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Alexander Hollaender

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PHYSICAL AND CHEMICAL FACTORS MODIFYING THE SENSITIVITY OF CELLS TO HIGH ENERGY AND ULTRAVIOLET RADIATION

by Alexander Hollaender

Biology Division, Oak Ridge National Laboratory

Microörganisms are in some ways ideal materials for obtaining immediate quantitative information on some of the basic aspects of radiation effects. It is well known that they can often be handled in very large numbers, thus giving statistical results on effects which are hardly recognizable on higher organisms. They are, in general, not suitable for studies of radiation effects on chromosomes which have been so well demonstrated by the previous speaker, (Norman H. Giles, Jr.), and for most genetical studies which will be discussed by the succeeding speaker (H. J. Muller).

Typical killing curves which have been obtained with fungi in our Laboratory are given in figure 1. They refer to a comparative study of X rays, and alpha particles and protons on Aspergillus terreus, (Stapleton and Martin, 1949). We are, however, not too enthusiastic about the use of these curves as the only basis for interpretation of the mechanism of lethal action of radiation, since "death" could be caused by a variety of mechanisms which are difficult to untangle.

There are many treatments which in themselves have little or no effect on the radiation sensitivity but, if given in supplement to ionizing radiation, produce striking effects. In general, these supplementary treatments have increased the sensitivity of cells to radiation rather than protected them; however, there are a few exceptions to this.

HEAT AND VISIBLE LIGHT

The first two factors to be discussed are the effect of heat and light

Fig. 1

Comparative lethal action of X rays (250 Kv), of protons, and of α particles on dry spores of Aspergillus terreus (from Stapleton and Martin).

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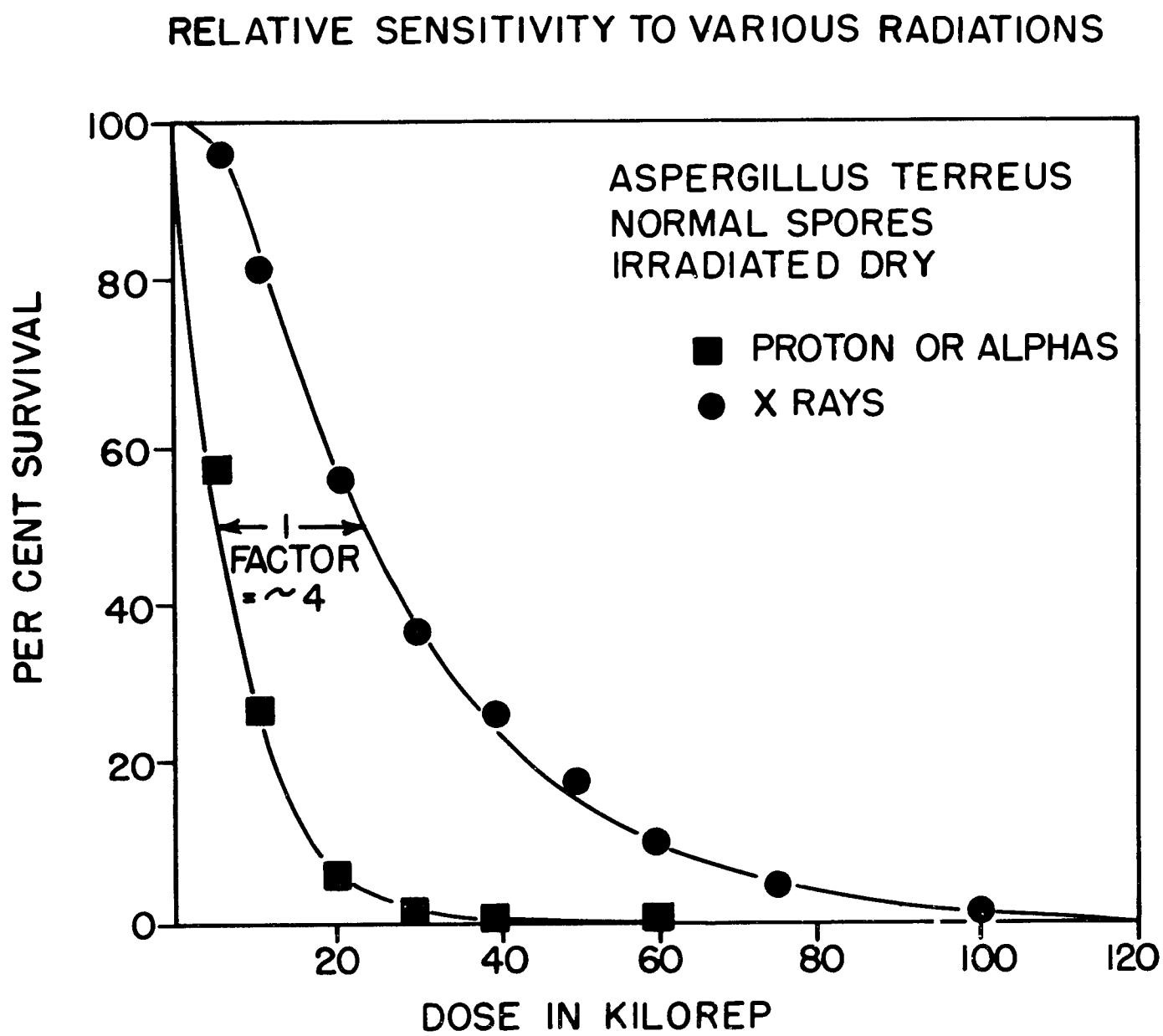

