with deeply staining, irregularly oval nuclei. Such cells are capable of phagocytosis, as illustrated by the rounded central macrophage in Fig. 3, taken from a culture previously exposed to rat erythrocytes infected with *Plasmodium berghei,* suspended in the liquid phase. The illustrated macrophage contains several erythrocytes in varying stages of digestion, as well as a number of rod-shaped granules of malarial pigment. No phagocytosis was seen in fibroblasts of 2-week cultures in which the macrophages were actively phagocytic.

Summary. A method for culturing a single layer of mononuclear agranulocytes from rat peritoneal exudate is described. Two-week cultures of this type consist chiefly of a network of fibroblasts interspersed with numerous macrophages, and are suitable for the maintenance of obligate intracellular parasites (ex.: *Trypanosoma cruzi,* leishmaniform stage). They may conveniently be used for drug testing, opsonic studies, or other tests requiring the exposure of a single layer of cells, normal or parasitised, to a supernatant liquid phase.

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Influence of Oxygen upon Genetic and Nongenetic Effects of Ionizing Radiation on *Paramecium aurelia.** (20150)

R. F. KIMBALL AND NENITA GAITHER. (Introduced by Alexander Hollaender.)
From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

It has been shown for a variety of organisms that X irradiation under low oxygen tension is less effective in producing biological damage than X irradiation under high oxygen tension. Hollaender, Baker, and Anderson(1) and Giles(2) may be consulted for review. It has been repeatedly suggested that this effect is caused by the reduced quantity of the active materials HO_2 and H_2O_2 produced at low oxygen concentration, implying that a considerable part of the biological effect of X rays must be due to these substances. It is important to find out how far this generalization is true and so to examine carefully any cases in which there is little or no oxygen effect. The present paper reports a series of experiments with *Paramecium aurelia* which show that under certain circumstances oxygen has little or no effect on the production of certain kinds of radiation damage, although under other circumstances it has a marked effect. It seems possible to interpret these findings in terms of changes in the importance of H_2O_2 in producing these effects. Evidence is also presented that oxygen may influence radiation damage in other ways than its effect upon H_2O_2 production.

Materials and methods. Stock 90 of variety 1 of *P. aurelia* was used throughout. The paramecia were selected to be in the first half of the interval between divisions. Shortly after treatment, a number of paramecia, usually 30, were isolated and continued as daily isolation lines.

The technique for measuring the genetic effect at doses of a few kiloroentgens has been described(3). In the present paper, the results are expressed as the percentage of normal exautogamous clones (clones which reach a maximum population in a limited quantity of culture medium in 4 days). This percentage becomes too small to be useful at about 10 kr. However, the percentage of exautogamous clones which survive 4 days, regardless of the

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amount of growth, decreases much more slowly as dose increases and is useful in the range from 10 to about 300 kr.

Nongenetic effects in *Paramecium* were investigated at doses above 30 kr. The two measures used were the number of divisions in the first day expressed as a percentage of the control and the percentage of survivors after one day. These two effects are discussed by Kimball, Geckler, and Gaither(4). In their paper, it is shown that the best general measure of division delay is the reciprocal of the time to the sixth division. With X rays, however, the effects after the first day are so small that, for most purposes, the number of divisions in one day is equally good. Since in some experiments only this measure was available, it has seemed best to use it throughout. In each experiment, unirradiated controls were exposed to high and low oxygen concentrations at the same time and in the same manner as the irradiated animals. There was no evidence from the data that the division rate of unirradiated paramecia was influenced by such relatively brief exposures to different oxygen concentrations. Therefore, the control value for the number of divisions in one day was obtained by averaging the two groups.

The X-ray source was a General Electric Maxitron 250 operated at 250 kvp and 30 ma. For higher doses, including all experiments in which nongenetic effects were investigated, no filters were added. This unfiltered beam is highly heterogeneous and contains a large soft component because of the thin beryllium window of the tube. The measurement of this beam involves difficulties which have not been completely overcome. A nylon Victoreen 250-r chamber was used and measurements were made in air. No attempt was made to correct for scatter and for the difference between the absorption by the walls of the chamber and the exposure containers. The intensity measured in this way was approximately 68 kr/min at 7.7 cm and 16 kr/min at 15.4 cm from the target, which were the two positions used. For some of the investigations of genetic effects at low doses, a 3-mm Al filter was added; and exposures were made at approximately 500 r/min. Other low-dose exposures were made with the unfiltered beam reduced to approximately this intensity by lowering the milliamperage. No significant difference was noted between genetic effects with the 2 types of radiation. High-dose exposures were also made in a Co^{60} γ source kindly made available to us by the Organic Chemistry Section of this laboratory. The source had an intensity of 16 kr/min.

