscopic interferences and fluctuation in flame temperature are sources of errors. A very good review on this subject was written by Gardner (101). Two photometers were used in this investigation: Baird Associates Flame Photometer and Beckman DU Flame Spectrophotometer.

The Baird Flame Photometer is a "double beam" instrument. The optical system consists of two sets of optical components, arranged at 90 deg. to each other. In each light path there is a barrier-layer cell type of photodetector and a filter that selects the characteristic flame radiation to be transmitted. The two photodetectors send the signals to the same galvanometer from opposite directions. These two signals can be brought into balance by adjusting the potentiometer. In analyzing with this instrument a calibration curve must be constructed.

One prepares a series of solutions containing various calibrated amounts of the element to be determined. To each sample is added a fixed quantity of lithium, which is used as an "internal standard." When the sample is placed at the position where the axes of the two optical components meet, one set of optics transmits the characteristic flame radiation of the element to be determined. The other set transmits the charactieristic flame radiation of lithium. The two signals are brought into balance and the potentiometer scale reading is noted for the corresponding concentration of the element in the sample. A curve is then plotted of the potentiometer readings against the concentrations, and this constitutes the calibration curve. To determine the concentration of the element in a sample, the same fixed quantity of lithium is added, the two signals of the two detectors are brought into balance, and the potentiometer reading is recorded. The concentration of the sample can be determined from the calibration curve. For Na and K in the range from 0 to 0.4 milliequivalent/liter the calibration curves are straight lines. The operating range of this instrument is from 0.1 to 50 ppm (parts per million) for both Na and K, or 0.004 to 2 meq/l for Na and 0.0025 to 1 meq/l for K (102).

The Beckman DU Spectrophotometer can be converted into a flame photometer by putting in the atomizer-burner attachment. The optical system has many advantages over the Baird Flame Photometer. It contains a prism that can separate the different emission lines. The fuel is hydrogen and the flame can be brought to a higher temperature with this instrument. The photodetector system is extremely sensitive. It consists of two phototubes connected to a photomultiplier unit. The two tubes are sensitive to two different ranges of the spectrum. The detection limit for sodium at 5893 A is stated to be 0.0002 ppm and that of K at 7665 A to be 0.001 ppm (103). To analyze with this instrument also a calibration curve must be constructed. In this case no internal standard is used. The plot of the dial readings versus the concentrations of an element constitutes such a curve. Only for very low concentrations is the calibration curve a straight line.

Since the Beckman flame photometer provides a high sensitivity for the detection of Na and K, practically all results were obtained with this instrument. Results are found to be reproducible and they could be obtained with good accuracy and in less time than with the Baird flame photometer or with the neutron-activation analysis.

RESULTS AND DISCUSSION

GROWTH CHARACTERISTICS

The diploid (X320), the tetraploid (X323), and the hexaploid (X362) cells showed similar growth characteristics. The average generation time for each culture during the exponential growth phase was found to be 1.2 hours. They all reached the stationary phase within 15 hours after inoculation, when the inoculum was adjusted to an initial cell concentration of 10^5 cells/ml. At 6 hours after inoculation the number of the cells showing doubles and buds reached a maximum, then it decreased rather rapidly. At the maximum these cells occupied about 50% of the population. The pH of the growth medium was 4.8 at the beginning. It remained constant at this value for about 6 hours and then decreased to 3.8 to 3.6 as the cells were reaching the stationary phase. During the stationary phase the pH remained constant for many hours. The growth curves and the pH curves for the three strains can be superimposed on one another, except that each reached a different maximal value at the stationary phase, cells of higher ploidy grew to a lower value. The packed-cell volumes were 6.2, 7.6, and 6.3 ml respectively. The growth characteristics of X320 are shown in Fig. 2.

The haploid strain (S163A) is a "clumpy" strain. The cells grew in clusters. During the exponential phase the clusters were rather large; some had more than 14 cells each. The clusters could not be broken up by shaking the suspension with glass beads, nor could they be dispersed by suspending the cells in equal volume of 20% maltose solution, as suggested by Eddy (104). However, at the stationary phase, both the numbers and the sizes of the clusters were much reduced, and they could be further reduced by diluting the suspension with water. The generation time was estimated to be 1.8 hours. Because of their slow growth the cells did not reach the stationary phase until 33 hours. The cell concentration and the packed-cell volume were much smaller than those of the other three strains.

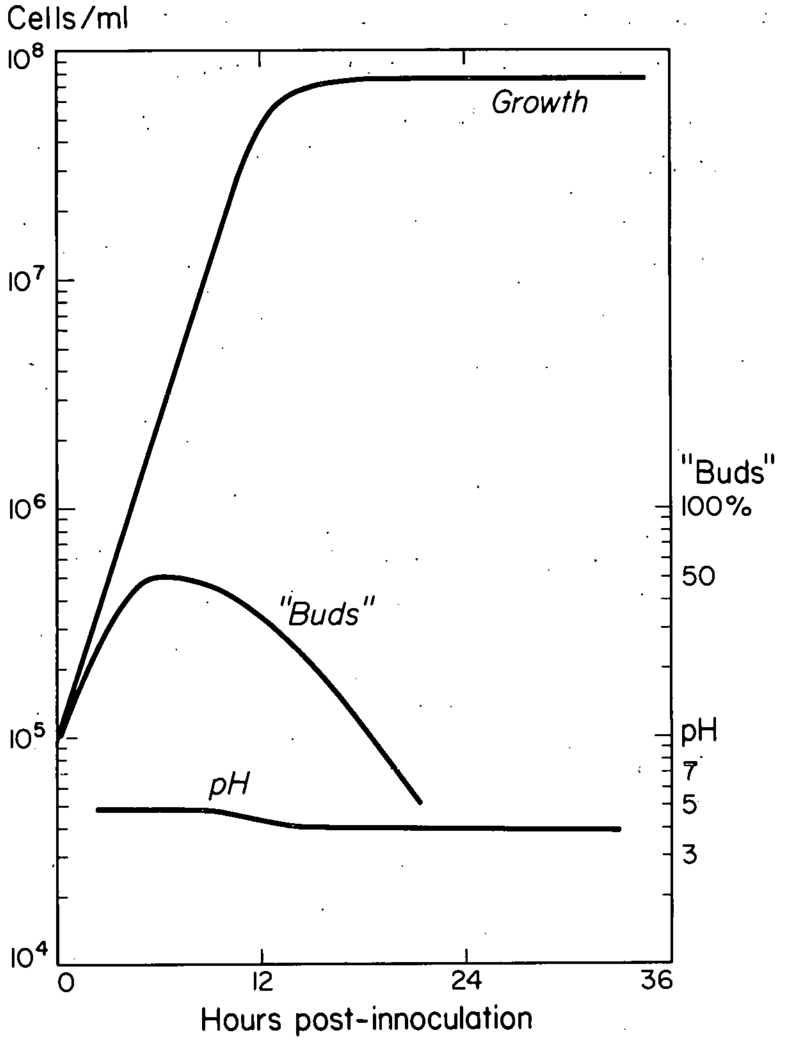

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Fig. 2. Growth characteristics of diploid (X320) in PDB medium.

To test the effect of PDB medium on the reduction of the buds, another haploid strain, S288C, which is closely related to the diploid X320, was grown both in PDB medium and in synthetic medium (Difco Nitrogen Base Special + dextrose + methionine). It was found that the cells grown in PDB medium had a much lower bud count than in the synthetic medium, 8% as compared with 40% at 26 hours. The growth characteristics are shown in Figs. 3 and 4. From the curves one notices that S288C grown in PDB showed growth characteristics similar to the diploid strain X320. The curves can be superimposed.

The data in Table I show a comparison of the characteristics of the different strains. The "buds" are the cells that appeared as doubles and those that had buds attached to them. The numbers in the column under "buds" are the ratios of the number of doubles plus buds to the total number of cells. The cell concentration at 36 hours for S288C was higher in the table than that given in the curve, because the cells were agitated differently during growth.

Table I

Characteristics of cells of different ploidies

Strain (Ploidy)	Cell concentration at 36 hrs (No./ml)	Buds (%)	Packed-cell volume (ml)
S288C (1n)	1.36×10^{8}	9	6.2
S163A (1n)	4.67×10^{7}	15	2.7
X320 (2n)	7.27×10^{7}	5	6.3
X323 (4n)	3.90×10^{7}	1	7.6
X362 (6n)	2.83×10^{7}	1	6.8

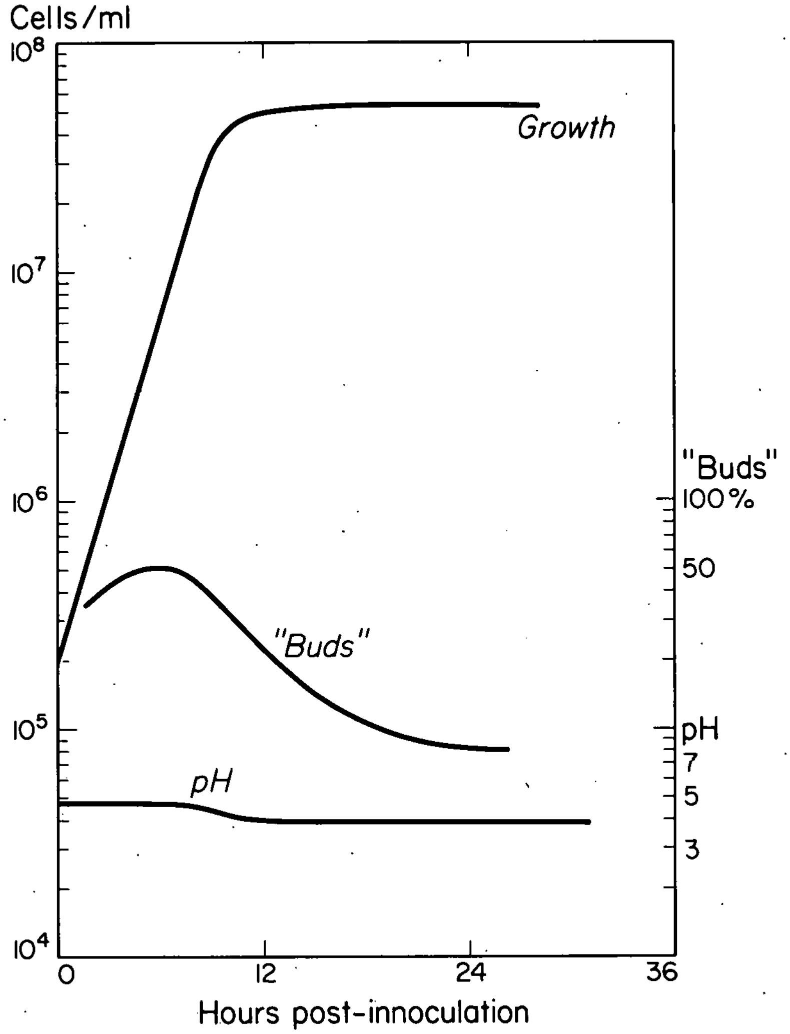

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Fig. 3. Growth characteristics of haploid(S288C) in PDB medium.

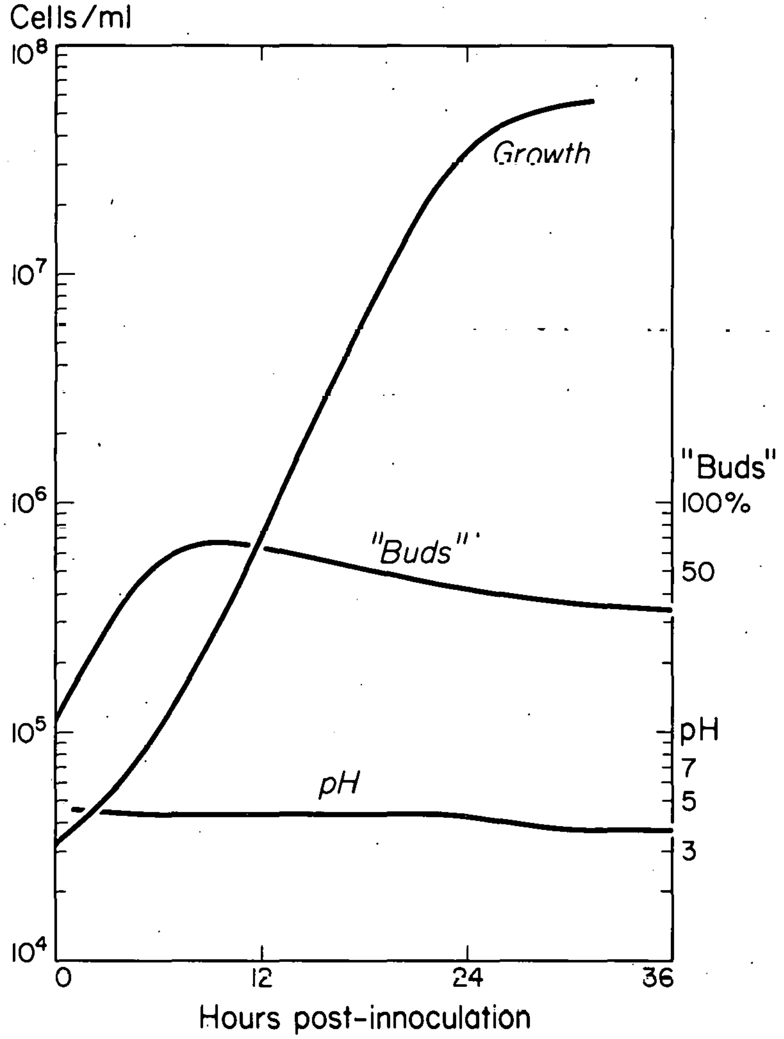

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Fig. 4. Growth characteristics of haploid(S288C) in synthetic medium.

DRY WEIGHT AND ASH WEIGHT

The dry weights and the ash weights of the different cultures are given in Table II. The figures within the parentheses give the number of determinations. For the diploid (X320) the dry weight was found to be 30% of the wet weight. The ratio of the ash weight to the dry weight is higher in the higher ploidies. It varies from 9% for the haploid to 11% for the hexaploid. The values for the haploid and the diploid fall within the range reported by White (105).

Table II

Dry weight and ash weight of cells of different ploidies

	Haploid S163A	Diploid X320	Tetraploid X323	Hexaploid X362
Dry weight ($\mu g/10^6$ cells)	17.2 ± 1.4 (7)	23.9 ± 0.4 (38)	47.6 ± 0.6 (8)	56.3 ± 2.4 (6)
Ash weight ($\mu g/10^6$ cells)	1.50 ± 0.06 (6)	2.12 ± 0.04 (39)	4.97 ± 0.19 (8)	6.25 ± 0.25 (6)
$\frac{\text{Ash weight}}{\text{Dry weight}}$	0.087 ± 0.011	0.089 ± 0.003	0.104 ± 0.005	0.111 ± 0.009

POTASSIUM AND SODIUM CONTENTS IN THE CELLS

The results for the potassium and sodium contents in the cells are given in Table III. As before, the figures within the parentheses give the number of determinations.

Table III

Potassium and sodium contents in the cells (in μg/10^8 cells)

	Haploid S163A	Diploid X320	Tetraploid X323	Hexaploid X362
K^+	23.36 ±0.84 (17)	62.39 ±0.57 (126)	165.8 ±1.4 (49)	201.4 ±2.5 (40)
Na^+	0.946 ±0.023(27)	0.508 ±0.010(147)	0.83 ±0.03(58)	1.16 ±0.01 (29)

If one plots the dry weight, the ash weight, the potassium content, and the sodium content of the cells versus the surface area of the cells (Fig. 5), or versus the cell volume (Fig. 6) calculated from the data obtained by Mortimer (95) (see Table IV), one finds that

(a) A straight line can be drawn through the three points representing the sodium contents of the hexaploid, the tetraploid, and the diploid and the origin in Fig. 5.

(b) The shape of the curves of the dry weight and the potassium content in Figs. 5 and 6 indicates that there might be a linear correlation between the two quantities. A plot of the potassium content versus the dry weight does show a straight line (Fig. 7).

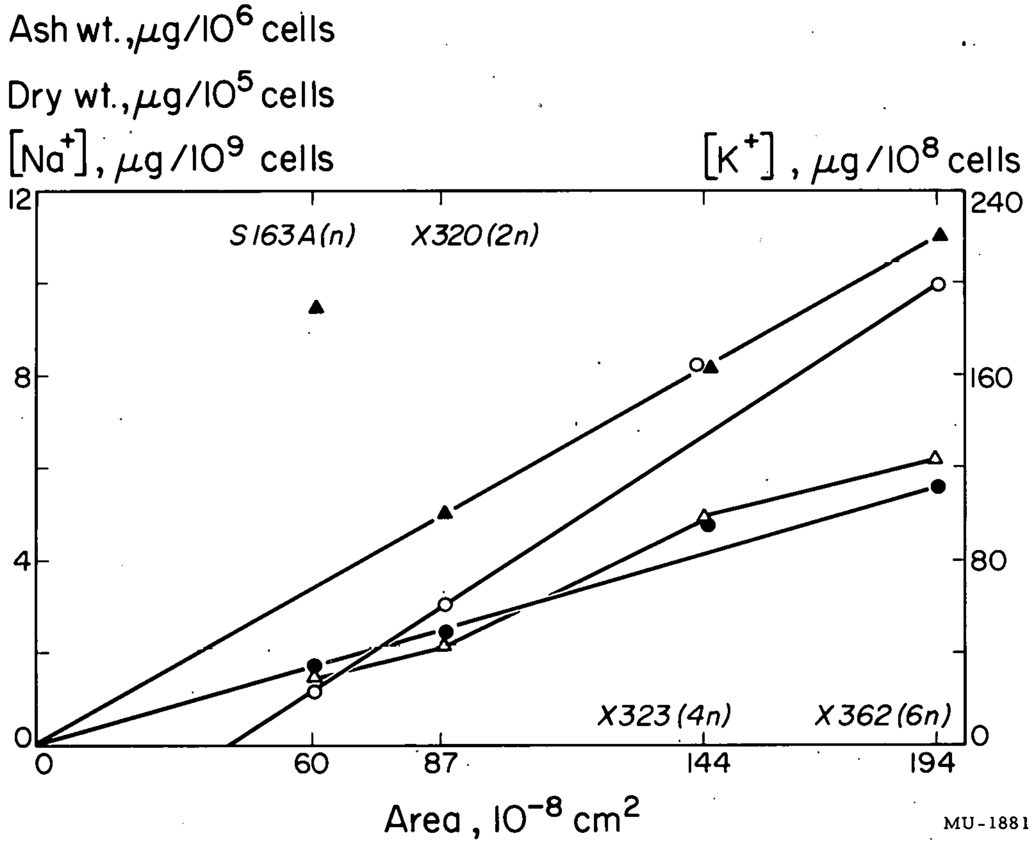

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Fig. 5. Relationship of the variables: the dry weight (•), the ash weight (△), the potassium content (○), the sodium content (▲), and the cell surface area.

