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Some effects of chronic radiation on a steady-state yeast population[1]
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Abstract

Saccharomyces cerevisiae in continuous culture has been subjected to chronic x-ray irradiation for many generations with apparently neither progressive deterioration nor acquired resistance to radiation. Detrimental effects on morphology and growth rate were observed, however, and measurements of the latter are presented graphically. Cycling of radiation on the continuous culture did not induce synchrony.

Key words for indexing:

Chronic radiation

Steady-state yeast

Continuous culture

Yeast

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INTRODUCTION

Because biological evolution has taken place in an environment including background radiation, probably not much different from that now existing, it is not unreasonable to assume that currently living forms are optimized in their ability to survive indefinitely in the presence of this radiation. Currently, the increase in exposure to chronic radiation from background and from use of radiation in medical diagnosis, and particularly of radiation industry workers and of space explorers, places renewed emphasis on questions of long-term effects. We may justifiably ask: What chronic dose is tolerable? And, further, what dose-rate increase above that now existing might lead to progressive deterioration such that a population could not ultimately survive? In addition, might not mutants appear, better able to withstand the deleterious effects of radiation and eventually dominate the population?

Ideally an attack on such questions should employ large populations and many generations, and, of course, preferably be performed with mammals. Relatively long life span and large investment in housing and care, however, limit what can be done with small mammals. However, duration-of-life exposure of mice has shown deleterious effects on longevity (2), and mutation rates to recessive visibles of 2.5×10^{-8} per R have been measured (3).

At the tissue level, continuous irradiation of gut has not produced any substantial change in cell proliferation characteristics (4). Similarly, red cell production, regenerative ability, and--to some extent--precursor populations of continuously irradiated rat appear remarkably normal (5).

Radiation in vitro over many generations of mouse leukemia cells (6) has produced no evidence of progressive deterioration. Chronic irradiation of E. coli (7) showed increased lag phase as well as a decreased growth rate very similar to that reported here. A linear rise in mutation rate (8) and increased induction of lysogenicity (9) have also been observed in E. coli in continuous-culture experiments.

In the work reported here, a population of yeast cells was maintained in continuous culture for many generations under continuous x irradiation at several different dose rates. Determinations were made of division-rate dependence on dose rate, evidence for possible increased radiation resistance was sought, and pulsed radiation was used in an attempt to induce synchrony.

CONTINUOUS-CULTURE PROPAGATION

Continuous-culture propagation of microorganisms requires that a culture in liquid suspension be maintained at constant volume, with equal rates of addition of fresh nutrient and removal of partially spent nutrient plus cells, mixing at all times being instantaneous and complete. It has been shown (10) that for a population of n cells per cm^3

$$dn/dt = (\alpha - \beta)\, n, \qquad (1)$$

where α is the growth rate and β is the washout rate; $\beta = w/V$, the nutrient flow rate divided by the growth-tube volume.

For steady-state operation with automatic control, such as in the Chemostat (10), the Bactogen (11), the Breeder (12), and the Turbidostat (13), $\alpha = \beta$ and the growth rate is determinable from physical constants of the apparatus. For continuous culture with manual

control of the flow rate, as in these experiments, an exactly steady state may not be obtained. It was determined experimentally, however, that if the change in population density in about 12 hours was less than a factor of two, the culture divided at the same rate as at steady state, so that steady-state division rate measurements were obtained even though the yeast culture was sometimes only near steady state. For the near-steady-state condition, from Eq. (1),

$$\ln n = (\alpha - \beta)\, t + c, \tag{2}$$

and substituting conditions as may be easily read from a semilog plot of n vs t, e.g., $n_2 = 2n_1$, in Eq. (2) leads to

$$\ln 2 = (\alpha - \beta)\, T_2, \tag{3}$$

where T_2 is the observed time for population to change by a factor of two. Then

$$\alpha = (0.693/T_2) + \beta, \tag{4}$$

and the growth rate is thus determinable from the physical constants of the apparatus and a rate-of-change measurement from the population density record. It should be pointed out that T_2 is a convenient time for calculations and that the population density was usually not allowed to change by as much as a factor of 2 during a measurement.

Chronic exposure to radiation slows division rate and concurrently inhibits division of a small percentage of the cells. These two effects may be separated as follows:

Let n = concentration of viable cells,

m = concentration of nonviable cells,

α = division rate of n,

β = washout rate for both n and m,

γ = rate of inhibiting division of n;

then $dn/dt = \alpha n - \beta n - \gamma n = (\alpha - \beta - \gamma)n$

and $dm/dt = \gamma n - \beta m$.

At steady state dn/dt and dm/dt are both zero, so

$$\alpha = \beta + \gamma$$

and $\gamma n = \beta m$;

then $$\alpha = \beta + \beta m/n = \beta(n + m)/n. \quad (5)$$

Thus the division rate of viable cells may be obtained from the washout rate and the fraction of viable cells, $n/(n + m)$.