FIG. 1

in the treatment after irradiation. Cells are more sensitive to heat after irradiation; this applies to X rays as well as ultraviolet (Gaulden, 1949). I would like also to refer you to the work of Curran (1938), and Anderson and Duggar (1941). E. H. Anderson (1950) in our Laboratory, found that a certain strain of Escherichia coli can be reactivated after exposure to ultraviolet several hundredfold, if incubated at 40°, as compared with incubation at 30°. This effect can be found to a slight degree after X-raying (about 5-fold). However, this heat recovery has been recognized with only strain B of Escherichia coli. In this connection it should be mentioned that Hayden and Smith, in their work with maize, found heat reactivation of seeds after X-ray exposure. Unfortunately, no repetition of this work has been reported. A careful test in our Laboratory has given negative results (Suskind, 1950).

PHOTOREACTIVATION

About two years ago Kelner (1949), and Dulbecco (1949), first reported "photoreactivation" after exposure to short ultraviolet. Most of the work reported until now has dealt with the primary irradiation of 2537 A. Some work by Carlson and McMaster (1950) in our Laboratory has shown that, in the nucleolus of the grasshopper neuroblast, the photoreactivation declines after exposure to wave lengths shorter than 2537 A. The photoreactivation itself is limited to wave length range of 3650 to 4500 A given immediately after "short ultraviolet" exposure. There is some difference of opinion in regard to the most effective wave length for different organisms or even different strains in one species of organism (Kelner, 1950; Knowles and Taylor, 1950). No significant photoreactivation has been found after exposure to X rays. It appears from the present information, that photoreactivation either destroys a toxic substance which is formed by irradiation with 2537 A or it reverses the destructive action of an essential compound

needed for the survival of the cell.

The fact that photoreactivation is found after exposure to short ultraviolet fits into the general picture of the behavior of cells after irradiation with ultraviolet as compared with X rays. Bacteria or fungi surviving ultraviolet have a much extended lag phase (Hollaender and Duggar, 1938). This extended lag phase has been determined very carefully, but it can also be recognized by observing plate cultures which have been incubated about 12 hours. There is very little of this extension after X-ray exposure. Our interpretation at present is that the extension of the lag phase is a non-chromosomal effect and that photoreactivation works mostly through the cytoplasm. Such an interpretation seems reasonable at first but would have to be checked experimentally.

