EFFECTS OF GAMMA IRRADIATION AND GASES ON THE SURVIVAL AND ACTIVITY OF *VIBRIO METSCHNIKOVII*

A THESIS

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We hereby recommend that the thesis prepared under our supervision by Huda Saleh Amash entitled Effects of Gamma Irradiation and Gases on the Survival and Activity of Vibrio metschnikovii

be accepted as fulfilling this part of the requirements for the Degree of Master of Science.

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INTRODUCTION AND REVIEW OF LITERATURE

The objective of this research was to investigate effects of gamma irradiation, resulting from exposure of Vibrio metschnikovii (Vibrio cholerae biotype proteus) to Cs-137 in the presence of atmospheres of Genetron-23 (fluoroform), oxygen, carbon dioxide, or compressed air.

Vibrio metschnikovii ATCC# e 7708 (originating from the University of Maryland, strain 503, R. Hugh) was chosen as the organism to be used in this research for the following three reasons. First, the characteristics of this organism are similar to Vibrio cholerae which is the causative agent of classical asian cholera. Second, the presence of Vibrio metschnikovii in water sources is of practical importance as an indicator organism for the effectiveness of radiation sterilization procedures (Myasnik and Morozov 1976). Third, there is a notable lack of information in the literature concerning the sensitivity of the cholera pathogen to ionizing radiation and chemical treatment.

The available literature concerning the effects of gases and gamma irradiation on biological systems of other microorganisms is likewise not very extensive. The relatively few references that relate to these studies are cited as follows.

Most studies concerning interactions of environmental gases and exposure to radiation on bacteria were performed on Escherichia coli. Beningo (1941) pointed out that nitrous oxide can be bactericidal or bacteriostatic for E. coli. Seifriz and Pollack (1949) reported that exposure of E. coli to nitrous oxide affects the protoplasm of the bacterial cells by gelatinizing it.

Vas (1953) concluded from his studies that the cytoplasmic membrane of E. coli cells was attacked by sulfur dioxide, resulting in an increase in permeability of the cell to amino acids.

Fluoride provides a logical material for investigations since it is inexpensive, commercially important, and also is recognized as a source of air pollution. Stokinger and Coffin (1968) stated that fluorides in the air result from the production of phosphate fertilizers, the manufacturing of aluminum, the leaking of fluorinated hydrocarbon refrigerants into the atmosphere, and the widespread use of aerosol propellants in insecticides and hairsprays, which expose man and other organisms to the effects of fluoride.

Brandt and Heck (1968) reported that fluorides act as cumulative poisons in plants, and Stokinger and Coffin (1968) cited the occurence of delayed manifestation of fluoride accumulations in animals. Landry and Fuerst (1968) observed mutagenic effects of several gaseous fluorinated hydrocarbons on E. coli B and E. coli Sd-4 11143.

Fuerst and Landry (1967) pointed out that nitrous oxide, carbonyl sulfide, perfluoropropane, and carbon tetrafluoride caused mutagenic effects in E. coli 4157, E. coli B and E. coli K-12. These workers found that sulfur dioxide was completely inhibitory, trimethylamine, monomethylamine, and methylmercaptan were bactericidal, and carbonyl sulfide was lethal to both E. coli K-12 and E. coli 4157.

Foltz and Fuerst (1974) reported that the fluorinated hydrocarbons were found to significantly increase mutation rates in the progeny of treated Drosophila over the control levels. Genetron-23 appeared to be the most mutagenic gas tested. Garrett and Fuerst (1974) observed that treatment with perfluorobutene-2 induced a recessive lethal mutation rate in Drosophila of 1.7%, as compared to 0.25% for compressed air.

Stephens et al. (1971) studied genetic and phenotypic responses to selected mixture of gases in Neurospora crassa. Fluoride containing gases and their analogous hydrocarbons, as well as oxygen, were tested for their effects on conidia formation, perithecia production, or mutagenicity.

Investigations concerning the influence of gases and irradiation on bacterial phages were performed. Appelmans (1922) found that infectivity of bacterial phages can be inactivated by irradiation. The effect of ultra violet light on the phage particles was a subject of intensive studies.

Adams (1959) investigated the effect of ultra violet light on bacterial phages. He concluded that ultra violet causes damage to the exposed phages including a growth delay in the phage particles surviving irradiation, a stimulation of genetic recombination and mutagenicity, physiological and genetic changes (Adams 1959).

Gates (1930) worked on Staphylococcus and found that ultra violet light had lethal effects on Staphylococcus phage at wave lengths from 2,300 to 2,970 A.

Cohen and Arbogast (1950) worked with UV-inactivated phages and came to the conclusion that these phages can stop the synthesis of bacterial DNA, RNA or protein. Kornberg et al. (1959) and Dirksen et al. (1960) found that UV-inactivated T2 phages can also elicit the synthesis of the phage specific enzymes.

Wacker et al. (1960), Wacker (1961), and Benker and Berends (1960) showed that the absorption of ultra violet light by DNA produces a variety of chemical modifications in the purine and pyrimidine residues of the irradiated polynuclectide chains. These investigators also found that modification of two adjacent thymine residues belonging to nucleotides on the same DNA polynucleotide chain were produced, and this thymine-thymine dimer was responsible for the death of the irradiated phage particles.

Setlow and Carrier (1966) and Logen and Whitmore (1966) discovered that ultra violet irradiation of DNA and polynucleotides of various purine and pyrimidine composition, yielded a variety of decomposition products as follows. The primary products in the irradiated DNA were thymine-thymine, cytosine-thymine, or cytosine-cytosine dimers. These dimers interfered with the nuclease activity of the cell or inhibit DNA synthesis (Logen and Whitmore, 1966).

Freifelder (1965) and Ginoza (1967) found that if bacteriophages are irradiated while suspended in nutrient broth, much of the lethal damage was due to strand breakage of the DNA backbone by the action of the radiation energy inside the phage, and the broth protected the phage from the radiation effects.

Dewey and Stein (1970) stated that it had been assumed that when biological material is inactivated by ionizing radiation, the damage is caused by direct absorption of energy to the largest molecule. In special cases large molecule damage is due to radical formation caused by the absorption of radiation in water. These radicals included hydrogen atoms, hydrated electrons, and hydroxy radicals, in addition to the secondary products like molecular hydrogen, hydrogen peroxide, and the negatively charged oxygen radicals (Dewey and Stein 1970). According to Dewey and Stein (1970), bacteriophages are much more sensitive to ionizing radiation

in pure water than in nutrient broth, because of the hydrogen peroxide and radicals that are formed in the water.

In a study of T7 phage Dewey and Stein (1970) demonstrated that the phage was more efficiently inactivated by hydrogen atoms in the presence of ultra violet light when the phage was suspended in a highly purified aqueous suspension, while hydrated electrons formed less inactivation, and OH or O_2 radicals caused no inactivation. These investigators concluded that some hydrated electrons reacted with phage without inactivating it, rendering the phage sensitive to hydrogen peroxide inactivation. While the hydroxyl radicals reacted with the phage with high efficiency, these ions did not inactivate or sensitize it.

Hollaender (1971) pointed out that many chemicals, as well as ionizing radiation and ultra violet light, produce mutagenic effects. He indicated that base analogs and intercalating agents mutate only replicating DNA while alkylating and radical-producing agents alter resting DNA. Hollaender (1971) suggested that DNA can be attacked by free radicals. He also stated that the lethal or chromosome breaking effect of X-ray (as a free radical producing agent) is greatly increased in the presence of oxygen which produces breakdown products of DNA bases and causes backbone breakage.

Krivankova et al. (1971) studied ultra violet light induced damage on virulent polyvalent Staphylococcus phages.

They found that the lethal effect of UV light was produced through the dimerization of thymine and other photoproducts on the polyvalant phages of Staphylococcus aureus 812, ϕ 131, A/5, and PA.

Gampel-Jobaggy et al. (1972) found that radiation sensitivity of T7 phage was strongly dependent on chemical additives. The OH radical was an important damaging species for T7 phage, while oxygen protected the phage against the irradiation induced damage.

Dewey and Stein (1968) irradiated inactive phage with gamma rays after treatment with hydrogen. They discovered that hydrogen atoms had more effect on the outer protein coat of bacteriophage T7, leading to the release of DNA into the solution, and the loss of phage infectivity.

Fuerst and Stephens (1970) found that carbon dioxide and sulfur dioxide inhibited ascospore germination and perithecia production completely in some strains of Neurospora crassa. They suggested that gamma irradiation caused damage but some protection was given by Genetron-23 or by ethane, while oxygen enhanced the radiation damage. These workers also found that in the presence of perfluorobutene-2, Co^{60} gamma irradiation was completely fungicidal, while the gas alone did not affect the organism. Perfluorobutene-2 provides protection against gamma irradiation induced effects in Drosophila, Serratia marcescens and in Escherichia coli (Fuerst and Stephens 1970).

According to Gould (1970), halogen free radicals cause toxicity to Bacillus cereus spores which were inactivated by gamma irradiation more effectively in the presence than in the absence of a variety of iodine compounds.

Mainland (1971) studied the mechanism of protection of M1 phage provided by some gases during gamma irradiation. Oxygen was found to protect M1 phage against gamma irradiation, while CH_4 and CO_2 released molecular oxygen upon exposure to gamma irradiation. Not much information is available in the literature about effects of radiation on the growth and activity of bacteria of the genus Vibrio. Sokurova (1974) investigated the sensitivity of many gram negative bacteria to radiation. He found that the genus Vibrio constitutes, most likely, the most radiosensitive group of microorganisms studied.

In another investigation concerning comparative sensitivities of some Vibrio species and E. coli to ultra violet light and ionizing radiation, Myasnik and Morozov (1976) reported that Vibrio metchnikovii and "water vibrios 46" were highly sensitive to gamma irradiation and did not differ in this respect from the cells of the hypersensitive E. coli mutant Bs-1. These investigators obtained the same results in a comparative study of sensitivity to X-rays of E. coli B_{s-1} and the pathogen of classical cholera, Vibrio cholera asiatica 150. In the same investigation Myasnik and Morozov (1976) reported that V. cholera asiatica 150 and V. cholera El Tor

601 are much more resistant to ultra violet light than E. coli Bs-1. They found that these vibrios were twice as sensitive to ultra violet light as cells of the wild strain of E. coli. Myasnik and Morozov (1976) suggested that there are strictly specific forms of inactivation inherent in each mutant strain of E. coli that has lost the capacity to repair DNA injuries induced by radiation. Phase contrast observations of cells exposed to gamma irradiation and ultra violet light exposed cells revealed that lysed cells constitute the main form of inactivation.

Additional experiments were conducted involving incubation of gamma irradiated vibrios in phosphate buffer, pH 8.6 (the optimum pH for vibrios). Sokurova (1978) studied the recovery system in species of Vibrio from lesions induced by gamma radiation. He found that V. cholerae biotype proteus, and aquatic Vibrio species 362 have no repair systems to eliminate radiation lesions when incubated in a liquid non-nutrient medium, or in pH 8.6 phosphate buffer for times up to 20 h. The number of the viable cells increased in both the non-irradiated controls and the irradiated samples. Sokurova (1978) demonstrated that this survival level is probably the M concentration which he defined as the number of cells at the stationary phase of growth, for this medium.