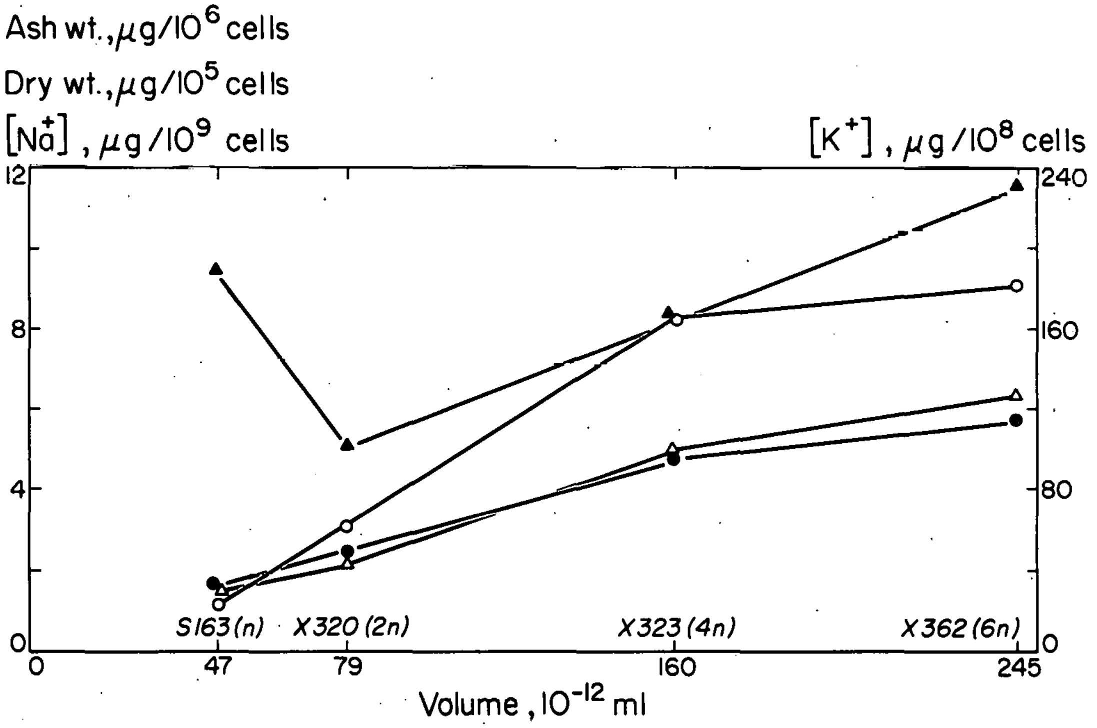

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Fig. 6. Relationship of the variables: the dry weight (•), the ash weight (△), the potassium content (○), and the sodium content (▲) to the cell volume.

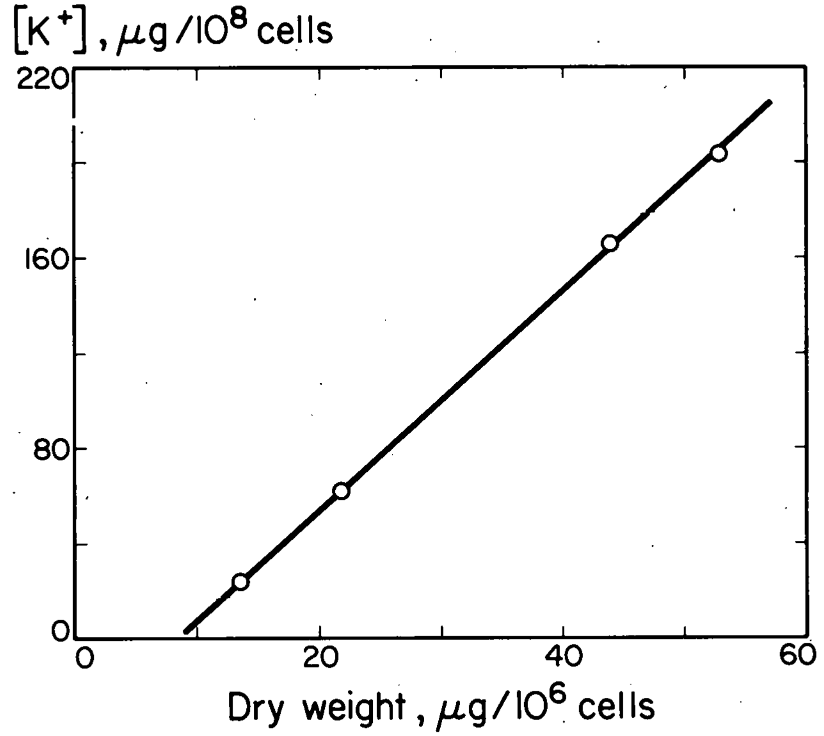

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Fig. 7. Relationship between the potassium content and the dry weight.

Table IV

The linear dimensions, the surface area and the volume of cells of different ploidies

	Haploid S163A	Diploid X320	Tetraploid X323	Hexaploid X362
Long axis 2a (μ)[a]	4.72	5.95	7.97	10.1
Short axis 2b (μ)[a]	4.35	5.04	6.20	6.80
a/b	1.08	1.18	1.20	1.48
Average volume[a, b]	54.8	82.5	165	253
Volume (μ^3)[c]	46.8	79.2	161	245
Area (μ^2)[d]	60.0	87.5	144	194
Area/volume[c]	1.28	1.10	0.896	0.792

[a] The figures were taken from Mortimer (95).

[b] The average volume is the average value of the volume of single cells.

[c] The volume is calculated from the equation $V = \frac{4}{3} \pi b^2 a$ using the average values of a and b.

[d] The surface area is calculated from the equation

$$A = 2\pi b^2 + 2\pi \frac{ab}{e} \sin^{-1} e,$$

using the average values of a and b.

DISCUSSION

That a straight line can be drawn through the three points and the origin in Fig. 5 indicates that the sodium content of the cell is proportional to the surface area of the cell for the diploid, the tetraploid, and the hexaploid. The deviation from the straight line for the haploid S163A can be explained either by (a) the "genetic" difference between the haploid and the other three strains, or (b) the difference in the growth characteristics as described on p. . The second explanation is supported by the fact that in S311B, another haploid strain of Saccharomyces cerevisiae, the sodium content of the cells harvested in the exponential growth phase was found to be much higher than that of the cells harvested in the stationary phase.

S163A not only differs from the other strain in having a high sodium content and a slow growth rate, but also its ash is different in appearance. The mass was flaky and could not be dissolved completely in 0.1 N HCl. The anomaly of S163A in its sodium content may also be associated with the clumpiness of the strain.

The straight-line relationship also stronglysuggests the existence of sodium pumps in the cell wall, or in the lipid inner membrane as suggested by Rothstein (64). Since the function of the sodium pump is to extrude the extra amount of sodium ions from the cell, the sodium content in the cell is thus regulated by the pump according to the physiological state of the cell. As the data show a proportionality between the sodium content and the surface area of the cell, it is conceivable that the sodium pump can be pictured as distribution uniformly over the cell wall, and that the "surface efficiency" pumping action per unit area is the same for all three strains (diploid, tetraploid, and hexaploid).

The linear relationship between the potassium content and the dry weight indicates that the potassium uptake of the cell is associated with some constituent in the dry matter. Since potassium has been found to be bound by muscle protein (106), by nucleotides in duck red blood cells (107), by rat liver cell microsomes (108), and by rat liver mitochondria (109), it is conceivable that potassium could be bound by protein, nucleotides, and other cellular particules in yeast. Northcole and Horne found that the protein content in the yeast cell wall was 13% by weight (110), and Ashikawa estimated that the dry matter in the microsome fraction containing soluble protein was 15% of the dry cell mass (private communication). The potassium content in the cell may thus consist of both "bound" and "free" ions distributed in some proportion in the cell wall and in the cytoplasm.

THE SURVIVAL CURVES

The survival curves of the different strains are shown in Fig. 8. The value of the percent survival is obtained from the ratio of the viability of the irradiated cells to that of the control cells, viability being defined as the ability of the cells to form colonies after treatment. The order of the radiation sensitivity of the different strains is in agreement with that reported by Mortimer (95), except that much higher dose was required to produce the same effect for all cells in this investigation. The "tail" of the haploid survival curve indicates the existence of the more radioresistant budding cells as demonstrated by Beam et al. (111). The difference in the radiation sensitivity of the cells in this and the earlier study (95) may be due to the difference in (a) the growth medium, (b) the dose rate, (c) the linear energy transfer, (d) the mode of irradiation (on agar plate or in liquid medium), and (e) the oxygen tension. Unpublished data of Mortimer and Beam ruled out the factors (a) and (b).

The results of Sayeg indicated no difference in the radiation sensitivity of the haploid yeast (112) when the value of the linear energy transfer (LET) falls below 50 Mev cm^2/g, or 5 kev/μ, taking the density of the yeast a 1 g/ml. This range corresponds to the value produced by X-rays at 50-kv or higher (113). Gunter demonstrated that the survival of diploid yeast increased from 37% to 70% when the

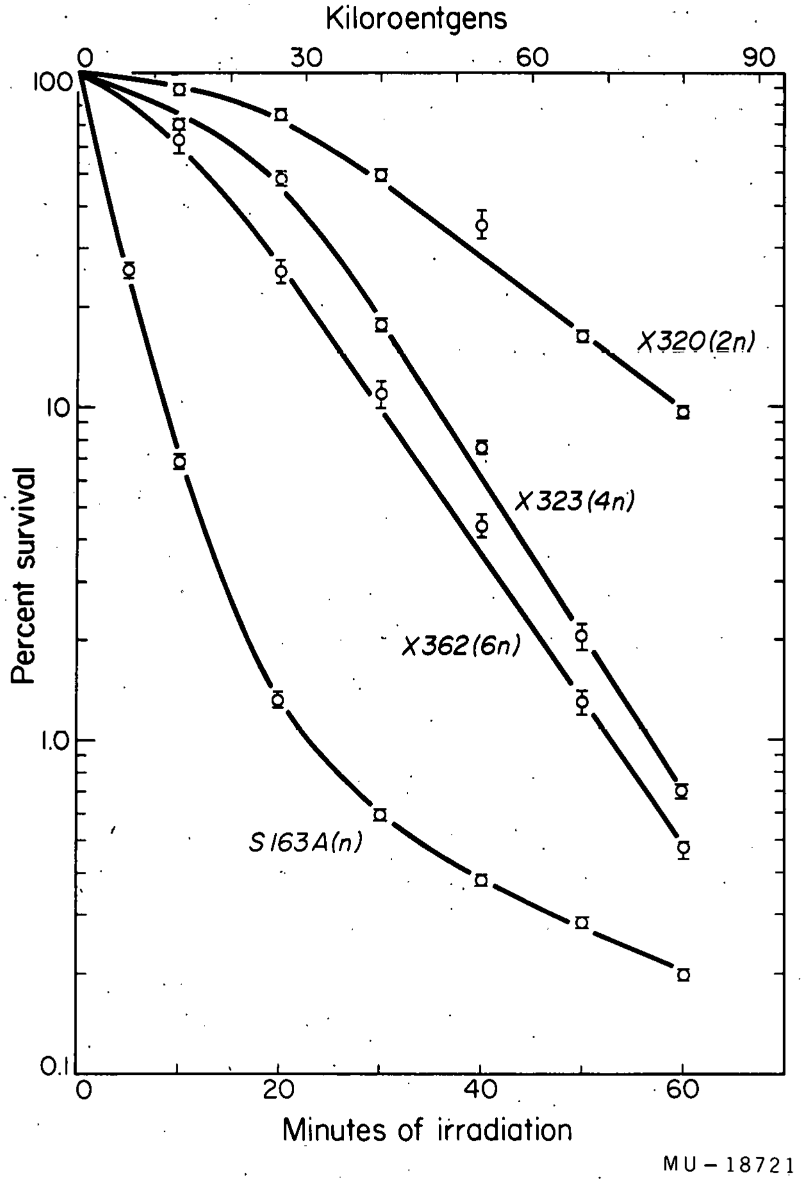

Fig. 8. Survival curves of the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362) grown in PDB.

cell concentration varied from 2×10^7 to 2×10^8 cells/ml, while the survival of haploid yeast did not change for cell concentration up to 10^9 cells/ml (114). As the endogenous respiration of the diploid was found to be much higher than that of the haploid cells, the increased survival at high cell concentration was interpreted as being caused by the decreased oxygen tension, which resulted from an imbalance between the endogenous respiration of the cells and the diffusion of oxygen into the suspension. Mitra reported that an autotetraploid yeast produced a more rapid fermentation (up to 30% more) than the diploid (115). (No genetic evidence for tetraploid state.) It is safe to assume that the endogenous respiration is higher in the higher ploidy. The cell concentration in this investigation for all cell strains, was more than 2×10^8 cells per ml. Comparing the LD_{50} dose with that reported by Mortimer (95), one finds that for haploids the radioresistance is the same in both cases, while for diploids it has increased 42%; for tetraploid, 78%, and for hexaploids, 170%.

POTASSIUM LEAKAGE

The results of the experiments on the potassium leakage from the cells to the medium are shown in Figs. 9 through 15. The curves indicate that the amount and the rate of leakage vary with ploidy and radiation dose. They are higher for larger cell and higher dose. The variation of the leakage in the control cells of the same strain was due to the oxygen which was used in stirring the suspension during the period of irradiation. The effect of oxygen upon the leakage is discussed later. The series of curves for X320 (Figs. 10-12) shows the variation of the amount and the rate of leakage with cell concentration. They were lower for higher cell concentration. The dependence is evident in the pure diffusion process. At higher cell concentration, the cells being closer to one another reduces the concentration gradient across the cell membrane at a much faster rate and thus reduces the leakage. The series of curves for X362(Figs. 14-15) shows a variation of the

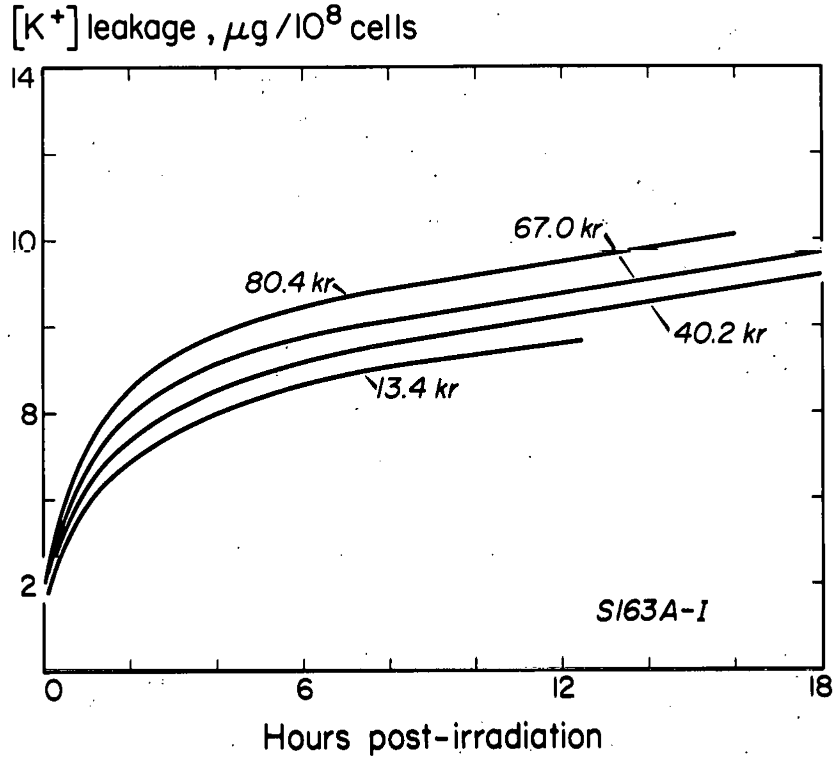

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Fig. 9. Potassium leakage from haploid (S163A). Cell concentration 5.53×10^8/ml. Top diagram is for the irradiated cells. Bottom is for the control cells.

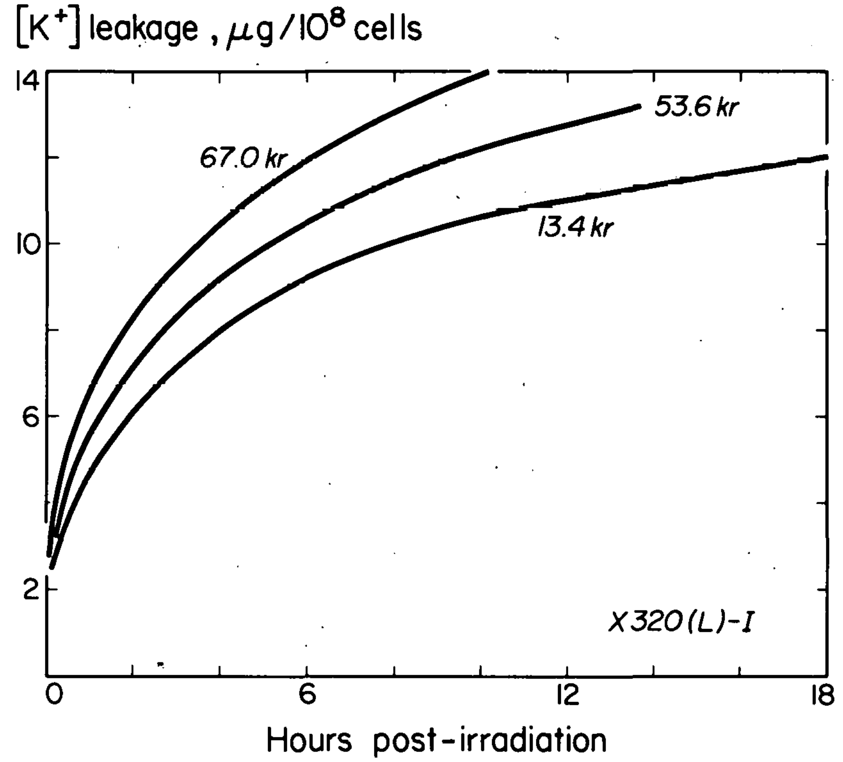

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Fig. 10. Potassium leakage from diploid (X320). Cell concentration 6.68×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells.