INFRARED

We reported several years ago, first in cooperation with Kaufmann (1946), and later with Swanson (1946), that infrared around 10,000 A given before (*Drosophila*, *Tradescantia*, and *Aspergillus terreus*) or after X radiation (*Tradescantia*) will increase the effectiveness of X radiation in producing chromosomal rearrangements and chromatid breaks and mutations (*Aspergillus terreus*). The effect is somewhat more pronounced in regard to chromatid breaks as Giles and Beatty (unpublished) have found in our Laboratory. The infrared alone given under carefully controlled conditions will produce no recognizable chromosome changes. It is important to point out that a carefully designed experimental technique must be used so as not to raise the temperature in these biological materials to a level in which heat damage could appear. This infrared work indicates that, in addition to the

usually recognized damage, there must be some "potential" damage in the chromosomes produced by X radiation which is not obvious under normal conditions and which can be repaired if X radiation is given alone. These additional effects of infrared radiations have also been observed in regard to nitrogen mustards by Swanson and by Kaufmann. These experiments indicate that X radiation causes an unstable condition in chromosomes which can be made obvious by infrared treatment.

WATER CONTENT

I am sure you are acquainted with the work on the sensitivity of plant viruses to X rays, depending on the water content of the virus crystals. Similar work has been done by Stapleton in our Laboratory in regard to irradiated Aspergillus terreus spores, (1) suspended in water, (2) freshly removed from an agar slant culture containing about 40-50% water, and (3) spores, dried and desiccated for 3 days, which contain only about 20-25% water. There is a striking increase of resistance to X rays of very dry spores. Water apparently sensitizes the spores to X rays by bringing in direct contact some substances formed in the water by X rays. However, what we call "dry" spores in our experiments still have a water content which should be an important factor in the sensitivity.

In connection with the irradiation in water, we should discuss the work of the Texas group (Wyss, Stone, and Clark, 1947), who found that by irradiating the medium with wave lengths shorter than 2537 A, bacteria and fungi grown in this irradiated medium would show a somewhat increased mutation rate. Hydrogen peroxide produced the same effect. Certain organic peroxides have been found to produce increased mutation rates, as reported

by a California Institute of Technology group (Dickey, Cleland and Lotz, 1949).

These findings point to the possibility that hydrogen peroxide, or certain organic peroxides which may be produced by radiation in the medium or inside living cells, might be an important factor in regard to the radiation sensitivity.

Further evidence is also brought out in the following discussion.

OXYGEN TENSION

The effect of oxygen tension on the sensitivity of chromosomes to X radiation has been reported by the previous speaker. R. S. Anderson reported nine years ago that yeast irradiated in the presence of oxygen was much more sensitive to X rays than when irradiated in the absence of oxygen. We have carried out similar studies in regard to Escherichia coli, the experimental details of which, although important, will be omitted here for the sake of brevity. All data presented are based on plate counts of bacteria grown aerobically after irradiation. Figure 2 illustrates results obtained with aerobically grown bacteria irradiated in oxygen, air, and nitrogen. This graph shows that the ratio of survivors at 60,000 r under two different gases is

$$\text{nitrogen/oxygen} \Rightarrow 1000$$

for aerobically grown Escherichia coli (B/r) whereas the relative sensitivity for oxygen-treated samples, as compared with nitrogen-treated ones, is approximately threefold.

The same modification of sensitivity has been demonstrated on Escherichia coli, strain B, which has been reported to be more sensitive to radiation than B/r. Since this strain B is more sensitive to X rays, the rate of killing is greater under both conditions studied, nevertheless the ratio of sensitivity of oxygen-treated cells to nitrogen-treated cells is approximately equal to that found for B/r. Nitrogen can be replaced with helium, hydrogen, or carbon

Fig. 2

Anaerobic and aerobic refer to the mode of growing the organisms. Oxygen, nitrogen refer to type of suspension in which bacteria were kept during irradiation.

