# EFFECTS OF X-IRRADIATION ON SUCCESSIVE GENERATIONS OF *Heterakis gallinarum* (Schrank, 1788)

by

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# INTRODUCTION

Radiobiology began with the discovery of X-rays by Roentgen in 1895. Since that time a great deal of effort and money have been spent on determining the effects of radiations other than those of the visible spectrum on biological systems both simple and complex.

The principal means by which energy is dissipated by radiation is through ionization. Ionization occurs when electrons are ejected from atoms through which the radiation passes. An atom which has had electrons ejected from it becomes positively charged and is then referred to as an ion. Molecules are held together by electro-chemical bonds which constitute shared electrons between atoms. The removal of such bonding electrons by ionization leads to dissociation or other chemical changes.

Another means by which radiations dissipate energy in matter is by excitation. Excitation occurs when an electron in an atom is raised from a level of lower energy to one of higher energy. Excitation is a much less drastic process than ionization hence chemical and biological changes produced are much less pronounced.

Ionizing radiations include alpha, beta and gamma-rays, the shorter wave length X-rays, protons and neutrons, while the non-ionizing radiations include the longer wave length X-rays and ultra-violet light. The radiant energy used in this investigation was of the ionizing type, ie X-rays, with a wave length of 0.12 to less than 3.00 Angstroms. The objective of this work was to determine the effect of X-irradiation on successive generations

of Heterakis gallinarum with special emphasis on the possibility of producing a radio-resistant strain of Heterakis.

Strains of plants and animals survive in nature because different allelomorphs of a particular gene are present. Occasionally a pure-breeding strain will spontaneously give rise to an organism having a different allelomorph of a particular gene than the rest of the strain. Such changes are called gene mutations, and with individual genes occur spontaneously with a frequency of the order of $10^{-5}$ to $10^{-6}$ per generation. X-rays and other ionizing radiations cause mutations at a rate greatly in excess of the spontaneous rate. Since ionizing radiation does increase the rate of mutation the solution appears to be simple; irradiate and mutations should appear.

Mutations can doubtless occur, spontaneously or by irradiation, in any cell, but a mutation in a single cell of an adult organism would be practically impossible to detect. If a mutation occurs, however, in reproductive material such as sperm or ovum, then every cell of the adult organism growing from the germ cell will carry the mutant gene.

Even with X-ray induced mutation, the rate at which any given mutational step can occur is very low, since the X-ray dose which can be given without producing sterility is limited and the yield of even the most frequent occurring mutations is only about $10^{-5}$ per one-thousand roentgens (Lea,1962). Therefore, the amount of radiation given is a very important factor in obtaining and producing the desired results. Cell division can be delayed with as little as 4 r. Gene mutations can be produced with 50 r or lower;

chromosomal aberrations such as fragments, bridges, rings and other configurations with 100 to 10,000 r; a change in cytoplasmic viscosity and cell permeability with 10,000 to 50,000 r, spindle dissruption with 60,000 r; a change in DNA viscosity, 56,000 to 168,000 r; and enzyme inactivation occurs with doses of 90,000 to 300,000 r.

If radiation induced mutation does take place, other problems are encountered which often hide or destroy the evidence of mutation. Among the visible mutations in Drosophila, for example, recessives are several times more frequent than dominants, and therefore must exist in the homozygous state to be visible. Not only are most mutations recessive, but most are also deleterious. This is to be expected on purely theoretical grounds for the reason that a mutation is essentially a random change in a complicated mechanism that is already functioning reasonably well. There are also a great many recessive mutations which, when homozygous, are lethal, so that the adult stage is never attained. Often the number of recessive lethals is much greater than the number of visible mutations and therefore even though visible mutations might have existed, they would never be detected because of the lethals. According to Grosch (1965) gene mutations can be produced with as little as 50 r or lower. Dosages used in this work (48,000 to 74,000 r) are therefore capable of causing and producing many more changes in cells than mutations.

# LITERATURE REVIEW

## Life History

Heterakis gallinarum, the cecal nematode, is common in the domestic chicken and other gallinaceous birds (Madsen,1950, 1952). Other heterakids have been reported in reptiles and mammals. The life cycle is direct involving only one host which ingests infective eggs that hatch during passage through the digestive tract. The hatched larvae migrate via the lumen to the cecum where they mature to adults.

Various times for eggs to develop to infective larvae have been reported; 9-11 days (Dorman,1928), 14-17 days (Clapham,1933) and 6 days (Osipov,1957). A description of the embryogenesis of H. gallinarum is given by Clapham (loc.cit.). The development of larvae to adults within the host usually occurs in the lumen of the cecum (Dorman,1928 and Clapham,1933). However, Riley and James (1921), Tyzzer (1934), and Roberts (1937) reported that larvae may be found in the cecal gland epithelium but rarely in the cecal mucosa. More worms develop in the left than in the right cecum (Lund,1959; Vatne,1963; Larson,1964; Ostlind,1966).

The period of prepatency has been reported as 24 days (Riley and James,1921), 56-61 days (Uribe,1922), 36 days (Dorman, 1928), 30 days (Baker,1933), 24 days (Clapham,1933) and 25-35 days (Osipov,1957). Variations in the period of prepatency could be related to the close association of Histomonas meleagridis with Heterakis (Baker,1933 and Vatne,1963). In naturally occurring populations the sex ratio of Heterakis is 1:1 (Vatne,

1963 and Ostlind,1966).

## Transmission of Histomonas

Histomonas meleagridis, the protozoan causing enterohepatitis (blackhead) in turkeys, has long been associated with Heterakis. Graybill and Smith (1920) produced histomoniasis in turkeys by feeding them larvated Heterakis eggs. Tyzzer and Fabyan (1922) superficially sterilized heterakid eggs and still were able to produce blackhead, which showed that the causitive organism was inside the shell. Lund and Burtner (1957) estimated that less than 1 out of every 200 heterakid eggs was positive for Histomonas. Vatne (1963) found 50 per cent of the chicks positive for blackhead when given a dose of 100 eggs, and 75 per cent positive when given a dose of 200 eggs. With a dose of 600 eggs he found 90 per cent positive for histomoniasis. Ostlind (1966) reported an average incidence of 10 to 24.6 per cent for histomoniasis among his experimental chickens. The 10 per cent incidence may have been due to storage of the eggs in the refrigerator for almost a year (7-9°C).

Madsen (1952) reported no significant differences in weight gain between chickens infected with Heterakis for five weeks and un-infected controls. Larson (1964) found, after frequent weight tabulation during the development of Histomonas, that birds failed to gain or even lost weight. The initial weight loss, usually on the tenth or eleventh day after infection, was followed by weight gain. Both Ostlind and Rasmussen (personal communication,1965) noted weight losses in birds that were

simultaneously infected with Heterakis and Histomonas. These researchers as well as Larson (1964) considered this change in normal weight gain to be a relatively good indicator of the presence of Histomonas.