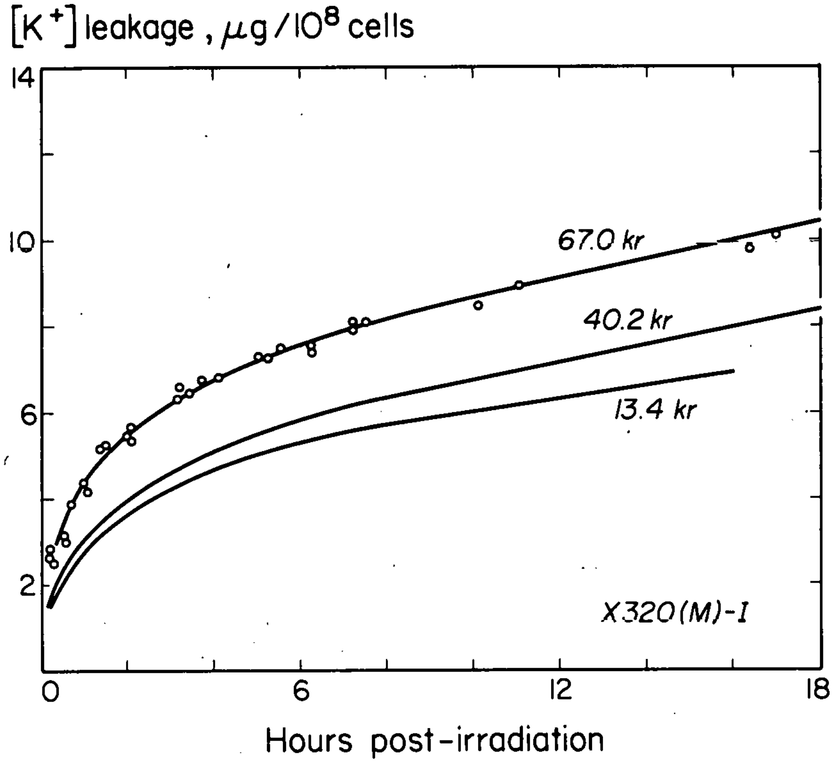

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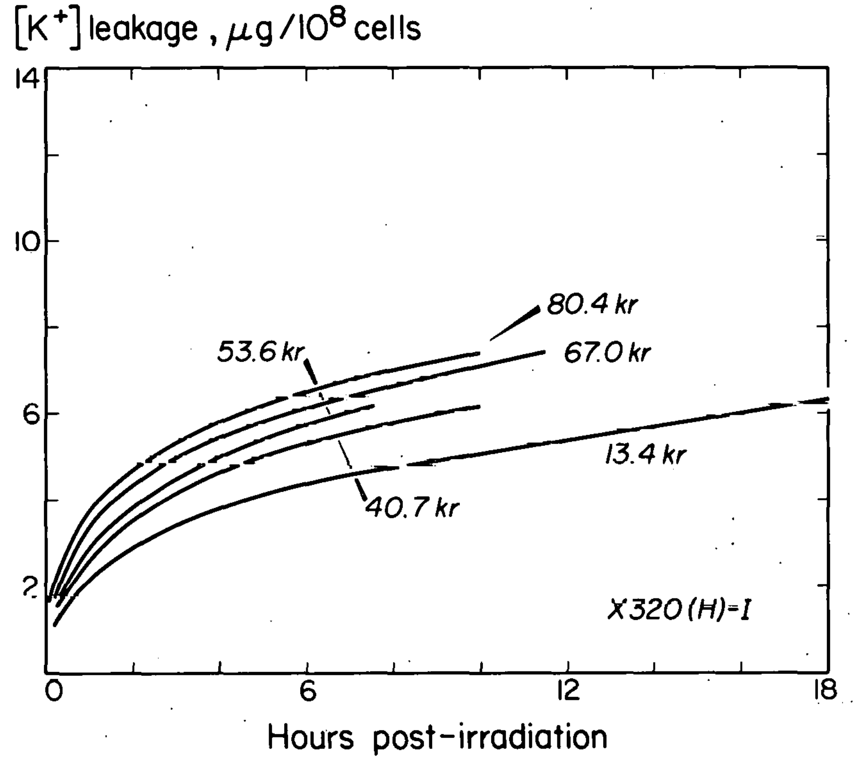
Fig. 11. Potassium leakage from diploid (X320). Cell concentration 9.10×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells.



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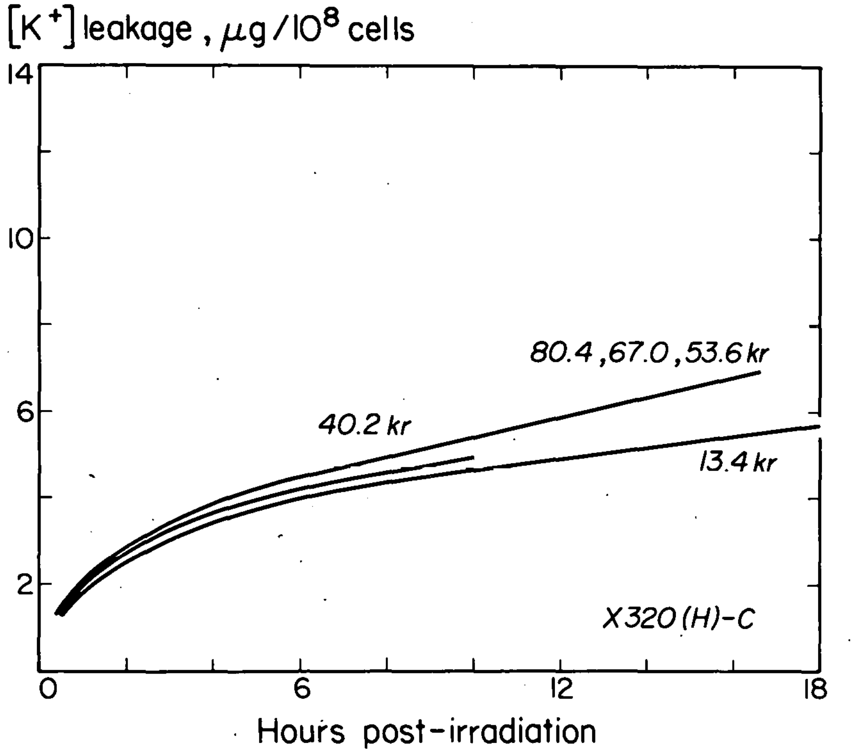

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Fig. 12. Potassium leakage from diploid (X320). Cell concentration 10.4×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells.

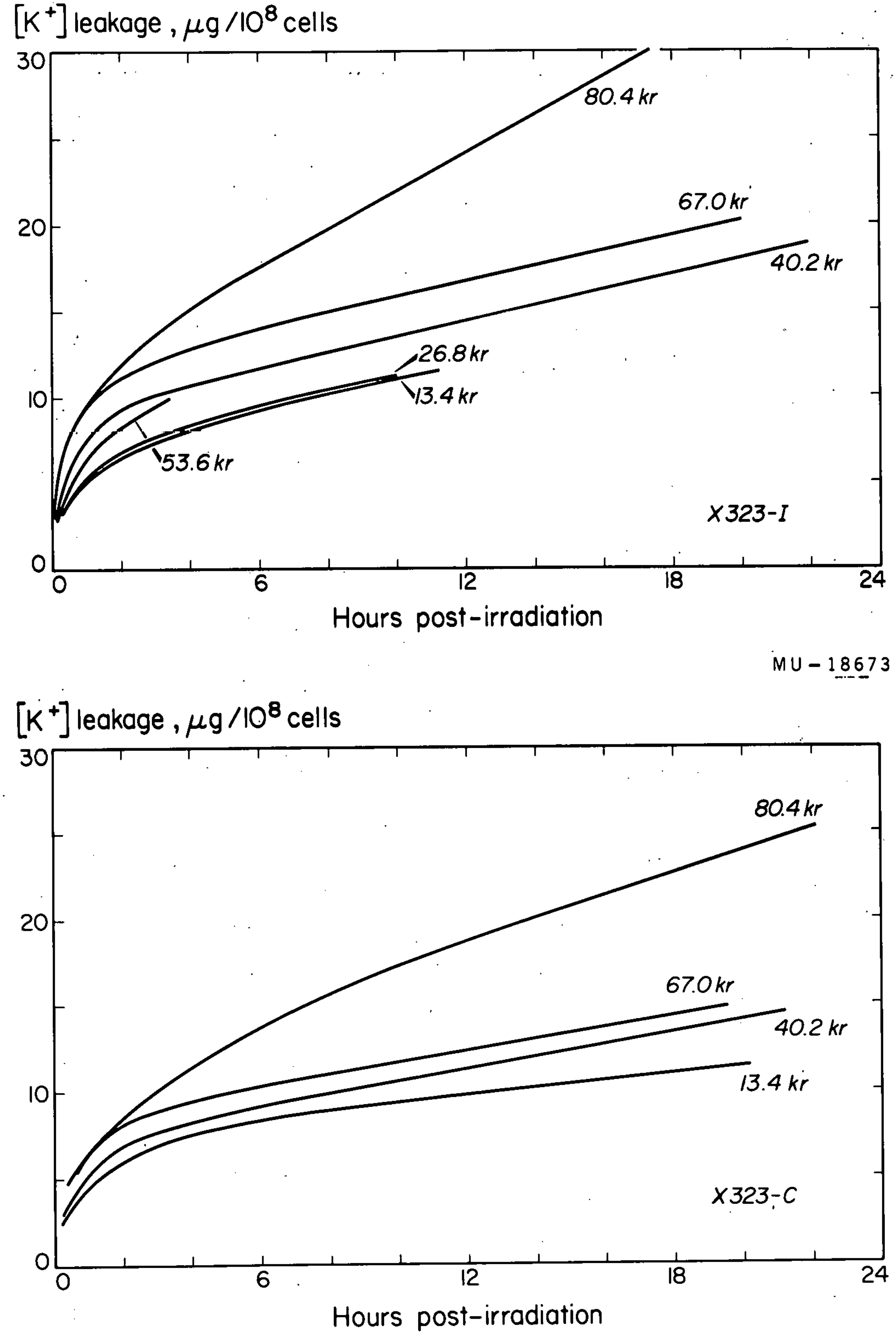

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Fig. 13. Potassium leakage from tetraploid (X323). Cell concentration 4.47×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells. Curves for 80.4 kr were obtained with plastic tubes.

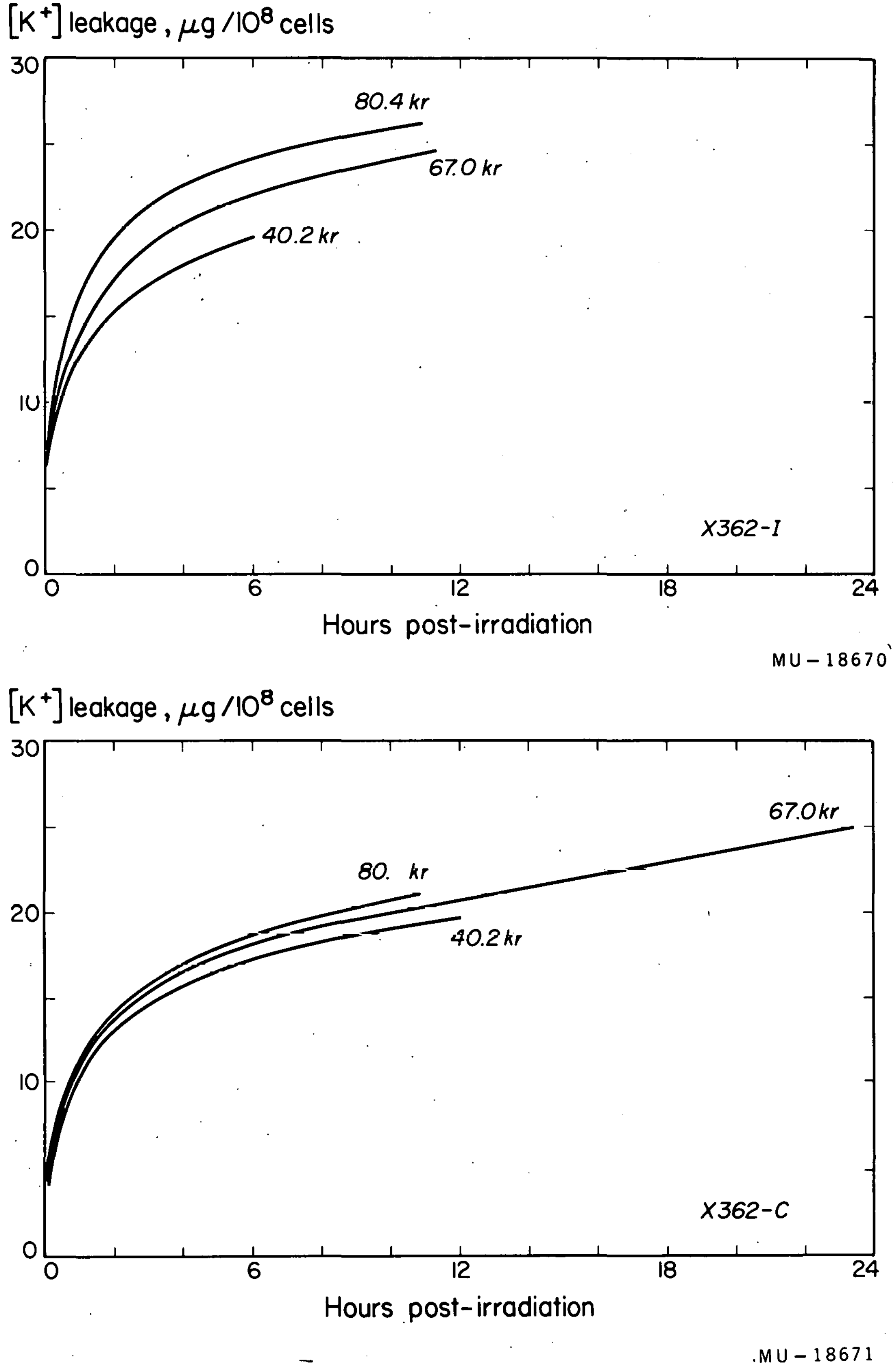


Fig. 14. Potassium leakage from hexaploid (X362). Cell concentration 3.68×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells.

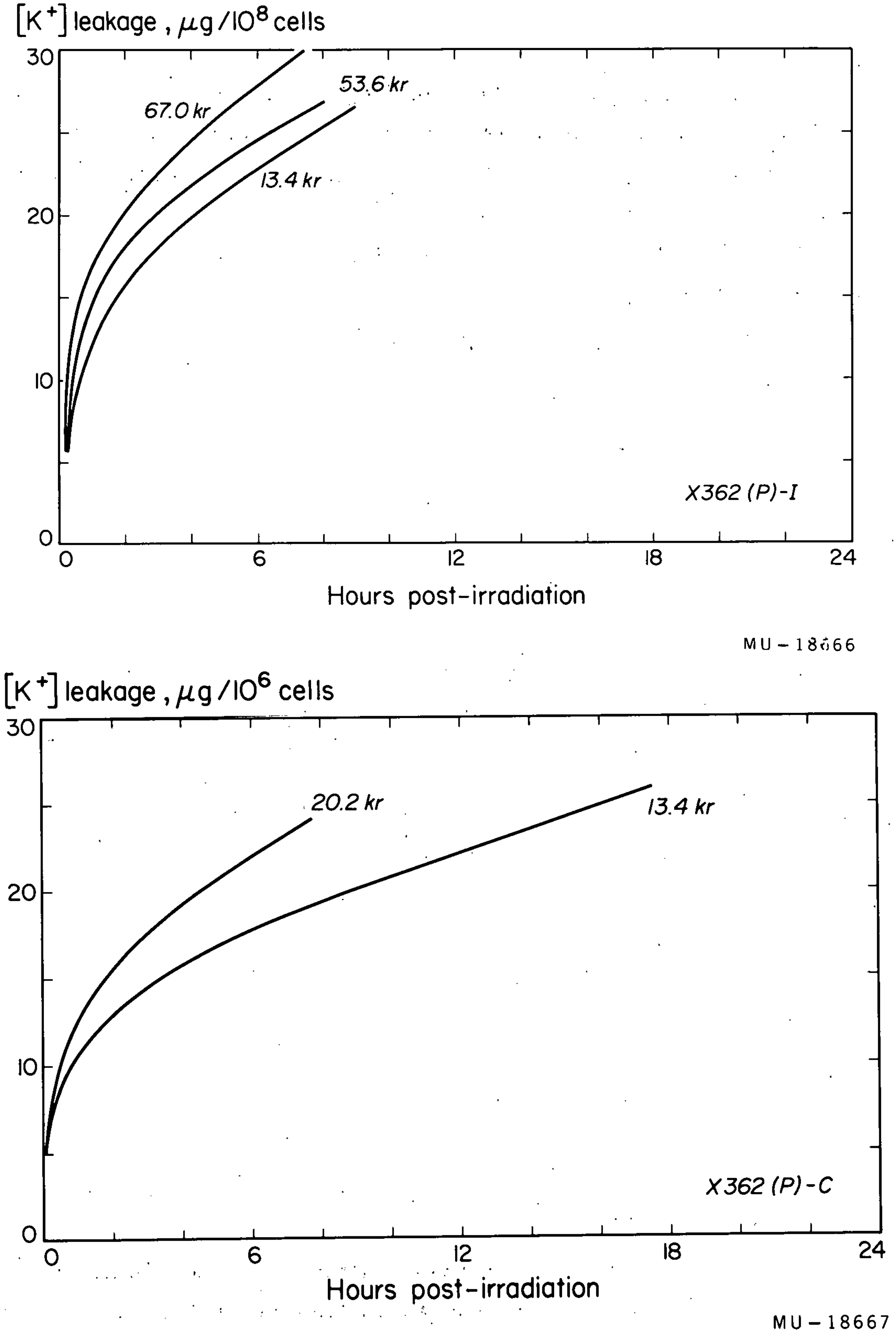


Fig. 15. Potassium leakage from hexaploid (X362). Cell concentration 3.43×10^8/ml. Top diagram is for the irradiated cells. Bottom diagram is for the control cells. All curves were obtained with plastic tubes.

leakage rate with the shape of the centrifuge tube. The cells in the plastic tubes (Fig. 15) lost potassium faster at a later time. This phenomenon was caused by the slower rate of sedimentation of the cells to the bottom of the tube. The plastic tube bottom is round and has parallel walls, while the bottom of the glass centrifuge tube is conical, and the cone has a capacity of 3 ml. Since only 2 ml of the suspension was used in this series of experiments, the cells in the glass tube settled down at a much faster rate and the leakage was thus much reduced. That the leakage was dependent upon the cell concentration and the shape of the container demonstrates rather strongly that physical diffusion processes dominate the physiological processes in potassium leakage.