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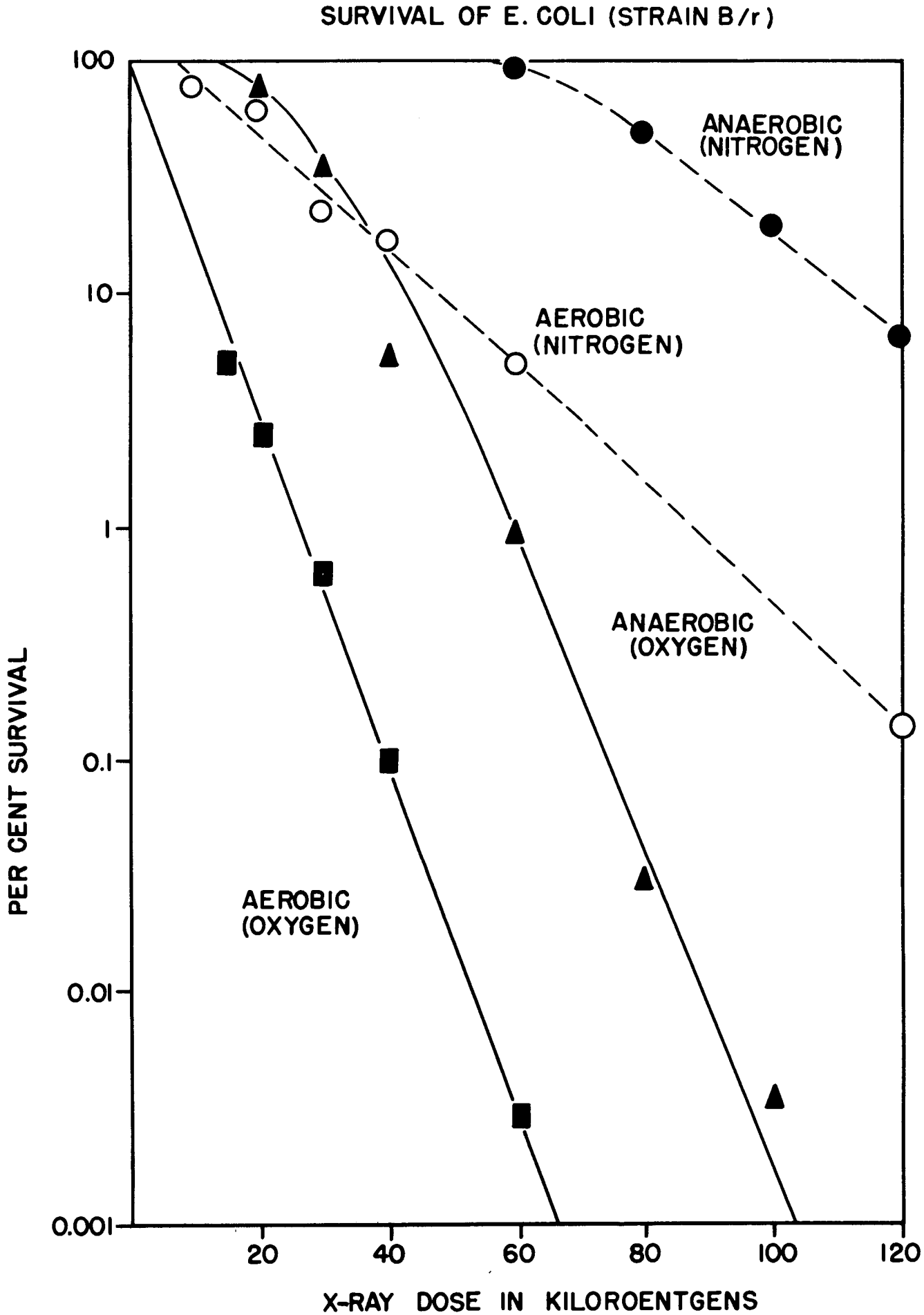

FIG. 2

dioxide without significantly changing sensitivity.

Sensitivity of aerobic versus anaerobic Escherichia coli, (B/r). Since it is well established that E. coli is a faculative anaerobe, it was decided to compare the relative sensitivity of this organism grown anaerobically previous to irradiation, with the same strain grown under strictly aerobic conditions. The bacteria grown anaerobically before irradiation and irradiated anaerobically were found to be extremely radioresistant.

The ratio of survival for the cells grown anaerobically prior to radiation is

$$\text{nitrogen/oxygen} = 10^5$$

The ratio of sensitivity between oxygen- and nitrogen-treated anaerobic cells is again, perhaps fortuitously, very close to three.

If the sensitivity of the extreme cases is compared, that is, cells grown aerobically prior to irradiation and irradiated in the presence of oxygen with those grown anaerobically before irradiation and irradiated in the absence of oxygen, a factor of ten is found.