Several different exposure containers and arrangements for achieving the desired oxygen tension were used. For experiments with filtered X rays and with γ rays, 2-ml volumetric flasks, fitted with bubbling tubes, were used. The gas was bubbled through the tubes for 15 min before irradiation, and, in the case of X rays, they were sealed off by stopcocks. Gas was bubbled throughout the exposure in the case of γ rays. The arrangement for X rays was the same as that used by Hollaender and his collaborators in this laboratory for work with microorganisms. Modifications were made so that 2 tubes for different gases could be exposed simultaneously in the limited space within the γ source. Exposure to unfiltered X rays was made in one of 2 kinds of containers. The first was made by cementing, with DeKhotinsky cement, a piece of mica approximately 25 μ thick to a lucite ring 2.5 cm high and 5.5 cm in diameter. This dish was filled with mineral oil to a depth of 1.5 cm, and the paramecia were placed in one or more small drops under the oil on the mica bottom. Irradiation was carried out 7.7 cm from the target with the beam directed upward through the mica bottom. Special boxes were designed which made it possible to bubble with the gas a small volume of fluid containing the paramecia, and to transfer a drop of this fluid to the oil dish and seal off the dish—all under the desired atmosphere. The oil in the dish was also bubbled with the gas. The sealed dishes were removed from the box and irradiated. These same boxes were adapted for exposure of paramecia to H_2O_2 under a desired atmosphere. Small volumes of fluid containing animals and similar volumes of solutions of H_2O_2 were placed in separate, open containers in the box, gas was passed through the box for 20 min, and then the peroxide was added to the animals with a pipette extending through the wall of the box. At the end of 10 minutes, culture fluid con-

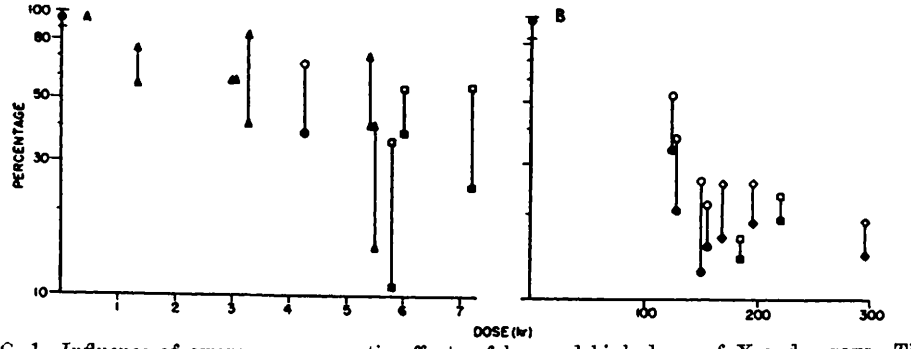

FIG. 1. Influence of oxygen upon genetic effects of low and high doses of X and γ rays. The pairs of points connected by lines represent high- and low-oxygen groups simultaneously given the same dose. Most of the points are average values for 20 irradiated animals with 25 exautogamous clones from each irradiated animal. A. % normal exautogamous clones at low doses. B. % clones surviving 4 days after autogamy at high doses. Open symbols are low oxygen tension; solid symbols, high. Circle = hanging drop. Square = oil dish. Diamond = γ rays. Triangle = 2-ml volumetric flasks and filtered X rays.

taining many living bacteria was added by means of another pipette, thus rapidly destroying the peroxide and ending the exposure. The other exposure dish consisted of a small block of lucite with a well drilled in its center. Two tubes on opposite sides made it possible to pass any desired gas through the well. The paramecia were placed in small hanging drops on a piece of mica 25 μ thick, and this was sealed over the well with hot paraffin. The gas was passed through the chamber for 15 min, the chamber sealed off with stopcocks, and the exposure made. In all cases, the low- and high-oxygen groups for any one dose of radiation were exposed simultaneously.