## Irradiation of Unsegmented Eggs

Although unsegmented eggs were irradiated in this study, the emphasis of the present work was in the production of a radio-resistant strain of Heterakis gallinarum, and not on the specific effects of irradiation on unsegmented eggs. Therefore, a literature review of the effects of irradiation on unsegmented eggs will not be undertaken here but the reader is referred to the works of Babero, (1952), Holthusen (1921a, 1921b), Kumagai (1961), Payne (1931), Perthes (1904), Ruff (1966), Ruff et al. (1965), Seide (1925), Shikhobalova et al. (1957a, 1958a,1958b), Shikhobalova and Paruzhinskaya (1959,1960,1961, 1962), Soeno (1961), Varga (1964a,1964c), and Villella et al. (1958), and Rasmussen (1966).

## Radio-Resistance

The development of radio-resistance in parasites and other organisms as well as cells and tissues has been reported by several authors. Gould et al. (1955a) reported that 10,000 r of $Co^{60}$ gamma-irradiation given Trichinella spiralis larvae resulted in sexual sterilization of most developing young adults and 18,000 r prevented most larvae from developing to adults. They concluded that irradiation with $Co^{60}$ neither produced radio-

resistance in the larvae of T. spiralis nor was there evidence that a radio-resistant strain could be produced. Alicata (1956) X-irradiated (5,000 r) fresh trichinous rat muscle, not thicker than 5 mm, wrapped in cellophane. Following irradiation, the muscle was fed to ten healthy young rats and six days after infection, four of the rats were killed to determine the percentage of sterile females (adult worms) in the intestinal tract. The other rats were killed about five weeks after infection. Infected muscle tissue was prepared from these rats, irradiated and fed to a second group of young rats. This procedure was repeated five times for a total of six generations. His criterion for determining resistance to irradiation was the degree of sterility noted among the irradiated adult female worms. It was thought that if resistance did develop, the females in subsequent generations would show increasingly less sterility than those of the previously irradiated generation. The author found that 65.7 per cent of the adult females were sterile in the sixth generation. This was slightly higher than the percentage of sterility (61.0) found in irradiating the stock strain for the first time. The author concluded that development of radio-resistance in T. spiralis was not possible by the method described above.

Job (1962) consecutively exposed four and then eight generations of cysticercoids of Hymenolepis diminuta in Tribolium confusum, to 15,000 r of gamma-irradiation. Three groups of adult worms were examined for length, number and size of proglottids, and for anomalies in proglottids and reproductive organs after 18

days of growth in the rat. The first group received no irradiation, the second received 60,000 r and the third received 120,000 r of irradiation. The author found that cysticercoids among the three groups were equally infective. Mean length of worms was greatest in the 120,000 r group. Variation was greatest in the 60,000 r group. Twenty-five worms in each group were examined and all in the control group had mature embryos, while five worms in the 60,000 r group and one in the 120,000 r group did not have mature embryos. Gravid proglottids were largest in the 120,000 r group. This group also showed an increase in the number of proglottids with fewer but larger embryos. Variation was less pronounced in the 120,000 r group than in the less heavily irradiated groups. Job (loc.cit.) concluded that the stabilizing factor of selection may have effectively opposed radiation damage and that there was development of radio-resistance.

Betz et al. (1958) X-irradiated lymphoid and myeloid tissue with a dose of 500 r and found that nearly all cells were destroyed. After regeneration the tissue was irradiated again and damage was not nearly as severe as the first time.

Christensen et al. (1965) irradiated two air dried strains of Streptococcus faecium with $Co^{60}$ gamma-irradiation. Selected substrains were found to have lower resistance to ionizing radiation than those of the parent strain, while some had the same and others still had greater resistance.

Ogaki et al. (1966) found that after reciprocal cross matings of two radio-resistant (relatively) strains of Drosophila melanogaster with two radio-sensitive strains, resistance to

irradiation was dominant to sensitivity.

Mice from 15 generations of X-irradiated males and 10 generations of X-irradiated females followed by six generations of no irradiation were comparatively less resistant to both protracted gamma-ray and fractionated X-ray exposure than were control mice (Spalding et al.,1963). Again in 1966, Spalding et al. showed that after irradiated 25 successive generations of mice, irradiated mice showed decrements in fitness by producing and weaning fewer progeny, by exhibiting a greater tendency toward cannabalism and sterility and by producing still births in greater numbers than control mice.

Takano et al. (1962) found that on the basis of growth character and dose analysis, radio-resistant cell lines were derived from the "HeLa" human cell strain by repetative irradiation with 2,000 r of $Co^{60}$ gamma ray. A relatively radio-resistant strain of mouse cell ($R_1$) was isolated from strain L cells (Whitfield and Hixon,1960). The authors found further selection by irradiation did not alter radio-resistance, however, further derivitives of $R_1$, ie, $R_2$ and $R_3$ retained their colony forming ability to a greater extent than irradiated L strain cells. Survival after a dose of 1,000 r was four to five times greater in the resistant lines than in strain L.

## MATERIALS AND METHODS

### Preparation of Egg Cultures

The source of eggs for all experiments was adult female Heterakis. The worms were collected from chicken viscera obtained from a local poultry processing plant in Manhattan, Kansas. Worms were recovered from the ceca by using an adaptation of the Ackert and Nolf (1929) hydraulic method. After the tip of the distal end of the cecum was cut, a small water hose equipped with a medicine dropper was inserted into the hole. The cecal contents were flushed onto a 40-mesh screen and the worms were washed free of debris. Sexes were separated with the aid of a stereozoom dissecting scope and stored in 0.85 per cent saline and 0.005 per cent merthiolate[1] solution in a refrigerator (7-9°C).

When a supply of eggs was needed, about 500 female worms were macerated by using a mortar and pestle and then treated with a slightly warmed artificial digestive solution (1.0 per cent pepsin and 0.5 per cent hydrochloric acid) for five minutes. The mixture was then filtered through an 80-mesh screen into four clean but non-sterile wetted Petri dishes (60 X 15 mm). The eggs were allowed to settle for five minutes before the supernatant was decanted. Distilled water was carefully added in order to minimize disturbing the eggs on the bottom of the dish. To assure the recovery of any eggs re-suspended by this operation, the

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[1]Eli Lilly and Company, Indianapolis, Indiana.

contents were allowed to settle for another five minutes. This procedure was repeated four times so as to remove the digestive solution.

An aqueous 0.2 per cent formalin solution was used as a culture medium in all egg cultures. If the egg cultures were not immediately used after preparation, they were refrigerated at 7-9°C.