Use of organic compounds (amino acids) as protective agents. It was noticed that bacteria (E. coli, B/r), were more sensitive to X rays when exposed in phosphate buffer than in nutrient broth (Difco) 8 g/liter. Since broth contains a wide variety of amino acids it was decided to test the protective action of amino acids, first in groups and then individually, if the goup tests appeared promising.

The effect of amino-acid solutions as protective agents was studied from two points of view; (a) the effect of amino-acid concentration on bacterial survival; (b) the protective action of optimum concentration of amino acids as obtained from survival (a) as a function of X-ray dose.

The results indicate that only glutamic acid and cysteine afforded increased protection over the range of concentrations used in these experiments (Fig. 4).

It appears from the data presented here that there are two different effects: (1) radiation-produced changes in the medium which can be reduced by lowering the oxygen tension of the medium; (2) the studies with anaerobic cells, on the other hand, indicate that essentially complete removal of oxygen from the cells also results in lowering of their X-ray sensitivity. The combination of two protective systems results in extreme resistance of the organisms to radiation.

The possibility that respiration is tied up with the radiation sensitivity seems to be indicated by these tests.

DISCUSSION

The data from Fig. 2 were recalculated on the basis of the target theory (see Lea, 1946), and are given in Fig. 3. A purely physical interpretation on the basis of the target theory of the effects of X rays on E. coli leads one to the conclusion that it is possible to vary the number of "targets" at will, by means of adjustment of growth conditions or conditions during irradiation. Whereas this type of physical interpretation may be a helpful tool in the study of radiation effects, the chemical approach appears more promising at the present time. The hypothesis that hydrogen peroxide formation induced by radiation is responsible for radiation effects on chromosomes is in many ways attractive, as the previous speaker has so well shown. However, this hypothesis

Fig. 3

Data from Fig. 2 recalculated for "target" determination. See discussion in text.