DESCRIPTION OF THE CONTINUOUS-CULTURE PROPAGATOR

A diagram is shown in Fig. 1. Associated with the growth tube G (also shown in Fig. 2) are the five service systems: (a) nutrient supply, (b) used nutrient removal, (c) aeration and agitation, (d) population density measurement, and (e) x-ray irradiation.

The culture in G was fed from the nutrient supply NS1 contained in a 2.5-liter rubber bag within a water-filled closed 6-liter flask W2. Pump 1, continuously variable and precisely adjustable (14), transferred water from reservoir W1 to W2, thus displacing nutrient into G. This displacement procedure makes unnecessary sterilization of the pump. Nutrient (0.1% yeast extract and 1% dextrose) was prepared and autoclaved in NS2 and transferred to NS1 with removal of an equal volume of water from "W out."

Constant growth volume was maintained by level-control contacts C1 in the side arm of G. They were connected to a vacuum tube relay R1, which in turn operated V1. Valve V1 was a pinched rubber

tube, spring-closed, and opened by a solenoid. Bubbles issuing from air inlet A1 for aeration and mixing raised the liquid level in the growth tube and, in order to maintain a constant liquid volume, the bubble size and hence air pressure were held constant. The air pressure from pump 2 was controlled by contacts C2 (in a conducting water column), relay R2, and bleeder valve V2. Valve V3 was opened by timer switch SW for 5 sec of each minute for the aeration.

During 55 sec of each minute a measure of the relative density of the culture was recorded by the system diagrammed in the upper right of Fig. 1. Light from the stabilized source L was scattered by the suspension in G and picked up by photocell P. Rotating shutter RS chopped the beam at 330 Hz so that an ac band-pass amplifier, Amp, could amplify the signal from P. Amp had voltage-regulated power, Pwr 1, and in addition, the ac power for the entire system was regulated by Pwr 2. As a result of these several precautions, no instability problems developed throughout the course of the measurements.

The culture was irradiated with a Universal portable 85-kV x-ray machine which was also powered by the regulated source Pwr 2. For dose-rate calibration, a 100-R Victoreen ionization chamber was placed in the growth tube and several timed doses correlated with x-ray tube currents. A recording milliammeter in the x-ray tube plate circuit then served to monitor the dose rate during the chronic irradiation runs.

EFFECTS OF CHRONIC X-RAY IRRADIATION

A steady-state culture of Saccharomyces cerevisiae SC-6 (15), when subjected to continuous irradiation, always became non-steady-

state. That is, the population level decreased and eventually would have washed out if the nutrient flow rate had not been reduced to match the decreased division rate. In addition, an examination under 400× of samples from the growth tube showed marked change in the sizes of the cells. In the unirradiated culture they were of relatively uniform size and grouped approximately 95% in pairs and the rest in fours. Groups of three or more than four, as well as singles, were rare. But when the culture was exposed to radiation, a spectrum of sizes up to approximately twice that of the unirradiated was apparent and at 6150 R/generation, the maximum available, the doubles dropped to about 90%, with ones and fours about equally divided for the remainder. As with the unirradiated culture, groups of three and more than four were rare. The groups of two and four agree with previous observations (16) that mother-daughter cohesion is very strong prior to the inception of budding, and drops markedly thereafter. Further, budding by both of a pair is nearly synchronized. Thus fours break into twos shortly after budding starts and remain as a pair while the daughter matures.

Steady-state division rates at 21 dose rates were obtained. Viability at three of these dose rates is shown in Fig. 3. For this, samples from the growth tube were sprayed with an air brush onto 1-cm^2 agar blocks to give 300 to 600 pairs, and, after one day of incubation at room temperature, were counted with the aid of a microscope. Groups of less than 40 cells were classified as nonviable. In Fig. 3 the bars indicate standard deviations and the number at each point indicates the number of measurements. The line of Fig. 3 was then used with Eq. 5 to calculate the growth rate of the viable cells.

These data for the 21 growth rates with chronic radiation are plotted in Fig. 4. (The data for E. coli in Fig. 4 will be discussed later.)

Linear change of division rate with dose rate of 1.0×10^{-4} $hr^{-1}R^{-1}$ generation describes the data very well out to about 2500 R per generation time, after which the division rate changes at a decreasingly slower rate up to a dose rate of 6150 R/generation, the maximum available. Three curves are drawn among the points of the first, second, and third through fifth experiments. A lowered division rate, such as appeared in the first run, occurred when an overgrown culture was subsequently brought to a steady state. Since the three curves appear to follow the same slope, the separation probably indicates a variation in the normal population and not a radiation effect. In run No. 1 the culture became overgrown twice, due to equipment malfunction, before a steady state was obtained, and then the division rate stayed within 2% of $\alpha = 0.443\ hr^{-1}$ for 49 generations before the x rays were turned on. Run No. 2 was inoculated from No. 1 and shows the same radiation effect except that the division rates were all higher, indicating a sort of recovery. Run 3, inoculated from 2, was apparently normal in comparison with Run 4, which was started from a fresh culture. Run 5 was also started from a fresh culture, but it attained a density of about 3×10^7/ml before it was adjusted to a steady state at 5×10^6/ml. (Above 1.8×10^7/ml growth was no longer in the log phase.)