## Calibration of X-ray Unit

Prior to all calibration and irradiations, the X-ray unit[1] was operated until current and temperature fluctuations ceased. All irradiations were performed at 90 kv and 4 ma without external filters. An adjustable radiation block, (Ostlind and Hansen,1966) shaped somewhat like the X-ray unit cone was used to support the thimble of a Victoreen r-meter[2] so that it received just as much irradiation as would eggs in Petri dish cultures. An average of four, 1.5 second readings was taken to determine the dose rate from which the time required for a specific dose of irradiation could be calculated. These calibration procedures were done before each series of irradiations.

## Irradiation Procedure

All culture dishes were taken from the refrigerator before

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[1]Picker X-Ray Corp., Greb X-Ray, Kansas City, Mo.

[2]Victoreen Instrument Company, Cleveland, Ohio.

irradiation and warmed to room temperature. The old culture medium was replaced with fresh medium and adjusted to a depth of 2 mm and the temperature was recorded. Eggs were then exposed to pre-determined dosages (74,000 r or 48,000 r) of irradiation according to experimental design. Immediately after irradiation, the temperature was recorded, culture medium was again replaced and egg cultures were incubated (31°C) until the eggs became infective.

## Developmental Counts

The eggs in Petri dish cultures were examined using a compound microscope equipped with a Petri dish adapter for the mechanical stage (Ostlind and Hansen,1964). The eggs were classified according to their degree of development using the following developmental criteria reported by Ackert (1931) for Ascaridia galli;

Stage 1. The fertile egg to and including the four cell stage.

Stage 2. The five cell stage to and including the last stage of the morula with large blastomeres.

Stage 3. The morula stage with small blastomeres to and including the last stage before becoming a tadpole larva.

Stage 4. The tadpole stage to and including the infective stage.

One-hundred viable eggs were observed and classified as to degree of development in both irradiated and non-irradiated (control)

cultures every three days for a total of fifteen days.

## Experimental Animals

The experimental chickens were day-old straight-run White Rocks obtained from commercial hatcheries[1]. Immediately after arrival all chicks were intranasally inoculated with Newcastle Disease Vaccine[2] and placed in electric brooders. When two weeks old, they were weighed, wing-banded, distributed into groups of equal weight (Gardiner and Wehr,1950) and placed in growing batteries. All birds were fed a balanced antibiotic-free diet.

## Infecting Animals

A modification of the egg administration method of Hansen et al. (1954,1956) and Larson(1957) was used. All culture fluid was carefully decanted from the culture dish and replaced with five ml of a 1.25 M sucrose solution. The eggs were scraped from the bottom of the dish with a rubber policeman and the entire sugar-egg suspension was poured into a small bottle. By the use of an automatic calibrated pipette, three drops of the suspension were delivered to a glass microscope slide and all larvated eggs were counted. By adding eggs or sucrose solution as necessary to the stock solution a desired dose of 100±10 eggs discharged.

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[1]Deforest Hatchery, Peabody, Kansas, and Stillwater Hatchery, Stillwater, Oklahoma.

[2]Live Virus $B_1$ Strain, Lederle Laboratories, Pearl River, New York.

## Determination of Histomoniasis

Birds simultaneously infected with Heterakis gallinarum and Histomonas meleagridis show a reduced worm burden (Ostlind and Rasmussen, personal communication,1965). Therefore, birds with histomoniasis were not included in calculations of worm burdens in the present study. To determine the presence of histomoniasis, all birds were weighed from the sixth to the fifteenth day after infection because birds infected with both Heterakis and Histomonas show only a slight gain, no gain, or a loss in weight.

## Prepatant Period

To determine the length of the prepatant period, a Willis flotation (1921) was performed to detect the presence of eggs. Nineteen days after infection, samples of cecal feces (pasty and greenish brown in color) were collected each morning from the dropping pans. Approximately two grams of cecal feces were put into shell vials (15 X 50 mm) which were then filled one-half full of a saturated aqueous sodium nitrate solution (sp. gr. 1.35-1.40). Feces were mixed with the flotation fluid and allowed to stand until the air bubbles rose to the surface. The suspension was then strained through a 30-mesh screen into a similar vial and filled with enough sodium nitrate to form a meniscus above the rim of the vial. A cover glass was carefully applied on the meniscus and allowed to remain in contact for fifteen minutes after which it was carefully removed and placed on a glass microscope slide and examined for eggs

using low power of a compound microscope. This procedure was repeated daily for both irradiated and control groups until eggs were found in the feces indicating patency of the infection.

## Necropsy

Thirty days after infection all birds were killed and all worms were collected according to the method described by Ostlind (1966). The abdominal cavity was cut open with a scissors and the ceca were removed and placed in separate 2-oz. bottles half filled with 0.85 per cent saline. The contents of each bottle were poured into a 9-inch fingerbowl and each cecum was opened with an enterotome beginning at its proximal end. Fecal matter was shaken from the cecum by agitating the organ in a quick reciprocating motion using a pair of forceps. Each cecum was returned to its 2-oz. bottle, filled half full of water, capped and shaken vigorously for 5-10 seconds. The contents of the bottle were rinsed into the finger bowl and the cecum was carefully removed. The contents of the finger bowl were poured through a 12-inch funnel into a wide mouth quart jar, saline was added to dilute to 0.85 per cent, then jar and contents were refrigerated overnight. The next day the jar was capped and shaken to break up the fecal matter. After standing for 30 minutes, the supernatant fluid was decanted with a glass "j" tube connected to a water faucet aspirator. The contents were poured and rinsed into Pilsner glasses and allowed to settle. Approximately 250 ml remained in the quart jar containing the sediment. After 15 minutes each glass was decanted

by the "j" tube method and rinsed into 4-inch finger bowls. The entire contents were examined under low power of a stereozoom dissecting scope and worms were removed with the aid of a bent teasing needle and placed in saline (0.85 per cent).

## Infectivity and Sex Ratios

Since all birds received a dose of 100±10 eggs, the actual number of worms recovered at necropsy was the per cent infectivity. With the aid of a stereozoom dissecting scope the worms were sexed and worm burdens were calculated (average number of worms per bird, females per bird and male to female ratios).

## Worm Measurement

For measurement and examination purposes 50 worms were selected from each sex, each group, each generation, in each experiment.

The outside of a Petri dish bottom (100 X 15 mm) was ruled off with a diamond point pencil in square centimeters. Only entire squares were numbered from 1 to 35 (horizontally - left to right). By means of a random number table, squares were selected at random and blackened with ink. The entire worm burden (in 25 ml of saline) of one group (sexes separate) was poured into the Petri dish. The dish was swirled around rapidly a few times to insure a random distribution and 50 worms were taken from those areas which had been blackened. Selected worms, mounted in saline on 2 X 3 inch glass slides, were placed in a slide holder beneath an enlarger bellows.

The enlarged image of the worms was projected on frosted glass and traced on onion skin paper. A Dietzgen map measurer, calibrated in centimeters, was used to measure lengths. Average lengths for male, female and combined male and female lengths were recorded.