In other experiments, dealing with the survival of gamma irradiated Vibrio cells incubated on various nutrient media, Sokurova (1978) found that V. cholerae biotype proteus and aquatic Vibrio species 362 do not grow on minimum glucose-saline medium, while they grow on the surface of the alkaline agar and Fish bone agar which contain high nutrient qualities. He concluded that: "When incubated on the surface of agar media, bacteria of the genus Vibrio do not recover from lesions induced by gamma irradiation."

Since V. cholerae is the causative agent of classical Asian cholera, which is still out of control in some Asian countries, many questions may be raised with respect to the methods for controling the growth of this microorganism and the spread of the disease. In order to answer some of these questions, the research reported in this thesis was conducted to determine effects of environmental gases including Genetron-23, oxygen, carbon dioxide, and compressed air on the survival rate of V. metschnikovii. The influences of exposure to gamma irradiation on growth, carbohydrate fermentation abilities, and antibiotic sensitivity of the organism were also investigated.

MATERIALS AND METHODS

Strain History

The bacterial strain used in this research was a culture of *Vibrio metschnikovii* (*Vibrio cholerae* biotype *proteus*), ATCC # e 7708. It was obtained as a lyophilized culture from the American Type Culture Collection, Rockville, Maryland. This strain was used in all experiments. It was maintained under refrigeration at 4 C on 2% nutrient agar slants containing 1% NaCl.

Growth Media Used

All *Vibrio metschnikovii* cultures were maintained in 18 x 150 mm test tubes on agar slants consisting of 32 g of Difco nutrient agar (containing 15 g Difco agar), 5 g of Difco agar and 10 g of NaCl, per liter of distilled water. The organims were grown in 150 mm Petri dishes. Broth medium was prepared by adding 8 g of Difco Bacto nutrient broth and 10 g of NaCl to one liter of distilled water. All dehydrated media used were obtained from Difco Laboratories, Detroit, Michigan, U.S.A. One percent carbohydrate media were prepared by adding 10 g of each carbohydrate (Eastman Kodak Company, Rochester, N.Y.) either glucose, maltose, fructose or starch to 32 g of Difco nutrient agar, containing 5 g of Difco agar and 10 g of NaCl per liter of distilled water. All media

and test tubes were sterilized in the autoclave at 124 C at 18 lb pressure for 10 minutes. The Petri dishes and pipettes were sterilized in the hot air oven at 180 C for 2 h.

Preparation of the Inoculum for Treatment

Vibrio metschnikovii was grown for 24 h at 28 C on 1% nutrient agar slants. Ten ml of sterile distilled water were used to wash the cells of each of four slants and to resuspend the combined washings in less than 160 ml water. From this suspension serial dilutions were prepared, and the absorbance of each dilution was determined with a Bausch and Lomb Spectronic 20 Spectrophotometer at a wave length of 620 mμ. One ml of each suspension was inoculated into a Petri plate and a nutrient agar medium was poured into each plate. All plates were incubated at 28 C for 48 h. The number of colonies in each plate were counted, and a curve was plotted of absorbance vs. the plate counts of the bacterial suspensions. The concentration of the cells that gave the best plate count was approximately 1.5×10^4 cells/ml.

Gases Employed

The gases used in this study included Genetron-23 (Fluoroform), oxygen, carbon dioxide, and compressed air, obtained in lecture bottles from Matheson Gas Products, a division of Well Ross, Inc., East Rutherford, New Jersey and La Porte, Texas. The description of the gases is presented in Table 1.

Table 1--Gases tested for biological effects on Vibrio metschnikovii.

Names of Gases*	Formula	Molecular weight
Oxygen	O_2	32.00
Genetron-23 (Fluoroform)	CHF_3	70.00
Carbon dioxide	CO_2	44.00
Compressed air		

*Descriptive data published by Matheson Gas Products (1969), East Rutherford, New Jersey.

The Gassing Procedure

Modified Pyrex Turner bulbs (11 1/2 x 4 1/2 cm), with a 100 ml liquid capacity, were used as gassing chambers. After calibration of the Hasting Mass Flowmeter scale for each gas, as measured by water displacement in an inverted graduate cylinder, the flow rate of each gas used in the experiments could easily be determined as the amount of gas that would displace 20 ml of water per min. The gas was delivered from the lecture bottle through copper tubing to the Hastings Mass Flowmeter and through a sterile drying tube into the Turner bulb to another drying tube. From there the effluent of gas went out through the chemical exhaust hood. For all experiments a 10 ml aliquot of an aqueous bacterial suspension $(1.5 \pm 0.1) \times 10^4$ cell/ml was pipetted into a sterile Turner bulb. Two hundred ml of the gas to be tested was bubbled through the suspension at a rate of 20 ml/min. The gaseous atmosphere was preserved by turning the top of the Turner bulb, thus sealing the gassing chamber from the external environment. Standard sterile procedures were used for all experiments performed.

The Irradiation Procedure

After the bacterial suspension had been exposed to a selected gas atmosphere, the samples to be irradiated in a selected gaseous environment and the non gas treated controls

were placed into a Cs-137 gamma irradiator one h after the gas had been delivered to the Turner bulbs. Each 10 ml of bacterial suspension was irradiated with 8.66×10^4 R/h for 9 min, as based on previous experimental determinations.

The Biochemical Tests

The ability of Vibrio metschnikovii to ferment glucose, maltose and fructose was tested by inoculating each of the irradiated samples and the non treated controls into 10 Petri dishes for each carbohydrate medium. All plates were incubated at 28 C for 48 h. The ability of the tested organisms to ferment a carbohydrate was apparent from the changes in color of the media containing phenol red indicator, which changes color from red to yellow as acid is produced by the fermentation of carbohydrate.

Starch Hydrolysis Test

The ability of Vibrio metschnikovii to hydrolyze starch was tested by inoculating each of the irradiated samples and the non treated controls into 10 Petri plates of starch agar medium. All plates were incubated at 28 C for 48 h. Few drops of 1% potassium iodine (Baker Chemical Co., Phillipsburg, N. J.) were added onto the medium in each plate. The appearance of clear, uncolored zones around the colonies indicates the ability of the organism to hydrolyze starch (Blair et al. 1970).

The Antibiotic Media

To obtain antibiotic liquid media, concentrated antibiotic solutions were sterilized by filtration through 20 micron pores of a Nalgene Filter Unit (Nalge Sybron Corporation, Rochester, N.Y., U.S.A.). From this stock solution, various dilutions were prepared. For example, the quantities of streptomycin sulfate (Sigma Chemical Company, St. Louis, Mo., U.S.A.) required were 8.5, 17.0, 25.5, 34.0, 42.5, 85.0, and 170.0 mg of the antibiotic per 100 ml of distilled water. From each dilution, one ml was pipetted into 500 ml of sterile nutrient 1% NaCl broth, resulting in 0.01, 0.02, 0.03, 0.04, 0.05, 0.10 and 0.20 units/ml of streptomycin liquid medium. Five ml of each medium were pipetted into 18 x 150 mm sterile plugged test tubes.

Chloramphenicol liquid media dilutions were prepared in a similar way, using different concentrations of the antibiotic as required.

Antibiotic Sensitivity Tests

The sensitivity of V. metschnikovii to streptomycin and chloramphenicol was tested after exposure of the organism to 8.66×10^4 R/h of gamma irradiation for 7 min in atmospheric air and Genetron-23, which were delivered at a flow rate of 20 ml/min for 10 min. One-tenth ml of the exposed and unexposed organisms was inoculated to each test tube that

contained the antibiotic medium. All test tubes were incubated at 28 C for 48 h. The concentration of cells was determined with a Bausch and Lomb Spectronic 20 Spectrophotometer at a wave length of 620 mμ. A curve was plotted showing absorbances vs. antibiotic concentrations.

The sensitivities of V. metschnikovii to various antibiotics, including sulfadiazine, tetracyclin B, chloromycin, streptomycin, terramycin, coly-mycin, penicillin G, neomycin and polymyxin B (Difco laboratories, Detroit, Michigan) were also tested on a solid medium. Both the exposed and unexposed control organisms were inoculated by cross-streaking a swab full of each on a 1% NaCl nutrient agar plate. Paper discs, which contained standardized concentrations of the antibiotics were aseptically placed equal-distances apart on the inoculated agar surface using alcohol flamed sterilized forceps. The plates were incubated inverted at 28 C for 48 h. The inhibition of bacterial growth was determined by measuring in cm the total diameter of each zone surrounding each disc from the outer edge across the disc to the edge where growth starts on the other side. These experiments were repeated five times.

EXPERIMENTAL RESULTS

In preliminary studies methods were developed to grow Vibrio metschnikovii ATCC# e 7708 from lyophilized culture stock. The organism belongs to the family Vibrionaceae, consisting of gram negative rods, ranging in size from 1.3 to 2.1 μm by 0.4 to 0.7 μm. The axis of the cell is slightly curved. The organism is actively motile by means of polar flagella.

V. metschnikovii is a halophilic aerobic, or facultatively anaerobic organism. Optimum growth is obtained on alkaline media at pH 8.4, at 28 to 30 C. It utilizes peptone as a principle source of nitrogenous nutrients, producing ammonia (Hendrie et al. 1971). V. metschnikovii is sensitive to chloramphenicol, streptomycin, and polymyxin while it is insensitive to penicillin (Hendrie et al. 1970).

The organism is oxidase and catalase positive, and has the ability to hydrolyze starch. Acid, but no gas, is formed in carbohydrate fermentation media containing glucose, fructose, galactose, mannose, maltose, dextrin and starch (Hendrie et al. 1970).

