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The Effect of Gamma Irradiation on Growth of Seven Strains of *Trypanosoma avium*

A Thesis

Presented to

the Graduate Faculty

Central Washington State College

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

R. Wesley Leid, Jr.

June, 1970

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Robert H. Brown

ACKNOWLEDGEMENTS

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INTRODUCTION

Trypanosomes are classified by Honigberg, et al. (1964) as follows: Phylum Protozoa; Subphylum Sarcomastigophora; Superclass Mastigophora; Class Zoomastigophorea, Order Kinetoplastida; Suborder Trypanosomatina; Family Trypanosomatidae and Genus Trypanosoma. All possess one flagellum which is either free or attached to the body through an undulating membrane. Every species within this suborder is parasitic and multiplies by either binary or multiple fission. Trypanosoma avium Danilewsky 1885, as the name implies, is limited to birds. It has been recorded from many species of birds and the life cycle and fine structure have been well defined (Baker-1956a, Baker-1956b, Baker-1956c, Baker-1966, and Baker and Bird-1968).

The present investigation had its origin in an irradiation study of a strain of Trypanosoma avium in the American Robin, Turdus migratorius (Cordell-1968). This study proposed radiation sensitivity as a possible means or tool for determining speciation in trypanosomes.

The prime motivating factor in the present study was the desire to show significant differences in radiosensitivities within six strains of Trypanosoma avium. A seventh strain was later added for the last two irradiation treatments. Gross morphology and differences in growth curves associated with varying temperatures suggested that the strains being cultured were not the same (Oliver-1967,

Cordell-1968, and Clark-pers. comm.) With these differences in mind, the strains were subjected to different dosage levels of gamma irradiation.

LITERATURE SURVEY

Radiation and its effects have been effectively documented by many authors. The literature has been thoroughly reviewed from 1935 to 1950 by Kimball (1955) and from 1950 to 1963 by Giese (1967). These two reviews are outstanding in their presentation of the pertinent literature.

Radiation has been used on many living plants and animals. The main emphasis of the research, however, has been to increase man's knowledge concerning the biological effects of this radiation. It has not been used to any extent as a tool in Taxonomy. Wichterman (1961) used four species of Paramecium in an experiment to test the radioresistance of the species. He noted differences between the species with regard to radiation sensitivity. His study was very important to this investigation as it provided the ideas and impetus for subjecting Trypanosoma avium to gamma irradiation.

Pershad and Bowen (1961) subjected eight different forms of Lilium to gamma irradiation from a cobalt 60 source. They found that closely related forms showed similar sensitivities to radiation while distant forms exhibited dissimilar sensitivities.

Anellis and Kock (1962) compared two strains of Clostridium botulinum with regard to their radiosensitivities. Strain A had a higher overall resistance to gamma irradiation than did Strain B. There was some overlapping, however.

Work has also been done using radiation as a tool to lower the infectivity of parasites (Kimball-1955 and Giese-1967). Miller (1964) subjected Ancylostoma caninum larvae to ionizing radiation. The x-irradiation caused a decrease in the infectivity of the larvae. There was a direct correlation between increasing dosage and decreasing infectivity. The radiation seemed to have a greater effect on the males.

Fitzgerald (1968) subjected Eimeria bovis oocysts to ionizing radiation from a Cobalt 60 source. It took only 10 krads to show some loss of infectivity, but it took 100 krads to completely destroy infectivity.

Kobayashi and Jacobs (1963) compared two strains of Toxoplasma gondii as to loss of infectivity after irradiation. One strain was able to withstand a higher dose of radiation before this loss of infectivity than did the other.

Vanderberg, et al. (1968) subjected Plasmodium berghei sporozoites to x-irradiation. They showed that the higher the dose of radiation the lower the number of mice developing an infection. Sporozoites which received more than 10 krads were unable to produce any blood infection.

The problem of regeneration has also been favorably approached through the use of radiation (Kimball-1955, Giese-1967, and Burchill-1968). Burchill (1968) irradiated Stentor coeruleus in various stages of oral regeneration. The primary response was one of interruption in the regeneration cycle. This commenced soon after irradiation and

led to a delay in development.

Trypanosomes have been little studied and all of the work in irradiation has been concerned with those species which directly affect man. Trypanosoma avium has not been subjected to irradiation although it is readily obtainable and non-pathogenic.

Halberstaedter (1938) subjected Trypanosoma gambiense to varying dosages of x-irradiation. He demonstrated a loss of infectivity at 12 krads, a visible change at 100 krads, and a loss of motility at 200 krads in a few forms. Emmett (1950) investigated Trypanosoma cruzi and found a decreased infectivity at 10 krads, but no visible changes at 100 krads. Patel (1936) investigated the effects of soft x-irradiation upon Trypanosoma brucei and demonstrated that 6 krads was enough to decrease infectivity in this species.

METHODS AND MATERIALS

Seven cultured strains of Trypanosoma avium obtained from birds of the families Corvidae and Turdidae were used in this irradiation study. Representatives of the family Corvidae consisted of the following species: Steller's Jay (Cyanocitta stelleri), common crow (Corvus brachyrhynchos), black-billed magpie (Pica pica), Clark's nutcracker (Nucifraga columbiana) and the gray jay (Perisoreus canadensis). In the family Turdidae the American robin Turdus migratorius was the only species used. Two morphologically distinct strains were obtained from the black-billed magpie. The trypanosoma strain from the gray jay was only recently obtained (November, 1969); therefore, it was used only in the last two irradiations. The remaining six strains have been subcultured for two years or longer.

The cultures were obtained as described by Oliver (1967) and Cordell (1968). The desired bird was sacrificed, the tibio-tarsus removed, and all feathers, muscles and fascia removed. The scissors and the bone end were sterilized, the bone cut, and a piece of the marrow aseptically removed and placed in culture tubes. The tubes were then placed in an incubator at room temperature (24-26°C.). The subcultures were made from these originals.