For acute sublethal irradiation of single cells, the commonly measured effect is division delay. This has been measured in diploid S. cerevisiae (16) and interpreted on the basis that delay is proportional to the fraction of a specific number of delay sites inactivated by the

radiation and that repair follows zero-order kinetics, i.e., the rate of re-formation of active sites is constant.

In order to compare the chronic and acute radiation data, an equivalence between dose rate and acute dose needs to be established. It was shown that practically all the delay was associated with the second division after irradiation, and that there were a sensitive stage and a relatively insensitive stage which appeared to represent conditions throughout the division cycle. In the absence of delay data covering all stages of the division cycle, it was assumed that each of the two stages accounted for a half generation time, and the expected delay was calculated as if exposure were made continuously. The two points that fall within the range of the continuous exposure experiments are plotted as "B ▽" in Fig. 5. There the data are the same as in Fig. 4 except that the growth rates have been converted to generation times and then the generation time with no exposure has been subtracted, which leaves a time that is interpreted as average delay. The dashed curve connecting the two B▽ points fits the inactivation-of-site model and has the formulation for the delay d in hours in terms of the dose rate D in kR/generation

$$d = 3(1 - e^{-0.3D}) - 0.8e^{-0.3D} \qquad (6)$$

The general expression previously reported (16) was of the form

$$d = c(1 - e^{-kD}), \qquad (7)$$

to which an additional term was necessary to fit the chronic irradiation data. This term is most important at low dose rates, where it indicates a threshold. That the continuous radiation data do not fit this expression at the low end is indicative of different time-dependent rules of

damage and repair.

On the assumption that the re-formation of sites proceeds at a constant rate, whereas damage is proportional to dose rate, there would exist a dose rate below which the ability to re-form sites would exceed the number inactivated, so that no damage would have to "wait for its turn" to commence repair. This would lead to smaller delay times than Eq. 6 indicates, giving way to Eq. 7 at and above a dose rate at which the repair mechanism was fully occupied. This dose rate would correspond to the flex point which in Fig. 5 occurs at about 1500 R/generation, and one can speculate that the region where repair to all damage could commence immediately could have special properties, such as perhaps no ultimate limitation (by radiation) on the survival of the culture.

Sigmoid-type data such as in Fig. 5 may often fit the Gompertz equation, $y = ab^{c^x}$: in fact, the lowest curve is described by

$$d = 1.52(0.0611)^{0.612^{(D/500 - 1)}}.$$

This function has been useful in interpreting mortality data, and may prove useful with microorganisms as further chronic irratiation data become available.

The results of chronic irradiation of E. coli over several generations in batch culture were reported (7) as no apparent effect on growth rate; only the length of the lag phase was increased by radiation. Close inspection of the data presented, however, discloses small changes in growth rate which, when plotted in Fig. 4, (--▽--symbol) give results remarkably similar to those obtained with yeast. The two curves are for different inocula--1% of a 16-hour culture for the upper,

and 1% of a 6-hour for the lower. Also the data for the upper curve were identical for E. coli B and B/R. The straight line from zero to 2500 R/gen has the slope of $1.0 \times 10^{-4} hr^{-1} R^{-1}$ gen found with yeast, and indicates a similarity of division delay mechanisms for the two organisms.

RECOVERY AFTER CONTINUOUS IRRADIATION

As with the establishment of a steady state with continuous exposure to x rays, so also the recovery to normal division rate takes several generations of time after turning off the x-ray machine. Somewhat analogous observations have been made elsewhere (4, 6, 9). Figure 6 shows the division rate as a function of the elapsed time after termination of chronic irradiation at 6150 R/generation. Cells in early stages after division, when presumably all delay damage had been repaired, would have accumulated the least amount of delay damage at termination of irradiation. These cells then lead the division procession and constitute a dominant part of the population when it has regained normal division rate.

ABSENCE OF ACQUIRED RESISTANCE TO ACUTE X-RAY EXPOSURE

Exposure of yeast to sublethal radiation greatly enhances mitotic crossing over (17), which is in yeast a major cause of genetic variation. When the exposure is continuous for many generations, the possibility exists for the selection of a variant better able to resist the damaging effects of radiation. Accordingly, percent survival was measured after acute exposure to 25 kR of several samples of unirradiated and long-term-irradiated cells taken from the continuous culture. The results are summarized in Table I.

Table I. Viability after 25 kR acute radiation exposure.

Dose rate (R/generation)	Generations of chronic irradiation	Number of samples	Percent viable	σ
0	-	2	56	1.0
2515	75	5	55	2.3
6150	115	1	59	-

There was apparently no dramatic change in radiation resistance as a result of the long chronic exposure and, if any small change exists, it is masked by the variation in the data. In experiments of comparable nature, no indication of a lowering of radiosensitivity was found in E. coli irradiated continuously for 60 generations (9), but mouse leukemia cells after about 6 months of irradiation became more resistant to both chronic and acute irradiation (6). A continuous culture of yeast should be irradiated for a longer period before a definitive conclusion can be reached.