ANALYSIS OF THE DATA

To get a numerical expression for the characteristic of a diffusion process, one could calculate the permeability constant "h" in each case. The permeability constant is defined by D/δ, where D is the diffusion constant and δ is the thickness of the membrane across which the material is carried by the force due to the concentration gradient. In mathematical form the diffusion constant D is defined by the equation

$$\frac{\partial C}{\partial t} - \nabla \cdot \underline{(D \nabla C)} , \qquad (1)$$

where C is the concentration of the material in question. Equation (1) can also be written as

$$\int \frac{\partial C}{\partial t} \, dV = \phi_S \, \underline{D \nabla C} \cdot \underline{dS} . \qquad (2)$$

This means that the change in the mass within a closed volume V is proportional to the integral over the whole surface area of the product of the surface element dS and the normal component of the concentration gradient ∇C. The proportionality constant D is defined as the diffusion constant. The quantity $D \nabla C$ has the dimension of $ml^{-2}t^{-1}$, which represents the amount of substance passing through a unit area per unit time. If the boundary surface is a thin membrane of thickness δ, $D \cdot \nabla C$ can be written as

$$D \cdot \nabla C = \frac{D}{\delta} (C_1 - C_2) = h (C_1 - C_2), \qquad (3)$$

where C_1 and C_2 are the concentrations of the substance in the two regions separated by the membrane. For a system in which the concentration gradient is a constant and always perpendicular to the surface element, Eq. (3) can be written as

$$\frac{dm}{dt} = aH (C_1 - C_2) , \qquad (4)$$

where A is the total surface area. This equation defines the permeability constant "h", which has the dimension of lt^{-1}, or cm/sec in the cgs system of units. From Eq. (1) we see that C is a function of the space coördinates and time. To solve the equation for a spherical cell immersed in a liquid medium, one may assume that the system is composed of three regions: The cytoplasm, the cell membrane, and the liquid medium. Each region is satisfied by Eq. (1) with its own diffusion constant. In addition, one may assume that the concentration and the flux are continuous at the boundary. For a given initial condition and additional boundary conditions the equation may be solved. In general, solutions of this kind are quite complex. Since the problem is similar to that of heat conduction, one may compare the case to that of composite spherical solids in heat conduction. The solution to the problem may be found in Carslaw's "Conduction of Heat in Solids" (116). Rashevsky solved the steady-state problem of diffusion processes accompanied by chemical reactions in a spherical cell (117). Roughton gave the solution in a more complete form by including the time component (41), although the conditions imposed by the two authors were not exactly the same.

Unfortunately, none of the solutions is applicable to the case in question, because (a) yeast cells are not spherical in shape and (b) no consideration was given to the interference due to the neighboring cells. The cell concentration used in this investigation was quite high, and the intercellular distance was less than three times the long axis of the ellipsoid.

In spite of the complexity of the problem, one may still be able to make some estimation of the permeability constant from Eq. (4). If the suspension is well stirred the concentration in the medium is a function of time only and the concentration inside the cell may be considered to vary with time and not with space.

In this investigation, only the potassium content in the medium was measured. It was determined in μg/ml and was converted to the amount leaked out per 10^8 cells.

If m_1 = amount of potassium inside the cell,

m_1 = amount of potassium leaked into the medium per cell,

n = the cell concentration in number per ml,

and

V = the volume of the cell,

then $m_2 = C_2/n$,

and Eq. (4) can be written as

$$-\frac{dm_1}{dt} = Ah\,\left(\frac{m_1}{V} - nm_2\right) = \frac{dm_2}{dt} \quad , \tag{5}$$

where

$$m_1(t) + m_2(t) = m, \tag{6}$$

and m can be considered as the "free" potassium initially present inside the cell.

Substituting Eq. (6) into Eq. (5), we have

$$\frac{dm_2}{dt} = \frac{Ah}{V}\left[m - (1+nV)m_2\right] . \tag{7}$$

If immediately after irradiation the cell had already leaked out an amount equal to $(m_2)_0$, the solution to Eq. (7) is given by

$$\frac{m}{(1+nV)} - m_2 = \left[\frac{m}{(1+nV)} - (m_2)_0\right] \exp\left[-Ah\,(1+nV)t/V\right]. \qquad (8)$$

If the experimental conditions are satisfied by the equation, the semilog plot of the quantity $\frac{m}{(1+nV)} - m_2$ versus time t gives a straight line. The two unknown quantities h and m can be determined graphically from Eqs. (7) and (9) by successive approximation.

As was discussed in the preceding section, the situation was complicated by the sedimentation of the cells and the fact that the medium cannot be considered as infinite. Thus the semilog plot of the quantity $m/(1+nV) - m_2$ versus time and the plot of dm_2/dt versus m_2 did not give straight lines. However, from the leakage curves obtained with plastic tubes and glass tubes, as in X323 and also in X362 (see Figs. 13-15), we see that the two corresponding curves almost coincide with each other in the first hour. There was a good deal of handling and transfer of materials; settling down of the cells was observed after 40 minutes. It is therefore safe to consider that the settling effect did not interfere seriously during the first 40 minutes. In the analysis of the data only the first 45 minutes was taken into account. The values of the permeability constant h are given in Table V. Since they are dependent upon the values of m, which were of great uncertainty and were affected by the sedimentation of the cells, they are not too accurate and should not be taken seriously. However, they are within the range of 10^{-9} and 10^{-8} cm/sec. If one did consider that the sedimentation of the cells affected the leakage in the very beginning of the measurements, and analyzed the curves obtained from the semilog plots as two-component "decay" curves, the values of h would be increased not more than fivefold. Their order of magnitude would still fall within the range mentioned.

Table V

The percent survival and the permeability constant of the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362).

Strain (Ploidy)	Description	Dose (kr)	Survival (%)	Permeability constant h (10^{-9} cm/sec.)		
				Irradiated	Control	Ratio
S163A (1n)	Cell concentration 5.5 x 10^8/ml	80.4	0.2	8.79	-	-
		67.0	0.26	8.32	6.46	1.29
		40.2	0.6	7.92	6.46	1.23
		13.4	7.7	7.30	6.00	1.22
X320 (2n)	Cell concentration 6.7 x 10^8/ml	67.0	17	7.98	6.42	1.24
		53.6	29	7.98	-	-
		13.4	97	7.36	-	-
	Cell concentration 9.1 x 10^8/ml	67.0	17	8.89	7.53	1.18
		40.2	46	7.52	6.50	1.16
		26.8	72	7.26	6.50	1.12
	Cell concentration 10.4 x 10^8/ml	80.4	10	8.82	4.98	1.77
		67.0	17	8.32	-	-
		53.6	29	7.50	-	-
		40.2	46	7.48	4.75	1.57
		13.4	96	4.57	3.82	1.20
X323 (4n)	Cell concentration 4.5 x 10^8/ml	67.0	2.1	12.0	11.7	1.03
		53.6	6.0	11.4	-	-
		40.2	17	8.87	8.48	1.06
		26.8	46	8.56	-	-
		13.4	76	7.68	7.66	1.01
X362 (6n)	Cell concentration 1.68 x 10^8/ml	80.4	0.36	15.3	12.3	1.25
		67.0	1.3	12.8	11.4	1.10
		40.2	9.4	12.3	11.1	1.11
	Cell concentration 3.43 x 10^8/ml in plastic tubes	67.0	1.3	12.9	10.8	1.20
		33.6	3.5	11.2	10.8	1.04
		26.8	26	10.7	10.2	1.05

Another method to illustrate the effect of radiation upon the leakage is to determine the net increase in the potassium content of the medium within the first 45 minutes after irradiation, and compare the value obtained for the irradiated cells with that for the control.

The results of the potassium leakage within the first 45 minutes after irradiation are plotted in Figs. 16-18. The plot of the net leakage (Fig. 19) for the complete series for cells receiving a dose of 67 kr is found to be a straight line versus the surface area. This, again, suggests that the leakage is largely a physical diffusion process.

DISCUSSION

On examining Fig. 18, one notices that for cells of the same strain large loss of potassium is associated with low survival. This leads one to question whether the loss is the cause or the effect, or merely coincident with the cell "death". In the foregoing sections it has been demonstrated that the leakage of potassium was largely due to a physical process; death however, probably involves a physiological phenomenon. It seems likely that the potassium loss is the the result of physiological change and is an expression of radiation damage not directly associated with nor causing death. The following facts may support the view.

When the cells were suspended in distilled water, more potassium was found to leak out. This process could be repeated many times. Pulver found that 30% of the potassium could be washed out in this manner (118). In X320, the cells after being harvested and washed, were kept in distilled water suspension from 1 week to 2 months. They were either resuspended or were heated in boiling water. The loss of potassium was found to be 30% for cells kept in the distilled water for 1 week, and 90% for the cells kept in the distilled water for 2 months. The corresponding values for the sodium were 57% and 96% respectively. If one assumes that after this long period the cells were all unable to

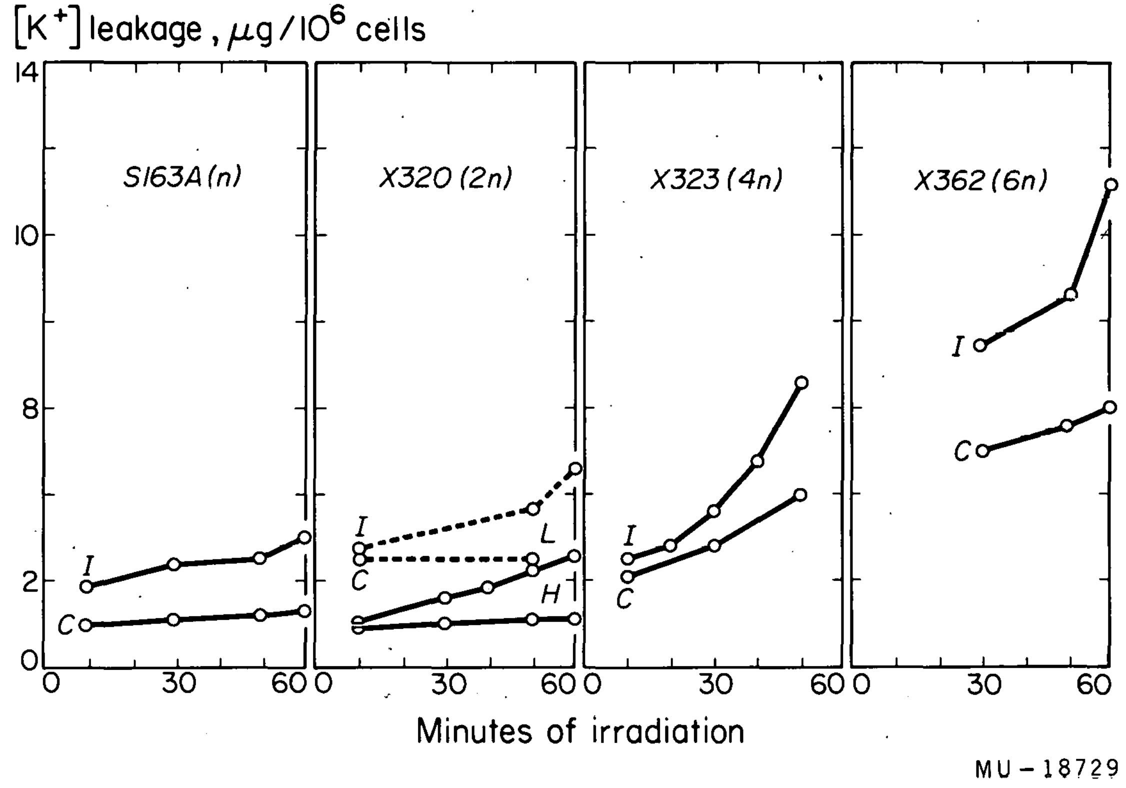
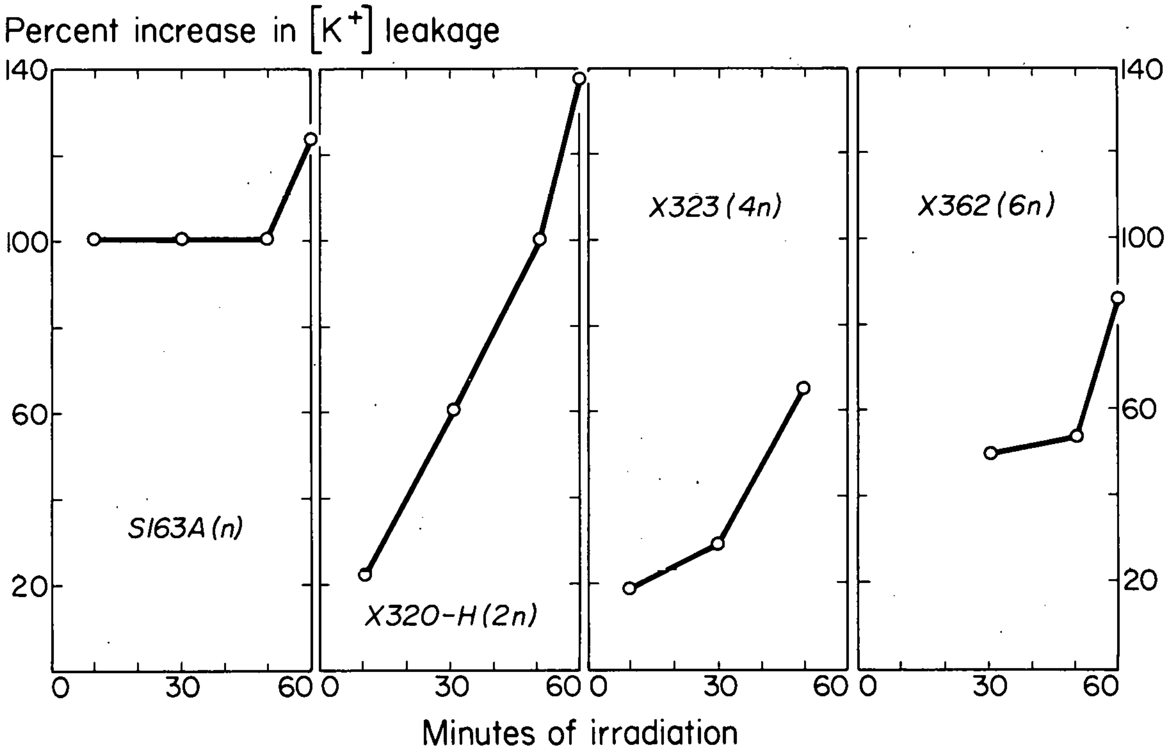


Fig. 16. The net potassium leakage within the first 45 minutes after irradiation from the haploid(S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362).



MU – 18728

Fig. 17. The percent increase in the net potassium leakage of the irradiated cells over that of the control for the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362).

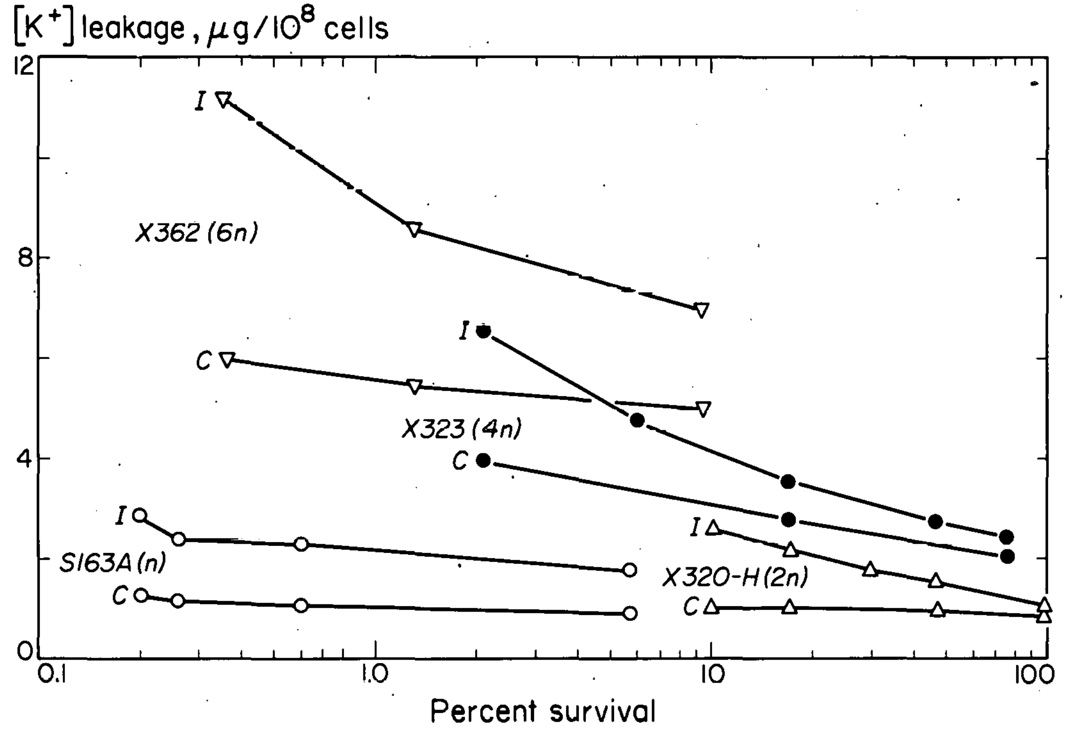


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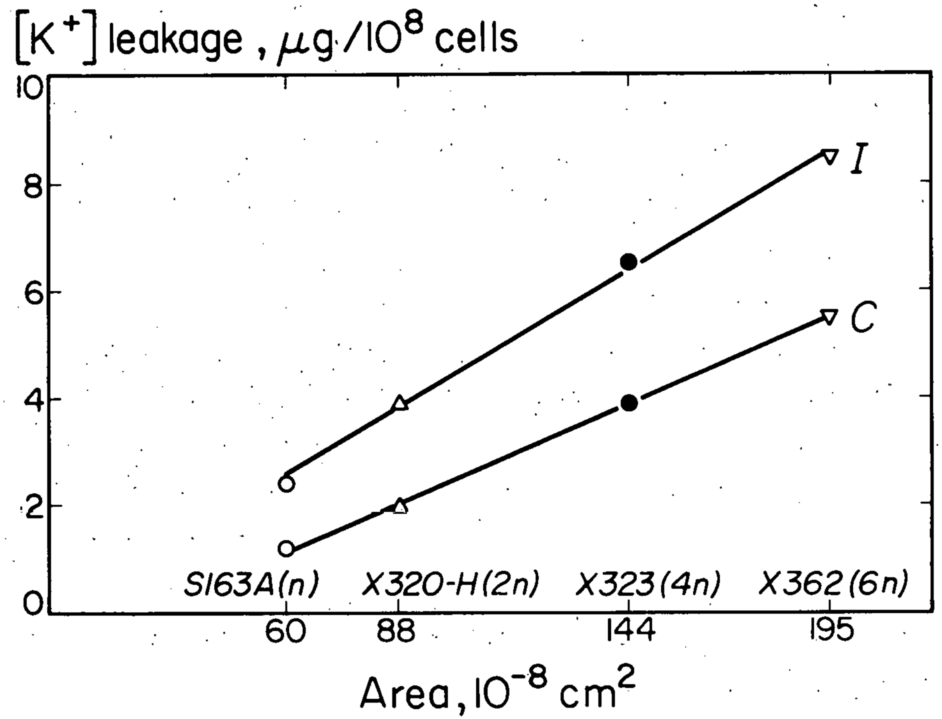
Fig. 18. Relationship between the potassium leakage and the percent survival for the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362).