The NNN media was prepared as described by Baker (1966). The constituents and their proportions are listed

below:

> NaCl, 6g.; agar, 16g.; distilled water, 950 ml. This solution was transferred in 5 ml. aliquots to 22 ml. screw-cap vials and autoclaved. After autoclaving and cooling, 1 ml. of sterile, fresh rabbit blood was added to each tube. This mixture constituted the base of the media and was allowed to solidify on a slant. After solidification, 1 ml. of the liquid overlay was added. The overlay was a Locke's solution containing 2.86g. of streptomycin sulfate and added to the slant. Locke's solution was prepared by combining: NaCl, 8g.; KCl, 0.2g.; KH_2PO_4, 0.3g.; glucose, 2.5g.; and water, 1 liter. The tubes were then incubated at 37°C. for twenty four hours. After incubation they were stored in refrigeration at 5°C. until needed.

In the present study, instead of using 5 ml. aliquots, 1 ml. aliquots of NNN media were placed in small vials. This was a necessary modification due to the size limitations of the available radiation source. These vials were of flint glass, short form, screw-cap vials having a capacity of 6 ml. and obtained from Kimble Scientific Supplies. One-half ml. of sterile rabbit blood (warmed to room temperature) was placed in these same vials. Warming of the blood was a necessity as the blood would coagulate the media when taken directly from the refrigerator and added. When this happened, the blood was improperly mixed with the agar. As a result, one was unable to count the organisms on the hemocytometer because of the large number of red blood cells. After allowing the blood and agar to solidify, 1 ml. of Locke's solution was added as an overlay. The vials were then incubated, refrigerated and used as needed. This media was made up at least one day before, and not over ten days prior, to innoculation.

It was decided that the cultures were to be irradiated while the organisms were somewhere in the lower half of the logarithmic growth phase. During the first irradiation the magpie #1 strain was transferred four days prior to irradiation while the others were transferred twenty four hours previous to the same irradiation. This did not result in enough organisms within the fluid to allow for consistent results. After the first irradiation it was determined that the magpie #1 strain would have to be innoculated at least one week prior to the date of irradiation because of its slow growth rate. The other strains had to be innoculated at least four days prior to this date if they all were to reach approximately the same levels in their growths. These times were established and proved to be successful.

The innoculated vials were placed in a 12" X 9" X 2" sheet of styrofoam in which holes had been bored by a #7 cork borer. In the styrofoam sheet the vials were arranged by strains horizontally, and by dosages vertically (Fig. 1). The screw-caps of each vial were color coded and numbered to further eliminate any chance of error. Each vial was counted on the morning of irradiation. Each vial was shaken by hand approximately thirty times to insure an even distribution of organisms. The cap was removed, the top flamed, and a loopful of the fluid removed by a platinum loop aseptically. The top was flamed again and the cap replaced. The resultant loopful of fluid was transferred to a hemocytometer and counted. The organisms were counted

under a phase contrast microscope (American Optics, Phase-star, Series 10) with only motile forms included in the count. Also only those trypanosomes found in 1 sq. mm., which corresponds to 0.1 cu. mm. of fluid, were counted. The vials were counted the day after the irradiation and each successive day until the count of the organisms in the vials reached zero through four consecutive days. In some cases, if the cultures in the irradiated vials had reached a peak and then declined, leveled off and become stable, they were also terminated.

For irradiation the vials were transferred into a three-layered styrofoam holder (Fig. 1). The vials were rotated from level to level and from facing into the chamber to facing outward (Fig. 2). This spacing was done in order that all combinations would be used as the radiation chamber had a plus or minus value of seventeen per cent for the radiation dosage depending upon the location within the chamber.

The vials were irradiated at the United States Department of Agriculture Research Station in Yakima, Washington. This station possessed a Cobalt 60 source (Model 120M, American Nuclear Corporation, Oak Ridge, Tennessee). This source had a vertically moveable irradiation chamber that, when in down position, was completely surrounded by Cobalt 60 slugs. This machine (Fig. 3) was located in a building in which the temperature was maintained at a constant 3°C. during the time of irradiation. The control

FIGURE 1.

This photograph shows both the styrofoam holders used in the experiment. The three layered holder was used to hold vials while they were irradiated. The flat holder was used to hold the vials after irradiation and during the succeeding counts.

FIGURE 1.