## Morphological Examination of Worms

All saline mounted nematodes were examined under a compound microscope immediately after tracing. The description of Heterakis gallinarum as given by Clapham (1933) and Baker (1935) was used for determination of any abnormal characteristics. Percentages of worms showing any abnormalities were calculated.

## Experimental Protocol

Experiment Ia. Four egg cultures were prepared (P generation). Two were irradiated (74,000 r, $ED_{50}$) and two were not (control). The X-ray dose was 653.0 r/min. (90 kv and 4 ma).

Experiment Ib. Four egg cultures were prepared from the progeny ($F_1$ generation) of Experiment Ia. Two plates were prepared from the irradiated group and were again irradiated (74,000 r). Two other plates were prepared from control groups and were not irradiated. The X-ray dose was 707.2 r/min. (90 kv and 4 ma). No new egg cultures were prepared from the progeny ($F_2$ generation) of the irradiated group in Experiment Ib because too few worms were recovered for re-infection. The dose used in Experiment I ($ED_{50}$-74,000 r) was considered too high and was lowered ($ED_{33}$-48,000 r) for Experiments II and III.

Experiment IIa. Four egg cultures were prepared (P generation). Two were irradiated (48,000 r-$ED_{33}$) and two were not (control). The X-ray dose was 700.0 r/min (90 kv and 4 ma).

Experiment IIb. Four egg cultures were prepared from the progeny ($F_1$ generation) of Experiment IIa. Two plates were prepared from the irradiated group and were again irradiated (48,000 r) while two were prepared from the control group and were not irradiated. The X-ray dose was 638.0 r/min (90 kv and 4 ma).

Experiment IIc. Four egg cultures were prepared from the progeny ($F_2$ generation) of Experiment IIb. Two plates were prepared from the irradiated group and were again irradiated (48,000 r) while two were prepared from the control group and were not irradiated. The X-ray dose was 668.0 r/min (90 kv and 4 ma). No new egg cultures were prepared from the progeny ($F_3$ generation) of the irradiated group in Experiment IIc because too few worms were recovered for re-infection.

Experiment III(a,b and c). This experiment was a replication of Experiment II(a,b and c). The X-ray dosages for Experiment III were 642.0, 574.8 and 656.0 r/min, respectively, (90 kv and 4 ma). Again no new egg cultures were prepared from the progeny ($F_3$ generation) of the irradiated group in Experiment IIIc because no worms were recovered for re-infection.

# RESULTS

Determination of the $ED_{50}$ and the $ED_{33}$. Embryogenesis was progressively retarded when eggs were exposed to X-ray doses from 0 (control) to 90,000 r (Table 1, Fig. 1). Table 1 shows that the percentage of eggs reaching Stage 4 in their development decreased from 89 per cent (control) to 34 per cent (90,000 r). From these data a graph was constructed to determine the $ED_{50}$ and the $ED_{33}$ (Fig. 1). Extending the points of 50 per cent and 33 per cent to intercept the plotted line, the $ED_{50}$ and the $ED_{33}$ were 74,000 r and 48,000 r respectively.

Effect of X-rays on embryogenesis. The embryological development of controls remained relatively constant throughout all experiments. However with each irradiated generation there was an increased susceptibility to irradiation as shown by a lower percentage of larvae reaching Stage 4 after 15 days of incubation (Table 2, Figs. 2-9).

Effect of X-rays on the transmission of *Histomonas*. Ostlind (1966) showed that the total incidence of histomoniasis was dependent on the total worm burden and not on the dosage of irradiation. Results (Table 3) were erratic and gave no indication that irradiation had caused a reduction in histomoniasis. However, when the total (control plus irradiated) incidence of histomoniasis is compared to the total worm burden a similar trend is seen, indicating that the reduction of histomoniasis is dependent on the reduction in total worm burden (Figs. 10-12).

Table 1. Effect of Roentgen dosages on the percentage of eggs reaching Stage 4.

| Roentgen Dosage | Incubation (Days) | | |
|---|---|---|---|
| | 3 | 9 | 15 |
| 0 | 47 | 80 | 89 |
| 25,000 | 42 | 77 | 78 |
| 30,000 | 28 | 69 | 77 |
| 40,000 | 42 | 74 | 75 |
| 55,000 | 0 | 63 | 65 |
| 65,000 | 1 | 53 | 58 |
| 90,000 | 0 | 30 | 34 |

EXPLANATION OF FIGURE 1

Determination of $ED_{50}$ and $ED_{33}$ dosage of X-ray for unsegmented eggs of *Heterakis gallinarum*.

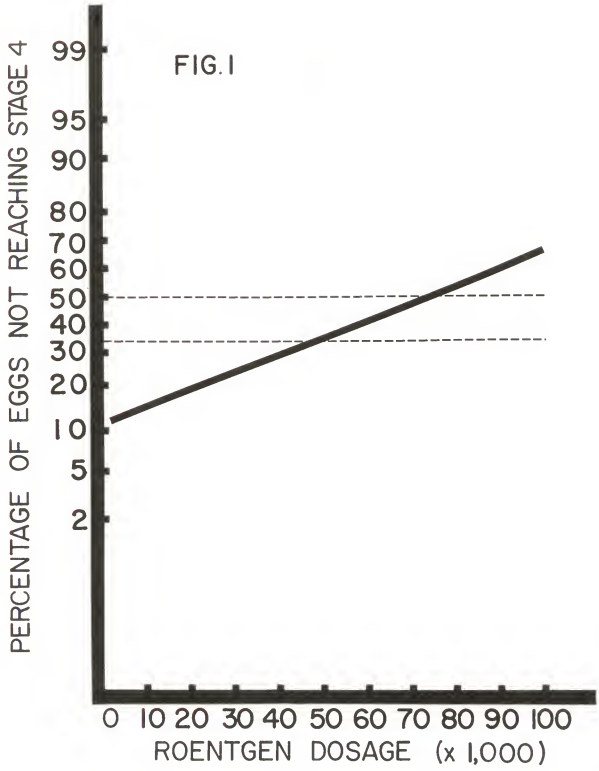


FIG. I
PERCENTAGE OF EGGS NOT REACHING STAGE 4
99
95
90
80
70
60
50
40
30
20
10
5
2
0 10 20 30 40 50 60 70 80 90 100
ROENTGEN DOSAGE (x 1,000)

Table 2. Percentages of non-irradiated and irradiated eggs reaching Stage 4.