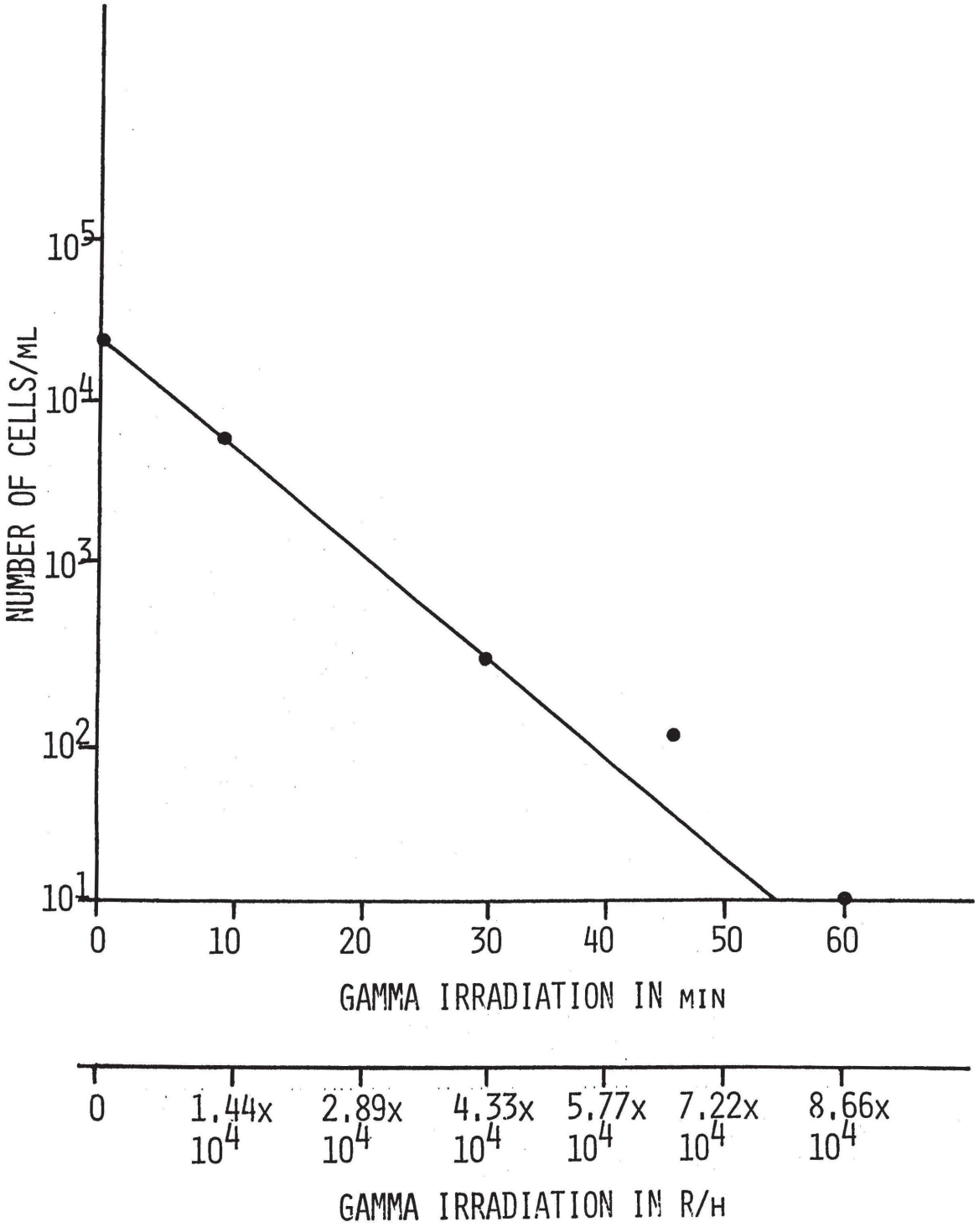
A number of experiments were performed testing these activities of V. metschnikovii. The organism was exposed to different environmental conditions and variations of gassing with and without irradiation. It was also necessary to

determine the optimal concentration of the bacterial cells for the contemplated research, by growing the organism on four nutrient agar slants containing 1% NaCl for 24 h, after which the cells were suspended in 200 ml of sterile distilled water. In order to establish the concentration of the bacteria, absorbance of the suspension was determined with a Bausch and Lomb Spectronic 20 Spectrophotometer at a wave length of 620 mμ. The concentration of the cells in all experiments was approximately 1.5×10^4 cells/ml.

An attempt was made to determine the effect of exposure of V. metschnikovii suspended in sterile distilled water to Cs-137 gamma irradiation in atmospheric air for various time periods. Ten ml of the bacterial suspension was pipetted into each Turner bulb. These samples were exposed to 8.66×10^4 R/h gamma irradiation for 10, 30, 45, and 60 min. Subsequently, one ml of each treated solution was inoculated into a Petri plate, and 15 ml of sterile nutrient agar containing 1% NaCl were added by pouring the medium at 50 C over the cells, with proper mixing. All plates were incubated at 28 C for 48 h. The number of colonies per plate were counted by using a Darkfield Colony counter (American Optical Company, Buffalo, N.Y.). The data obtained was recorded as number of cells vs. irradiation time.

As shown in Figure 1, the growth of V. metschnikovii decreased as the irradiation time increased. It can be noted

Figure 1--Effects of exposure to 8.66×10^4 R/h of Cs-137 gamma irradiation for various time intervals on the survival of Vibrio metschnikovii.


NUMBER OF CELLS/ML
10^5
10^4
10^3
10^2
10^1
0
10
20
30
40
50
60
GAMMA IRRADIATION IN MIN
0
1.44x 10^4
2.89x 10^4
4.33x 10^4
5.77x 10^4
7.22x 10^4
8.66x 10^4
GAMMA IRRADIATION IN R/H

that 30 min of exposure inhibited 98.7% of the growth, and only 0.4% of the cells survived after 45 min of irradiation. A treatment of the organism with irradiation for 60 min, inhibited the growth completely. It was found that an LD/70 dose for V. metschnikovii can be obtained after 9 min of gamma irradiation.

An experiment was performed to determine if there was any difference in effects on V. metschnikovii due to an exposure of the organism to 8.66×10^4 R/h of gamma irradiation in compressed air, as compared to control irradiations in atmospheric air. Compressed air was introduced to the Turner bulb for 10 min at a rate of 20 ml/min. After the organism was exposed to 8.66×10^4 R/h of gamma irradiation for 7 min, instead of 9 min because of the high sensitivity of the organism to radiation, the atmosphere was retained for one hour. One ml aliquots of the exposed and unexposed organisms were inoculated into each Petri dish, to which 15 ml of 2% nutrient agar containing 1% NaCl were added. In order to mix the organisms with the medium, each plate was rotated by hand several times. After an incubation at 28 C for 48 h, the numbers of colonies per plate were counted by using a Darkfield Colony counter. The results are shown in Table 2. It was found that 2.8×10^4 cells/ml survived when the organisms were treated with compressed air, while 1.4×10^4 cells/ml grew when the organisms were incubated in atmospheric air.

Table 2--Survival of Vibrio metschnikovii ATCC# e 7708 after irradiation with Cs-137* in atmospheric and in compressed air.

Exposure to gamma irradiation min	Compressed air** cells/ml***	Atmospheric air cells/ml
0	2.8×10^4	1.4×10^4
7	4.9×10^2	9.0×10

*The organisms were irradiated with a dosage of 8.66×10^4 R/h.

**Ten ml of bacterial suspension were gassed for 10 min in Turner bulbs, the flow of air was controlled by a Hasting Mass Flowmeter.

***1 ml of Vibrio metschnikovii suspension was poured into a Petri dish, 2% of nutrient agar containing 1% NaCl was added. After 48 h of incubation at 28 C the number of colonies in each plate was counted. Each reading is the average of three experimental replications.

As expected, irradiation damage occurred in either atmosphere. The number of cells dropped from 2.8×10^4 cells/ml for the samples which were exposed to compressed air only, to 4.9×10^2 cells/ml for the irradiated cells in compressed air, and from 1.4×10^4 cells/ml for the samples that were exposed to atmospheric air only, to 9.0×10 cells/ml for the cells that were irradiated in atmospheric air. As can be noted, compressed air provided some protection against irradiation induced damage.

Effects of compressed air, oxygen, carbon dioxide, or Genetron-23 on the growth of V. metschnikovii, were compared to the survival rate of the organism with and without subsequent exposure to irradiation. One ml of the exposed and unexposed organisms was inoculated into Petri dishes, using the same inoculation technique as described previously.

All plates were incubated at 28 C for 48 h. The total number of colonies in each plate were counted. Figure 2 shows the results of this experiment. It can be noted that none of the gases had a protecting effect against gamma irradiation induced damage. Carbon dioxide with and without irradiation inhibited the growth of V. metschnikovii completely. Table 3 presents the data of Table 2 in terms of percentages of the control (non-irradiated atmospheric air). The compressed air was found to enhance the growth of the organism to 200%, and Genetron-23 increased the survival rate

Figure 2--Survival of Vibrio metschnikovii after prior treatment with gas followed by 8.66×10^4 R/h of gamma irradiation in a Turner bulb. The gas was dispensed for 10 min at a rate of 20 ml/min, and allowed to remain in the bulb for one hour before exposure to irradiation for 7 min.

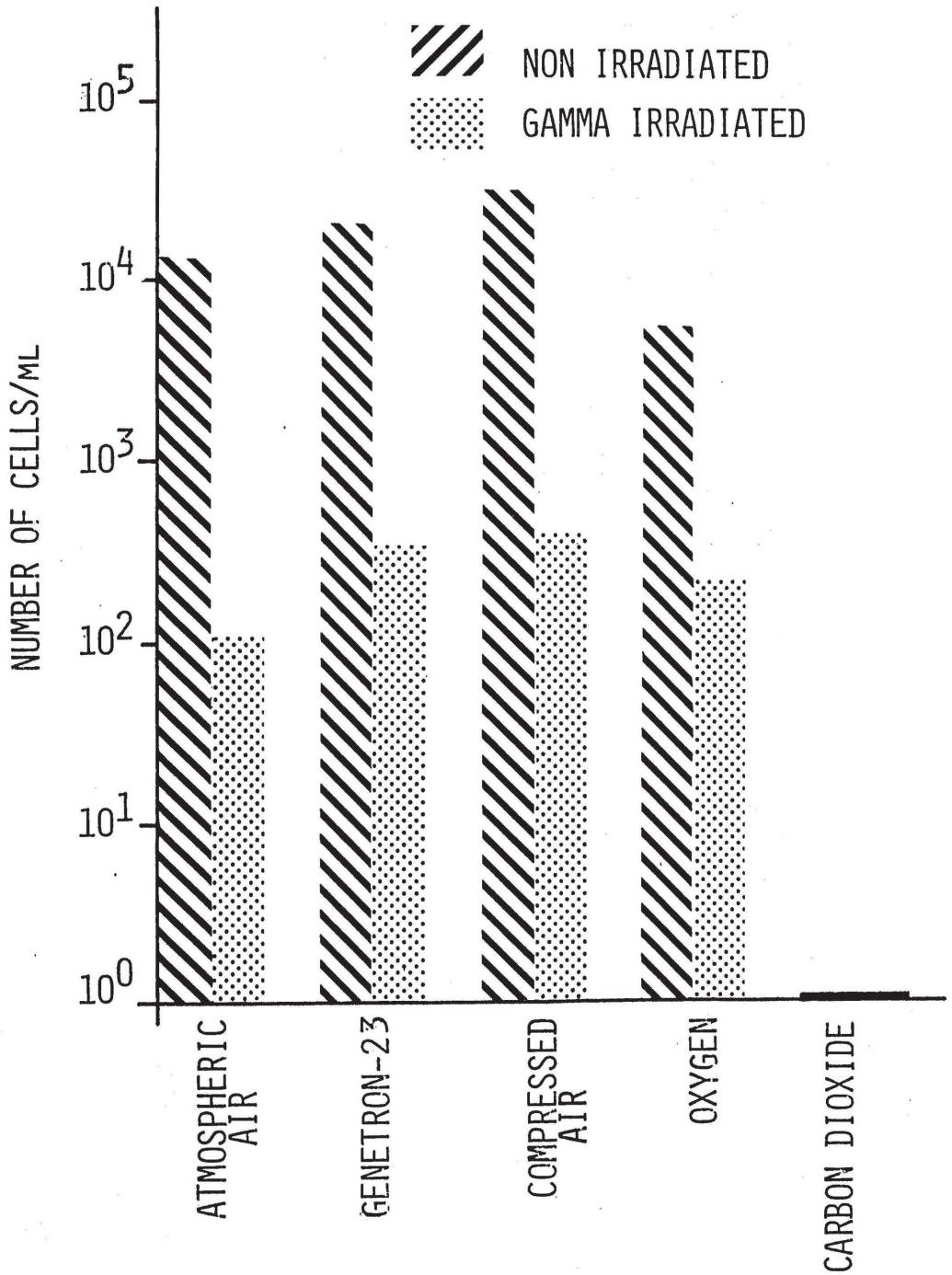

NON IRRADIATED
GAMMA IRRADIATED
NUMBER OF CELLS/ML
10^5
10^4
10^3
10^2
10^1
10^0
ATMOSPHERIC AIR
GENETRON-23
COMPRESSED AIR
OXYGEN
CARBON DIOXIDE