SURVIVAL ABILITY

By extention of measurements of acute radiation effects it is reasonable to conclude that the irradiated yeast in continuous culture is subjected to division delay (16), to dominant lethal mutations (18), and to recessive lethal mutations (15) with partial dominance (19) and mitotic crossover (17), which leads to homozygosity and elimination of lethals. Sensitivity variation with stage of division cycle (20) further complicates the picture. In spite of this onslaught the experimental culture continued to propagate apparently without progressive deterioration for at least 115 generations, by which time the dose accumulation

was approximately 300,000 R. It seems reasonable to conclude, therefore, that a continuously operating repair mechanism must be an integral part of the system. In addition, the repair mechanism itself is probably subject to radiation damage, which could provide the ultimate limit on survival of a continuously irradiated population.

DIVISION-SYNCHRONY ATTEMPT

If large populations of cells dividing synchronously were available, they would constitute a very useful tool for experiments designed to correlate changes in chemical composition with the various stages of cell division. A hypothesis that cell division occurs only when (a) a critical mass of protoplasm is reached, and (b) a division-trigger mechanism is set off, suggests that synchrony might be achieved by subjecting the cells first to one part of a cyclic treatment, in which the trigger act could be delayed until all cells had reached the critical mass, then to the other part, in which the divisions would be triggered in synchrony. This has led to synchronization of Tetrahymena by temperature cycling (21), of yeast by starvation and feeding (20), and of a thymidine-requiring yeast by periodically feeding thymidine (22).

Division delay of yeast by radiation has been shown to vary widely with maturation stage (16). Although the dependence is not known, it seems plausible to give periodic radiation pulses synchronized with the division rate of a growing culture and hope that the more sensitive cells would be delayed selectively and bunch at a time when least affected by radiation. This was tested in the propagator with automatically controlled intermittent x-ray exposures. The data are summarized in Table II.

Table II. Times and doses of intermittent-exposure experiments

Division time (hr)	Irradiation period (hr)	Dose delivered (R/pulse)	Duration of run (generations)
1.5	0.17	650	16
1.5	0.17	860	16
1.75	0.20	990	4
2.0	0.25	1240	12
2.0	0.33	1660	11

Since a dose rate of 5000 R/hr was the maximum that could be obtained, the pulses were relatively long so that, even though no cycling of population density with time was observed, the question is not considered closed.

SUMMARY

A steady-state culture of Saccharomyces cerevisiae, when subjected to chronic x-ray irradiation, experienced an increase in average cell size and a decrease in division rate covering several generations before a new steady state was established. Twenty-one steady states with radiation gave a linear dependence of division rate with dose rate of 1.0×10^{-4} hr^{-1} r^{-1} generation out to about 2500 R/generation. Then the curve tended to level off up to 6150 R/gen. Eighty-seven percent of the cells were viable at this dose rate. After removal of the radiation the culture returned to normal in several generations.

After 115 generations during which 300 000 R accumulated to the ancestral populations no evidence of progressive deterioration was observed and no acquired resistance to acute x-ray exposure was obtained.

Simple cycling of x-ray exposure does not appear to lead to division synchrony.

The results of these experiments indicate that a population as a whole may not be adversely affected in its ultimate ability to survive indefinitely under chronic exposure to irradiation. This conclusion holds for the yeast for dose rates delivering in a generation time up to approximately one-fourth of the LD_{50} for acute exposure. Obviously the individuals so exposed are detrimentally affected in their metabolic and reproductive abilities and longevity, as attested by decreased division rates and the presence of dead cells in the irradiated steady-state populations. But the ability of organisms to produce several progeny before dying has to be reduced drastically before their capacity to continue to fill their environment is destroyed.

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FIGURE CAPTIONS

Fig. 1. Diagram of the continuous-culture propagator.

Fig. 2. Growth tube of the continuous-culture propagator.

Fig. 3. Viability of steady-state yeast culture exposed to chronic irradiation

Fig. 4. Dependence of division rate on dose rate for diploid S. cerevisiae exposed to chronic x-ray irradiation, and for E. coli exposed to β radiation.