| Experiment | Group | Days after irradation | | | | | Generation |
|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | |
| Ia | Control | 23 | 68 | 81 | 71 | 85 | P |
| | Irradiated | 0 | 32 | 44 | 47 | 51 | |
| Ib | Control | 26 | 42 | 49 | 62 | 83 | $F_1$ |
| | Irradiated | 0 | 1 | 3 | 5 | 3 | |
| IIa | Control | 23 | 68 | 81 | 71 | 85 | P |
| | Irradiated | 0 | 56 | 61 | 68 | 71 | |
| IIb | Control | 28 | 45 | 64 | 64 | 81 | $F_1$ |
| | Irradiated | 5 | 23 | 26 | 31 | 29 | |
| IIc | Control | 31 | 47 | 63 | 76 | 84 | $F_2$ |
| | Irradiated | 1 | 11 | 18 | 20 | 18 | |
| IIIa | Control | 30 | 46 | 71 | 72 | 85 | P |
| | Irradiated | 0 | 16 | 50 | 53 | 64 | |
| IIIb | Control | 21 | 28 | 42 | 59 | 78 | $F_1$ |
| | Irradiated | 2 | 3 | 14 | 19 | 37 | |
| IIIc | Control | 12 | 23 | 47 | 61 | 79 | $F_2$ |
| | Irradiated | 0 | 7 | 13 | 26 | 27 | |

EXPLANATION OF FIGURES 2 AND 3

Fig. 2. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment Ia.

Fig. 3. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment Ib.

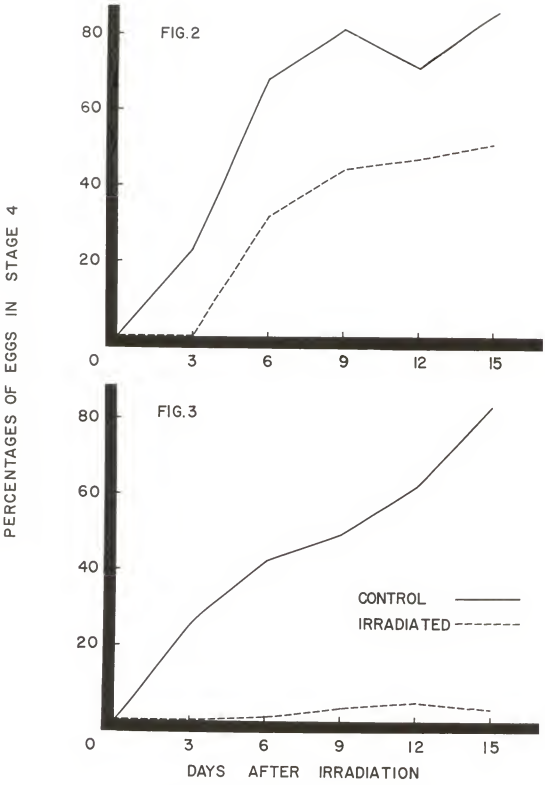


FIG.2
80
60
40
20
0
3
6
9
12
15
FIG.3
80
60
40
20
0
3
6
9
12
15
CONTROL
IRRADIATED
PERCENTAGES OF EGGS IN STAGE 4
DAYS AFTER IRRADIATION

EXPLANATION OF FIGURES 4, 5 and 6

Fig. 4. Percentages of eggs in Stage 4 in control groups and irradiated groups in Experiment IIa.

Fig. 5. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment IIb.

Fig. 6. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment IIc.

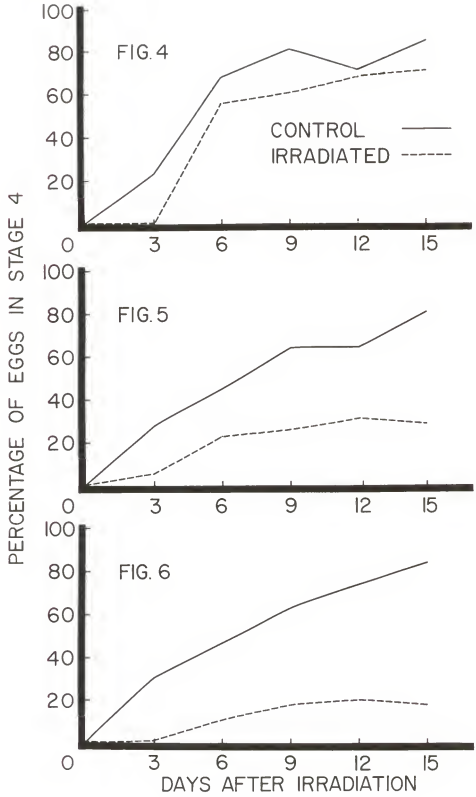


FIG. 4
CONTROL
IRRADIATED
FIG. 5
FIG. 6
PERCENTAGE OF EGGS IN STAGE 4
DAYS AFTER IRRADIATION
0
20
40
60
80
100
3
6
9
12
15

EXPLANATION OF FIGURES 7, 8 and 9

Fig. 7. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment IIIa.

Fig. 8. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment IIIb.

Fig. 9. Percentages of eggs in Stage 4 in control and irradiated groups in Experiment IIIc.

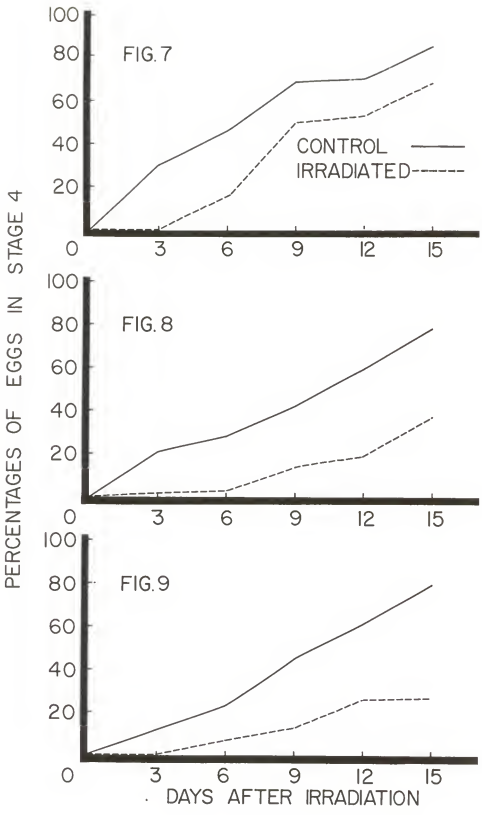


FIG. 7
FIG. 8
FIG. 9
CONTROL
IRRADIATED
PERCENTAGES OF EGGS IN STAGE 4
DAYS AFTER IRRADIATION
0
20
40
60
80
100
3
6
9
12
15

X-ray effect on the prepatant period of development. The prepatant period is the time needed for Heterakis larvae, freed from the egg shell in the host, to develop to maturity. In all three experiments (Table 4) patency of infection was attained in birds fed non-irradiated eggs either earlier or at the same time as among birds fed irradiated eggs. "Abnormal" eggs (eggs without shells) occurred in the feces of birds fed irradiated eggs in Experiments I, IIa and c and IIIb.