MU-18726

Fig. 19. Relationship between the net potassium leakage and the surface area for the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362). The irradiated cells received an X-ray dose of 67 kr.

divide, and therefore dead, one may consider some of the potassium (and sodium) was "bound" to the cells. If such is the case then the potassium ions that leak out can be considered as "free". If certain change, or damage, occurs in the cells that causes the potassium (or sodium) to be released from the bound state, the free ions can then migrate under some chemical or physical force, and the movement can be described as diffusion. Although the exact nature of the binding and of the binding sites are not known, Rothstein repeatedly demonstrated that the cation binding of the yeast cells is at the surface, and he proposed that the cell surface binds cations in much the same way as an ion-exchange resin (64-68).

If one defines retentivity as the ratio of the potassium leakage of the control cells to that of the irradiated cells, or the ratio of the corresponding values of the "permeability" constants, and plots the value versus the dose, one observes that the sensitivity of the potassium retentivity is of a different order from that of the survival. (Compare Fig. 20 with Fig. 8). This variation in the order of radiation sensitivity indicates two different modes of interaction of radiation with the cells. The differential effects on the phenomena obtained by Bruce in yeast with X-rays, uv, and visible light led to the same conclusion (93).

The values of the retentivity are shown in Table VI and the curves are represented in Fig. 20.

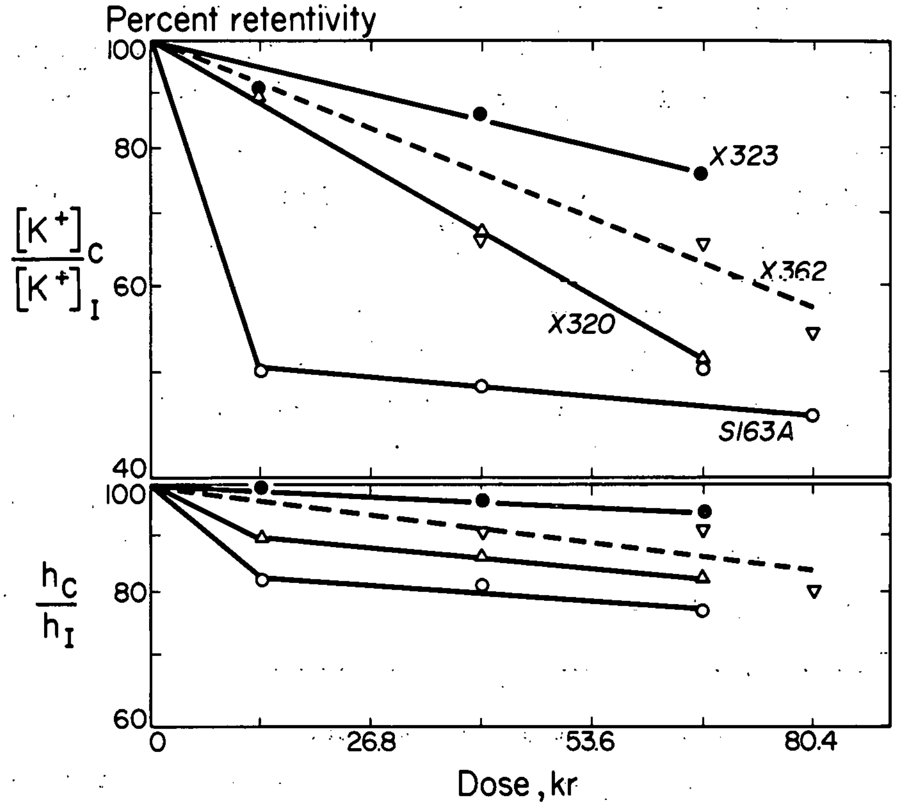


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Fig. 20. Relationship between the retentivity and the dose for the haploid (S163A), the diploid (X320), the tetraploid (X323), and the hexaploid (X362). The upper diagram gives the ratio of the K^+ leakage from the control cells to that of the irradiated cells. The lower diagram gives the ratio of the permeability constants. The symbols used in the two diagrams are the same.

Table VI

Potassium retentivity of cells of different ploidies

	$\frac{K^+ \text{ leakage in control cells}}{K^+ \text{ leakage in irradiated cells}}$						$\frac{\text{"h" in control cells}}{\text{"h" in irradiated cells}}$					
Dose (kr)	S163A (1n)	X320* (2n)			X323 (4n)	X362 (6n)	S163A (1n)	X320* (2n)			X323 (4n)	X362 (6n)
		L	M	H				L	M	H		
80.4	45	-	-	-	-	54	-	-	-	-	-	80
67.0	50	51	61	50	52	65	77	81	85	87	57	91
53.6	-	-	-	-	-	-	-	-	-	-	-	90
40.2	48	69	71	62	86	66	81	-	86	64	95	-
26.8	-	-	-	-	-	-	-	-	-	-	-	-
13.4	50	78	100	82	91	-	82	-	89	83	100	-

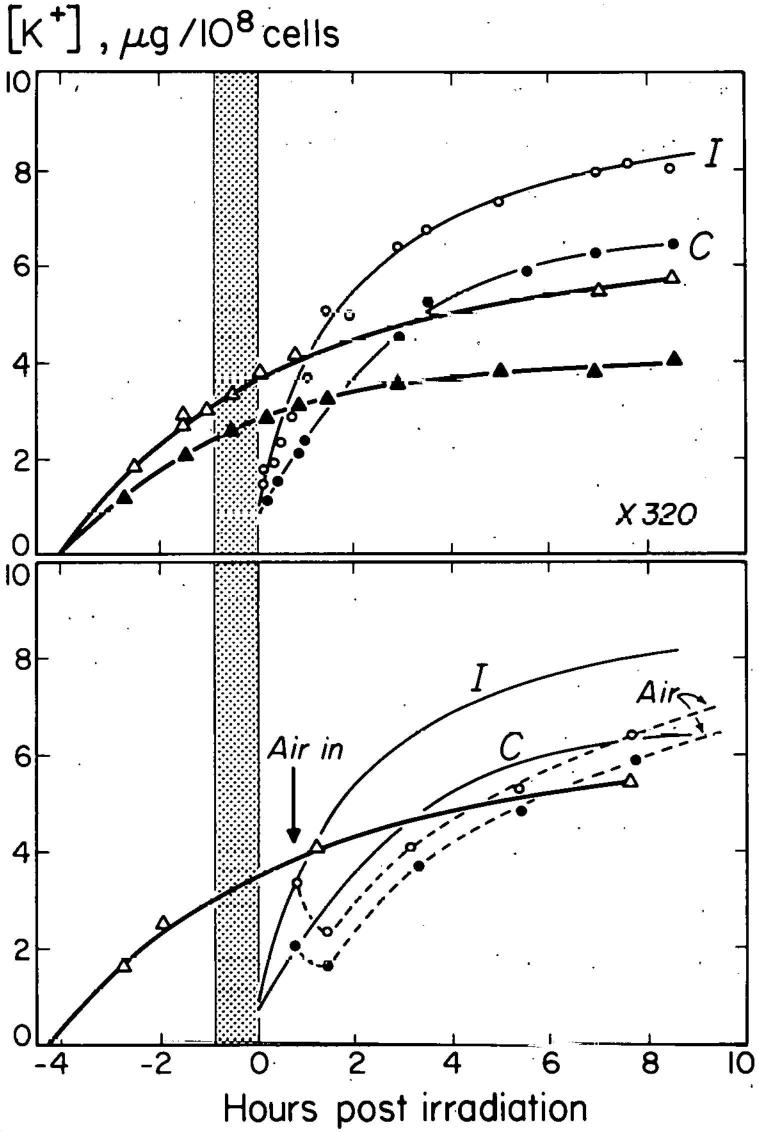
*X320 L M H are the symbols given to the curves (Figs. 16-19) indicating that the values were obtained with a low (L), medium (M), or high (H) cell concentration in the suspension.

EFFECT OF TEMPERATURE AND AERATION ON THE POTASSIUM LEAKAGE

Figures 21A and B show the results of the earlier experiments. As in the routine procedure, the cell suspensions were kept in the refrigerator ($5^{\circ}C$) until they were to be irradiated. The volume of the aliquot for each centrifugation process was 4.5 ml instead of 2 m. Curves 1 and 2 are the leakage curves for the irradiated and the control cells respectively. Since a certain amount of potassium had leaked out between the time the cells were washed and the time they were irradiated, parallel experiments were performed to determine the leakage during the period. Curves 3 and 4 were obtained for the cells kept at room temperature (20° to $21^{\circ}C$) and under refrigeration ($5^{\circ}C$). All centrifugation was done at room temperature. One observes the Curve 4 at all times gave lower values than Curve 3, but it gave a much higher value than Curves 1 and 2 at zero postirradiation time. The low values in Curve 4 are to be expected if one associates the potassium leakage with the metabolic rate of the cells. The depressed value in Curves 1 and 2 is interpretated as due to the oxygenation of the suspension during irradiation, that was the only difference in the treatment of the cells in the two cases. Curves 5 and 6 confirmed the interpretation. The dips were caused by the oxygen in the air used for aeration. The effect of oxygen on the potassium leakage and transport is discussed later. The shape of Curves 1-4 (when extended to 24 hours, not shown in Fig. 21) indicates an exponential rise in the potassium content in the supernatant fluid, and the amount of K^{+} did asymptotically approach a characteristic value 9 hours after irradiation. Each curve could be made to approximately fit the equation

$$K^{+} = K_{i} (1 - e^{-\lambda_{i} t)} \quad \text{for } i = 1, 2, 3 \text{ and } 4.$$

The semilog plot of the values $K_{i} \exp(-\lambda_{i} t)$ with time gave a straight line in each case (Fig. 22). The respective half lives were found to be



MU-18814

Fig. 21. Potassium leakage curves from X320, Curves 1 and 2 are denoted as I and C in both diagrams: I, for the irradiated cells and C, for the control cells. Curve 3 (△) was obtained for cells kept in room temperature and Curve 4 (▲) for cells under refrigeration (5°C). Curves 5 (○) and 6 (●) are the leakage curves obtained with aeration. The shaded area indicates the time of irradiation.

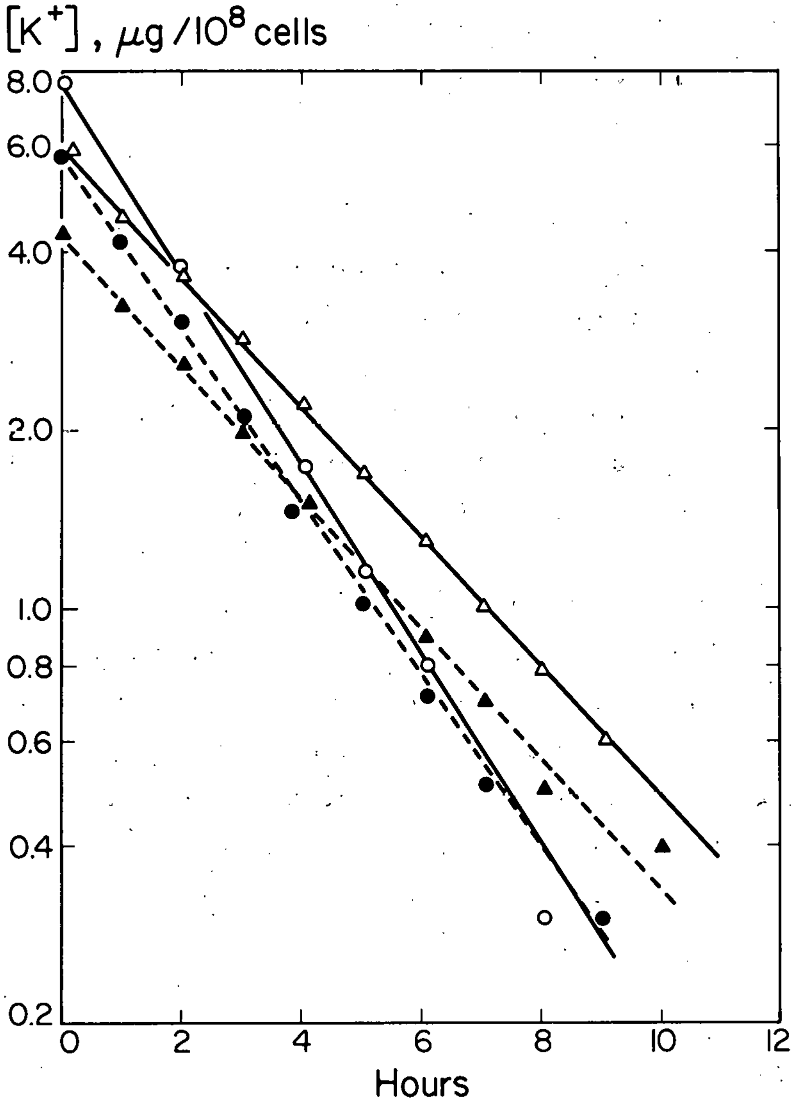


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Fig. 22. Semi-log plot of the potassium leakage versus time after irradiation. (○) for irradiated cells, (●) for the control cells, (△) for cells kept at room temperature, and (▲) for cells kept under refrigeration.

(a) irradiated cells, 1.9 hours;

(b) control cells, 2.1 hours;

(c) cells in room temperature, 2.8 hours;

and

(d) cells under refrigeration, 2.8 hours.

The increasing values for half life of cells in Conditions (a), (b) and (c) are understandable. However, the agreement for cells in Conditions (c) and (d) is hard to explain. If, as discussed earlier, the potassium leakage at later time in the experiment was affected by the settling down of the cells the agreement may be accidental. Even if the percentage rate of leakage was the same in the two cases, the absolute rate was quite different. It is also interesting to note that for cells in Conditions (b) and (c) the net loss was the same but the rate were different. Oxygenation of the suspension before determination of the potassium content accelerated the loss. If the asymptotically approached value of the potassium leakage were not affect by the settling of the cells one would attempt to conclude that cells kept at the same room temperature (and same physiological and physical conditions) had the same amount of "free" potassium ions available to be diffused out and that oxygenation accelerated the loss.

EFFECT OF NITROGEN ON THE POTASSIUM LEAKAGE

In the section on the analysis of data it was mentioned that the problem could be made simpler if the suspension were sell stirred. Since the oxygen caused the active transport of potassium, nitrogen was used. Nitrogen was passed through a flask of sterile water before it went to a manifold with 16 outlets, at a rate of 480ml/minute. Each outlet was led into a centrifuge tube containing 2 ml of cell suspension. A total of only six experiments was performed, three with the diploid (X320) and three with the hexaploid (X362). The results of one experiment each are shown in Figs. 23 and 24. Because the nitrogen was bubbled through the suspension some time after irradiation, the increased rate of potassium leakage was exaggerated. On the average, the net loss of potassium in the first 45 minutes after nitrogen bubbling was found to be a little more than that in the first 45 minutes after irradiation. The difference was not much, a little more in the irradiated cells than in the control. The increase in leakage probably was not due to the intrinsic effect of nitrogen but largely due to the stirring of the suspension. However, there was an interesting observation for X362. In all three cases, there was a slight delay in the potassium leakage in the control cells. The rise in the leakage was not so sharp in the first 5 minutes. The effect was more obvious if the results were plotted in percent increase (see Fig. 24B). Only in one case for X320 this effect was observed. Probably in the process of introducing nitrogen into the suspension enough oxygen was also introduced to initiate potassium transport. The concentration of X362 was much lower than that of X320.

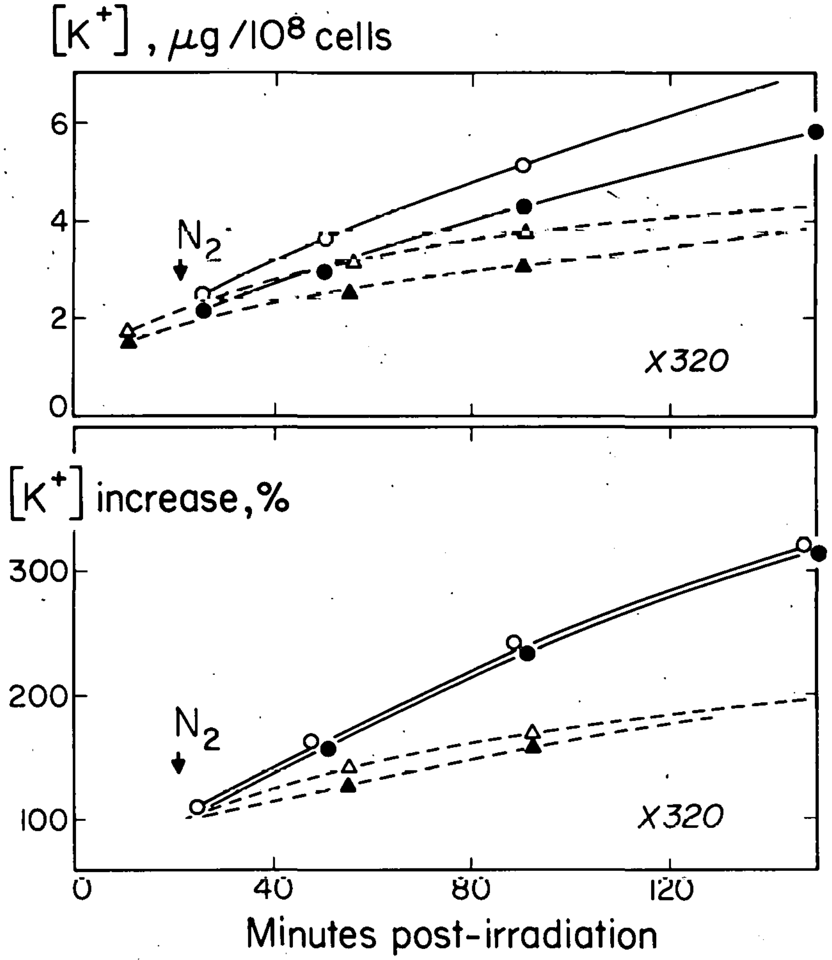

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Fig. 23. Effect of nitrogen on the potassium leakage (1).