The gases used were Linde nitrogen (99.9% pure), a commercial mixture of 2% oxygen and 98% helium, a mixture of 20% oxygen and 80% nitrogen, compressed air, and pure (commercial tank) oxygen. In the following, the first 2 will be called low oxygen and the last 3 high oxygen. No consistent differences within these 2 groups have been found.

Genetic effects. The influence of oxygen tension upon the genetic effects of low and of high doses are shown in Fig. 1. In this and other figures, each pair of points representing separate comparisons is connected with a vertical line. Fig. 1 shows that the low-oxygen group is consistently less affected than the high. Exact statements about the magnitude of the effect are not possible but some approximation can be made. Other experiments have shown that both criteria, percentage normal and percentage survival for 4 days, are nearly exponentially related to dose. The curve for the former shows a small upward convexity when plotted on semilog paper whereas that for the latter shows some upward concavity. Thus the ratio of the log of the percentage at high oxygen tension to that at low oxygen tension is a fairly good measure of the oxygen effect. This ratio was obtained for each pair of points separately, and averaged. The average for the low-dose group was 2.6; for the high-dose group, 1.3. This suggests that oxygen has more effect at low dose than at high but does not exclude the possibility that the effect is the same for the 2 groups, since the deviations from a simple exponential relation are such that the value for the low-dose group is an overestimate, the value for the high-dose group, an underestimate.

Nongenetic effects. Fig. 2 shows the data for survival for one day after irradiation. There is reason to believe that in any one experiment the dose curve has a very long shoulder and then drops rapidly from complete survival to complete death(5). The sharp drop, together with variations from experiment to experiment, explains the great scatter of the points. A careful examination of the figure indicates that, at all doses where there was any appreciable difference between the low- and high-oxygen groups, there was more death in the former. This is so for both soft X rays and γ rays. Thus hypoxia increases this effect or does not influence it at

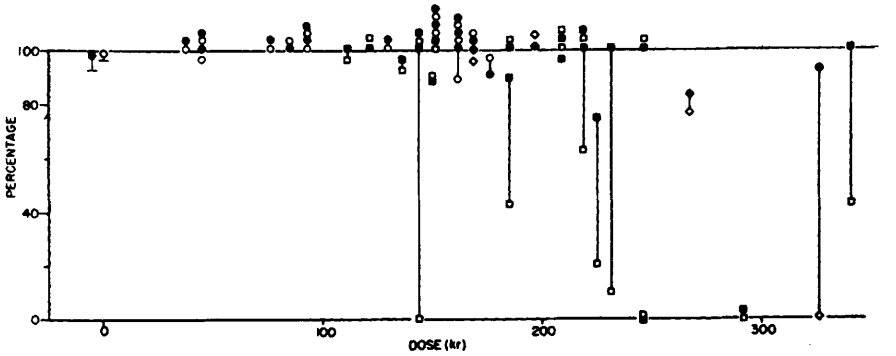

FIG. 2. % survival one day after high doses of X rays. The pairs of points connected by lines represent high- and low-oxygen groups simultaneously given same dose. Most of the points are values for 30 irradiated animals. Average values and ranges are shown for controls. Open symbols are low oxygen tension; solid symbols, high. Circle = hanging drop. Square = oil dish. Diamond = γ rays.