Table 3--Survival percentage of Vibrio metschnikovii ATCC# e 7708 after irradiation with Cs-137* in atmospheric air, compressed air, Genetron-23, oxygen, and Carbon dioxide.**

Treatment	Number of cells/ml***	Percentage of cells/ml
Atmospheric air	1.4×10^4	100.000
Irradiated in atmospheric air	9.0×10	0.643
Compressed air	2.8×10^4	200.000
Irradiated in compressed air	4.9×10^2	3.500
Genetron-23 (Fluoroform)	1.9×10^4	135.714
Irradiated in Genetron-23	3.3×10^2	2.357
Oxygen	5.6×10^3	40.000
Irradiated in oxygen	2.2×10^2	1.571
Carbon dioxide	0	0
Irradiated in carbon dioxide	0	0

*The irradiated samples were exposed to a dosage of 8.66×10^4 R/h for 7 min.

**Ten ml of bacterial suspension were gassed for 10 min in Turner bulbs, the flow of air was controlled by a Hasting Mass Flowmeter.

***One ml of V. metschnikovii suspension was poured into a Petri dish, 2% of nutrient agar containing 1% NaCl was added. After 48 h of incubation at 28 C the number of colonies in each plate was counted. Each reading is the average of three experimental replications.

to 135%, while oxygen decreased it to 40%. At the same time, oxygen provided the best protection against irradiation induced damage, as compared to the other gases. Compressed air and Genetron-23 gave the same amount of protection, while atmospheric air did not seem to have any effect against irradiation induced damage.

The ability of V. metschnikovii to ferment glucose, fructose, and maltose in the presence and absence of gamma irradiation, was examined. The results in Table 4 show that a dose of 8.66×10^4 R/h for 9 min weakened the growth of the organism and inhibited the fermentation of glucose, fructose, and maltose in carbohydrate medium during the first 24 h. Positive fermentation of these carbohydrates appeared after 48 h of incubation. When the irradiation time was decreased to 7 min, positive fermentation of glucose was enhanced during the first 24 h of incubation. The growth of the organism increased from 14 to 52 colonies/plate of glucose agar, from an average of 4 to 27 colonies/plate of fructose agar, and from 26 to 70 colonies/plate of maltose agar after 48 h of incubation. Best growth was obtained on maltose agar and was associated with a production of heavy mucoid material. The unexposed controls showed heavy growth and positive fermentation for all of these carbohydrates after 24 h of incubation.

In a set of experiments the influence of exposure to gamma irradiation on the ability of V. metschnikovii to

Table 4--Effect of exposure to 8.66 x 10^4 R/h of gamma radiation produced by Cs-137 on V. metschnikovii ATCC# 3 7708.

Treatment	Media streaked in petri plates*			
	Nutrient agar	Glucose agar	Fructose agar	Maltose agar
Exposure to Cs-137**				
Exp.#1, 24 hr incub.	weak growth	no ferm. 12 col/plate	no ferm. 6 col/plate	no ferm. 25 col/plate
48 hr incub.	7 x 10^3 cells/ml	pos. ferm. 15 col/plate	pos. ferm. 6 col/plate	pos. ferm. 30 col/plate
Exp.#2, 24 hr incub.	weak growth	no growth	no growth	no growth
48 hr incub.	10 x 10^3 cells/ml	pos. ferm. 2 col/plate	pos. ferm. 1 col/plate	pos. ferm. 5 col/plate
Exp.#3, 24 hr incub.	weak growth	no growth	no growth	no growth
48 hr incub.	2.5 x 10^3 cells/ml	pos. ferm. 25 col/plate	pos. ferm. 5 col/plate	pos. ferm. 45 col/plate
Exp.#4, 24 hr incub.	no growth	no ferm. 2 col/plate	no growth	no ferm. 15 col/plate
48 hr incub.	9 x 10^3 cells/ml	pos. ferm. 14 col/plate	pos. ferm. 3 col/plate	pos. ferm. 25 col/plate
Exp.#5, 24 hr incub.	no growth	pos. ferm. 12 col/plate	no growth	no ferm. 15 col/plate
48 hr incub.	9 x 10^3 cells/ml	pos. ferm. 52 col/plate	pos. ferm. 27 col/plate	pos. ferm. 70 col/plate
Unexposed control				
Exp.#1, #2, #3, #4, and #5, 24 and 48 hr incub.	seeded	pos. ferm. seeded	pos. ferm. seeded	pos. ferm. seeded

*For each medium in each experiment five petri plates were used.
**The cells in Exp.#1 to #4 were irradiated with 8.66 x 10^4 R/h for 9 min, in Exp.#5 for 7 min.

hydrolyze starch was investigated. Ten ml of the bacterial suspension was exposed to 8.66×10^4 R/h of gamma radiation for 7 min in a Turner bulb. One ml of each of the exposed and unexposed controls was pipetted into a Petri plate which contained 1% starch agar and 1% NaCl. The organisms were spread over the surface by using an alcohol flame sterilized L shaped glass rod. All plates were incubated at 28 C for 48 h. The number of colonies in each plate were counted.

The results of this experiment are shown in Table 5. As expected, irradiation decreased the survival of V. metschnikovii on nutrient agar medium from 2.5×10^4 cells/ml for the unexposed organisms, to 6.9×10^3 cells/ml for the irradiated bacterial cells. When the unexposed controls were inoculated on starch agar medium a good growth of 2.0×10^4 cells/ml was obtained. All colonies were tested for their starch hydrolysis abilities by adding few drops of Gram's iodine onto the starch agar surface, the appearance of a clear zone around each colony indicated that the organism has the ability to hydrolyze the starch.

Only one colony of the irradiated V. metschnikovii suspension grew on the starch agar medium. It was isolated and tested for starch hydrolysis and carbohydrate fermentation. When the morphology of the colony was examined, it was found to have the same characteristics of the cells of V. metschnikovii strain that were used in this research.

Table 5--Effect of exposure to Cs-137 gamma irradiation on the starch hydrolysis ability of Vibrio metschnikovii ATCC# e 7708.*

Treatment	Tested on agar media in petri plates	
	Nutrient agar	Starch agar
min	cells/ml**	cell/ml
0	2.5×10^4	2.0×10^4
7	6.9×10^3	0

*A dose of 8.66×10^4 R/h of gamma irradiation was used.
**One ml of bacterial suspension was pipetted onto the starch agar medium in a Petri plate, and spread with an alcohol flame sterilized L shaped glass rod. All plates were incubated at 28 C for 48 h. The number of colonies per plate were counted. Counts are means of five replicates.

Several experiments were performed to test the influence of streptomycin on V. metschnikovii in the presence and absence of gamma irradiation and Genetron-23. Figure 3 shows the sensitivity of V. metschnikovii to streptomycin media. The results in Figure 3 indicate that growth was enhanced in the presence of 0.01 and 0.02 units/ml of streptomycin in the media (with or without Genetron-23); then as the concentration of the antibiotic increased, the growth dropped. A concentration of 0.20 unit/ml inhibited all growth completely.

Figure 3 shows the effect of Genetron-23 on the sensitivity of V. metschnikovii to streptomycin. The results indicate that the gas enhanced the resistance of the organism to streptomycin. At a concentration of 0.10 units/ml of the antibiotic, the absorbance of the samples treated with Genetron-23 was 0.10, while the absorbance of the samples exposed to atmospheric air was 0.07, indicating that Genetron-23 provided some protection against streptomycin induced damage. A concentration of 0.20 units/ml of streptomycin in liquid medium inhibited the growth of V. metschnikovii completely when the organism was treated with Genetron-23 and atmospheric air. It can be noted in Figure 4 that a concentration of 0.04 unit/ml of streptomycin inhibited the growth completely resulting in 0 absorbance when the organism was exposed to 8.66×10^4 R/h of gamma radiation for 7 min in atmospheric

Figure 3--Effects of exposure to Genetron-23 on the sensitivity of Vibrio metschnikovii to various concentrations of streptomycin measured in units of streptomycin per ml of nutrient broth. This experiment was repeated five times.