X-ray effect on infectivity of eggs. The initial irradiation of eggs (first generation) had no effect on their infectivity to the host because comparable numbers of worms were recovered from control and experimental birds (Table 5, Figs. 13-15). However, the second generation of eggs given 74,000 r (Experiment I) and the third generations given 48,000 r (Experiments II and III) lost their infectivity. The eventual complete loss of infectivity in irradiated groups is attributed to the detrimental effects of the irradiation.

X-ray effect on male to female ratios. Vatne (1963) and Ostlind (1966) showed that the sex ratio among Heterakis populations was usually 1:1. In all irradiated groups except in Experiments Ib, IIc and IIIc, where sample sizes were too small for statistical analysis, sex ratios were significantly different from 1:1 (P 0.10). Sex ratios of control groups were not significantly different from 1:1 (P 0.10) except in Experiments IIa and IIb (Table 6). These results indicated that male Heterakis were more susceptible to irradiation than were females. Ruff and Hansen (1967) reported a similar

Table 3. Incidence of histomoniasis.

| Experiment | Group | Histomoniasis (%) |
|---|---|---|
| Ia | Control | 36.00 |
| | Irradiated | 28.00 |
| Ib | Control | 20.00 |
| | Irradiated | 00.00 |
| IIa | Control | 26.67 |
| | Irradiated | 26.67 |
| IIb | Control | 10.00 |
| | Irradiated | 14.28 |
| IIc | Control | 15.79 |
| | Irradiated | 4.17 |
| IIIa | Control | 4.17 |
| | Irradiated | 37.50 |
| IIIb | Control | 4.76 |
| | Irradiated | 11.11 |
| IIIc | Control | 3.70 |
| | Irradiated | 00.00 |

Table 4. Prepatant periods for Heterakis gallinarum.

| Experiment | Group | Days after infection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| Ia | Control | - | - | - | - | + | + | + | + | + | + |
| | Irradiated | - | - | - | - | A | + | + | + | + | + |
| Ib | Control | - | - | - | - | - | + | + | + | + | + |
| | Irradiated | - | - | - | - | - | A | + | + | + | + |
| IIa | Control | - | - | - | - | + | + | + | + | + | + |
| | Irradiated | - | - | - | - | A+ | + | + | + | + | + |
| IIb | Control | - | - | - | - | - | - | + | + | + | + |
| | Irradiated | - | - | - | - | - | - | - | + | + | + |
| IIc | Control | - | - | - | - | + | + | + | + | + | + |
| | Irradiated | - | - | - | - | - | - | - | - | - | A |
| IIIa | Control | - | - | - | - | - | + | + | + | + | + |
| | Irradiated | - | - | - | - | - | - | + | + | + | + |
| IIIb | Control | - | - | - | - | + | + | + | + | + | + |
| | Irradiated | - | - | - | - | - | - | - | A+ | + | + |
| IIIc | Control | - | - | - | - | - | + | + | + | + | + |
| | Irradiated | - | - | - | - | - | - | - | - | - | - |

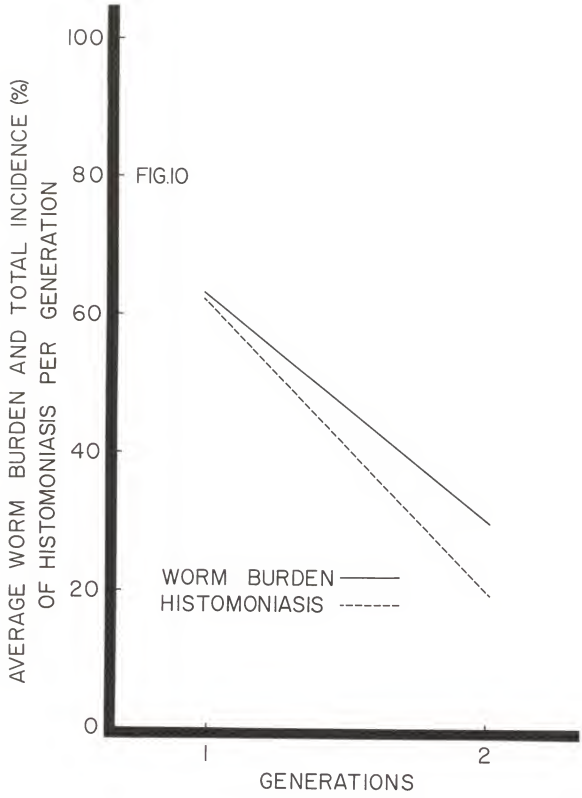
A = Abnormal eggs
+ = First recovery of eggs

Table 5. Infectivity of control and irradiated eggs of *Heterakis gallinarum*. All birds given 100±10 eggs.

| Experiment | Group | Worms/bird | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| Ia | Control | 18.73 | 18.50 | 36.87 |
| | Irradiated | 5.57 | 23.52 | 29.10 |
| Ib | Control | 14.67 | 14.75 | 29.42 |
| | Irradiated | 0.13 | 0.63 | 0.75 |
| IIa | Control | 12.63 | 27.09 | 39.72 |
| | Irradiated | 11.91 | 25.82 | 37.72 |
| IIb | Control | 1.83 | 3.94 | 5.78 |
| | Irradiated | 2.00 | 7.43 | 9.43 |
| IIc | Control | 4.53 | 5.59 | 11.12 |
| | Irradiated | 0.00 | 0.27 | 0.27 |
| IIIa | Control | 16.65 | 16.13 | 32.78 |
| | Irradiated | 11.00 | 20.27 | 31.27 |
| IIIb | Control | 7.90 | 9.67 | 17.57 |
| | Irradiated | 1.13 | 2.00 | 3.13 |
| IIIc | Control | 7.28 | 7.68 | 14.96 |
| | Irradiated | 0.00 | 0.00 | 0.00 |

EXPLANATION OF FIGURE 10

A comparison of the total worm burden and the total incidence of histomoniasis for each generation in Experiment I.


AVERAGE WORM BURDEN AND TOTAL INCIDENCE (%)
OF HISTOMONIASIS PER GENERATION
100
80
60
40
20
0
FIG.IO
WORM BURDEN
HISTOMONIASIS
1
2
GENERATIONS

EXPLANATION OF FIGURE 11

A comparison of the total worm burden and the total incidence of histomoniasis for each generation in Experiment II.