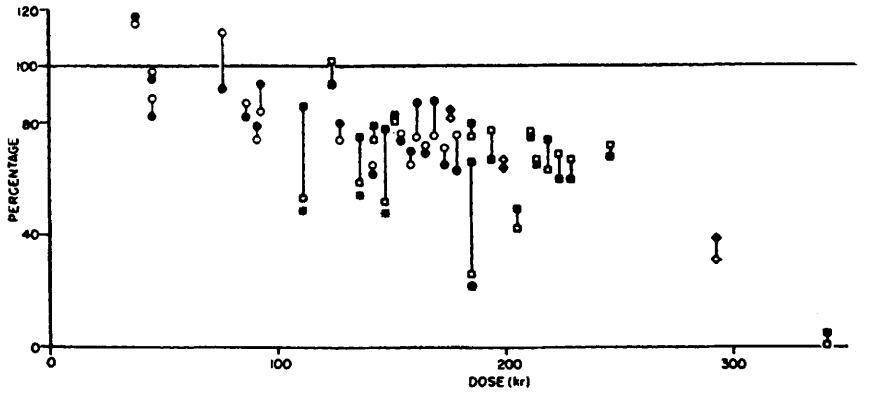

FIG. 3. Divisions in one day among survivors as % of control. The pairs of points connected by lines represent high- and low-oxygen groups simultaneously given the same dose. Many of the points are averages for 30 irradiated animals, but the number was less when survival was low. Open symbols are low oxygen tension; solid symbols, high. Circle = hanging drop. Square = oil dish. Diamond = γ rays.

all. It certainly does not decrease it.

The data in Fig. 3 are for divisions in the first day expressed as a percentage of the control. They agree with data in Fig. 2 in suggesting that, in some cases, hypoxia increases the effect. The main evidence for this comes from the 4 pairs of points marked with asterisks. The high- and low-oxygen groups for each pair are significantly different from one another. However, these 4 points represent all the data from just 2 experiments, one with one dose and one with 3. The rest of the data are consistent with the hypothesis that there was no effect of oxygen; and the whole body of data taken together shows no significant effect. However, the apparent nonrandom distribution of unusual deviations among experiments combined with the evidence in Fig. 2 suggests that the data are really heterogeneous in that a few cases show more effect at low oxygen tension while in the majority the oxygen tension is without influence. In any case, the important point is that there is no evidence that hypoxia decreases the effect.

Irradiation in medium containing few bacteria. The results reported in the foregoing sections were obtained by irradiating paramecia suspended in a culture medium containing many living *Aerobacter aerogenes*. It has been shown(6) that much of the non-genetic damage to paramecia irradiated in a medium containing few bacteria can be attributed to H_2O_2 formed outside the cell. It would be

TABLE I. Effect of Oxygen Tension upon Paramecia Irradiated in Hanging-Drop Preparations in Medium Containing Few Bacteria.

Exp. No.	Dose, kr	Nitrogen N*	Nitrogen S†	Nitrogen D‡	Oxygen N*	Oxygen S†	Oxygen D‡
1	0	0	—	—	30	100	4.1
	92	22	91	2.8	30	0	—
	153	27	0	—	26	0	—
2	0	30	100	4.0	26	96	4.2
	45	30	100	4.0	30	0	—
	91	30	100	2.6	30	0	—
3	0	30	100	3.5	30	100	3.5
	45	30	93	2.7	27	33	0.7
	89	30	83	1.5	30	0	—
4	0	30	100	3.7	30	97	3.1
	38	14	100	3.8	26	54	2.4
	77	29	100	3.0	30	10	1.3

* No. of treated paramecia.
† % survival for one day.
‡ Avg No. of divisions in first day among survivors.

TABLE II. Influence of Oxygen upon Damage by H_2O_2.

Exp. No.	H_2O_2, μg/cc	Nitrogen S*	Nitrogen D†	Air S*	Air D†
1	0	100	4.6	100	4.5
	8	97	3.1	23	2.4
	10	47	1.4	0	—
2	0	100	4.0	100	4.0
	8	50	1.9	30	1.3
	10	23	1.6	20	0.7
3	0	100	3.5	100	4.0
	8	100	3.4	93	2.8
	10	100	3.2	93	2.6
4	0	97	4.3	100	4.3
	8	87	2.3	33	1.6
	10	10	2.3	13	0.8

* % survival for one day, usually out of 30 treated paramecia.
† Avg No. of divisions in first day among the survivors.

expected that paramecia irradiated under the latter circumstances should show a typical oxygen effect on nongenetic changes because of the importance of oxygen in peroxide production. This is shown to be true in Table I. Kimball and Gaither(6) were unable to find any influence of the presence of bacteria upon the genetic effects of X rays.