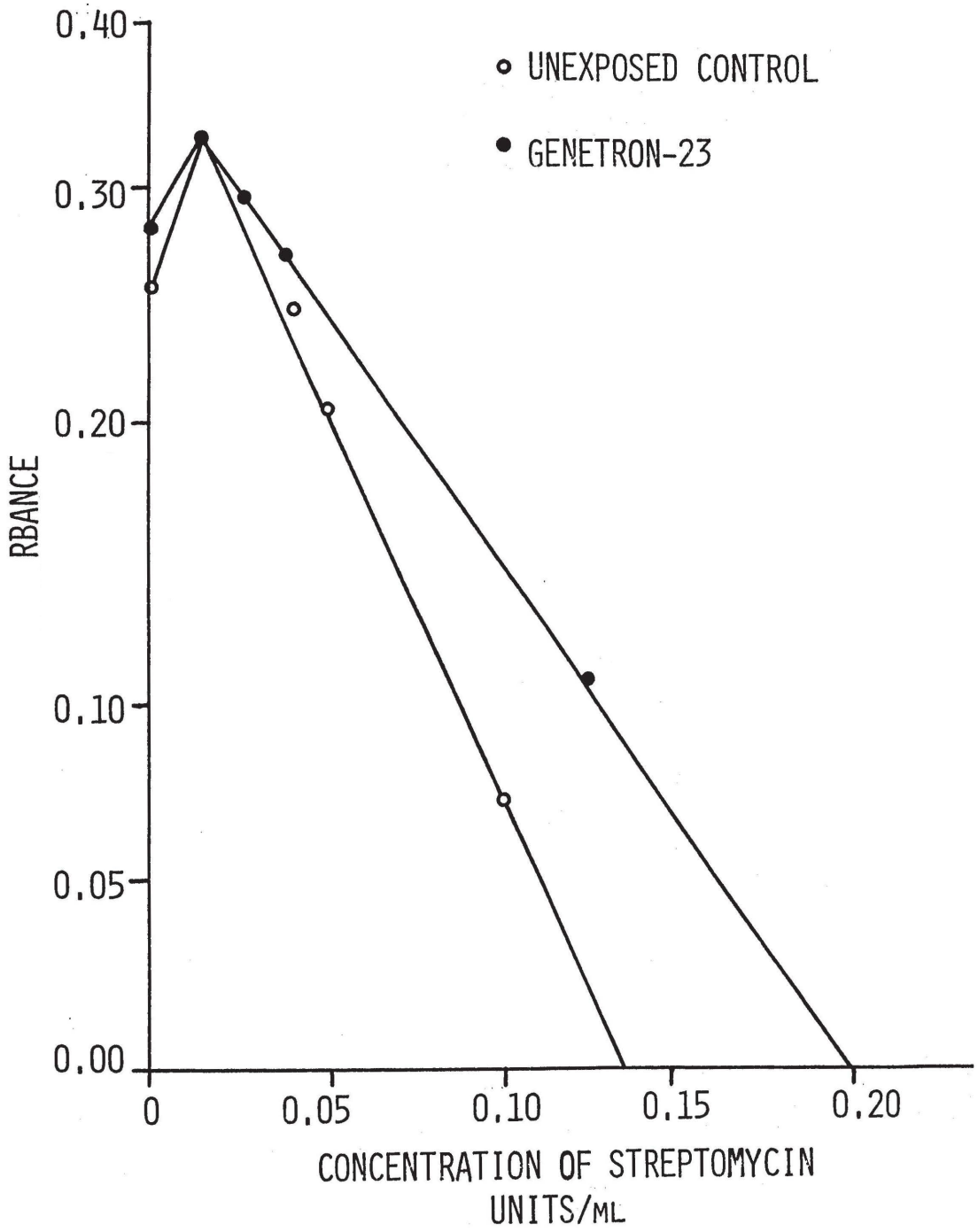
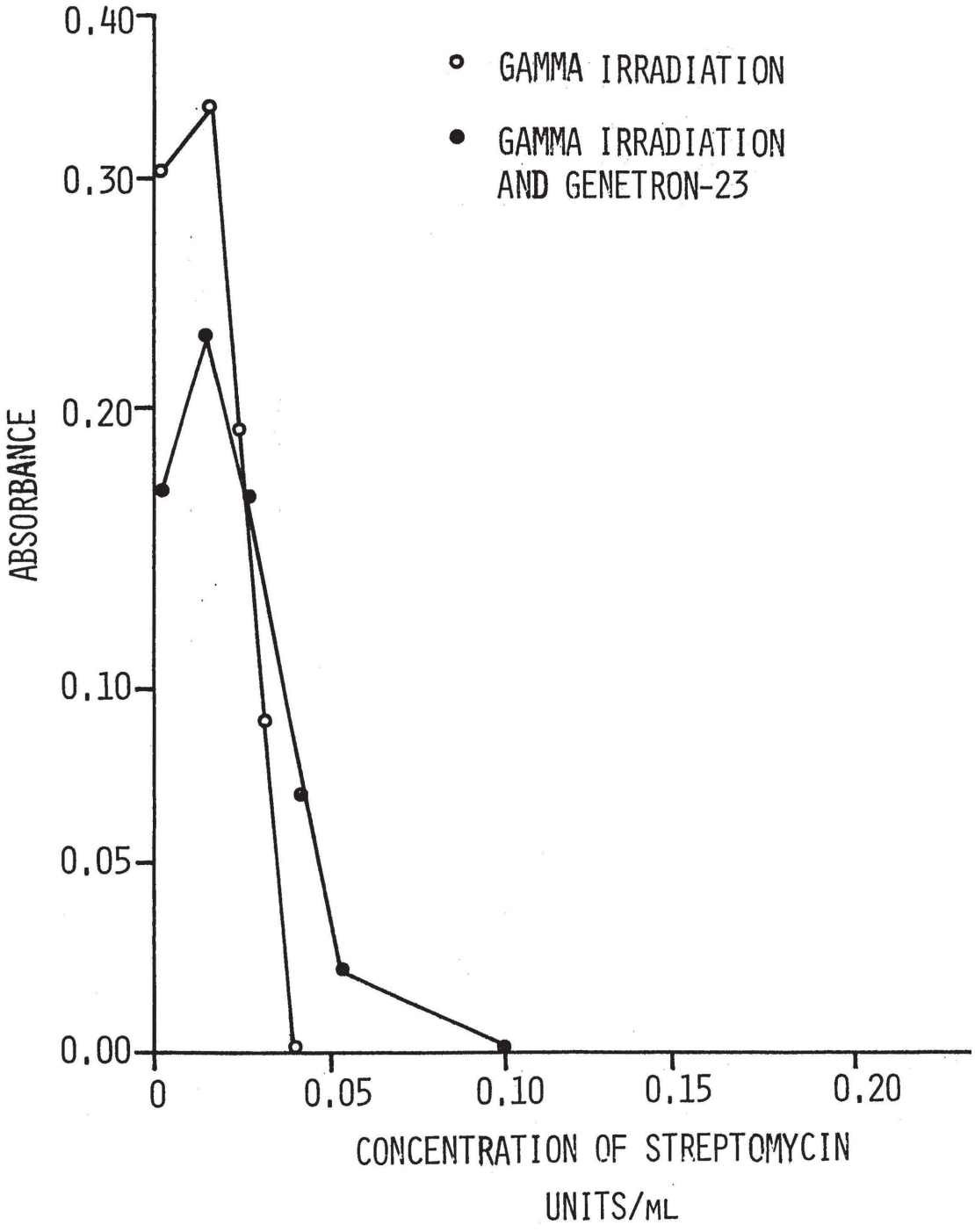

0.40
0.30
0.20
0.10
0.05
0.00
RBANCE
UNEXPOSED CONTROL
GENETRON-23
0
0.05
0.10
0.15
0.20
CONCENTRATION OF STREPTOMYCIN
UNITS/ML

Figure 4--Effects of exposure of Vibrio metschnikovii to 8.66×10^4 R/h of gamma irradiation in Genetron-23 and atmospheric air on the sensitivity of the organism to various concentrations of streptomycin as measured in units/ml of nutrient broth.

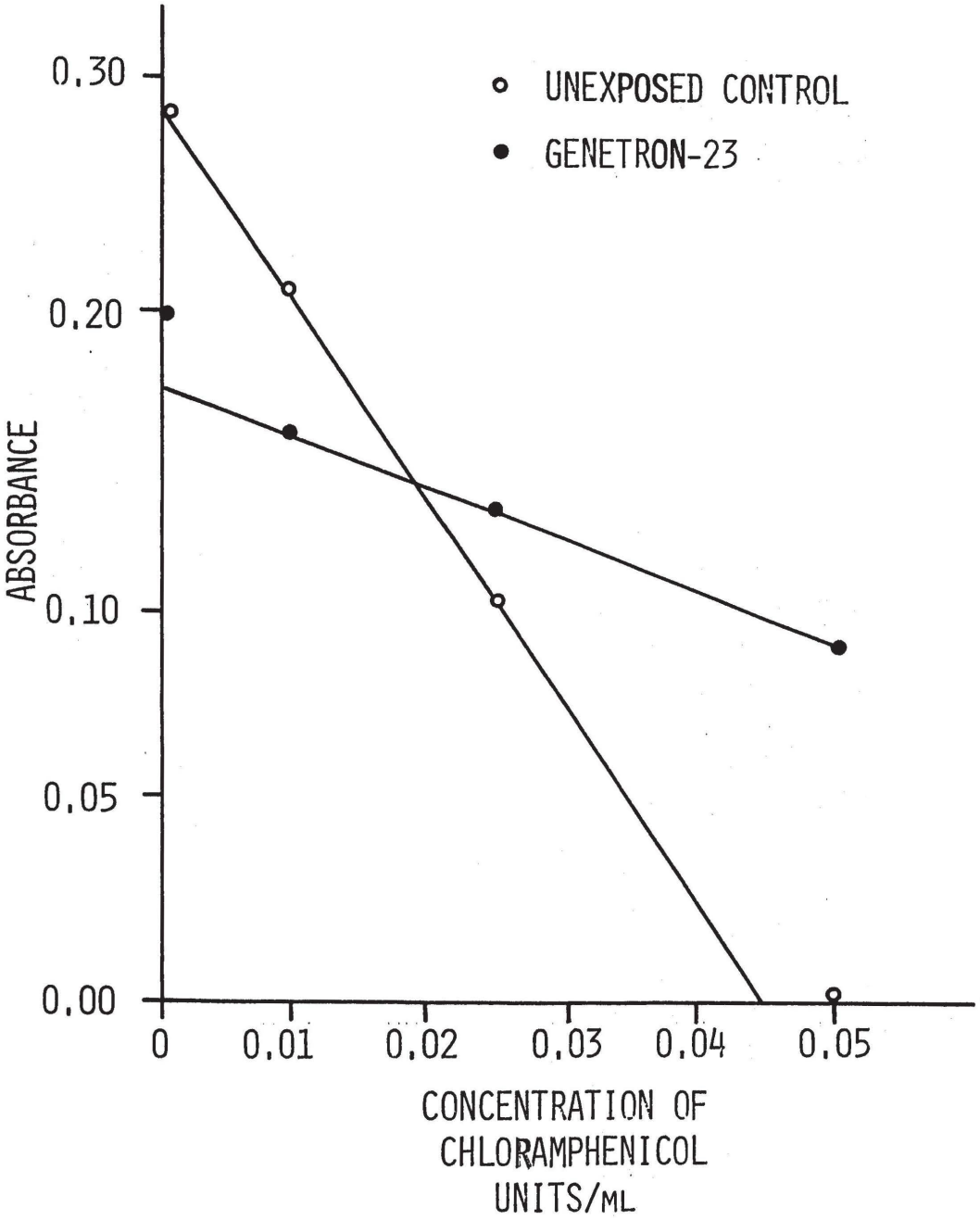

0.40
0.30
0.20
0.10
0.05
0.00
ABSORBANCE
GAMMA IRRADIATION
GAMMA IRRADIATION AND GENETRON-23
0
0.05
0.10
0.15
0.20
CONCENTRATION OF STREPTOMYCIN
UNITS/ML

air. While Genetron-23 decreased the sensitivity of V. metschnikovii to streptomycin when the organism was irradiated in the gas atmosphere, growth continued and 0.07 absorbance was obtained. A concentration of 0.10 units/ml of streptomycin inhibited the survival of the organism completely.

The effect of Genetron-23 on the sensitivity of V. metschnikovii to chloramphenicol was studied. As shown in Figure 5, the gas increased the resistance of the organism to chloramphenicol. At a concentration of 0.01 units/ml of the antibiotic, the absorbance of the samples that were exposed to atmospheric air dropped about 29%, while the absorbance of the samples that were treated with Genetron-23 decreased 22%. As the antibiotic concentration increased to 0.025 units/ml, the absorbance of the cells, exposed to atmospheric air, dropped 65% as compared to 28% for the Genetron-23 treated cells. Growth of V. metschnikovii exposed to atmospheric air was completely inhibited at a concentration of 0.05 units/ml of chloramphenicol, while the growth of the cells treated with Genetron-23 continued at an absorbance of 0.09.

Gamma irradiation had a modified effect on the sensitivity of V. metschnikovii to chloramphenicol when the cells were irradiated with 8.66×10^4 R/h for 7 min in atmospheric air. However, Genetron-23 provided some protection against radiation and chloramphenicol effects, as shown by the data

Figure 5--Effects of exposure of Vibrio metschnikovii to Genetron-23 on the sensitivity of the organism to various concentrations of chloramphenicol as measured in units/ml of nutrient broth.