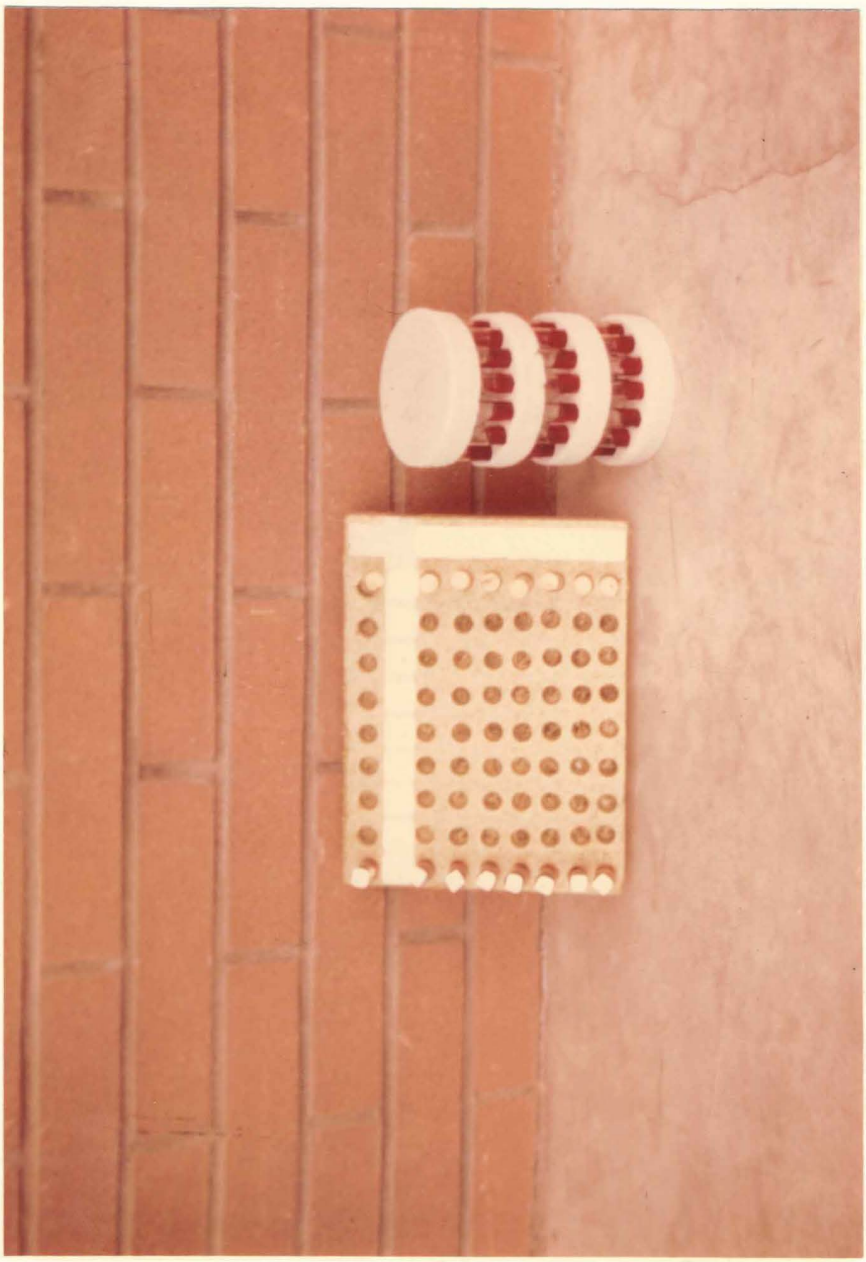


FIGURE 2.

This diagram shows the placement of the three layered styrofoam holder within the irradiation chamber for each irradiation treatment. 1=50 Krads, 2=100 Krads, 3=150 Krads, 4=200 Krads, 5=250 Krads, and 6=300 Krads.

FIGURE 2.