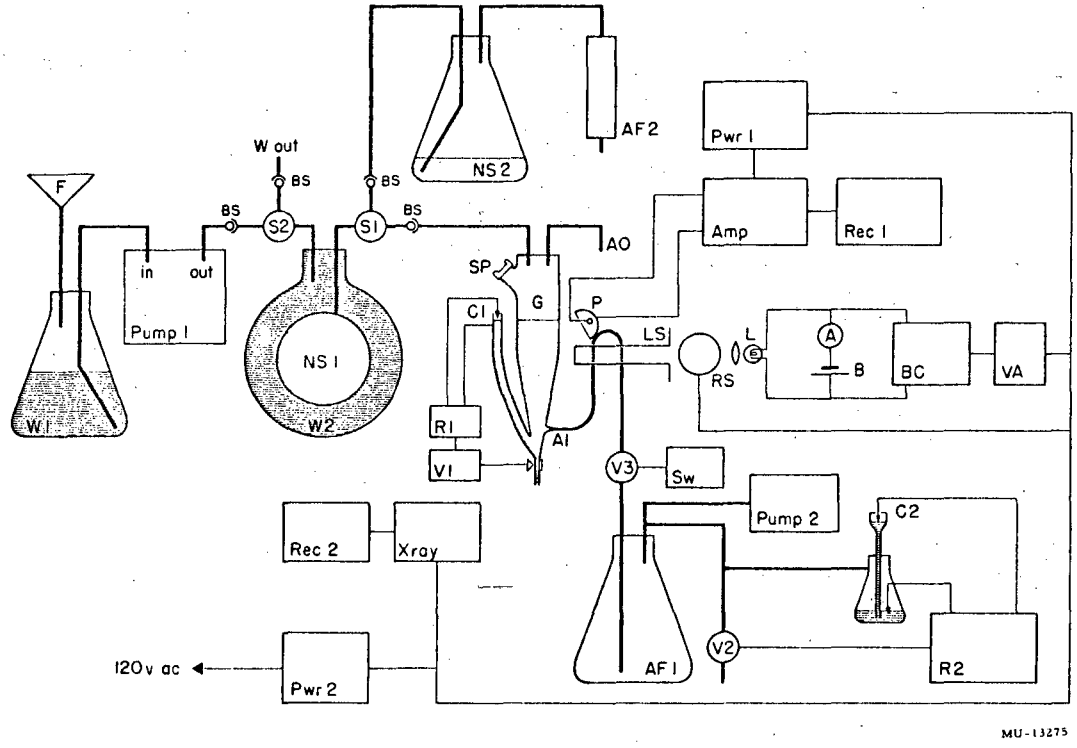
Fig. 5. Dependence of division delay on dose rate for diploid S. cerevisiae exposed to chronic x-ray irradiation.

Fig. 6. Recovery of yeast culture from depressed division rate at 6150 R/generation to normal division rate after x rays are turned off.

Key for Fig. 1: Diagram of the Continuous-Culture Propagator

A	ammeter, 0 to 10 A dc
AF 1	air filter, 1-liter flask stuffed with cotton
AF 2	air filter, 1.5 × 8-in. glass tube stuffed with cotton
AI	air inlet for stirring and aeration
AO	air outlet—glass tubing looped to prevent contamination of culture
Amp	stabilized, band-pass amplifier
B	two 6-volt 120 A-h storage batteries in parallel
BC	battery charger
BS	standard ball-and-socket connections
C 1	contacts in side arm of growth tube for volume control
C 2	contacts in water column for air-pressure control
F	funnel
G	growth tube
L	light source
LS	light shield
NS 1	nutrient-solution reservoir, 2.5-liter rubber breathing bag
NS 2	nutrient-solution sterilizing flask
P	photoelectric cell type 922
pump 1	water pump in medium supply system
pump 2	air pump, aquarium type
pwr 1	voltage-regulated dc power supply
pwr 2	voltage-regulated ac power supply
R 1, R 2	electronic relays
Rec 1	graphic recorder, Esterline-Angus 5-A ac

Rec 2	graphic recorder, Varian, 10-mv dc
RS	rotating shutter
S 1, S 2	three-way stopcocks
SP	sampling port
Sw	motor-driven switch, on for 5 sec, once per minute
V 1, V 2, V 3	magnetically operated valves
VA	variable autotransformer
W 1	water reservoir
W 2	water reservoir, 6-liter spherical flask
W out	connection for removing water in order to fill NS 1 from NS 2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BS
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BS
F
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NS 2
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Rec 1
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in
out
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NS 1
W2
W1
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R1
V1
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RS
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A
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Fig. 1