0.30
0.20
0.10
0.05
0.00
ABSORBANCE
UNEXPOSED CONTROL
GENETRON-23
0
0.01
0.02
0.03
0.04
0.05
CONCENTRATION OF
CHLORAMPHENICOL
UNITS/ML

plotted in Figure 6. At a concentration of 0.01 units/ml of the antibiotic, the absorbance of the irradiated organisms in atmospheric air was 0.12 as compared to 0.16 for the cells that were irradiated in Genetron-23 atmosphere. A concentration of 0.05 units/ml inhibited the growth of the irradiated samples in the atmospheric air completely, while the survival of the cells treated with irradiation in the presence of Genetron-23 continued, resulting in an absorbance of 0.01.

Another experiment was performed to determine the influence of gamma irradiation and Genetron-23 on the sensitivity of V. metschnikovii to sulfadiazine, tetracyclin B, chloromycin, streptomycin, terramycin, coly-mycin, penicillin G, neomycin, and polymyxin B. As may be seen in Figure 7, gamma irradiation increased the sensitivity of the organism to all antibiotics, while Genetron-23 provided some protection against the radiation and antibiotics effects. It can also be noted in Table 6 that chloromycin which produced an inhibition zone of 3.9 cm for the unirradiated-unexposed controls, and 5.1 cm for gamma irradiated samples was the strongest antibiotic tested. V. metschnikovii was resistant to sulfadiazine when it was exposed to atmospheric air or Genetron-23, while the samples which were exposed to 8.66×10^4 R/h of gamma irradiation for 7 min in the presence and absence of Genetron-23 were sensitive to this antibiotic, showing inhibition zones of 1.7 and 2.0 cm.

Figure 6--Effects of exposure of Vibrio metschnikovii to 8.66×10^4 R/h of gamma irradiation in Genetron-23 and atmospheric air on the sensitivity of the organism to various concentrations of chloramphenicol as measured in units/ml of nutrient broth.

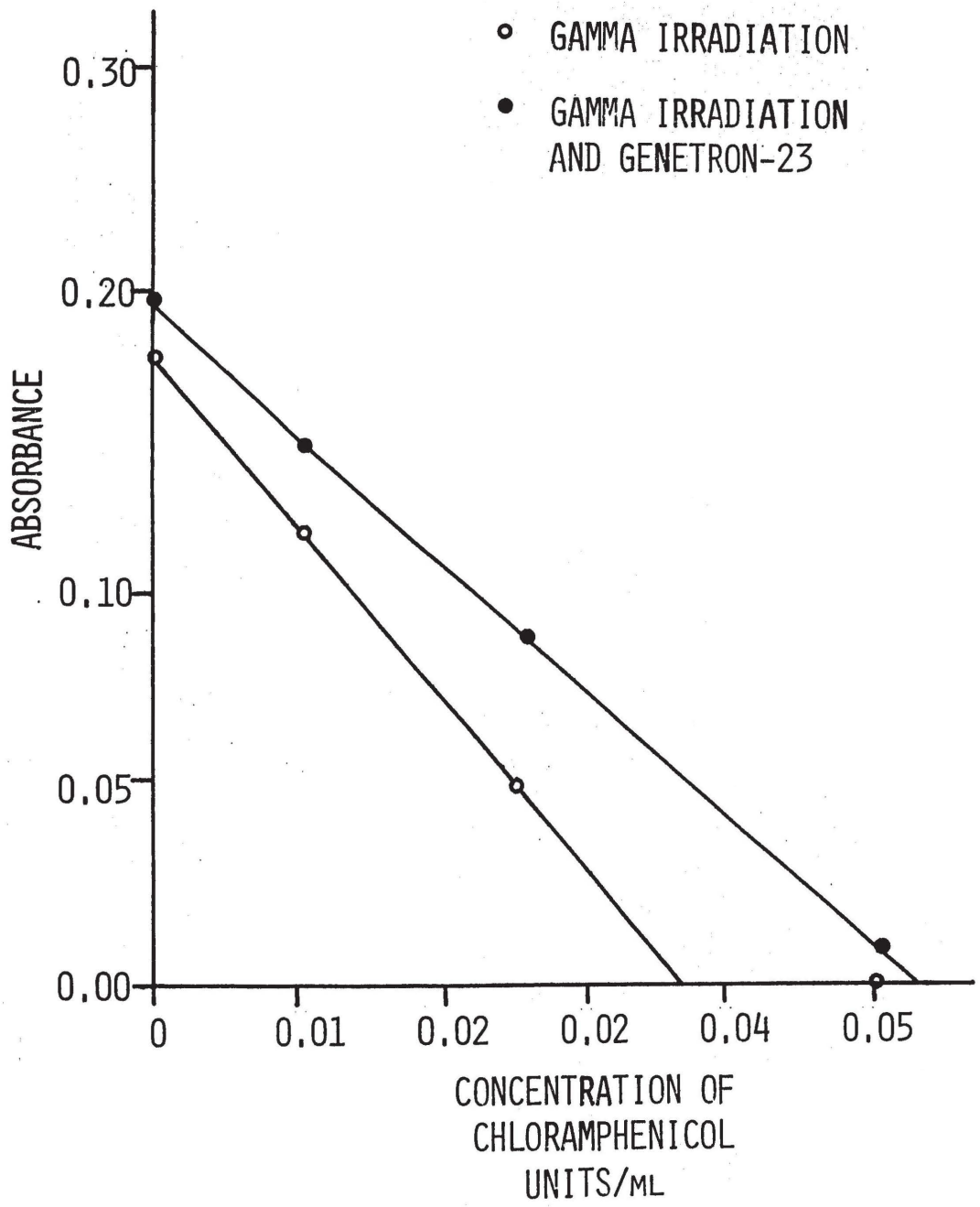
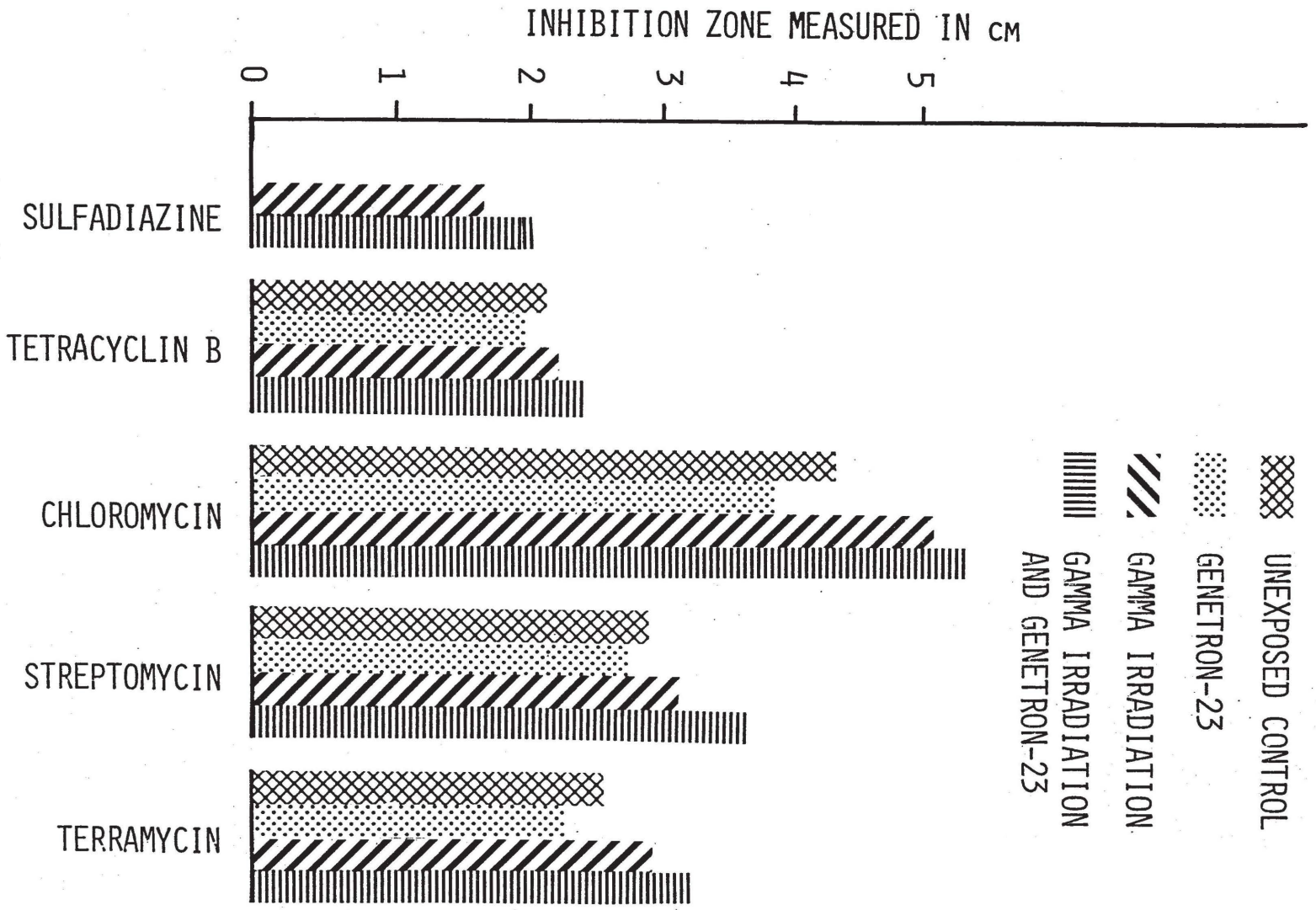

GAMMA IRRADIATION
GAMMA IRRADIATION AND GENETRON-23
0.30
0.20
0.10
0.05
0.00
ABSORBANCE
0
0.01
0.02
0.02
0.04
0.05
CONCENTRATION OF CHLORAMPHENICOL UNITS/ML

Figure 7--Effects of exposure of Vibrio metschnikovii to 8.66×10^4 R/h of gamma irradiation in Genetron-23 and atmospheric air on the sensitivity of the organism to several antibiotics.


INHIBITION ZONE MEASURED IN CM
0
1
2
3
4
5
SULFADIAZINE
TETRACYCLIN B
CHLOROMYCIN
STREPTOMYCIN
TERRAMYCIN
UNEXPOSED CONTROL
GENETRON-23
GAMMA IRRADIATION
GAMMA IRRADIATION AND GENETRON-23

Continuation of Figure 7--Effects of exposure of Vibrio metschnikovii to 8.66 x 10^4 R/h of gamma irradiation in Genetron-23 and atmospheric air on the sensitivity of the organism to several antibiotics.