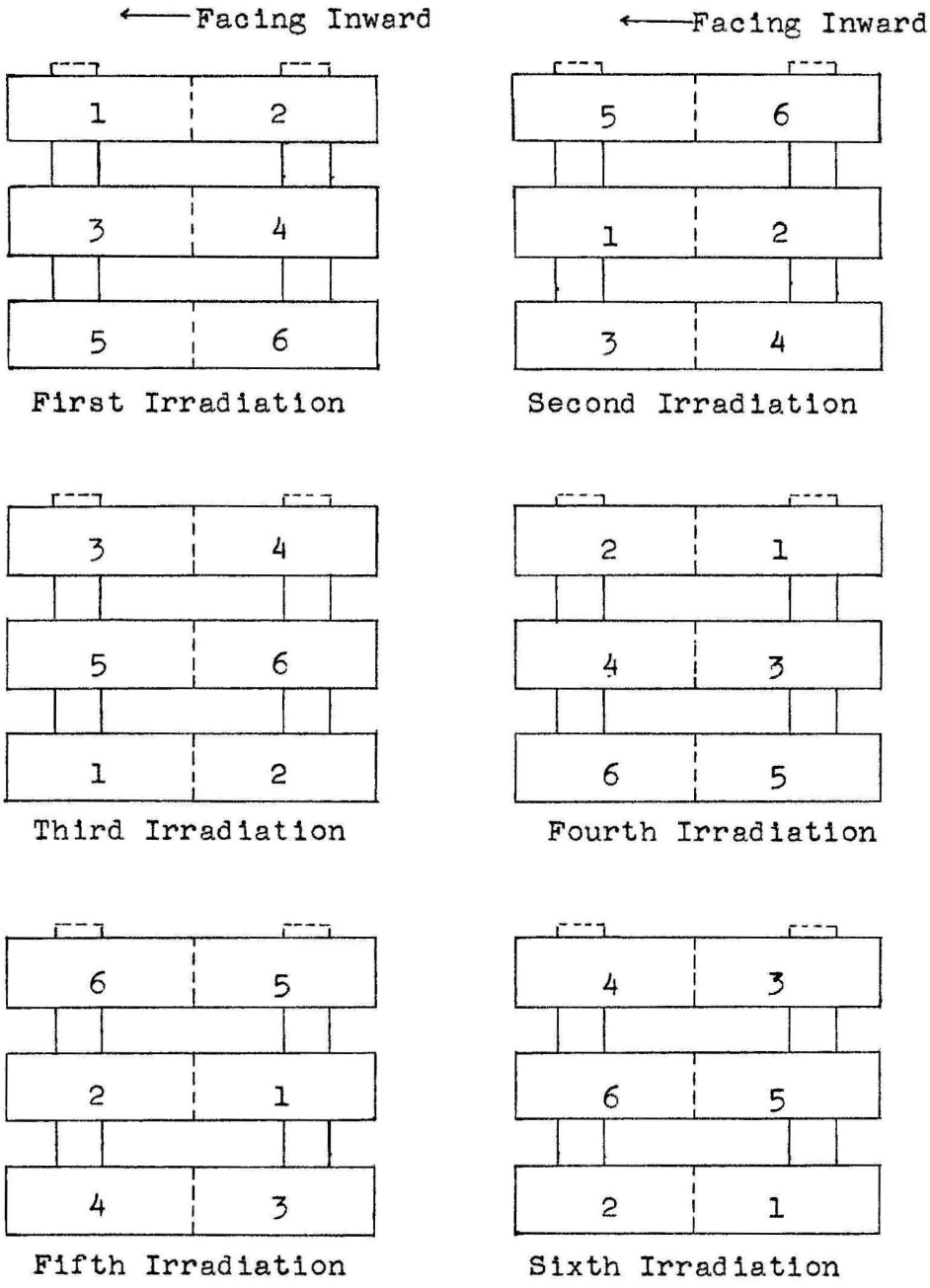
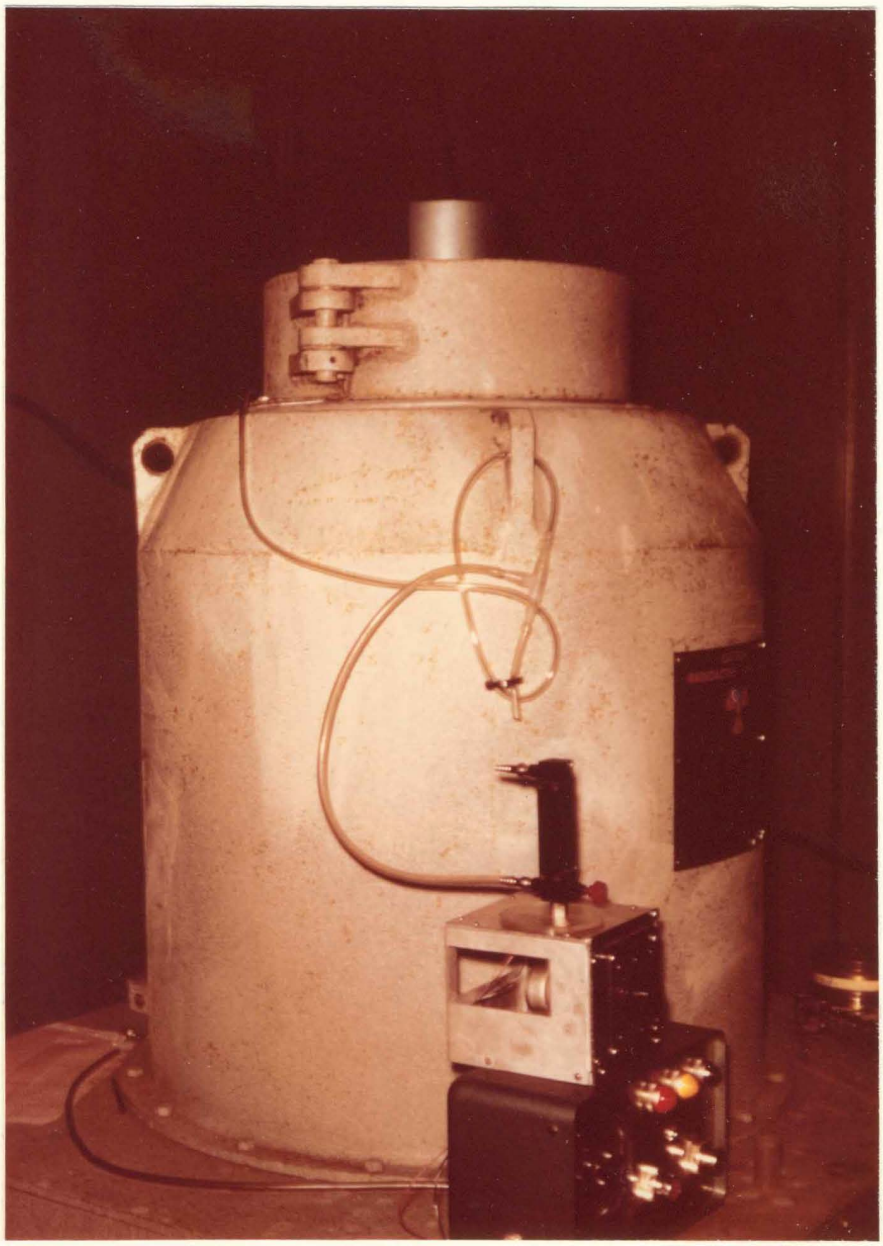

←Facing Inward
1
2
3
4
5
6
First Irradiation
←Facing Inward
5
6
1
2
3
4
Second Irradiation
3
4
5
6
1
2
Third Irradiation
2
1
4
3
6
5
Fourth Irradiation
6
5
2
1
4
3
Fifth Irradiation
4
3
6
5
2
1
Sixth Irradiation

FIGURE 3.

This photograph shows the Cobalt-60 source at the Department of Agriculture Entomology Research Station at Yakima, Washington.

FIGURE 3.



vials were kept in this room for the entire duration of the irradiation. The dosages were as follows: 50, 100, 150, 200, 250 and 300 krads. The time required to reach each of these dosages was predetermined by personnel at the research station. When the desired dosage level was reached, the chamber was raised and the vials for the particular dosage removed. To maintain the desired levels for the vials within the holder, clear vials of the same size replaced those that were removed. This was only necessary if the lowest dosages were on the first two levels. The dosages were checked at the end of the fifth treatment of irradiation by thermal luminescent dosimeters. These dosimeters were sent to Fargo, North Dakota, where it was determined that the calculated dosages had been twenty per cent low. This fact was taken into consideration for the sixth and final irradiation.

In order to confirm that the radiation was acting on the organisms and not the media, a non-innoculated tube was used at each level of radiation. These tubes were then innoculated upon arrival at Ellensburg. They were counted in the same manner as the irradiated vials.