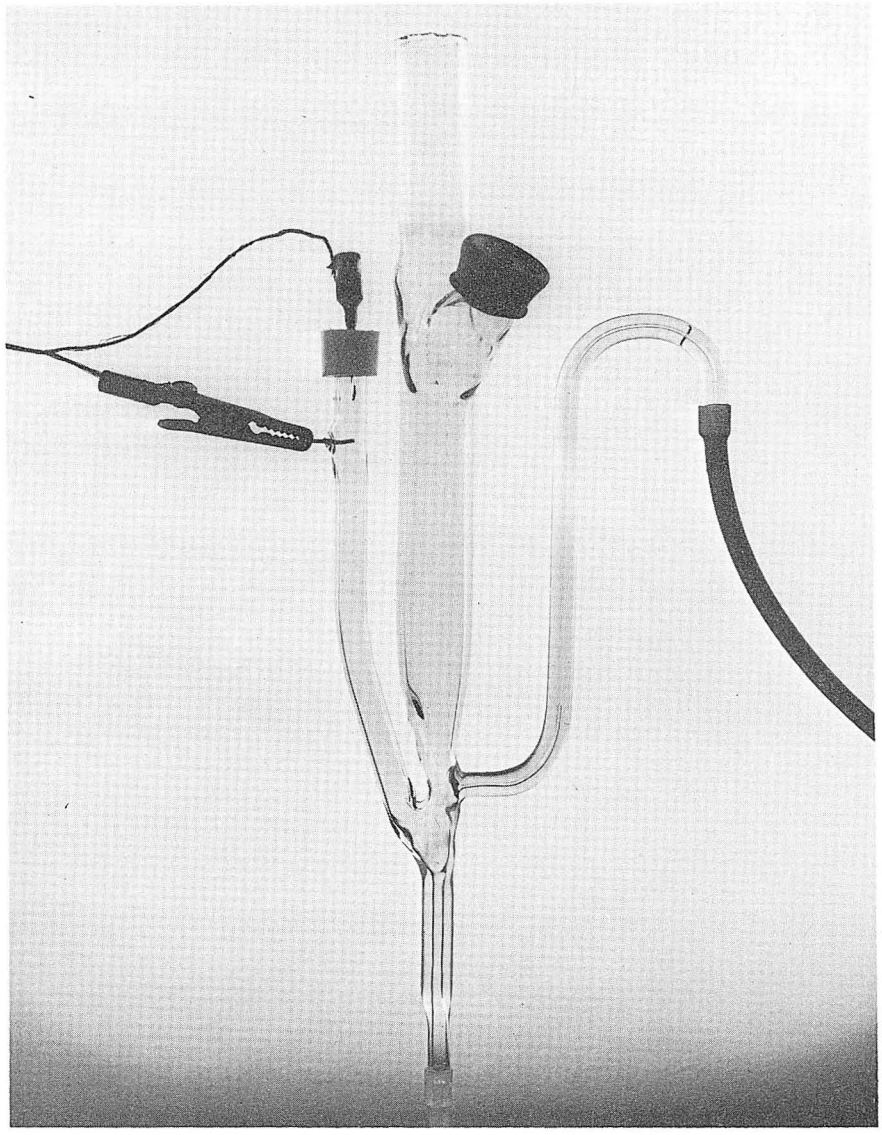

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Fig. 2

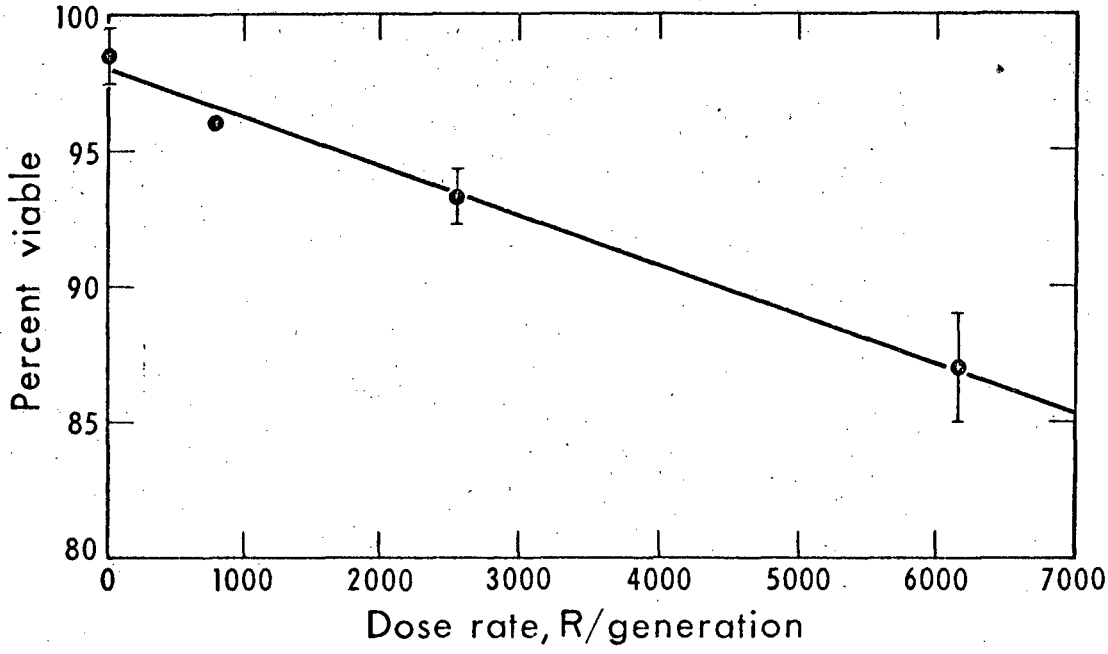

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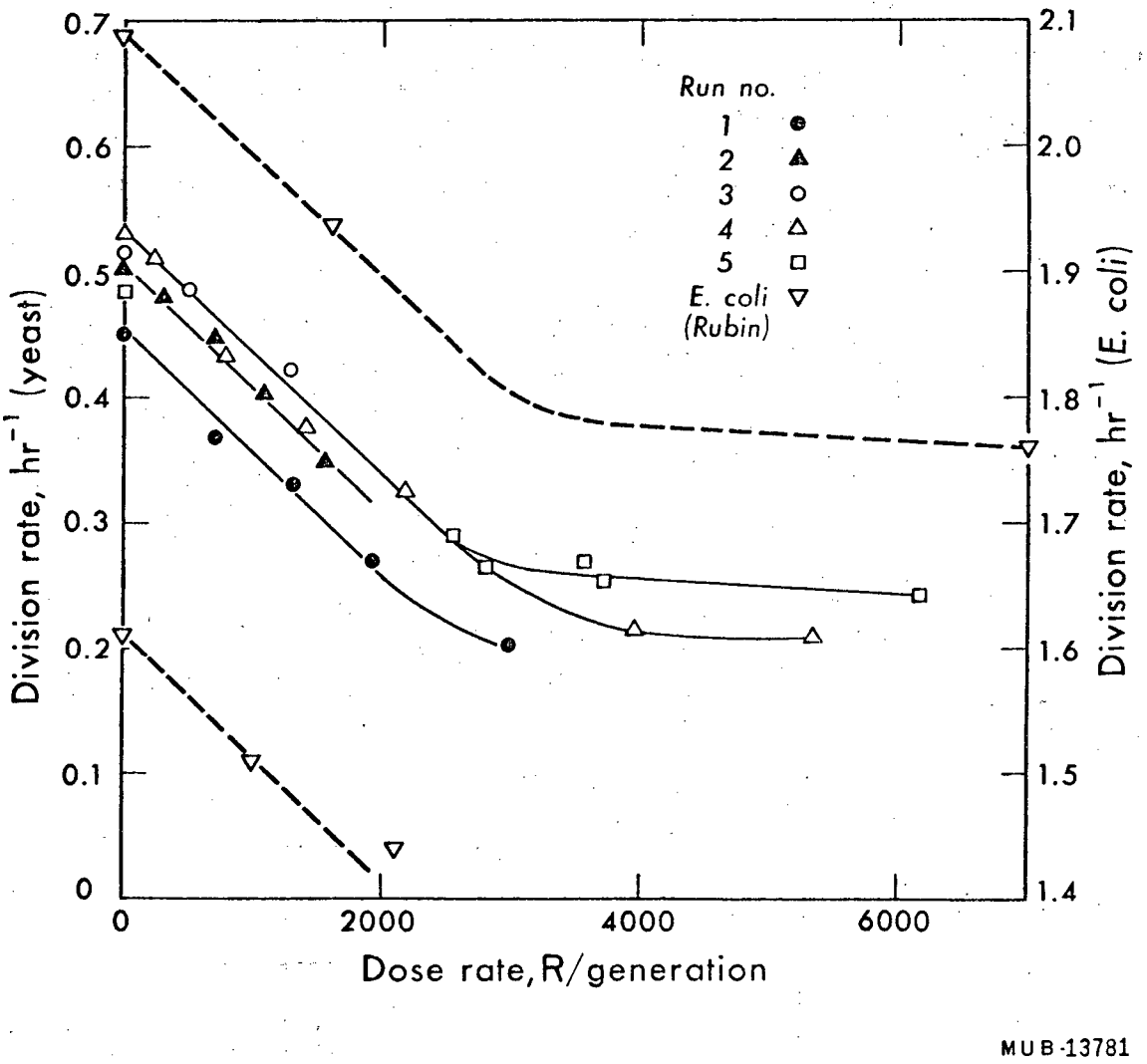
Fig. 3


Run no.
1
2
3
4
5
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Division rate, hr^{-1} (yeast)
Division rate, hr^{-1} (E. coli)
Dose rate, R/generation
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0.6
0.5
0.4
0.3
0.2
0.1
0
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
0
2000
4000
6000
MUB-13781