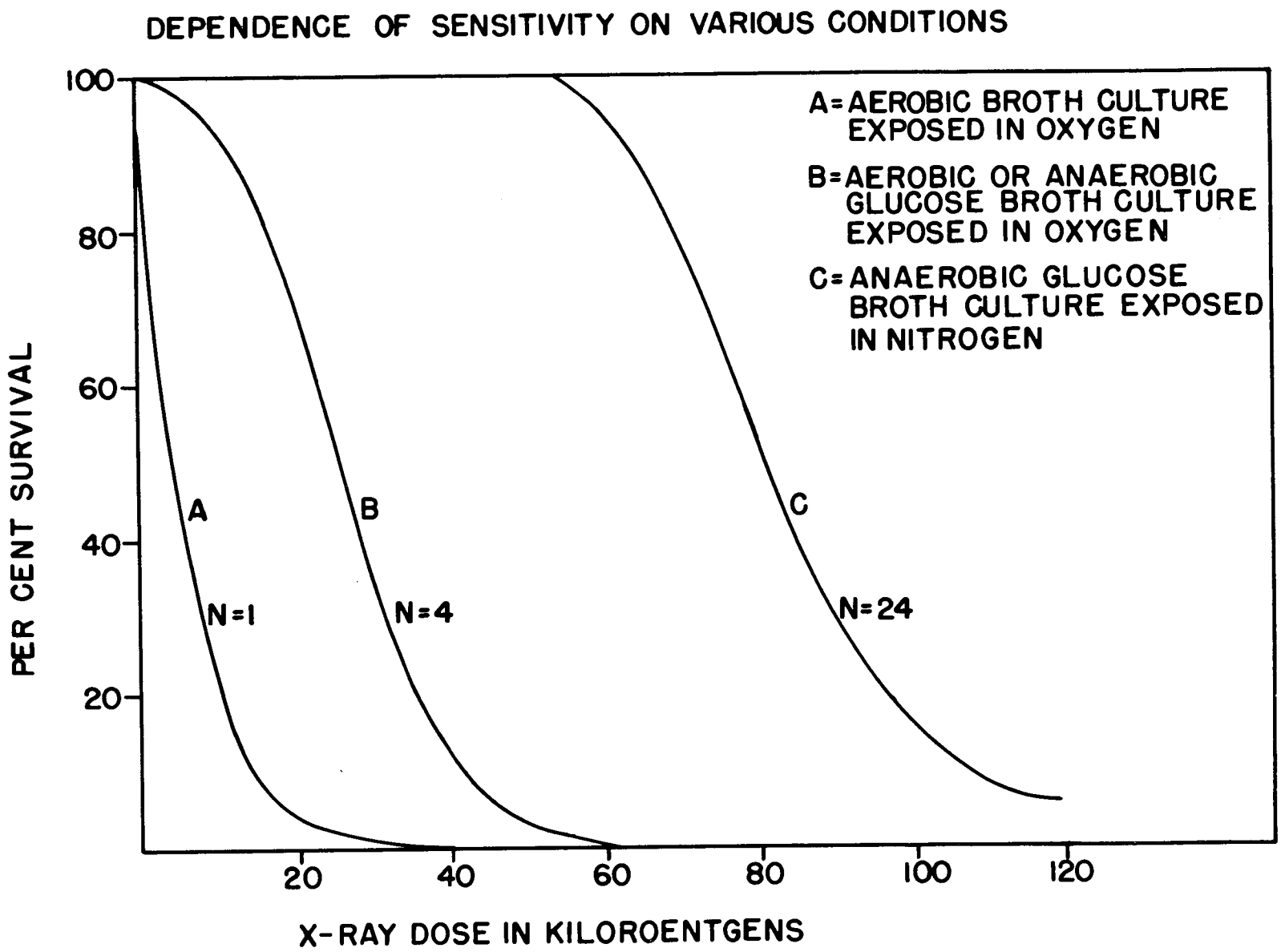
DEPENDENCE OF SENSITIVITY ON VARIOUS CONDITIONS
A=AEROBIC BROTH CULTURE EXPOSED IN OXYGEN
B=AEROBIC OR ANAEROBIC GLUCOSE BROTH CULTURE EXPOSED IN OXYGEN
C=ANAEROBIC GLUCOSE BROTH CULTURE EXPOSED IN NITROGEN
A
N=1
B
N=4
C
N=24
PER CENT SURVIVAL
100
80
60
40
20
20
40
60
80
100
120
X-RAY DOSE IN KILOROENTGENS

FIG. 3

Fig. 4

Effects of amino acids, prepared immediately before irradiation and added to the suspension to be irradiated, on the survival of B/r at 60,000 r (Hollaender and Stapleton).