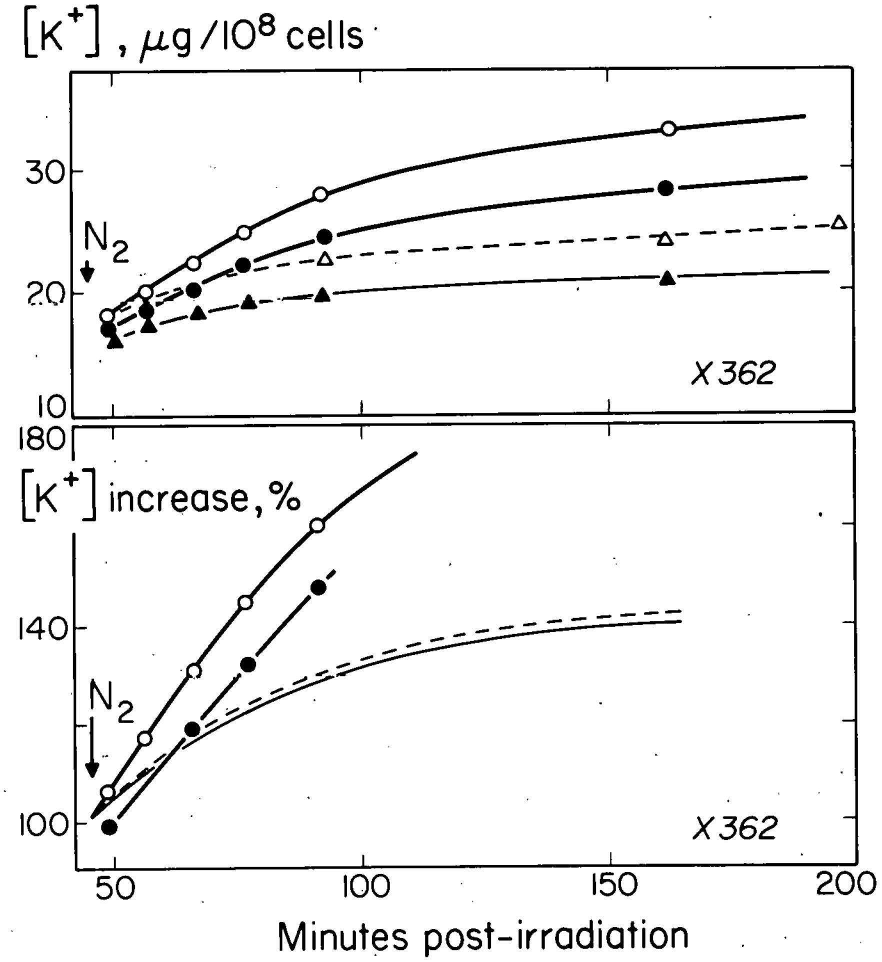

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Fig. 24. Effect of nitrogen on the potassium leakage (2).

EFFECT OF OXYGEN ON THE POTASSIUM TRANSPORT AND LEAKAGE

It was found previously that the air used in aeration had caused some potassium to be pumped back into the cells. A few experiments were therefore performed to study the transport phenomenon as well as the leakage. Oxygen was introduced into the cell suspension in the same way as nitrogen, but at the rate of 295 ml per minute. Two representative results are shown in Fig. 25. In the first case oxygen was introduced 35 minutes after irradiation. Practically all the potassium that had leaked out was transported back into the cells within 25 minutes. In the first 15 minutes, the irradiated cells transported in 0.78 μg/10^8 cells whereas the control transported 0.29 μg/10^8 cells. Since the number of cells was not corrected for those unable to form colonies, the irradiated cells were found to have done more work than the control. From the initial slope of the potassium uptake curve (drawn in enlarged scale for this purpose, but not shown in the figure), the rate of transport was found to be 0.064 μg/10^8 cells per minute and 0.044 μg/10^8 cells per minute for the irradiated and the control cells respectively. When oxygen was introduced between 3 and 4 hours after irradiation, more potassium was transported back into the cells. In this particular experiment, as shown in the second case, not all potassium was transported in. From the curves, it was found that the irradiated cells transported 3.38 μg/10^8 cells per minute, (initial rate, 0.24 μg/10^8 cells) and the control cells transported 2.43 μg/10^8 cells per minute, (initial rate, 0.17 μg/10^8 cells). In both cases, the irradiated cells pumped in more potassium per cell (not corrected for survival), thus performed more work than the control cells and at an increased rate, 45% higher. This seemed a little surprising, though, for one would expect that the cells having been "damaged" by radiation would impair their capacity to do work. The leakage, as shown in the case of aeration, was increased with oxygen bubbling.

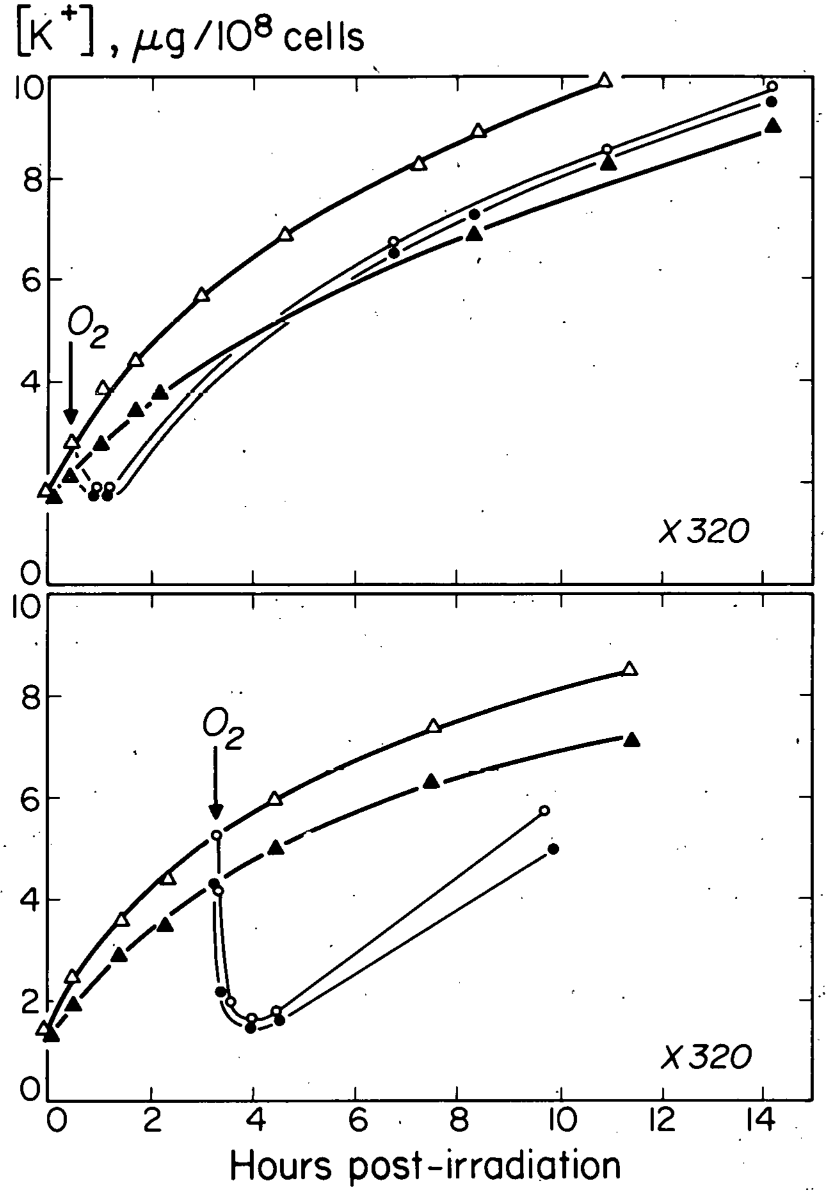

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Fig. 25. Effect of oxygen on the potassium leakage.

EFFECT OF DEXTROSE ON POTASSIUM TRANSPORT AND LEAKAGE

In the preceding section, it was evident that the oxygen had provided the energy for the potassium transport. The following experiments were carried out to study the potassium transport in the diploid (X32) and in the hexaploid (X362). A stock solution of 10% dextrose (w/v) was prepared. For each experiment, 1 ml of the stock solution was added to 14.5 ml of the cell suspension, thus making a final solution containing 0.6 g of dextrose in 10 ml suspension. For comparison, parallel experiments were done with a diluted suspension, by adding 1 ml of distilled water to 14.5 ml of the cell suspension similarly prepared. Since the initial rate of uptake was very high the first few minutes of the experiment were very critical. In the preliminary experiment, 0.5 ml of the stock dextrose solution was added to 6.5 ml of the cell suspension immediately after irradiation. Aliquots of the suspension were centrifuged at 12, 23, and 40 minutes after the addition of dextrose. No uptake of potassium was observed, and the final curves showed a reduction in the leakage for the cells that had dextrose. The reduction was much greater in the irradiated cells. The rates of leakage, in $\mu g/10^8$ cells per hour were found to be 2.6 and 2.5 repsectively for the irradiated and the control cells when water was added; and 1.0 and 1.8 respectively when dextrose was used. When dextrose was added at a later time, the transport phenomenon was more prominent, since there were more potassium ions in the extracellular fluid. The general shape of the curve looked almost the same as that for oxygen, as shown in Fig. 25.

Figure 26 shows the potassium uptake curves obtained with oxygen and with dextrose. The experiments were carried on for 3 hours. The results of only the first 2 hours are shown. Both oxygen and dextrose were introduced into the cell suspensions at 3 hours after irradiation. The irradiated cells received 40.2 kr. It is rather difficult to compare the effects quantitatively because the exact amount of oxygen in the suspension was not known, even though the flow rate was recorded. It is interesting to note, however, that although oxygen

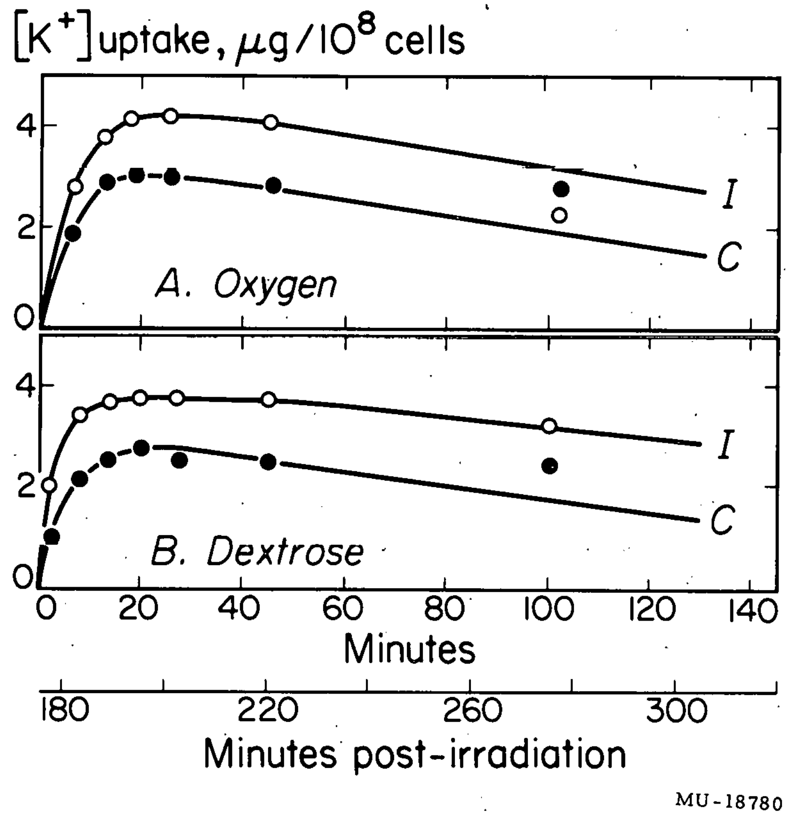

Fig. 26. Comparison of the effects of dextrose and oxygen on the potassium leakage. The experiments were carried on for three hours. Only the results of the first two hours are shown.

caused a higher uptake in both irradiated and control cells, the initial rate of uptake was little higher for dextrose. This increase in the initial rate may be an artifact because of the time difference in the measurement (one minute may cause a change in the apparent rate), or it may be genuine, as indicated in the curves, which were the results of the net movements of the potassium ions. Again in both cases, the irradiated cells performed more work and at a much higher rate. If one expresses the ratio of the amount of potassium transported to that originally present in the extracellular fluid as the "efficiency" of the "pump", the efficiencies of the irradiated cells were found to be 70% and 64% respectively with oxygen and with dextrose. The corresponding values of the control cells were 65% and 61% respectively.

The leakage of the potassium in the presence of oxygen was higher than in the presence of dextrose. This increase was due to the bubbling, which prevented the cells from settling, and also due to the intrinsic effect of oxygen demonstrated earlier. The higher rate of leakage in the controll cells in the presence of dextrose was accidental and an experimental error; in later experiments the irradiated cells showed a higher rate of leakage.

Figure 27 shows the results of the potassium uptake by the hexaploid cells. The irradiated cells also received a dose of 40.2 kr. Dextrose was added 5 hours after irradiation. As there were more potassium present in the extracellular fluid the uptake was consequently higher. The "efficiency" of the potassium "pump" in the irradiated cells was 63% and that in the control was 62%. It is interesting to note that the efficiency of the pump was practically the same for the control cells of both strains. (It was mentioned earlier that the efficiency of the sodium pump was the same in the diploid, the tetraploid, and the hexaploid cells.)

Figure 28 shows the results of the long-term study of the potassium pump of the diploid cells, to test whether the pump could be made to function again after the potassium had leaked out. Four

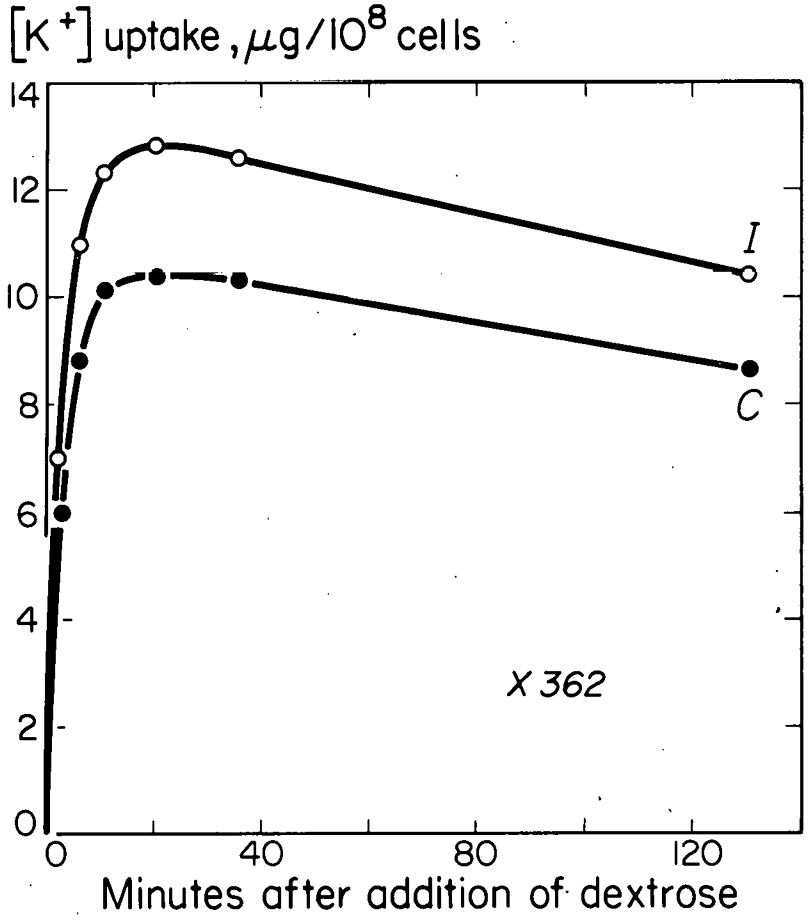

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Fig. 27. Potassium uptake by the hexaploid (X362). The cells received 107 kr of X-radiation. One ml of 10% dextrose solution was added 5 hours after irradiation.

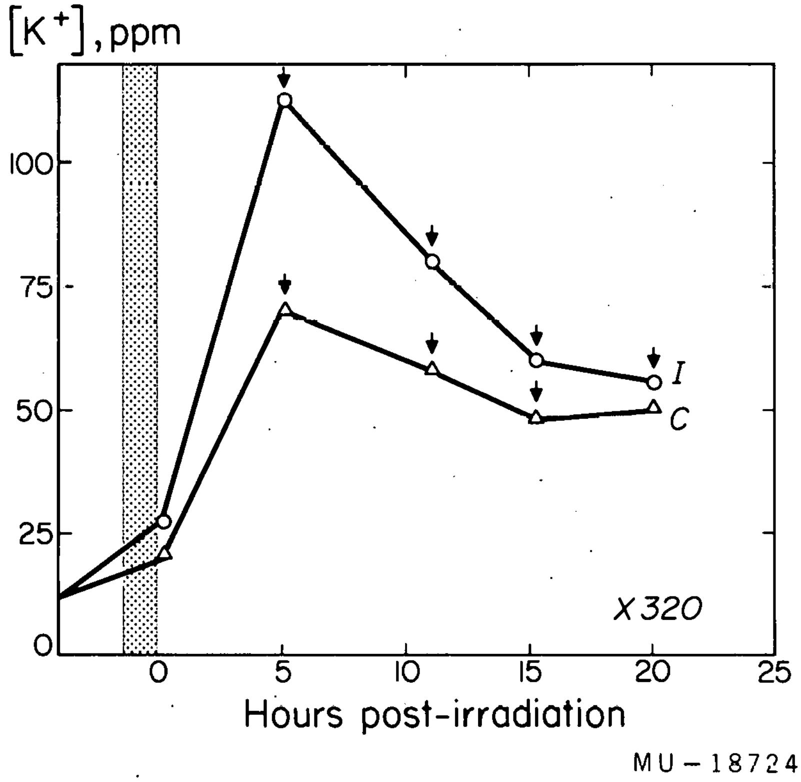


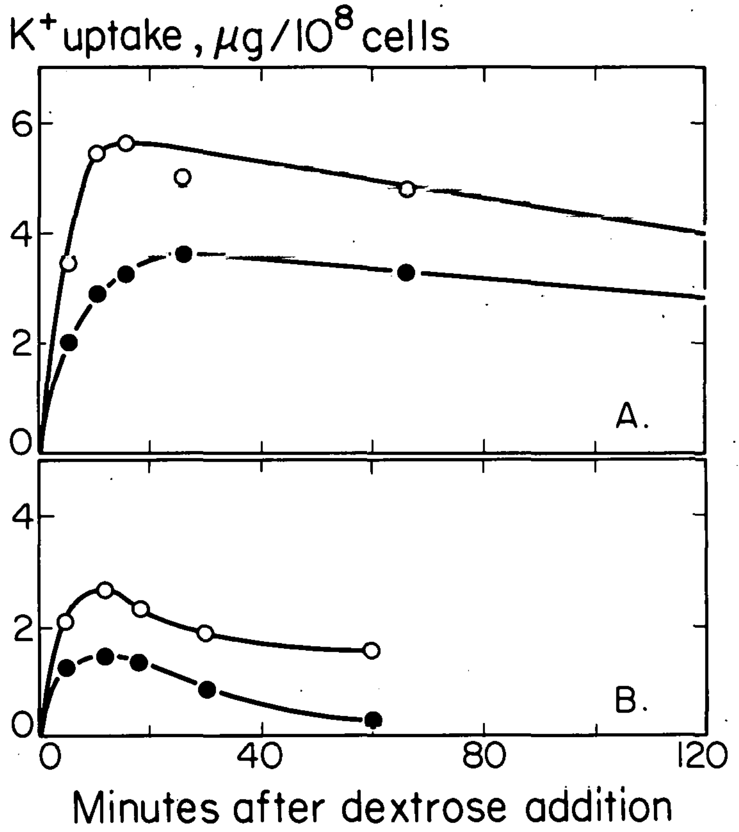
Fig. 28. Effect of successive additions of dextrose on the potassium leakage. Arrows indicate 1 ml of 10% dextrose solution was added to the cell suspension. The potassium concentration in the supernatant fluids is expressed in μg per ml. The shaded area indicate the time of irradiation.

successive 1-ml portions of dextrose solution were added at intervals of approximately 5 hours. The arrows indicate the times at which the portions were added. Immediately before the addition of dextrose an aliquot of the suspension was centrifuged and the supernatant was assayed for potassium and sodium. The potassium content in the extracellular fluid was expressed in μg per ml instead of $\mu g/10^8$ cells, because the cell concentration was different at each time and was more diluted after each addition of dextrose. The successive drops in the potassium content in the medium were caused by the fact that some potassium was still retained in the cells before the addition of dextrose and that it was also diluted upon the addition of dextrose.