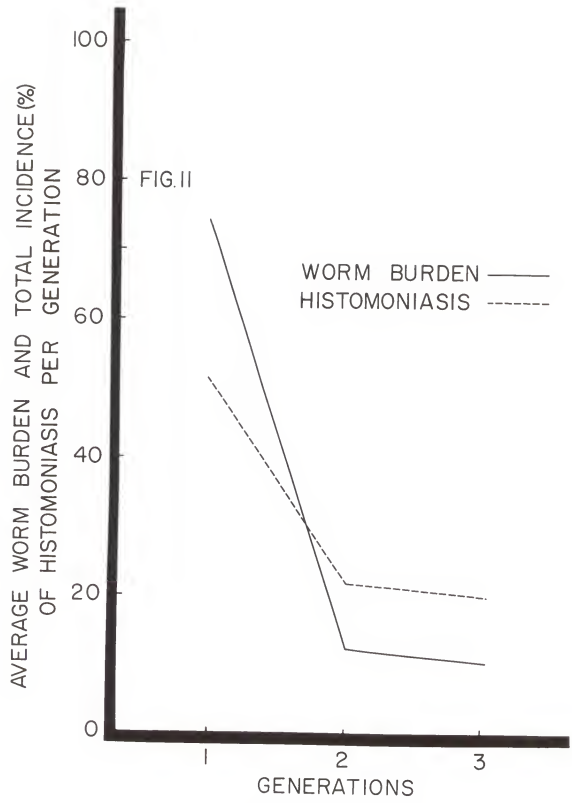


FIG. II
AVERAGE WORM BURDEN AND TOTAL INCIDENCE (%) OF HISTOMONIASIS PER GENERATION
100
80
60
40
20
0
WORM BURDEN
HISTOMONIASIS
1
2
3
GENERATIONS

EXPLANATION OF FIGURE 12

A comparison of the total worm burden and the total incidence of histomoniasis for each generation in Experiment III.

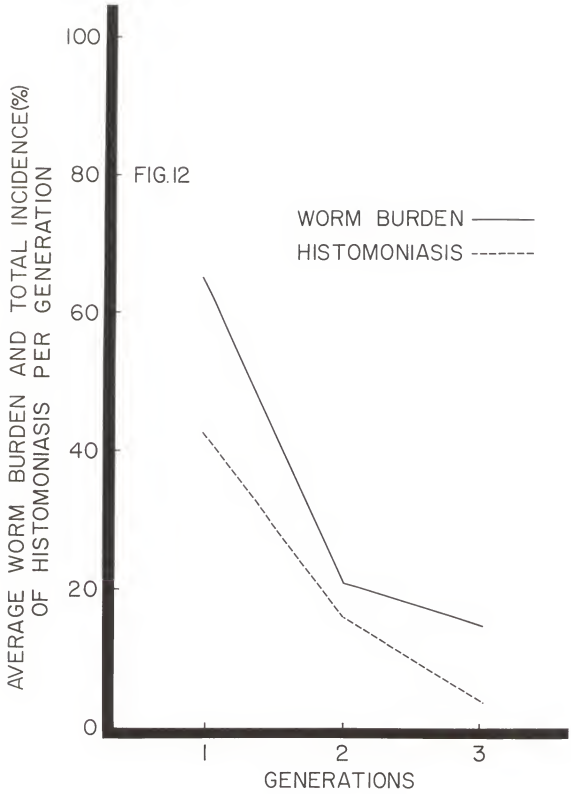


FIG.12
AVERAGE WORM BURDEN AND TOTAL INCIDENCE(%) OF HISTOMONIASIS PER GENERATION
100
80
60
40
20
0
WORM BURDEN
HISTOMONIASIS
1
2
3
GENERATIONS

EXPLANATION OF FIGURES 13, 14, AND 15

Fig. 13. Comparison of the average worm burden in control and irradiated groups in successive generations in Experiment I.

Fig. 14. Comparison of the average worm burden in control and irradiated groups in successive generations in Experiment II.

Fig. 15. Comparison of the average worm burden in control and irradiated groups in successive generations in Experiment III.

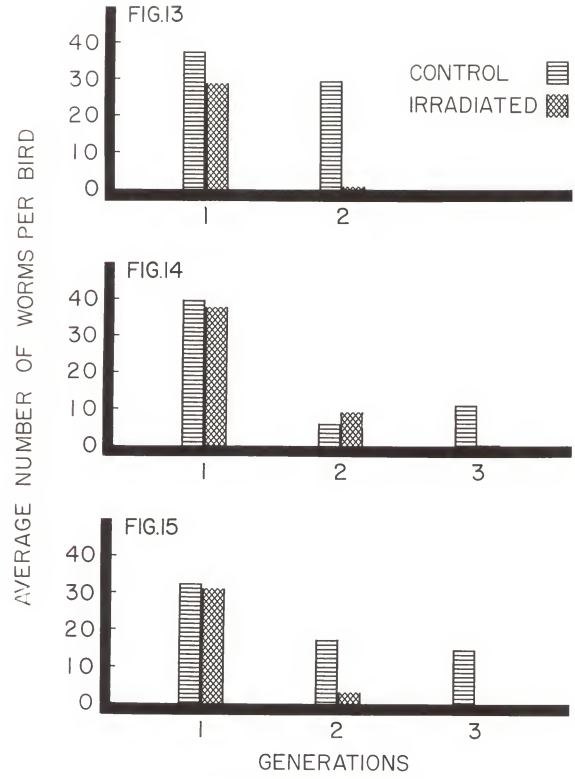


FIG.13
40
30
20
10
0
CONTROL
IRRADIATED
1
2
FIG.14
40
30
20
10
0
1
2
3
FIG.15
40
30
20
10
0
1
2
3
AVERAGE NUMBER OF WORMS PER BIRD
GENERATIONS

Table 6. Sex ratios for *Heterakis* populations.

| Experiment | Group | Males (Total) | Females (Total) | Ratio | Significance |
|---|---|---|---|---|---|
| Ia | Control | 294 | 296 | 1:1.01 | |
| | Irradiated | 106 | 477 | 1:4.27 | * |
| Ib | Control | 176 | 177 | 1:1.01 | |
| | Irradiated | 1 | 5 | 1:5.00 | ** |
| IIa | Control | 139 | 298 | 1:2.14 | * |
| | Irradiated | 131 | 284 | 1:2.17 | * |
| IIb | Control | 33 | 71 | 1:2.15 | * |
| | Irradiated | 28 | 104 | 1:3.71 | * |
| IIc | Control | 77 | 95 | 1:1.03 | |
| | Irradiated | 0 | 3 | ------ | ** |
| IIIa | Control | 383 | 371 | 1:0.97 | |
| | Irradiated | 165 | 304 | 1:1.84 | * |
| IIIb | Control | 166 | 203 | 1:1.22 | |
| | Irradiated | 18 | 32 | 1:1.78 | * |
| IIIc | Control | 182 | 195 | 1:1.05 | |
| | Irradiated | 0 | 0 | ------ | ** |

* Sex ratios significantly different from 1:1 (P 0.10)
** Sample size too small for statistical analysis

phenomenon when Ascaridia galli eggs were irradiated in the embryonated stage.