After the tubes had been irradiated, they were brought back to Ellensburg and kept in a 25°C. incubator for the duration of the counting period. This temperature had been determined earlier as the best temperature for the most optimum growth (Oliver-1967, Cordell-1968, and Clark-pers. comm.).

All growth results were subjected to an analysis of co-variance to determine their validity.

RESULTS

Table 1 represents the average number (x 10^4) of trypanosomes in one cubic centimeter of fluid at peak production. Figures in each column represent one treatment or run at that particular dose level of gamma irradiation.

In Table 2 each figure shown represents the average number of days for the trypanosomes to reach the peaks in Table 1. There is a one to one correspondence from Table 1 to Table 2. Day zero for Table 2 is taken to be the day on which the vials were irradiated. Day one is the day after irradiation and so on until the cultures were terminated.

Individual cultures were terminated when no organisms were observed through four consecutive days. They were also terminated after the cultures had reached their peaks, declined, and leveled off. The latter method was used in the majority of those cases when the trypanosomes were suppressed by the radiation for a time and then secondarily commenced growth and peaked.

In both Table 1 and Table 2 there are no data shown for dosage levels of 250 and 300 kiloroentgens (krads hereafter) respectively. None of the strains were able to survive these dose rates. As mentioned previously, the actual gamma irradiation received was equal to 300 and 360 krads respectively. Therefore, the highest level at which survival occurred was 200 krads, and when corrected by the

20 per cent error, stands at 240 krads.

A peak was counted only if it occurred on or after the fourth day from the time of irradiation. By doing this it was felt the resulting peaks would reflect more accurately the radiation effects on the trypanosomes. If figures for peaks on the first, second or third days were accepted, they could well be the result of growth properties inherent in the cultures rather than reflecting growth patterns after the irradiation took its full effect. In computing averages for a strain at a particular dosage, only those results within fifty (x 10^4) organisms of the highest figure at that level were included.

Data in Tables 1 and 2 shows that there are at least two and possibly three groups formed as a result of differential survival to the gamma radiation.

The Magpie #1 strain, which is a difficult strain to culture, could not survive any level of irradiation. There was a steady decrease in the number of organisms from the day of irradiation until the cultures were terminated.

The Magpie #2, Robin, and Steller's Jay strains were able to withstand up to 200 (240 actual) krads. There were no significant reductions in the total number of organisms at peak for any of these three strains. However, the total number of days necessary to attain this peak was increased over that required for the controls. The peak was delayed on the average approximately 1.5 to 5.2 times the average days necessary for the controls to reach their

peaks.

The little evidence obtained for the Gray Jay strain and the results available for the Common Crow and Clark's Nutcracker indicate that they fall into a natural grouping in radiosensitivity. With a single exception (Gray Jay strain) not one strain in this grouping was able to withstand any radiation higher than 50 (70 actual) krads. The peak was delayed on the average approximately 1.1 to 3.0 times the average days necessary for the controls to reach their peaks.

Results in both tables were subjected to an analysis of covariance. In the analysis for Table 1, it was shown that the Magpie #2 strain was not significantly different from the Robin, Steller's Jay, and Clark's Nutcracker strains at the 5 per cent level of significance. It was significantly different from the Gray Jay, Common Crow, and Magpie #1 strains at the 5 per cent level. No other significant results could be shown for counts in Table 1.

Table 2 presented special problems in that a preliminary test of significance showed that the regression between dosage levels and days to peak differed markedly between the strains. In this respect it appears as if the strains fell roughly into three classes. The assumption for a valid covariance analysis presupposes, however, that the regressions are the same for all strains. Thus further use of covariance was not warranted, and instead an analysis of variance was performed with levels of radiation used as

TABLE ONE.

This shows the average number of organisms (X 10^4) at peak for each run and at each indicated dosage or gamma irradiation. Also, the number expressed above when multiplied by the factor of 10^4 will express the total number of organisms in one cubic centimeter of blood, at peak.

TABLE ONE

DOSAGE / STRAIN	0 Krads	50 Krads	100 Krads	150 Krads	200 Krads
Magpie #1	341.6 325.0 1,500.0 500.0 362.5 750.0				
Magpie #2	975.0 775.0 1,750.0 1,350.0 1,200.0 1,006.3	1,425.0 625.0 850.0 1,400.0 450.0 1,037.5	525.0 850.0 875.0	500.0 850.0 450.0	950.0 950.0
Robin	405.0 512.5 1,875.0 625.0 737.5 437.5	900.0 425.0 850.0 500.0 712.5 575.0	425.0 331.3 500.0 687.5 1,100.0 550.0	365.0 550.0 208.3	400.0 750.0 466.6
Steller's Jay	606.3 1,000.0 1,625.0 750.0 750.0 600.0	700.0 1,000.0 506.3 600.0 750.0	208.3 250.0 650.0 420.0 950.0 300.0	425.0 1,125.0 600.0 975.0 283.3	925.0 1,025.0 425.0
Common Crow	337.5 566.6 1,050.0 925.0 562.5 300.0	325.0 1,125.0 80.0			
Clark's Nutcracker	825.0 1,125.0 1,750.0 950.0 925.0 750.0	1,000.0 200.0 825.0 200.0 126.0 825.0			
Gray Jay	550.0 750.0	750.0 100.0		200.0	

TABLE TWO.

This shows the average number of days for the organisms to peak per each run and at each indicated dosage of gamma irradiation. Day 0 is taken to be the day of irradiation and these figures represent days from that time.