The potassium and leakage curves were determined for two different conditions. One was 5 hours after irradiation, immediately after the first addition of dextrose, and the other at 20 hours, immediately after the fourth addition of dextrose. Two experiments were performed for the determination. The cells received a dose of 107 kr. The results are shown in Fig. 29A and B.

Comparing Fig. 29A and Fig. 29B, one observes that (a) the control cells show the same shape of curve, which is to be expected, and (b) the cells receiving 107 kr show a higher rate of leakage, which is also to be expected.

The curves in Fig. 29B are different from those in Fig. 29A. The uptake of the potassium was much less for the cells that had four doses of dextrose; the potassium available was less to start with. The "efficiency" of the pump was also much reduced. The efficiencies for the irradiated and the control cells are 69% and 64% in the first case and 34% and 31% in the second case. Although the initial rate of leakage was much higher in the second case, the rate of leakage was reduced an hour later. The rate was about 0.2 $\mu g/10^8$ cells per hour for both irradiated and control cells, compared with 1.3 $\mu g/10^8$ cells per hour and 1.0 $\mu g/10^8$ cells per hour, correspondingly for cells which had one ml of distilled water instead of one ml of dextrose at 20 hours after irradiation (results are not shown in the graphs).



MU - 18723

Fig. 29. Comparison of the potassium uptake by the diploid (X320) at different times after irradiation. The irradiated cells received 107 kr of X-radiation.
A: One ml of 10% dextrose was added in the cell suspension five hours after irradiation.
B: Four ml of 10% dextrose were added in the cell suspension at different times. Measurements of the uptake were taken after the 4th ml of dextrose added at 20 hours after irradiation.

DISCUSSION

In the above investigation, potassium leakage was not found to be affected by the nitrogen per se. A slight apparent increase was interpreted as due to the bubbling, which prevented the cells from settling to the bottom of the container. Rothstein, on the other hand, showed that in the absence of substrate, the K efflux under anaerobic conditions was reduced (119). The discrepancy in the two findings cannot be explained except perhaps by the fact that the techniques of measurement were different. In this investigation the net change of the potassium content was determined. Oxygen increased the potassium movements in both directions. It not only initiated the active transport of potassium, but also increased the rate of leakage. The later was manifested only when the rate of leakage is greater than the rate of active transport. The uptake rate was very much higher than the leakage rate. The increase in the leakage rate was associated with the rate of metabolism. The fact that cells kept under refrigeration lost less potassium than those kept at room temperature supported this association. Bruce and Stannard, studying effect of different substrates on the leakage rate, came to the same conclusion (86, 89), although they found a few exceptions. The factors that relate these two quantities are not known. Rothstein and Enns (62) and Conway and O'Malley (71) showed that the movements of potassium into the cells during fermentation were accompanied by the exchange of hydrogen ions, H; in leakage, the change in the potassium concentration in the extracellular fluid was also accompanied by a change in the H^+ concentration (Fig. 30). The change, however, did not indicate a one-to-one relation as demonstrated by Rothstein. A rough estimation showed that a change of 10^{-5} m/l in the H^+ concentration corresponded to a change of 2.8×10^{-4} m/l in the K^+ concentration. It is difficult to say which could be considered as the dependent variable. Rothstein and Bruce, studying the K efflux with different dyes, demonstrated that there was a definite

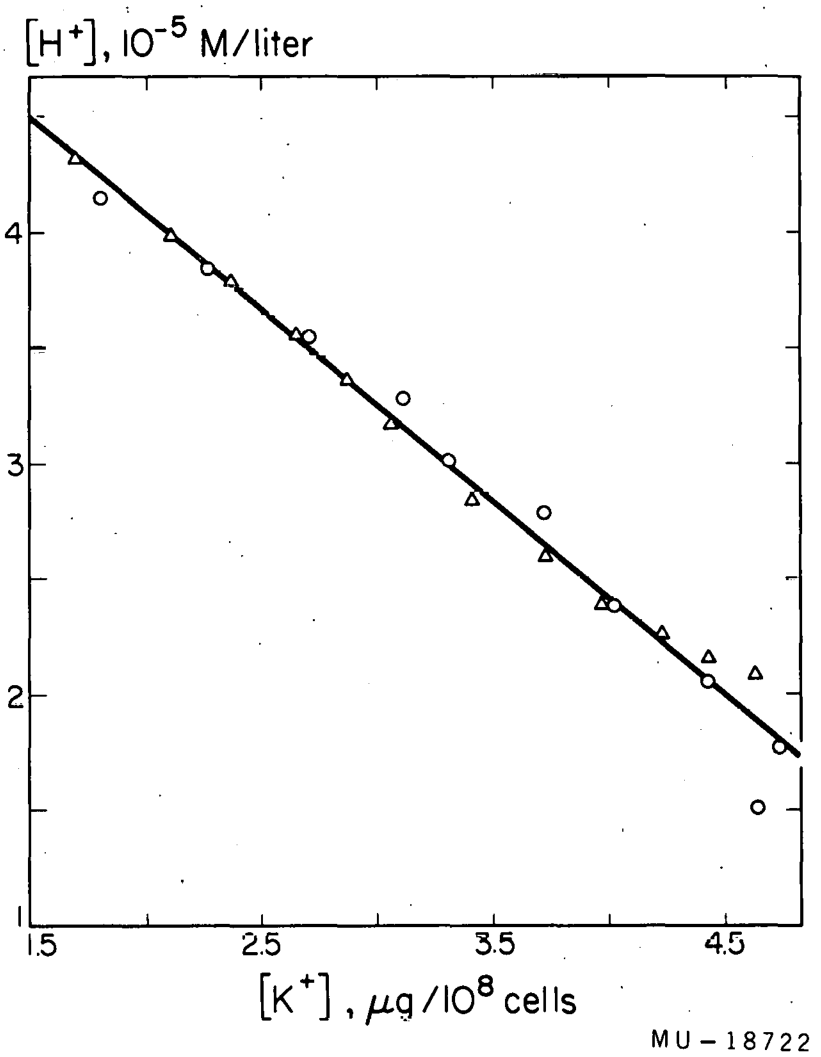


Fig. 30. Relationship between the hydrogen concentration and the potassium concentration in the supernatant fluid.

correlation of K efflux with redox potential, and suggested that the redox potential is a more important parameter than the metabolic rate per se (119). As the potassium leakage demonstrated more a physical diffusion phenomenon, one may like to think that the change in the redox potential regulated by the metabolism of the cells caused the release into the cytoplasm of some "free" potassium ions, which then moved out into the extracellular fluid by a diffusion process.

The induction of active transport by oxygen and dextrose indicates very clearly that energy is required in the process. As mentioned in the introduction, if energy is involved in the active transport, then the potassium uptake is expected to vary with the volume of the cells.

The potassium uptake was studied with both diploid and hexaploid cells. In order to compare the amount of uptake, one should take into consideration (a) the mass of cells used, (b) the amount of dextrose used, and (c) the time from irradiation until measurement. The mass has to be considered because the amount of the potassium content of the cell was found to be linearly related to the dry mass. The total mass calculated for the diploid to be 0.34 g and for the hexaploid 0.31 g, a difference of less than 10%. The amount of dextrose has to be considered because it determines the available energy. In all cases, the same amount of dextrose was used. The time of the measurement is important because the amount of the uptake depends upon the amount of potassium available. The measurements of uptake for the hexaploid were done 5 hours after irradiation, and for the diploid at 3 hours after irradiation. Corrections for the difference had to be made. The original data showed:

	Hexaploid	Diploid
Time of measurement	5 hours	3 hours
uptake, irradiated cells	12.9μg/10^8 cells	3.75 μg/10^8 cells
control	10.4 μg/10^8 cells	2.55 μg/10^8 cells
Efficiency, irradiated	63%	64%
control	62%	61%

The average "efficiency" of the potassium "pump" of the diploid cells, from three measurements taken between 3 and 5 hours, was 0.63 ±0.03 for the irradiated cells and 0.60 ±0.01 for the control cells. The leakage increased 1.23 times between 3 and 5 hours for the irradiated cells and 1.39 times for the control. Therefore the corrected values of the uptake are for the irradiated cells (corrected), $3.75 \times 1.23 \times 0.63/0.64 = 4.45$ μg/10^8 cells, and for the control (corrected), $2.55 \times 1.39 \times 0.60/0.61 = 3.48$ μg/10^8 cells. Thus for the irradiated cells we have

$$\frac{\text{Uptake by the hexaploid}}{\text{Uptake by the diploid}} = \frac{12.9}{4.45} = 2.90,$$

and for the control

$$\frac{\text{Uptake by the hexaploid}}{\text{Uptake by the diploid}} = \frac{10.4}{3.48} = 2.99 .$$

Now, from the data of Mortimer we find ,

$$\frac{\text{Area of the hexaploid}}{\text{Area of the diploid}} = \frac{194}{87.5} = 2.22 ,$$

and

$$\frac{\text{Volume of the hexaploid}}{\text{Volume of the diploid}} = \frac{244.6}{79.2} = 3.08 .$$

It is indeed very interesting to find that the ratio of the K^+ uptake for the control cells is so close to the ratio of the volume. Since the active transport of potassium took place in the presence of dextrose and was initiated by the introduction of oxygen, evidently energy is involved in this process. In such case, one would certainly expect the potassium uptake to be a linear function of the cell volume. The low ratio of the potassium uptake in the case of the irradiated cell is difficult to explain for the efficiency of the potassium pump was shown not to have been impaired at the dose used.

Potassium plays an important role in cell metabolism. It is an activator in many enzyme systems (120, 121). In yeast, it activates phospho-fructosekinase and phospho-glyceraldehyde dehydrogenase. Its function in carbohydrate metabolism has been studied by many investigators, such as Pulver and Verzar (118), Scott (122), Conway and O'Malley (71), and Rothstein and Enns (62); its effect on phosphorylation by Kotyk (123). The phenomena of potassium uptake and leakage by cells are well established, but the mechanisms are not fully known. In the presence of glucose, potassium ions are first actively transported into the cell and upon the utilization of the substrate the accumulated potassium returns to the medium. The active transport was interpreted by Pulver as associated with the formation of glycogen, by Conway with the formation of phosphate ester. Rothstein associated the retention of potassium with the synthesis of carbohydrate. When the carbohydrate reserves are used up the retained potassium is liberated from the bound state and diffuses out to the medium. Pulver demonstrated that the accumulated potassium returned to the medium when the glucose was depleted and that the active transport could be regenerated by addition of more glucose.

In this investigation, the active transport was regenerated successively four times. The uptake of potassium and the "efficiency" of the "pump" were much reduced. The leakage took place sooner and at an increased initial rate. All these observations indicate that the energy derived from glycolysis was less at 20 hours after irradiation upon the fourth addition of dextrose than at 5 hours following only one addition of dextrose. If the potassium leakage were associated with the depletion of the carbohydrate reserves, it would be hard to explain the increased initial rate of leakage. With more dextrose available in the cells more carbohydrate should be synthesized, unless the rate of depletion is higher than the rate of synthesis.

It is also difficult to explain the larger amount and the higher rate of leakage of the cells that, instead of receiving the fourth 1-ml addition of dextrose received 1 ml of water. It was shown by Rothstein and also in this investigation that glucose and dextrose increase the rate of potassium leakage.

With oxygen bubbling, the source of energy, i.e. oxygen, was available to the cells at all times, and yet the cells returned the accumulated potassium to the medium after a certain period. It is evident that the energy source for operating the pump is not the oxygen per se. Either the oxygen initiates the operation of the pump or some product in the tricarboxylic acid cycle supplies the energy to operate the pump. When this supply of energy is depleted some of the bound potassium is released and returns to the medium.

Whatever the mechanism responsible for potassium transport is, it is safe to say that the yeast cells obtain the energy for operating the potassium pump from glycolysis and respiration. Studies with inhibitors of potassium transport in different organisms have often produced conflicting results. The conclusion drawn by Tosteson and Johnson that Adenosine triphosphate was responsible for the movement of potassium seem to clarify some of the problems. If this is the case, it is easy to comprehend that the active transport in nerve depends only on aerobic metabolism, while that in human red cells depends on the energy from glycolysis (124).

SODIUM LEAKAGE

The behavior of the sodium ions in the leakage phenomena was quite different from that of the potassium ions. The amount of sodium lost from the cell to the distilled water did not show any obvious relationship to the ploidy of the cells, nor did it increase with dose. Unlike K^+ ions, which continuously diffused out from the cells at an appreciable rate after many hours, the rate of sodium leakage decreased sharply after 2 or 3 hours following irradiation.

Because of the sodium contamination from the glassware, the net amount of the sodium lost from the cells could not be determined very accurately. It is also because of this contamination that the sodium content in the supernatant fluid from the cell suspension that had been irradiated was found to be extremely variable--it could be either larger than, the same as, or smaller than that of the control. Table VII gives the cell suspension of the sodium content in the supernatant fluid from the cell suspension for cells of different ploidies.

Table VII

Average range of the sodium content in the supernatant from cell suspension of cells of different ploidies

Strain	Average range of Na content ($\mu g/10^8$ cells)	Percent of total Na content
Haploid (S163A)	0.2 - 0.3	21 - 32
Diploid (X320)	0.1 - 0.2	20 - 39
Tetraploid (X323)	0.3 - 0.4	36 - 48
Hexaploid (X362)	0.35 - 0.45	30 - 39

Figure 31 shows the results of three experiments on the sodium leakage from the haploid cells (S163A). The irradiated cells received

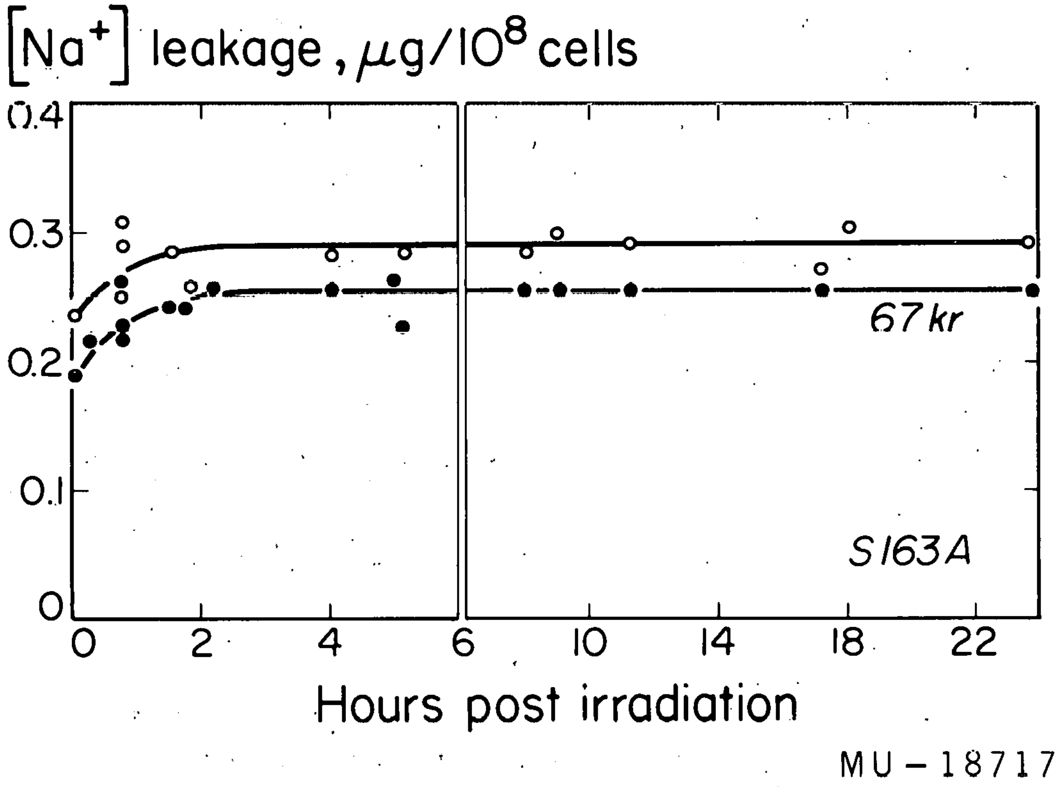


Fig. 31. Sodium leakage curves for the haploid (S163A).