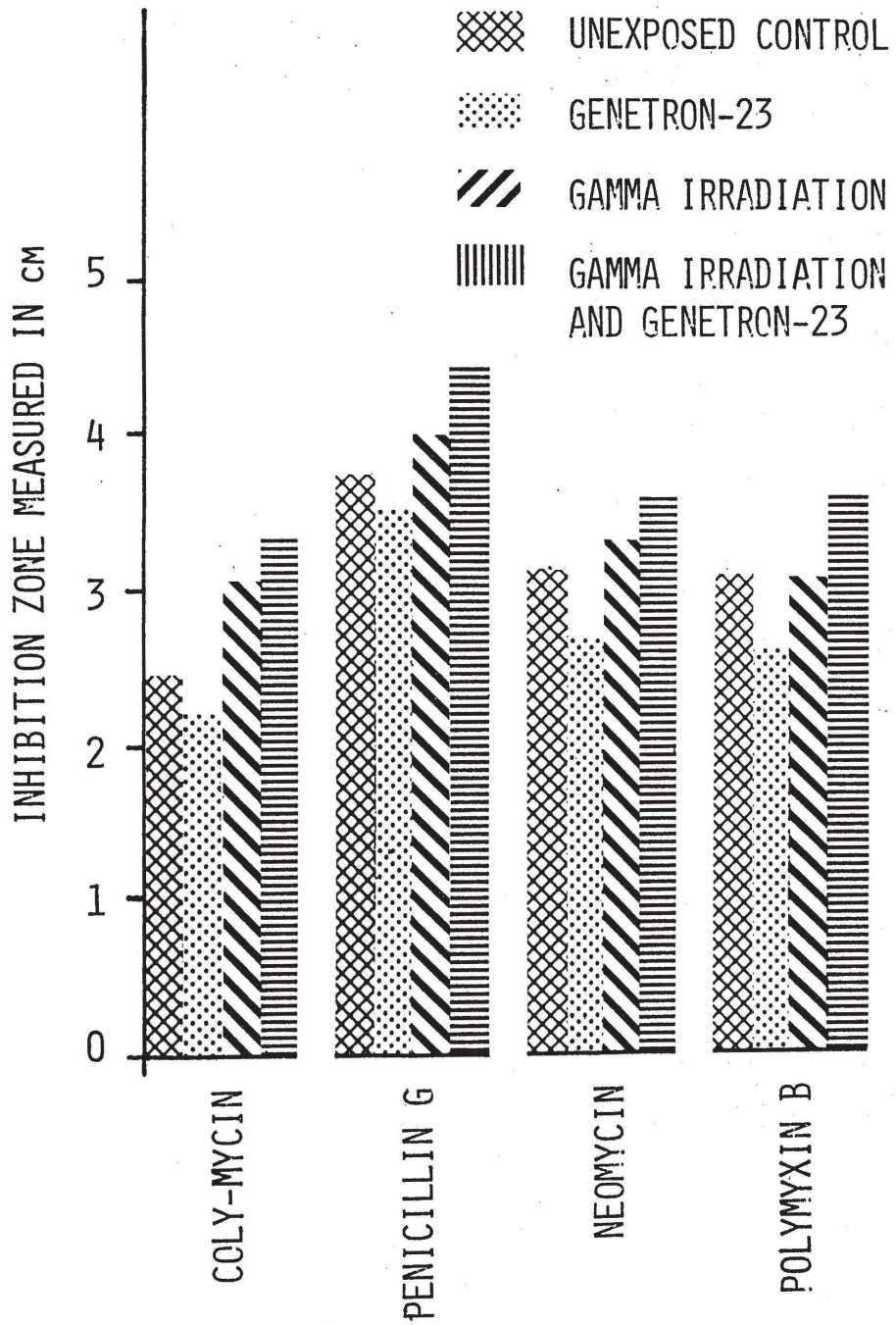
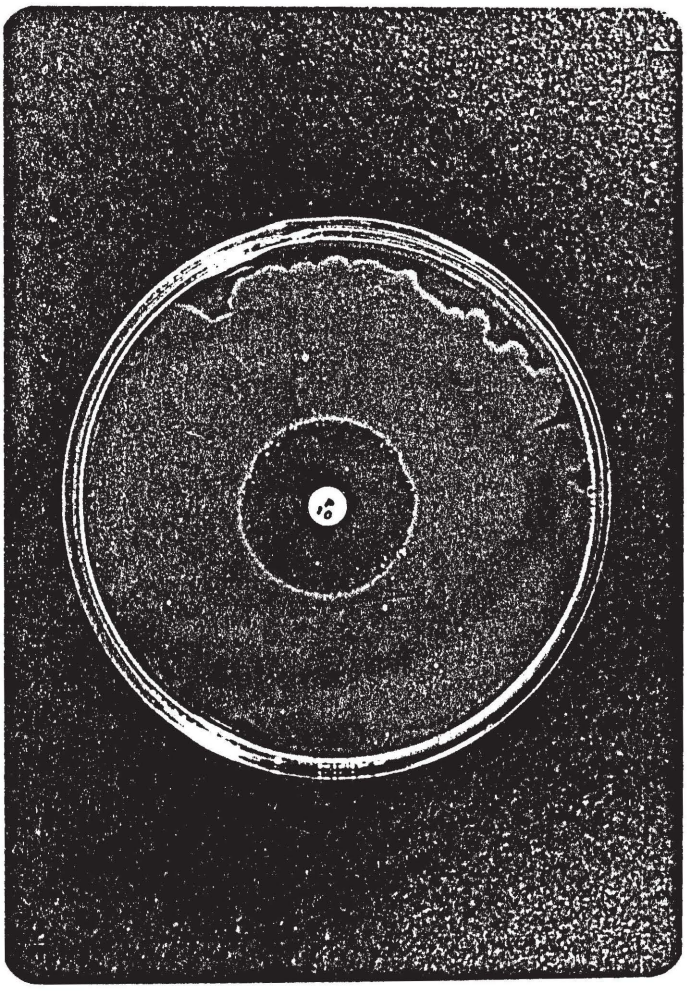

UNEXPOSED CONTROL
GENETRON-23
GAMMA IRRADIATION
GAMMA IRRADIATION AND GENETRON-23
INHIBITION ZONE MEASURED IN CM
0
1
2
3
4
5
COLY-MYCIN
PENICILLIN G
NEOMYCIN
POLYMYXIN B

Table 6--The sensitivity of Vibrio metschnikovii ATCC# e 7708 to antibiotics after irradiation with Cs-137* in atmospheric air and Genetron-23.**

Antibiotic	Treatment***			
	Atmospheric air	Irradiated in atmospheric air	Genetron-23	Irradiated in Genetron-23
Sulfadiazine	resistant	resistant	1.7 ±0.23	2.3 ±0.36
Tetracyclin	2.1 ±0.50	2.0 ±0.06	2.2 ±0.06	2.5 ±0.06
Chloromycin	4.2 ±0.60	3.9 ±0.06	5.0 ±0.40	5.1 ±0.16
Streptomycin	2.9 ±0.06	2.8 ±0.30	3.1 ±0.13	3.6 ±0.70
Terramycin	2.6 ±0.20	2.3 ±0.16	2.9 ±0.26	3.1 ±0.16
Coly-mycin	2.4 ±0.40	2.2 ±0.30	3.0 ±0.30	3.3 ±0.16
Pennicillin G	3.7 ±0.00	3.5 ±0.60	4.0 ±0.26	4.4 ±0.50
Neomycin	3.0 ±0.13	2.6 ±0.06	3.2 ±0.03	3.4 ±0.03
Polymyxin B	3.0 ±0.06	2.5 ±0.16	3.0 ±0.10	3.4 ±0.10

*The irradiated samples were exposed to a dosage of 8.66×10^4 R/h for 7 min.
**Ten ml of bacterial suspension were gassed for 10 min in Turner bulbs, the flow of gas was controlled by a Hasting Mass Flowmeter.
***Mean diameter of inhibition zone ± S.E., cm.

Figure 8--Photograph of the antibiotic sensitivity test. Vibrio metschnikovii was exposed to 8.66×10^4 R/h of gamma irradiation for 7 min in Genetron-23 and in atmospheric air. The antibiotic disc was placed on an inoculated nutrient agar Petri plate. The inhibition zone around the disc was measured in cm.



DISCUSSION

The results of the research reported in this thesis concern effects of gamma irradiation and/or several gaseous atmospheres on the survival, activity, and antibiotic sensitivity of Vibrio metschnikovii ATCC# e 7708. Two gases that were tested, oxygen and carbon dioxide, are normal constituents of the atmosphere; the other one (Genetron-23) is introduced into the air and may have an influence on living systems. (Qureshi 1972).

An experiment was performed to determine the effect of exposure to 8.66×10^4 R/h of gamma irradiation for different time intervals on the survival of V. metschnikovii. It was found that an LD_{70} dose can be obtained after 9 min irradiation with 8.66×10^4 R/h. Irradiation for 60 min inhibited growth completely. From the results shown in Figure 1 it can be noted that V. metschnikovii is highly sensitive to gamma irradiation. The results obtained are in agreement with the findings reported by Sokurova (1974). He found that bacteria of the genus Vibrio are, most likely, related to the most radiosensitive group of bacteria.

Other studies have been performed in relation to high bacterial sensitivities, to ionizing radiation and to DNA compositions. In a study with many bacterial species, including Pseudomonas fluorescens, Azotobacter agile, Escherichia coli B,

Pseudomonas aeruginosa, Serratia marcescens, and Micrococcus pyogenes, Kaplan and Zavarine (1962) stated that: "the incorporation of certain purine and pyrimidine base analogs into deoxyribonucleic acid (DNA) of bacterial and mammalian cells has been shown to augment their sensitivity to the lethal effects of ultraviolet and ionizing radiations. The fact that alteration (by the analogs) of DNA base composition can influence radiation response suggested that radiosensitivity of DNA might be a function of natural base composition." From this study, these investigators concluded that a correlation exists between ionizing radiation sensitivity and DNA base composition of these bacterial species. Myasnik and Morozov (1976) were not in agreement with this conclusion. These investigators studied the sensitivities of E. coli and several Vibrio species to ultra violet and ionizing radiation. They found that cells of V. metschnikovii and water vibrio 46 are highly sensitive to gamma irradiation and that these cells are similar in this respect to the cells of E. coli B_{s-1}. Myasnik and Morozov (1976) stated that if the high sensitivity of vibrios to ionizing radiation were due to their nucleotide composition, they should have expected to find a larger amount of GC pairs in vibrionic DNA than in E. coli, while the DNA of V. cholerae contains only 44% G + C. Since V. metschnikovii, water vibrio 46 and E. coli B_{s-1} differ in their nucleotide compositions, Myasnik and Morozov (1976) suggested that the

high sensitivity of vibrios to ionizing radiation is not due to nucleotide composition, but may be a genetic feature.

Another factor besides DNA that could determine the sensitivity of bacterial cells to ionizing radiation is the cell membrane. Shenoy et al. (1970) found that injury of the membrane leads to cell death. Myasnik and Morozov (1976) stated that the cell membrane and the cell wall are more labile in vibrios than in E. coli. Thus vibrios were found to be very sensitive to changes in osmotic pressure of the medium. These investigators stated that: "70% of the cells perished immediately after transferring El Tor vibrios from isotonic NaCl solution (0.85%) into distilled water, and only 0.5% of the cells remained viable after 60 min in distilled water. The unique morphology of vibrios, cells in the shape of commas, is also indicative of the structural distinctions of their cell wall." Since vibrios have labile cell membranes and cell walls, it can be concluded that this lability is one reason of the high sensitivity of vibrios to ionizing radiation.