TABLE TWO

DOSAGE / STRAIN	0 Krads	50 Krads	100 Krads	150 Krads	200 Krads
Magpie #1	6.3 14.0 3.0 3.8 6.3 9.0				
Magpie #2	5.5 5.0 9.0 4.0 3.0 8.0	10.0 5.0 7.5 14.0 12.5 13.5	11.0 10.0 9.0	15.5 14.0 16.0	19.0 22.0
Robin	7.6 4.0 1.0 5.0 3.5 3.5	9.0 5.0 10.0 7.0 12.3 14.0	20.0 10.0 17.0 12.5 17.0 11.0	16.8 14.0 18.0	17.0 23.0 16.6
Steller's Jay	5.8 1.0 1.0 6.0 5.0 4.0	9.0 14.0 13.0 21.0 9.0	16.3 11.0 14.5 17.2 19.0 9.3	10.0 14.0 13.5 20.5 14.0	15.0 19.0 14.0
Common Crow	5.5 5.3 3.0 3.0 15.0 4.5	4.0 19.0 21.0			
Clark's Nutcracker	4.0 8.0 2.0 10.0 2.5 7.0	9.0 11.0 14.0 5.0 25.6 27.0			
Gray Jay	2.0 15.0	15.0 4.0		11.3	

a factor in order to remove the effects of radiation before comparing days to peak for the different strains. Even after removing the effects of radiation, the differences between strains was not significant at the 5 per cent level (the differences were significant at the 25 per cent level). If there is no difference between strains, there is a one-in-four chance of falsely concluding that such a difference exists. The fact that the author has not been able to demonstrate a statistical difference at a small significance level is due in no small part to the paucity of data for several of the strains at the higher levels of radiation.

Tables 3 and 4 represent vials of media that were irradiated and then innoculated. Table 3 expresses the average number of organisms (x 10^4) at peak for each dose level. The vials were innoculated with a strain on the same day that the vials were irradiated. Table 4 expresses the average number of days necessary for the peaks attained in Table 3.

Based on data from Tables 3 and 4, it becomes evident that for the four strains used there were no significant changes in the media due to radiation effects. The radiation may have been affecting the media to some extent, but this was not readily ascertained.

There are, however, some depressed growth peaks in the Clark's Nutcracker, Common Crow, and Magpie #1 when compared to control peaks of the same strains in Table 3.

With these eight exceptions for the three strains mentioned above, there is, on the average, no significant depression in growth peaks. There is no increase in the number of days necessary to attain these peaks. The exceptions probably reflect the inconsistency of the cultures rather than the radiation changing the media to any extent. All strains in Tables 3 and 4, with the exception of the Robin, show inconsistencies in growth when subcultured.

No monster formation was observed in the irradiated cultures at any time during the experimental work.

TABLE THREE

This table represents the average number of organisms (X 10^4) at peak for media that was first irradiated and then innoculated.

TABLE THREE

STRAIN / DOSAGE	Clark's Nutcracker	Common Crow	Magpie #1	Robin
0 Krads	1,500.0 1,000.0	962.5	1,200.0	1,075.0
50 Krads	1,125.0 450.0	550.0	1.0	1,050.0
100 Krads	600.0 625.0	125.0	1,325.0	800.0
150 Krads	80.0 525.0	100.0	256.3	750.0
200 Krads	900.0 131.3	625.0	125.0	687.5
250 Krads	483.3 25.0	162.5	1,025.0	925.0
300 Krads	600.0 108.3	525.0	1,000.0	900.0

TABLE FOUR

This table represents the average number of days necessary to attain the peaks in Table 3 and in media that was first irradiated and later innoculated. Day 0 is equal to the day of innoculation. This day occurred on the same day of gamma irradiation, but after the vials had been irradiated.

TABLE FOUR

STRAIN / DOSAGE	Clark's Nutcracker	Common Crow	Magpie #1	Robin
0 Krads	14.0 11.0	12.5	7.0	5.0
50 Krads	13.0 16.0	8.5	6.3	5.5
100 Krads	17.0 8.0	10.0	4.0	6.0
150 Krads	25.0 15.5	9.0	12.3	5.0
200 Krads	13.0 16.8	8.0	7.5	5.5
250 Krads	24.3 6.5	15.5	5.5	7.0
300 Krads	13.5 12.6	8.0	15.0	5.0

DISCUSSION

Irradiation Effects

No monster formation was observed at any time during the present investigation. This is in accordance with Cordell's (1968) work on the trypanosome strain from the American Robin. He concluded it highly unusual that no drastic or aberrant forms were observed as the trypanosomes were subjected to extensive radiation of some three hours in duration. This was not the expected result since ionizing radiation is known to have a mutagenic effect on a number of protozoa (Wichterman-1957). There is also an extensive review on earlier studies by Mottram (1942). There are at least two possible reasons for the lack of monster formation.

Studies have shown that streptomycin has the apparent quality of reducing mutagenesis (Kimball-1961, Kimball, Gaither and Wilson-1957, and Kimball, Gaither and Wilson-1962). It is quite likely that the initial radiation damage is premutational. This is followed by an intermediate stage and finally, when the mutation is set into the cell pattern of the organism, it is spoken of as a terminal event (Kimball-1961, Kimball and Perdue-1962, Kimball, Gaither and Perdue-1961, and Kimball, Gaither and Wilson-1962). It appears as if the streptomycin, by retarding all growth, allows time enough for the organism to recover during the intermediate step of radiation induced

mutation (Giese-1967). This delay is thought to allow time for the affected organism to repair or at least modify the radiation lesion that is affecting its metabolic pathways, before that damage becomes a permanent part of its genetic makeup.

Streptomycin was used in the culture tubes to control the number of bacteria. An additional effect of the antibiotic could have been to delay the intermediate step mentioned above, thus allowing time for the trypanosomes to recover to such a degree that no gross morphological changes were observed.