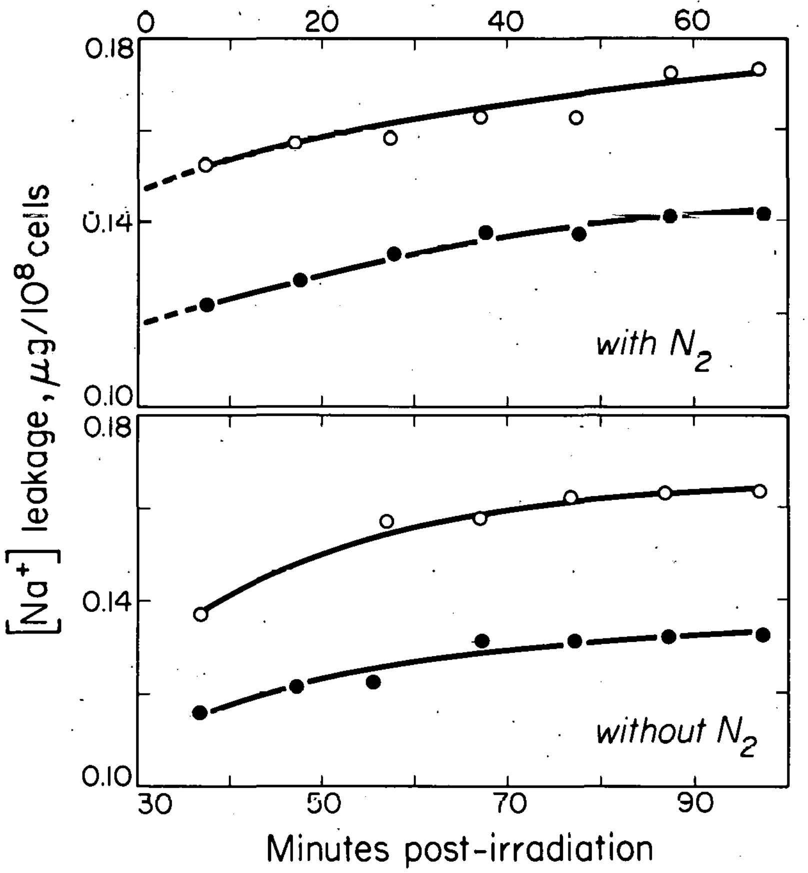
an X-ray dose of 67 kr. In this case, the sodium content in the supernatant fluid did not increase after 2 hours following irradiation. The net loss of sodium for the cells was found to be 0.05 $\mu g/10^8$ cells, for both the irradiation and the control cells. This indicates that radiation did not have any effect on the sodium leakage.

EFFECT OF NITROGEN, OXYGEN, AND DEXTROSE ON THE SODIUM LEAKAGE

The effect of nitrogen (anaerobiosis) on the sodium leakage was not conclusive. Figure 32 shows the result of one experiment with the diploid cells. The irradiated cells received an X-ray dose of 40 kr. In this particular experiment, the net increase of the sodium content in the supernatant fluid was found to be 0.02 $\mu g/10^8$ cells per hour for both the irradiated and the control cells in the presence of nitrogen. Without nitrogen, the net increase was found to be 0.035 $\mu g/10^8$ cells for the irradiated cells and 0.015 $\mu g/10^8$ cells for the control. Although the data indicate that in the presence of nitrogen the irradiated cells lost less sodium and the control cells lost more, the difference was not significant, for a difference of 0.1 $\mu g/10^8$ cells in the sodium content corresponded to a difference of only 1 ppm of sodium in the supernatant.

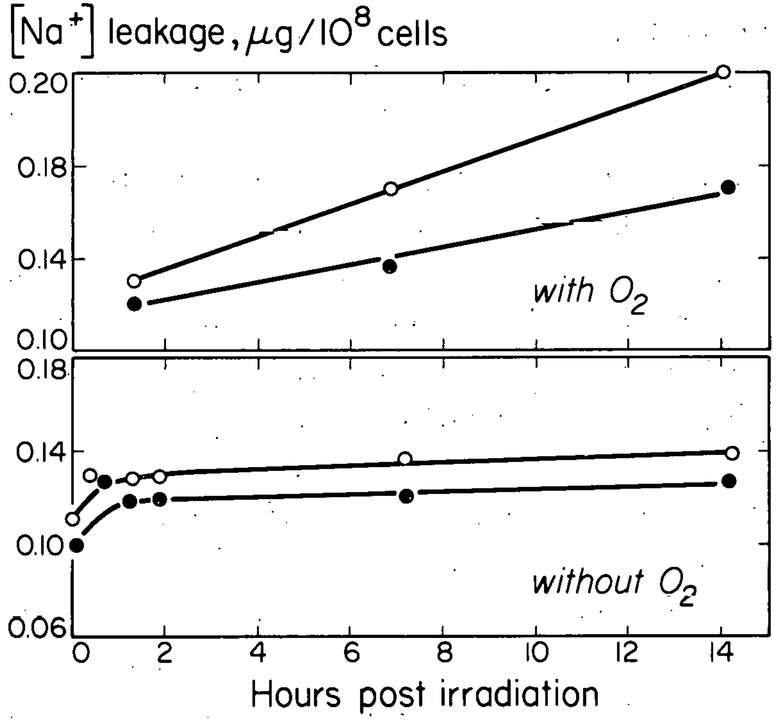
The bubbling effect per se of nitrogen, which caused an increased rate in the potassium leakage, was not observed here in the sodium leakage. The effect of oxygen on the sodium leakage was more evident.

Figure 33 shows the result of one particular experiment with the diploid. The irradiated cells received an X-ray dose of 40 kr. In the presence of oxygen, the irradiated cells lost 0.09 $\mu g/10^8$ cells in a period of 12.7 hours, while the control cells lost 0.05 $\mu g/10^8$ cells. These values correspond to a leakage rate of 0.007 $\mu g/10^8$ cells per hour for the irradiated cells and 0.004 $\mu g/10^8$ cells per hour for the control. It is interesting to note that in the absence of oxygen, both irradiated and control cells continuously lost some minute amount



MU 18718

Fig. 32. Effect of nitrogen on the sodium leakage. The irradiated cells (diploid) received an X-ray dose of 40.2 kr.



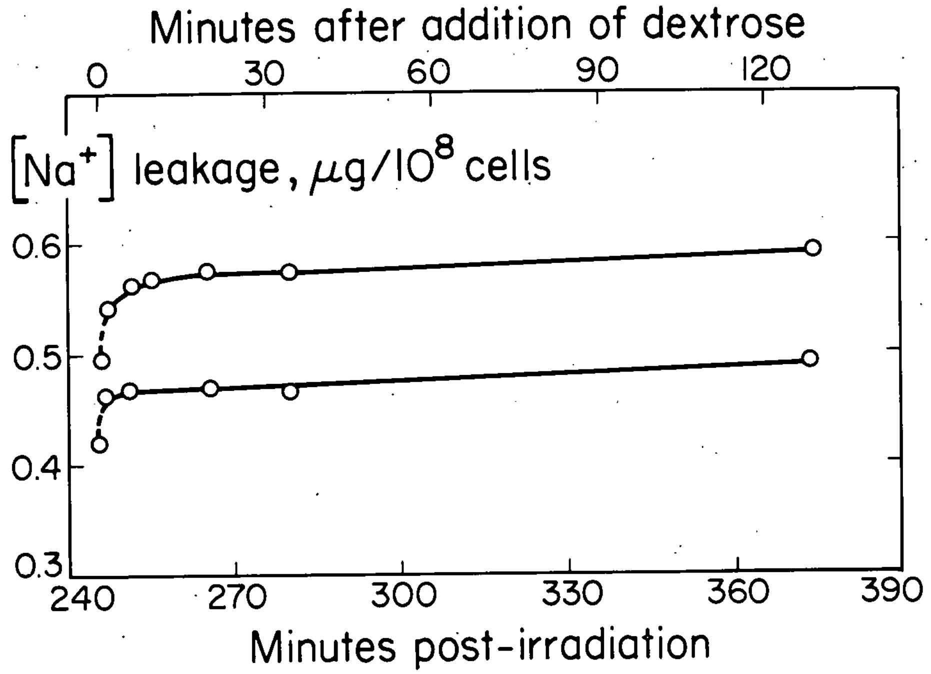
MU – 18715

Fig. 33. Effect of oxygen on the sodium leakage from the diploid. The irradiated cells received an X-ray dose of 40.2 kr.

of sodium to the distilled water medium 3 hours after irradiation. The amount was about 0.006 to 0.008 $\mu g/10^8$ cells in a period of 14 hours, which was much smaller than found in the presence of oxygen. The same behavior was also observed for cells of higher ploidy, but not in the haploid (S163A) as shown in Fig. 31. The difference, however small, may be due to the anomaly of the haploid (S163A) in its sodium content.

The effect of dextrose, if any, on the sodium content was very small. Figure 34 shows the result of an experiment with the hexaploid (X362). The irradiated cells received an X-ray dose of 40 kr. The increase in the sodium content, as indicated by the first two points in the graphs for both the irradiated and the control cells, was due to the introduction of sodium from the dextrose solution, therefore it should be disregarded. The loss of approximately 0.05 $\mu g/10^8$ cells in 2 hours after the addition of dextrose falls within the range shown in Table VII. Because dextrose was added 4 hours after irradiation--at which time, according to Fig. 33 (assuming that the hexaploid behaved the same way as the diploid), the rate of sodium leakage was very small--this increase of 0.05 $\mu g/10^8$ cells in a period of 2 hours is significant, and it must be due to the effect of dextrose. In addition, if one considers the straight-line portion of the leakage curve, one finds that the irradiated cells lost 0.02 $\mu g/10^8$ cells in about 100 minutes whereas the control cells lost the same amount in about 85 minutes. These values are large compared with the value obtained in the absence of dextrose (or oxygen). For the tetraploid (X323) in the absence of dextrose (or oxygen) the cells lost the same amount, i.e., 0.02 $\mu g/10^8$ cells, in a period of 8 to 16 hours.

Figure 35 shows the results of the successive additions of dextrose every 5 hours. (See Fig. 28 for a similar experiment on the potassium leakage.) Although the higher sodium content in the supernatant fluid from the cell suspension of the control cells was insignificant, the steady increase of the sodium content after four additions of dextrose



MU-18719

Fig. 34. Effect of dextrose on the sodium leakage from the hexaploid (X362). The irradiated cells received an X-ray dose of 40.2 kr.

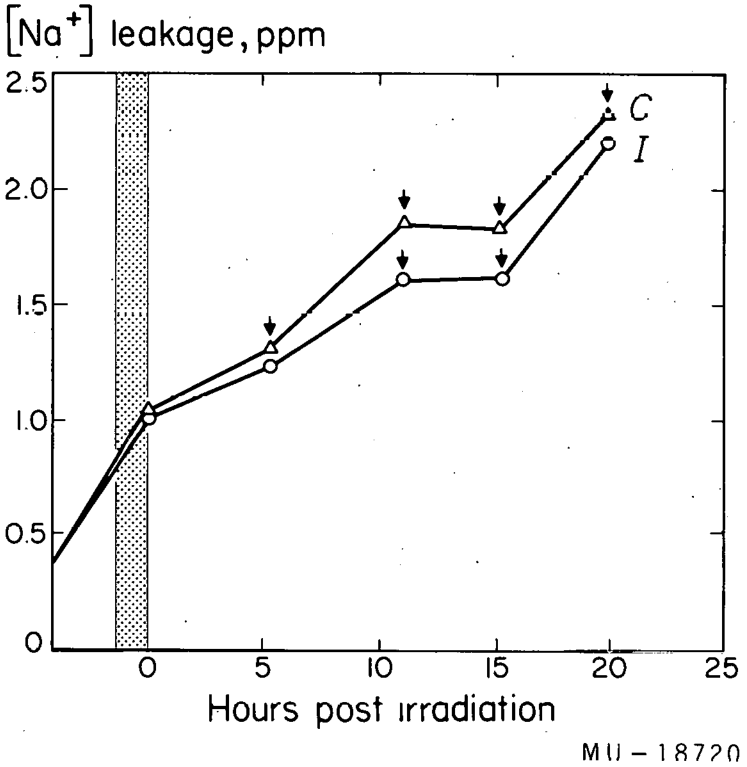


Fig. 35. Effect of successive additions of dextrose on the sodium leakage. The irradiated diploid cells received an X-ray dose of 107 kr. The arrows indicate the time at which dextrose was added. The shaded area indicates the time of irradiation.

may be meaningful. Although addition of dextrose had introduced some sodium into the cell suspension, as shown by the first two points in the curves in Fig. 34, the amount introduced was rather small. According to the data, the addition of 1 ml of dextrose to 14.5 ml of the suspension caused an increase of less than 0.1 ppm in the sodium content in the supernatant fluid. Four ml of dextrose would introduce a total increase of 0.4 ppm. Since the increase was shown to be more than 1 ppm, the effect of dextrose on the sodium leakage was not an artifact.

DISCUSSION

As mentioned in the preceding section, an abrupt reduction in the rate of the sodium leakage was observed 2 to 3 hours after the measurement, which was started immediately after irradiation. The same phenomenon was also observed if the measurement was started immediately after the cells were suspended in distilled water following the routine harvest and washing processes. The shape of the curve was similar to that given in Fig. 31, with the time coordinate shifted to an earlier time $(t - t_0)$, t_0 being the time elapsed between the suspension and the irradiation. The sodium level in the supernatant fluid was, on the average, lower than that obtained after the irradiation. Although the true nature of the extrusion of sodium is not known, the initial loss of sodium from the cells to the distilled water may be due to the diffusion process, since the driving force could be derived from the existing concentration gradient across the membrane. After the initial loss, the ion movements are governed by some physiological processes. This reasoning may explain the abrupt reduction in the rate of sodium leakage when the measurement was made immediately after the suspension in the distilled water.

In all experiments with radiation, the cell suspension was kept in the refrigerator (5°C) for from 3 to 4 hours before it was irradiated. During this period, some of the sodium had already leaked out, and by the end of the period the rate of sodium leakage had already decreased greatly. The leakage could be accounted for by the average initial sodium level immediately after irradiation, as shown in Table VII. From Figs. 31, 33B, and 34 one notices that the rate of leakage was rather high compared with what would be expected after the sodium had been leaking for 3 to 4 hours. This increased rate of leakage must be initiated by some processes other than the driving force due to the concentration gradient, unless that gradient was suddenly increased.

From the nature of the result of the oxygen effect on the sodium leakage, it seems probable that oxygen was the agent. Since the cell suspension was oxygenated during the irradiation process, the inference is not without support. The variation in the average initial value of the sodium level in the supernatant fluid for cells of different ploidies, as shown in Table VII, also supports this view. The haploid (S163A), having almost twice as much sodium in the cell, gave a higher average initial value (0.2 $\mu g/10^8$ cells, compared with 0.1 $\mu g/10^8$ cells).

In the study of Porphyra, Epply inferred that oxidative phosphrylating respiration is required for sodium exclusion (125). If such is the case in yeast, one can infer with certainty that in this investigation oxygen was the agent that initiated the increased rate of the outward movements of the sodium ions. In this study, the sodium concentration in the extracellular fluid was lower than the sodium concentration in the extracellular fluid) is higher than the concentration of the "fre" sodium ions in the cytoplasm, one can consider the outward movement as an active transport process.

The binding of sodium in the yeast has been studied extensively by Rothstein (64-65), by Conway and his associates (76-84), and by Takada and his associates (126-132). There is ample evidence that some sodium ions in the cell are bound to various sites and that these bound ions could be released by some compounds that interfere with the cell metabolism. Hiroaka and Takada (132) found that Na efflux was increased in the presence of ribonuclease. With nuclear spin-resonance technique Jardetzky found that some metabolites such as lactate, pyruvate, and citrate form specific complexes with sodium ions (133).

In many instances yeast cells were found to exchange potassium for sodium. One of the examples was given by Sanders and Giese (134). The slight increase in the rate of sodium leakage in the presence of dextrose (Fig. 34) and the steady increase in the sodium content in the supernatant fluid after successive additions of dextrose (Fig. 35) may be associated with the mechanism that governs the exchange of potassium with sodium ions, although the potassium uptake was found to be much faster than the sodium leakage. On the other hand, it may be caused by the available energy supplied by the glycolysis.

A sodium-rich yeast cell as developed by Conway (76) is in many ways a better organism to study the sodium ion transport phenomena. But the cell-volume change accompanied by the movement of sodium ions (135) may complicate the study, involving the correlation between leakage and transport with the size of the cell.

Despite the voluminous work by many investigators throughout all these years, the understanding of the basic mechanism of the movement of the sodium and potassium ions into and out of the cell cannot be achieved without further investigations.

SUMMARY

1. The sodium content of the yeast cell, Saccharomyces cerevisiae, was determined for the haploid (S163A), the diploid (X320), the tetraploid (X323), the hexaploid (X362) of a related polyploid series. With the exception of the haploid, which is a "clumpy" strain, the sodium content was found to bear a linear relationship to the cell surface area.

2. The potassium content of the yeast cell of the four ploidies studied was found to bear a linear relationship to the dry weight of the cell.

3. The amount and the rate of the potassium leakage from the cells to the distilled water medium varied with the cell concentration in the suspension and with the shape of the container in which the cells were suspended.

4. The amount and the rate of potassium leakage from the cells to the distilled water medium varied with the ploidy of the cell and with the X-ray dose. They were larger and higher in cells of higher ploidy and with higher dose.

5. The net potassium lost from the cells to the distilled water medium within the first 45 minutes after irradiation bore a linear relationship to the cell surface for both the control cells and the cells that had received an X-ray dose of 67 kr.

6. The order of the radiosensitivity of the potassium retentivity of cells of different ploidies does not follow the same sequence as that of lethality.

7. Cells stored under refrigeration lost potassium more slowly to the distilled water medium.

8. The effect of nitrogen on the potassium and sodium leakage was not obvious.

9. In the presence of dextrose, the cells reabsorbed most of the potassium ions lost to the distilled water medium but returned them again to the water after a certain period. The rate of leakage after reabsorption was found to be much higher.

10. The active transport process could be repeated many times by successive addition of dextrose. The active transport process was not impaired by radiation with the dose used, but the leakage was increased.

11. The ratio of the amount of potassium uptake by the hexaploid in the presence of dextrose to that of the diploid is 3:1, the same as the ratio of their respective cell volumes.

12. The effect of dextrose on the sodium leakage, if any, was very little.

13. In the presence of oxygen, both the irradiated and the control cells were able to reabsorb the potassium ions lost to the distilled water medium, as for dextrose. The leakage rate after the active transport process was increased. Radiation did not seem to impair the active transport process.

14. Oxygen was found to increase the rate of sodium leakage from the cells to the distilled water medium.

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