Fig. 4

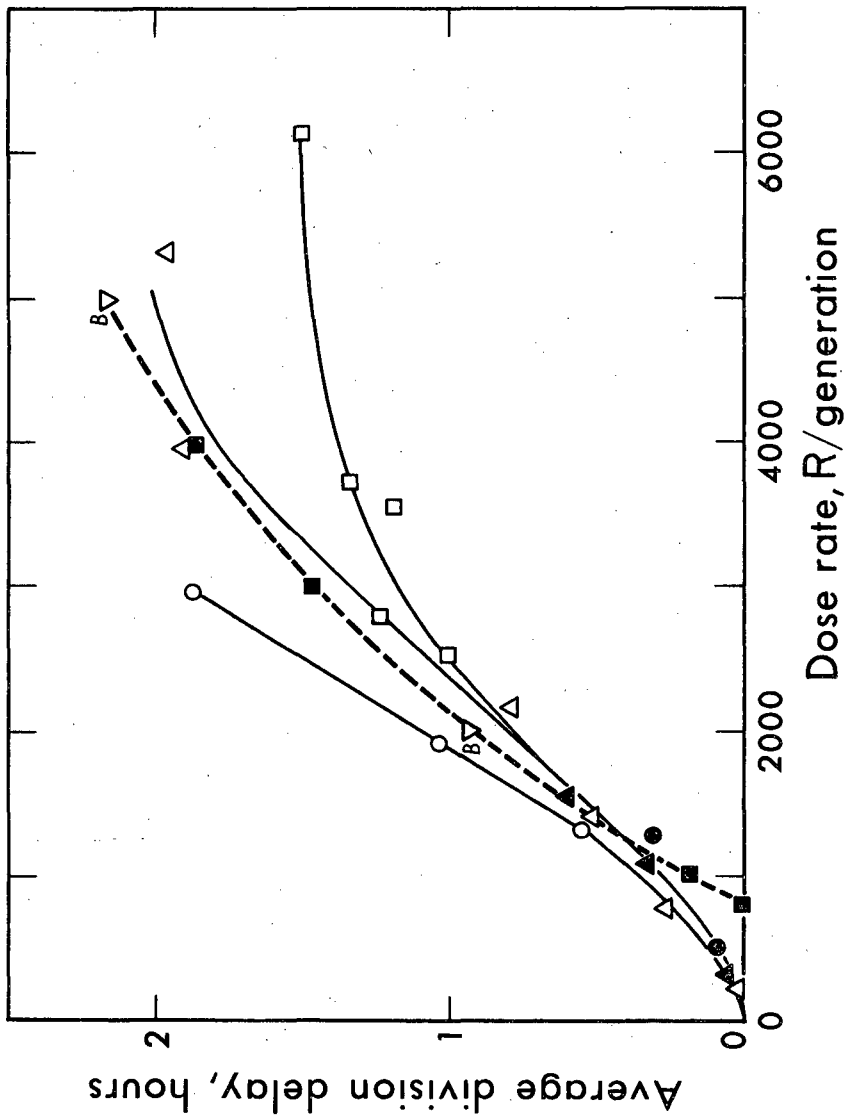

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Fig. 5

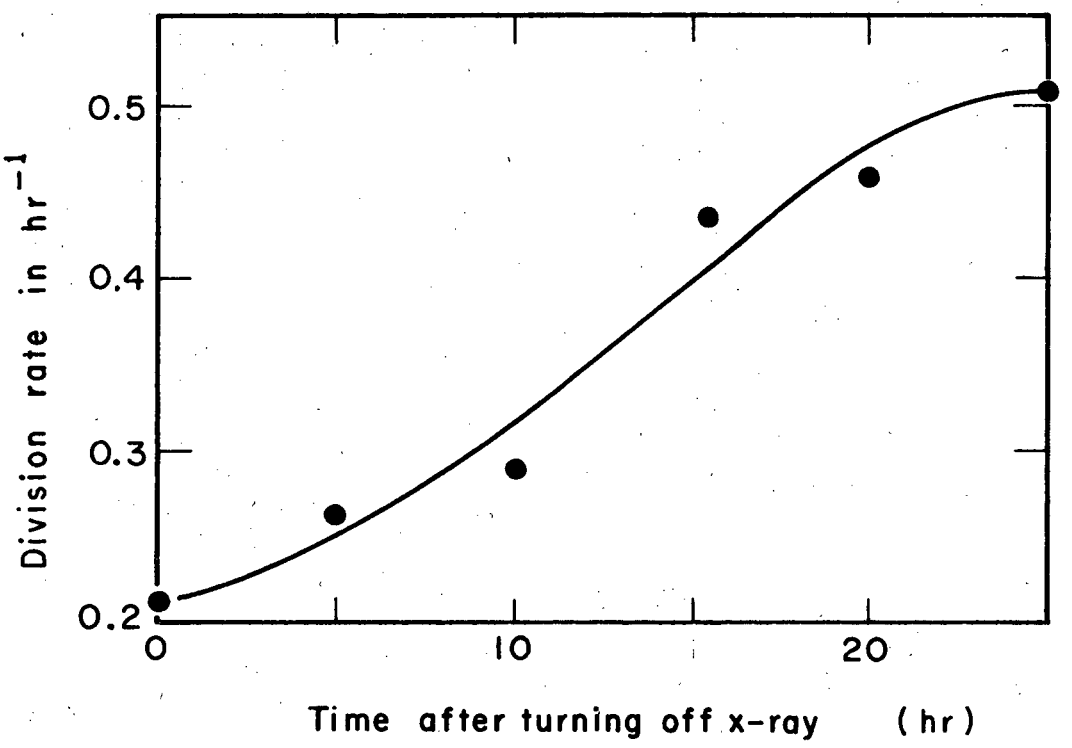

Fig. 6

XBL676-3242