It seems likely that there may be another reasonable explanation for the lack of gross changes. Most zooflagellates are diploid in their chromosome makeup (Kudo-1966). However, the chromosome number for Trypanosoma avium has not been ascertained. Mutations that occur (whether they are radiation induced, chemically induced or naturally occurring) give rise to recessive alleles rather than dominant alleles in the majority of cases (Giese-1967). It is also known that Trypanosoma avium divides asexually by either binary or multiple fission. If it is assumed that Trypanosoma avium is both homozygous dominant and diploid for the majority of its chromosomes, then it becomes evident why no bizarre forms were observed. As has already been documented, the mutations that occur give rise to recessive alleles. This assumed homozygosity of dominance along with the fact that any mutation which occurred would produce a recessive allele

limits any chance of the mutation being expressed. The only way for a mutation to become evident would be to have another mutation at the remaining allele. This is a highly unlikely event and would certainly account for the paucity of aberrant forms. Moreover, if the organism is assumed to be heterozygous for the majority of its chromosomes, then any mutation which occurred on the recessive allele would go unnoticed. If the mutation took place anywhere on the dominant allele, the end result might be lethal and no survivors would be left to transmit the mutation if that was the case.

However, the author does not consider it an either-or situation. Both the use of streptomycin and the possible diploidy of the trypanosome strains could be acting in tandem to reduce the chance of any mutation being expressed.

Due to the lack of karyotypes for Trypanosoma avium strains, it appears that this might be a new avenue of approach for learning more about the strains dealt with in this investigation. It seems probable that karyotypes before and after irradiation may show some interesting results.

There was observed throughout the experiment a delay in growth as is shown by Table 2 and also a complete cessation of growth above 200 (240 actual) krads. As trypanosomes divide by binary or multiple fission, in an actively growing culture, many different stages of growth were observed. One form in particular, a rosette, was of great interest. A rosette is formed by incomplete separation of dividing trypanosomes forming a mass of organisms all

attached at one end, so their bodies project outward from a single point. In a normal non-irradiated culture at the time of the next counting, a significant increase would be noticed as a direct result of these rosettes finishing their division and the resulting release of many active, motile forms. In the irradiated cultures these rosettes were also evident and slightly more numerous. However, one major exception was noted. There was not a breaking up of the rosettes with the resulting significant increase in organisms. The predictable increase of the normal non-irradiated cultures could not be shown. It can be postulated that the radiation affected in some manner, as yet undetermined, the metabolic pathways controlling division and prevented the process from being completed. If it was completed, the organisms produced had a relatively rapid death (death is determined by lack of motility). This damage to the metabolic pathways would help explain the lack of the increase so predictable in the controls. This abortion of the division processes was continued up to such time as the culture either recovered from the effects of the radiation and resumed its normal growth or the organisms lost their viability and the cultures declined until they were terminated. In those strains that recovered, the rosettes gave their predictable increases on the succeeding day's counts. Rosette production continued from the day of irradiation until the cultures were terminated. In the cases where the culture was declining, the total rosette production was much

lower. At the lower dosage levels of irradiation, the radiation induced changes in the cell pathways were not of sufficient magnitude to completely abort division to the extent that there was a death of the culture. The one obvious exception was that of the Magpie #1 strain, as it was unable to survive any level of irradiation. In this strain and at those levels at which the other strains were unable to recover, it is postulated that the cumulative radiation damages to the organism were of such a magnitude as to preclude any chance for survival and reproduction.

The streptomycin might have had an effect at the lower dosage levels by allowing time for radiation damages to be changed or at the very least modified, enabling the rosettes to finish division after a delay of x number of days. Somewhere between 200 and 250 (240 and 300) krads a point was passed at which the Steller's Jay, Magpie #2, and Robin strains were unable to recover from the assumed massive radiation damage to the metabolic processes of these strains. The organisms could not carry on the processes vital for life and thus perished.

Statistics

The results of this investigation show a significant difference between the Magpie #2 strain and the Gray Jay, Common Crow and Magpie #1 strains. They do not show any significant difference between the Magpie #2 strain and the Steller's Jay, American Robin, and Clark's Nutcracker

strains. It would seem a little presumptuous to erect a new species for one strain until such time as more data become available. To really analyse the effect radiation has on depressing the total number of organisms at peak and the increasing of the total number of days necessary to attain this peak more data are needed. To obtain these data the dosage levels should be lowered so more surviving cultures would be the result. It would seem that 25 krad increments could be nicely dealt with. This would give more data for the Common Crow, Gray Jay, and Clark's Nutcracker strains, and to some extent, the Magpie #1 strain.

The general grouping by radiation sensitivities, as evidenced by Table 2, into three divisions does have some morphological basis. The Magpie #1 strain is certainly unlike all the others in that it is relatively long and slender. The Steller's Jay, Magpie #2, and Robin strains are approximately one-half the length of the Magpie #1 strain, but are $2\frac{1}{2}$ to 3 times its width. The Common Crow and Clark's Nutcracker strains attain at least the length of the Magpie #1 strain, but their width approaches that of the Steller's Jay, Magpie #2, and Robin strains. The Gray Jay strain is intermediate between the Common Crow and Clark's Nutcracker strains and the Steller's Jay, Magpie #2 and Robin strains in both width and length.

It then becomes simply a matter of delaying any decision on the specific status of these seven strains of Trypanosoma avium until such time as more data become

available.

SUMMARY

1. Seven strains of trypanosomes from the bone marrow of the Magpie, Steller's Jay, Common Crow, Clark's Nutcracker, Gray Jay, and Robin were subjected to gamma irradiation of varying doses. The effects of this irradiation were observed with regard to delay in growth and to depression in the total number of organisms at peak growth. These results were subjected to statistical analysis. It was determined that the Magpie #2 strain differed at the 5 per cent level of confidence from the Gray Jay, Common Crow, and Magpie #1 strains. It did not differ from the Clark's Nutcracker, Robin, and Steller's Jay strains at the 5 per cent level.