Oxygen and the action of H_2O_2. The failure to find an intracellular oxygen effect for division delay might, conceivably, result from a balance between a decreased production and an increased effectiveness of H_2O_2 under hypoxia. This hypothesis was tested and ruled out by exposing paramecia to H_2O_2 under different oxygen tensions. The results are shown in Table II. It is clear that instead of an increased effect at low oxygen tension there is a decreased effect. Thus hypoxia should not only decrease the amount of H_2O_2 formed but should decrease its effectiveness. The balanced-action hypothesis is ruled out, unless the improbable assumption is made that H_2O_2 coming from outside the cell differs in this respect from H_2O_2 produced locally. As a further check on this conclusion, samples of H_2O_2 were bubbled with nitrogen and with air for 15 minutes. No difference in concentration could be detected colorimetrically using the starch-iodide method(6).

Discussion. The failure to find an intracellular oxygen effect of the typical kind for division delay and death in one day in *Paramecium* might mean one of 4 things. (1) Insufficient peroxide is produced locally within the cell to be effective. (2) Sufficient peroxide is produced but its formation is not influenced by oxygen tension. (3) The oxygen tension has not been lowered enough in the parts of the cell concerned. (4) Oxygen increases peroxide formation but decreases its effectiveness. The fourth hypothesis has been eliminated by data in the preceding section. The third hypothesis is unlikely in light of the consistent finding of an oxygen effect upon the genetic changes produced by high doses. It is made still more unlikely by the similarity of the results with different methods of removing oxygen. However, it cannot be finally eliminated since localized differences in oxygen concentration are conceivable. The second hypothesis might be true for soft X rays. It is possible that effective concentrations of H_2O_2 are formed only in the dense ionization at the ends of electron tracks and that most of the H_2O_2 in these regions is formed by combination of OH radicals rather than by reactions with oxygen. However, the similarity of the results with γ and soft X rays makes this hypothesis very improbable. Thus the first hypothesis is, by far, the most likely one.

It is made still more likely by the following considerations. We have found that 2 μg/cc of H_2O_2 added to the medium has no detectable effect even after exposures of several hours, whereas a 10-minute exposure to 6 μg/cc produces a distinct delay in division.

Thus there is a limiting concentration below which no effect is produced. Moreover, *Paramecium* possesses an active catalase which would prevent the accumulation of H_2O_2 within the cell, especially if the catalase were in close proximity to the material whose damage leads to division delay and death. Such a location of the catalase is quite possible if the suggestion(6) that division delay and death are the result of changes in the outer portions of the cytoplasm is correct.

Thus we come to the hypothesis that locally produced H_2O_2 is so rapidly destroyed that effective concentrations are not reached within the cell unless it is overwhelmed by large quantities from the outside. Only in this latter case does H_2O_2 become the most important cause of the damage, and only then is a typical oxygen effect found. When peroxide is prevented from accumulating outside the cell, it is even possible that oxygen may act to protect the cell against damage by H atoms by reacting with them to form H_2O_2, against which the cell is well protected. This may explain the greater effect which is sometimes found under low oxygen tension though it does not explain the heterogeneity of the results.

These findings for *Paramecium* agree with those for bacteriophage in suggesting that only when H_2O_2 is involved is the action of X radiation decreased by decreased oxygen tension. Watson(7) distinguishes 3 components to the action of X rays on phage, 1) a direct effect presumably due to energy absorbed within the phage, 2) an indirect effect which he suggests is due to active radicals of short half life, and 3) an after effect due to relatively stable substances. Alper(8,9) makes essentially the same distinction, gives positive evidence that H_2O_2 causes the after effect, and shows that oxygen is necessary for the manifestation of this effect. Hewitt and Read(10) show that the direct effect is not influenced by oxygen, and Alper(9) shows the same lack of effect upon the initial rate of inactivation of phage (presumably Watson's indirect effect) and upon the sensitization of phage to H_2O_2. She believes that this means that oxygen is not needed for the action of radicals during irradiation and is effective only through its role in H_2O_2 formation. The results with *Paramecium* can be considered to support this point of view, at least in showing that oxygen does not enhance the nongenetic effects of X rays except when the conditions are such that these effects are due mostly to peroxide.