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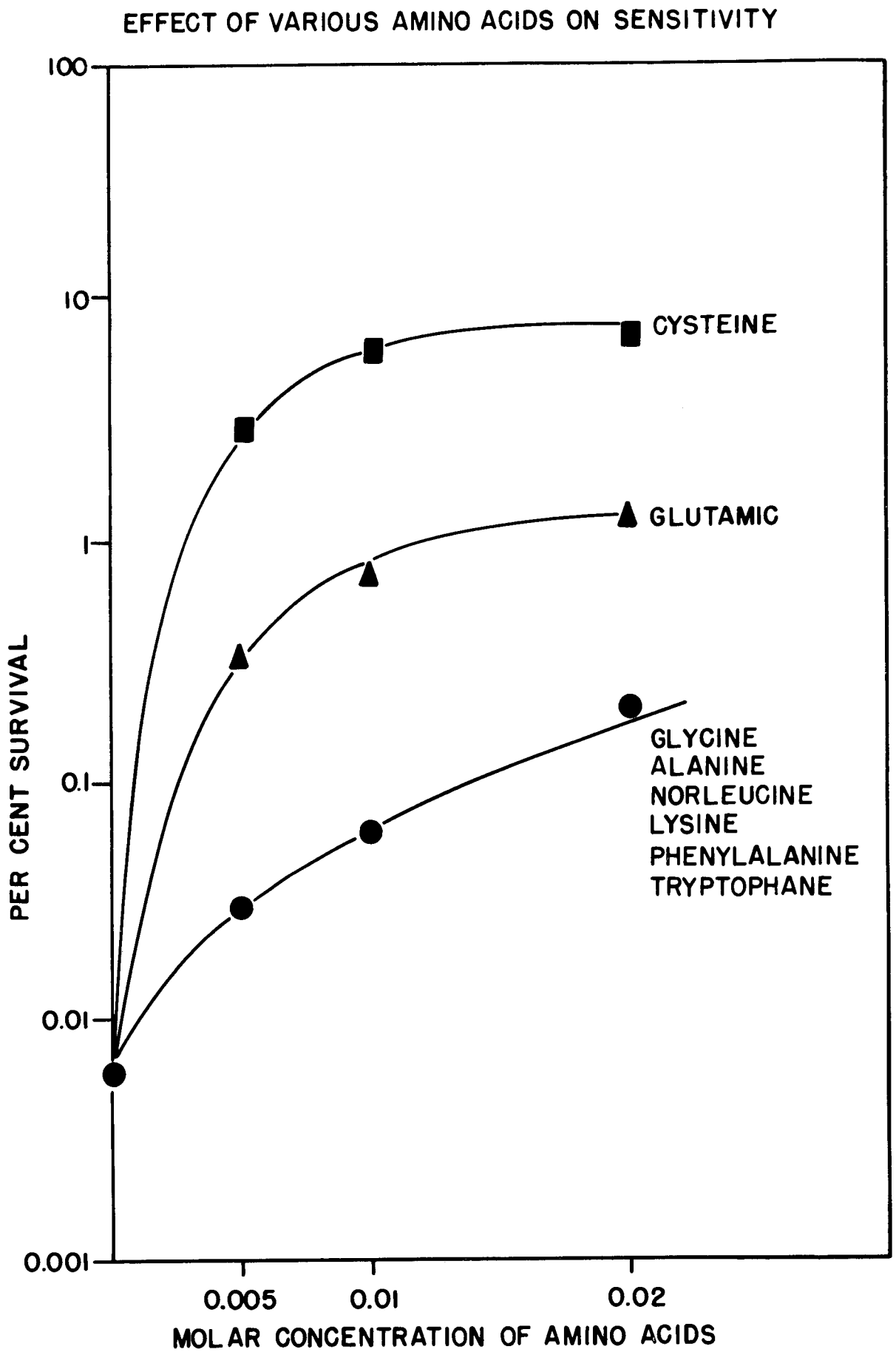

FIG. 4

is less attractive in the interpretation of lethal effects on bacteria and fungi for the following reasons. (1) Bacteria suspended in a medium which has been irradiated with X rays (60,000 to 80,000 r) will not be killed by this medium. The half life of hydrogen peroxide in buffer solution is of sufficient length that it should show a residual effect. (2) Hydrogen peroxide added to the suspension medium becomes toxic to bacteria only if concentrations are used which are far in excess of the ones produced by the usual amounts of radiation (> 200,000 r). (3) Organic peroxides, as well as hydrogen peroxide, should be produced by irradiation in broth suspension and amino-acid solutions. However, bacteria become more resistant if irradiated in the presence of these compounds. There is a possibility that the hydrogen peroxide attaches itself to the organic compounds, especially amino acids, and thus makes itself less available to the bacteria.

It appears that radiation probably produces a radical of relatively short life but which exists long enough to diffuse through the medium and into the bacteria or is produced in the cells themselves and distributed by diffusion. At present this radical could be HO_2. However, there may be other compounds formed by irradiation, the existence of which is not yet established.

We have mentioned in the introduction that radiation death in organisms could be effected by many factors. Hydrogen peroxide might be one of the contributing factors, but other radicals found in the decomposition of water by radiation may play a special role in contributing to the killing of the organisms. The effect of oxygen in regard to the sensitivity could be largely explained by such a hypothesis. I feel that we are basing our speculation too much on studies which have been conducted with pure water, whereas in living

cells, where really "pure" water does not exist, there must be a loose association of molecules which could be affected by these radiation-produced radicals. A typical example would be a nucleoprotein built into chromatin material. It appears somewhat promising that untangling metabolism mechanisms effected by oxygen under the influence of radiation will give us a clue to this problem. Finally, the inactivation of the sulphydril-requiring enzyme systems discussed by Barron may also play an important role.

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