2. Based on these observed results it appears that there are three natural groups with regard to radiosensitivity. The first group consists solely of the Magpie #1 strain. The second consists of the Steller's Jay, Magpie #2, and Robin strains. The last consists of the Clark's Nutcracker, Gray Jay and Common Crow strains. Morphologically, these strains fall into essentially the same grouping shown for the radiosensitivities.

3. No bizarre or aberrant forms were observed at any time during the entire experiment. This may be due in part to the role played by streptomycin in delaying all growth and allowing mutational damage to be modified. It may also be

due to the postulated homozygous dominance and diploidy of the organisms. If the animals were homozygous and diploid, any mutation occurring would result in a recessive allele. As the trypanosomes divide by binary or multiple fission, this mutation would not be expressed.

LITERATURE CITED

Anellis, A. and R.B. Kock. 1962. Comparative resistance of strains of Clostridium botulinum to gamma rays. Applied Microbiology. 10(4):326-330.

Baker, J.R. 1956a. Studies on Trypanosoma avium Danilewsky 1885. I. Incidence in some birds of Hertfordshire. Parasitology. 46:308-320.

_____. 1956b. Studies on Trypanosoma avium Danilewsky 1885. II. Transmission by Ornithomyia avicularia L. Parasitology. 46:321-334.

_____. 1956c. Studies on Trypanosoma avium Danilewsky 1885. III. Life cycle in vertebrate and invertebrate hosts. Parasitology. 46:335-352.

_____. 1966. Studies on Trypanosoma avium. IV. The development of metacyclic trypanosomes in cultures grown in vitro. Parasitology. 56:15-19.

_____, and R.G. Bird. 1968. Trypanosoma avium. Fine structure of all developmental stages. J. of Protozool. 15(2):298-308.

Burchill, B.R. 1968. Effects of radiations on oral regeneration in Stentor coeruleus. J. of Exp. Zool. 169(4):471-480.

Clark, G.W. Personal communication.

Cordell, W.G. 1968. Biological studies on various strains of Trypanosoma avium. M.S. Thesis, unpublished. Central Washington State College, Ellensburg, Washington. 1-38.

Emmett, J. 1950. Effect of X radiation on Trypanosoma cruzi. J. of Parasitol. 36:45-47.

Fitzgerald, P.R. 1968. Effects of ionizing radiation from Cobalt-60 on oocyst of Eimeria bovis. J. of Parasitol. 54(2):233-240.

Giese, A.C. 1967. Effects of radiation upon Protozoa. In: Research in Protozoology (Chen, T., ed.). Pergamon Press. New York. Vol. 2. pp. 267-356.

Halberstaedter, L. 1938. The effects of X rays on trypanosomes. Brit. J. Radiol. 11:267-270.

Honigberg, B., et al. 1964. A revised classification of the phylum Protozoa. J. of Protozool. 11(1):7-20.

Kimball, R.F. 1955. Effects of radiations on protozoans and the eggs of invertebrate animals other than insects. In: Radiation Biology (Hollaender, A., ed.). McGraw-Hill. New York. Vol. 2. pp. 285-331.

_____. 1961. Post-irradiation processes in the induction of recessive lethals by ionizing radiation. J. Cell. Comp. Physiol. 58(Suppl. No. 1):163-170.

_____, and S.W. Perdue. 1962. Studies on the refractory period for the induction of recessive lethal mutants by X-rays in Paramecium. Genetics. 47:1595-1607.

_____, N. Gaither and S.W. Perdue. 1961. Metabolic repair of premutational damage in Paramecium. In: Research in Protozoology (Chen, T., ed.). Pergamon Press. New York. Vol. 2. p. 340.

_____, N. Gaither and S.M. Wilson. 1957. Post-irradiation modification of mutagenesis in Paramecium by streptomycin. Genetics. 42:661-669.

_____. 1962. Reduction of mutation by post-irradiation treatment after UV and various kinds of ionizing radiations. Rad. Res. 10:490-497.

Kobayashi, A. and L. Jacobs. 1963. The effect of irradiation on Toxoplasma gondii. J. of Parasitol. 49(5):814-818.

Kudo, R.R. 1966. Protozoology. 5th ed. Thomas, C.C. Springfield. 1174 pp.

Miller, T.A. 1964. Effect of X-irradiation upon infective larvae of Ancylostoma caninum and immunogenic effect in dogs of a single infection with 40 krad irradiated larvae. J. of Parasitol. 50(6):735-742.

Mottram, J.C. 1942. The problem of tumours. The application of blastogenic agents to ciliates: a cytoplasmic hypothesis. Lewis and Co., Ltd. London.

Oliver, R.M. 1967. Effect of temperature upon reproduction and cyclic development of culture forms of Trypanosoma avium. M.S. Thesis, unpublished. Central Washington State College, Ellensburg, Washington. pp. 1-36.

Patel, C. 1936. Ueber die physikalische und chemische beeinflussbarkeit von Trypanosomen in Hinblick auf das Vorkommen abgeschwachter Erregerstamme. In: Radiation Biology (Hollaender, A., ed.) McGraw-Hill. New York. Vol. 2. p. 319.

Pershad, G.D. and C.C. Bowen. 1961. Differential sensitivity to gamma irradiation of Lilium varieties. J. of Heredity. 52(2):67-72.

Vanderberg, J.P., et al. 1968. Protective immunity produced by the injection of X-irradiated sporozoites of Plasmodium berghei. II. Effects of irradiation on sporozoites. J. of Parasitol. 54(6):1175-1180.

Wichterman, R. 1957. Biological effects of radiations on Protozoa. Bios. 28:3-20.

_____. 1961. Survival and reproduction of Paramecium after X-irradiation. J. of Protozool. 8:158-161.