Sokurova (1978) investigated the capacity for post-irradiation recovery of bacteria of the genus Vibrio when gamma-irradiated cells are incubated in various nutrient media. He found that bacteria of the genus Vibrio probably have no repair systems to eliminate radiation lesions, when these cells are incubated in a liquid non-nutrient medium, or when incubated on the surface of agar media.

In another experiment, V. metschnikovii was exposed to gamma irradiation in various gaseous atmospheres. From the results in Figure 2, it can be noted that Genetron-23 enhanced the growth of the organism, while oxygen decreased it, but carbon dioxide inhibited growth completely. Mainland (1971) speculated that carbon dioxide released molecular oxygen which attacked the DNA of phage M1, when the phage suspension was exposed to this gas. It can be suggested that the inhibition of V. metschnikovii by the action of carbon dioxide may be related to a lethal mutation produced as a result of the damage caused to the DNA.

In order to study the mechanism of the carbon dioxide action on V. metschnikovii cells, it is recommended to reduce the time of treatment of the bacterial suspension with this gas. Exposure of the organism to compressed air increased the survival rate about 100%. When V. metschnikovii was exposed to 8.66×10^4 R/h for 7 min in the presence of the gases tested, none of them produced a complete protective effect against radiation induced damage. However, oxygen was relatively most protective against irradiation induced damage. Genetron-23 and compressed air provided equal protection against irradiation damage. Other results were obtained by Fuerst and Stephens (1970) in their study of the effects of gases and gamma irradiation on Neurospora crassa Em5256A, Em5297A, and St. Lawrence 74A. They found that oxygen enhanced the damage pro-

duced by Co^{60} gamma irradiation, while Genetron-23 gave some protection against that effect. When the organisms were exposed to Genetron-23 alone, most of the recoverable mutants were produced (Fuerst and Stephens 1970). Landry and Fuerst (1968) reported that Genetron-23 and other fluorine containing compounds that were tested exhibited mutagenic activities in E. coli Sd-4. At the same time, they provided protection against exposure to ultraviolet light and Co^{60} gamma irradiation. According to these findings, Landry and Fuerst (1968) concluded that the fluorinated gases cannot be considered inert to the metabolic systems of biological organisms.

Since Genetron-23, a methane analog, enhanced the growth of V. metschnikovii and provided some protection against gamma irradiation induced damage, it may be speculated that the organism incorporated a molecular amount of this gas leading to an augmentation in the survival rate of V. metschnikovii.

Other experiments were performed to study the influence of exposure of V. metschnikovii to gamma irradiation on the abilities of the organism to ferment glucose, maltose, and fructose. As Table 4 shows, irradiation with 8.66×10^4 R/h for 9 min, inhibited the fermentation abilities of the organism for all three carbohydrates during the first 24 h of incubation. By decreasing the time of exposure to 7 min, positive fermentation of glucose appeared during the first 24 h of incubation. Since the glucose fermentation reactions in-

volving the conversion of glucose to fructose 1-phosphate followed by the production of pyruvate and then lactic acid are catalyzed by hexokinase, 6-phosphofructokinase, and pyruvate kinase (Lehninger 1976), it may be suggested that the delay of the glucose fermentation that resulted from the action of gamma irradiation was due to the inactivation of one or more of these three essential enzymes. The time of exposure of the organism to gamma irradiation acts as a direct factor in this inactivation.

According to Lehninger (1976), the fermentation pathways of fructose and maltose start with the conversion of these carbohydrates to fructose 1-phosphate and glucose which then enter the glycolysis pathway as described previously. Fructose is phosphorylated by the action of fructokinase to produce fructose 1-phosphate, while maltose is hydrolyzed to its monosaccharide component which is D-glucose. This reaction is catalyzed by α-glucosidase enzyme. Since the exposure of V. metschnikovii to 8.66×10^4 R/h of gamma irradiation for 7 min inhibited only the fermentation of fructose and maltose during the first 24 h of incubation, and since the glucose fermentation was not inhibited, it can be suggested that gamma irradiation caused damage to fructokinase and α-glucosidase. Perhaps this damage is responsible for the inhibition of the entry of fructose and maltose into the glycolysis pathway.

Exposure of V. metschnikovii to gamma irradiation inhibited the growth of the organism on starch agar which contained 1% NaCl. This suggests that the organism may have lost the ability to utilize and hydrolyze starch. Since α-amylase catalyzes the hydrolysis of starch yielding a mixture of glucose and maltose, (Lehninger 1976) it was postulated that irradiation might have destroyed α-amylase or inhibited its synthesis. Since the exposure of V. metschnikovii to gamma irradiation impaired the ability of the organism to utilize and hydrolyze starch, and since this exposure caused only a delay in ther fermentation of glucose, fructose, and maltose, it can be suggested that the starch hydrolysis pathway is the most sensitive of the tested carbohydrate fermentation reactions to gamma irradiation. This may be due to the assumption that α-amylase is the most sensitive enzyme to gamma irradiation as compared to other enzymes that catalyze the fermentation reactions of glucose, fructose, and maltose.

Only one mutant colony of V. metschnikovii was isolated, on starch agar. This mutant colony was tested for cellular and colonial morphology and carbohydrate fermentation abilities. It was found that these cells had the same characteristics of the cells of the V. metschnikovii strain that were used in this research.

Several experiments were performed to investigate the effects of streptomycin on V. metschnikovii in the presence

and absence of gamma irradiation and Genetron-23. It was found that the growth of the organism was enhanced when streptomycin was present in the medium in a concentration as low as 0.02 units/ml; then as the antibiotic concentration increased, the growth dropped. Since the used antibiotic was in the form of streptomycin sulfate, it can be concluded that V. metschnikovii utilizes the sulfate when it exists in the medium at low concentrations. An amount of 0.20 units/ml of streptomycin destroyed all V. metschnikovii growth completely, which according to Hash (1972) was a result of the inhibition of protein synthesis of the cell. These findings are in agreement with the work of this investigator who studied the mechanism of action of streptomycin on the molecular level of E. coli. Hash (1972) stated that streptomycin restricted the protein synthesis by attacking the 30S subunits of the ribosomes which are responsible for binding mRNA to the ribosome, resulting in the inhibition of protein synthesis.

The results in Figure 4 show that Genetron-23 enhanced the resistance of the irradiated V. metschnikovii cells to streptomycin. This resistance could be due to the stability that was provided by Genetron-23 to the 30S subunits of the ribsomes, which prevents streptomycin from attacking these proteins, leading to the rest of the protein synthesis to take its place in the cell.

The effect of chloramphenicol on V. metschnikovii cells was investigated. Figure 6 shows that a concentration of 0.05 units/ml of this antibiotic inhibited growth of the organism completely. These data indicate that V. metschnikovii is very sensitive to chloramphenicol which according to Hash (1972) causes a damage to protein synthesis inside the cells with a concomitant inhibition of either DNA or RNA synthesis, and binds aminoacyl and of tRNA and terminates protein synthesis at this point.

The results shown in Figure 6 prove that Genetron-23 decreased the sensitivity of V. metschnikovii to chloramphenicol. This result could be due to the protection that is provided by Genetron-23 to tRNA, which prevents the termination of the protein synthesis.

Based on the data shown in Figure 7 , gamma irradiation was found to increase the sensitivity of V. metschnikovii to sulfadiazine, tetracyclin B, chloromycin, streptomycin, terramycin, coly-mycin, penicillin G, neomycin, and polymyxin B. Genetron-23 provided some protection against the irradiation and antibiotics effects. According to Hash (1972), streptomycin, chloromycin, neomycin, tetracyclin, and erythromycin inhibit protein synthesis of the bacterial cell, while penicillin inhibits the synthesis of the cell wall by destroying transpeptidase and D-alanine carboxypeptidase. It can be suggested that Genetron-23 protected the protein synthesis

enzymes transpeptidase, and D-alanine carboxypeptidase against the antibiotic damage.

Since Genetron-23 provided some protection to V. metschnikovii cells against the effects of gamma irradiation and the tested antibiotics, further investigations may be suggested concerning the study of the mechanism of Genetron-23 action on the molecular level of V. metschnikovii cells by using these antibiotics as indicators.

SUMMARY

1. The objective of this research was to investigate the effects of exposure of Vibrio metschnikovii to Cs-137 gamma irradiation and/or selected gaseous atmospheres on survival, carbohydrate fermentation and sensitivities to antibiotics.
2. Vibrio metschnikovii (V. cholerae biotype proteus) ATCC# e 7708, is similar in its characteristics to Vibrio cholerae, the causative agent of the classical Asian cholera.
3. Turner bulbs with 100 ml capacity were employed for gassing and irradiation of the organism. Oxygen, carbon dioxide, Genetron-23, or compressed air were introduced into 10 ml bacterial suspensions at a rate of 20 ml/min, for 10 min under sterile conditions and maintained at room temperature for one hour prior to exposure to gamma irradiation, as were the controls that were gassed only. Gamma irradiation was applied to the bulbs at a rate of 8.66×10^4 R/h from a Cs-137 source.
4. An LD_{70} of V. metschnikovii was obtained after 9 min of irradiation with 8.66×10^4 R/h. A 60 min exposure inhibited growth completely. The time of treatment for most experiments was reduced to 7 min because of the high sensitivity of V. metschnikovii to gamma irradiation.

5. Exposure to compressed air or Genetron-23 increased the survival of V. metschnikovii, while oxygen decreased it, but carbon dioxide inhibited growth completely. When the organism was exposed to gamma irradiation in these gaseous atmospheres, oxygen was most protective against the induced damage, Genetron-23 and compressed air provided less, but about equal, protection.
6. A dose of 8.66×10^4 R/h of gamma irradiation for 9 min inhibited the fermentation processes of glucose, fructose, and maltose during the first 24 h of incubation. When the irradiation time was reduced to 7 min, the glucose fermentation process was enhanced during the first 24 h of incubation. Irradiation also inhibited the ability of the organism to hydrolyze starch, and to grow on starch agar medium. This suggests that the starch hydrolysis process may be most sensitive to gamma irradiation, compared to other carbohydrate fermentation pathways tested.
7. Exposure of V. metschnikovii to 8.66×10^4 gamma irradiation increased the sensitivity of the organism to chloramphenicol and streptomycin. Treatment with Genetron-23, with and without irradiation, enhanced the resistance of the bacteria to both antibiotics.
8. The sensitivities of a V. metschnikovii population to several antibiotic discs, were tested, including streptomycin, chloromycin, neomycin, polymyxin B, coly-mycin,

salfadiazine, penicillin, tetracyclin B, and terramycin. Chloromycin was found to be the most inhibitory antibiotic toward V. metschnikovii. As expected, Genetron-23 provided some protection against irradiation effects and decreased the sensitivity of V. metschnikovii to all antibiotics that were used.

9. Since Genetron-23 provided some protection to V. metschnikovii cells against the effects of gamma irradiation and the tested antibiotics, further investigations may be suggested concerning the mechanism of action of Genetron-23 on V. metschnikovii cells, using antibiotics as indicators.

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