The distinct oxygen effect which is found for genetic damage in *Paramecium* could well mean that a considerable portion of this damage is brought about by peroxide. There is no contradiction in the idea that peroxide is effective in the micronucleus but not in the cytoplasm since the catalase content of the micronucleus may be low and only one or a few molecules of peroxide may be sufficient. However, the evidence from *Paramecium* only makes peroxide a possibility and does not exclude other explanations of the oxygen effect.

Finally, we have shown that oxygen may have a 2-fold effect upon the production of radiation damage. It may influence the production of active substances such as peroxide, but it may also influence the biological effectiveness of these substances. Thus, the oxygen effect for paramecia irradiated with few bacteria present consists of at least 2 parts. The presence of oxygen increases the production by irradiation of H_2O_2 in the medium, and it also increases the biological effectiveness of this substance. Similar combined effects may exist in other organisms.

Summary. The nongenetic effects of ionizing radiation upon *Paramecium,* when many bacteria are present during irradiation, are not influenced or perhaps are slightly increased by hypoxia. However, when few bacteria are present, these effects are decreased by hypoxia. The latter finding is in accord with the previously obtained evidence that much of the nongenetic damage produced when few bacteria are present is due to H_2O_2 formed outside the cell. The former finding suggests that H_2O_2 formed inside the cell is not of importance in producing nongenetic damage. On the other hand, genetic damage is consistently less under hypoxia. Previous evidence has shown that this kind of damage is not produced by H_2O_2 formed outside the cell. However, it seems quite possible that H_2O_2 or HO_2 produced locally within the nucleus is of importance in producing genetic damage;

but the evidence from *Paramecium* does not rule out other interpretations; *e.g.*, an effect of oxygen itself upon the recombination of broken chromosomes.

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Adaptation of Type I Poliomyelitis Virus to Mice. (20151)

C. P. LI AND MORRIS SCHAEFFER.

From the Virus and Rickettsia Section, Communicable Disease Center, Public Health Service, Federal Security Agency, Montgomery, Ala.

Since the adaptation of the Type II (Lansing) strain of poliomyelitis virus to mice by Armstrong(1), numerous attempts have been made to accomplish this with other poliomyelitis virus strains. Success with the Type III (Leon) strain was reported by Li and Habel (2), employing the intraspinal route in mice (3). This has been confirmed by Casals *et al.*(4), but the low virulence of the adapted strain has limited its usefulness in laboratory and field studies. More recently, Li and Schaeffer(5) have been able to increase the adaptability of the Type III strain to a degree of virulence which enhances its satisfactory utilization in mouse neutralization tests for the detection of antibodies.

Although Steigman and Kokko(6) have shown Type I poliomyelitis virus to persist in the mouse brain, they have not demonstrated that multiplication of the virus takes place. This report will describe methods employed in the successful adaptation of the Type I (Mahoney) strain to mice and its propagation in the rodent host.

Materials and methods. Virus. The Mahoney strain of poliomyelitis was employed in this work. This virus was originally isolated by Dr. Thomas Francis, Jr., from a pool of 3 stools of asymptomatic cases of poliomyelitis in Cleveland in 1941 and was later classified as Type I. The virus, when furnished us by Dr. Jonas E. Salk, had been through a total of 14 monkey passages and 2 monkey testicular tissue culture passages. *Immune serum.* Hyperimmune serum was prepared in monkeys with adjuvants following the procedure of Salk *et al.*(7) against Type I (Brunhilde), Type II (Lansing), and Type III (Leon) monkey propagated stock viruses. These sera were checked for specificity both in mouse and tissue culture neutralization tests. Immune monkey sera prepared and tested against the 3 types of virus in other poliomyelitis laboratories were obtained and also were used in neutralization tests to prove the specificity of the adapted virus. *Animals.* In nearly all the experiments performed, CFW mice were used. These were 3- to 5-week-old mice in the earlier passages, but later 8- to 12-week-old mice were employed because of preliminary indications that the latter may be more susceptible. Hamsters, cotton rats, and C3H mice were tested on 2 occasions. When monkey infectivity tests were conducted, rhesus monkeys weighing 3 to 6 pounds were inoculated intracerebrally. The rodents were successfully infected only by the intraspinal route. Intracerebral passages failed, and combined intraspinal and intracerebral inoculations were not superior. *Tissue culture.* The technic employed in our tissue culture has been described previously(8). Essentially, it