X-ray effect on growth. These results were inconclusive as statistical analysis could not be made because too few worms were recovered from the irradiated groups in the second generation of Experiment I and the third generations of Experiments II and III (Table 7). There is an indication of a slight decrease in size in successive irradiated generations.

X-ray effect on morphology. In all generations the incidence of morphological abnormalities was greater in irradiated groups than in control groups even though the same type of abnormalities were found in the controls (Tables 9-16). The unusually high percentages of abnormalities in irradiated groups in Experiment Ib (Table 10) and Experiment IIc (Table 13) can be related to the few worms examined. The most frequently occurring abnormalities in irradiated groups were; abnormal bursa, abnormal bursal ray distribution, abnormal eggs, cervical cuticular blisters, coagulation, darkened reproductive organs, reduced number of eggs, retarded development of reproductive organs, shortened spicule, unfertilized eggs and vacuolate testes.

Table 7. Effects of irradiation on the size of adult worms.

| Experiment | Group | Sex | Avg. Length (mm) | Range (mm) | No. Measured |
|---|---|---|---|---|---|
| Ia | Control | M | 7.47 | 4.20-9.20 | 50 |
| | | F | 9.18 | 5.20-12.0 | 50 |
| | Irradiated | M | 7.44 | 3.00-9.40 | 50 |
| | | F | 9.60 | 6.00-12.0 | 50 |
| Ib | Control | M | 8.52 | 7.60-10.0 | 50 |
| | | F | 9.63 | 8.00-12.0 | 50 |
| | Irradiated | M | 4.40 | --------- | 1 |
| | | F | 10.20 | 9.00-12.0 | 5 |
| IIa | Control | M | 7.47 | 4.20-9.20 | 50 |
| | | F | 9.18 | 5.20-12.0 | 50 |
| | Irradiated | M | 7.63 | 4.20-12.4 | 50 |
| | | F | 8.96 | 7.80-10.6 | 50 |
| IIb | Control | M | 7.68 | 4.00-10.0 | 33 |
| | | F | 9.74 | 7.00-11.8 | 28 |
| | Irradiated | M | 6.75 | 5.00-9.60 | 50 |
| | | F | 8.18 | 5.00-10.4 | 50 |
| IIc | Control | M | 9.18 | 8.00-10.2 | 50 |
| | | F | 10.20 | 8.00-12.0 | 50 |
| | Irradiated | M | ---- | --------- | 0 |
| | | F | 9.93 | 8.60-10.8 | 3 |
| IIIa | Control | M | 8.13 | 7.20-10.2 | 50 |
| | | F | 9.84 | 7.20-11.6 | 50 |
| | Irradiated | M | 7.64 | 4.80-10.4 | 50 |
| | | F | 9.44 | 8.00-11.0 | 50 |
| IIIb | Control | M | 8.89 | 7.00-11.0 | 50 |
| | | F | 9.60 | 8.00-11.0 | 50 |
| | Irradiated | M | 7.13 | 5.20-8.40 | 18 |
| | | F | 9.34 | 6.40-11.2 | 32 |
| IIIc | Control | M | 8.64 | 5.60-9.80 | 50 |
| | | F | 9.98 | 8.20-11.8 | 50 |
| | Irradiated | M | ---- | --------- | 0 |
| | | F | ---- | --------- | 0 |

Table 9. Percentages of abnormalities in Heterakis. Expt. Ia.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 0 | - | 4 | - |
| Abnormal bursal ray distribution | 16 | - | 22 | - |
| Abnormal eggs | - | 0 | - | 12 |
| Cervical cuticular blister | 8 | 6 | 8 | 16 |
| Coagulated alae | 0 | 0 | 0 | 8 |
| Coagulated bursa | 0 | - | 2 | - |
| Darkened intestine | 0 | 0 | 0 | 0 |
| Darkened reproductive organs | 0 | 0 | 0 | 2 |
| Reduced bursa | 4 | - | 6 | - |
| Reduced number of eggs | - | 4 | - | 4 |
| Retarded development of reproductive organs | 0 | 4 | 0 | 10 |
| Short spicule | 0 | - | 4 | - |
| Stubby tail | 4 | 8 | 4 | 4 |
| Unfertilized eggs | - | 0 | - | 0 |
| Vacuolate intestine | 0 | 18 | 0 | 20 |
| Vacuolate ovary | - | 6 | - | 18 |
| Vacuolate testes | 0 | - | 4 | - |
| Vulval cuticular blister | - | 0 | - | 0 |
| Without eggs | - | 4 | - | 8 |

Table 10. Percentages of abnormalities in Heterakis. Expt. Ib.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 0 | - | 0 | - |
| Abnormal distribution of bursal rays | 22 | - | 20 | - |
| Abnormal eggs | - | 2 | - | 40 |
| Cervical cuticular blister | 8 | 2 | 0 | 40 |
| Coagulated alae | 6 | 0 | 0 | 0 |
| Coagulated bursa | 8 | - | 0 | - |
| Darkened intestine | 4 | 0 | 0 | 0 |
| Darkened reproductive organs | 0 | 4 | 0 | 20 |
| Reduced bursa | 0 | - | 0 | - |
| Reduced number of eggs | - | 0 | - | 20 |
| Retarded development of reproductive organs | 0 | 0 | 0 | 0 |
| Short spicule | 0 | - | 100 | - |
| Stubby tail | 6 | 4 | 0 | 0 |
| Unfertilized eggs | - | 0 | - | 100 |
| Vacuolate intestine | 0 | 0 | 0 | 0 |
| Vacuolate ovary | - | 0 | - | 0 |
| Vacuolate testes | 2 | - | 0 | - |
| Vulval cuticular blister | - | 0 | - | 0 |
| Without eggs | - | 0 | - | 0 |

Table 11. Percentages of abnormalities in Heterakis. Expt. IIa.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 0 | - | 38 | - |
| Abnormal distribution of bursal rays | 16 | - | 16 | - |
| Abnormal eggs | - | 0 | - | 6 |
| Cervical cuticular blister | 8 | 6 | 4 | 6 |
| Coagulated alae | 0 | 0 | 0 | 8 |
| Coagulated bursa | 0 | - | 16 | - |
| Darkened intestine | 0 | 0 | 0 | 0 |
| Darkened reproductive organs | 0 | 0 | 6 | 0 |
| Reduced bursa | 4 | - | 6 | - |
| Reduced number of eggs | - | 4 | - | 6 |
| Retarded development of reproductive organs | 0 | 4 | 2 | 6 |
| Short spicule | 0 | - | 0 | - |
| Stubby tail | 4 | 8 | 4 | 4 |
| Unfertilized eggs | - | 0 | - | 0 |
| Vacuolate intestine | 0 | 18 | 0 | 0 |
| Vacuolate ovary | - | 6 | - | 2 |
| Vacuolate testes | 0 | - | 0 | - |
| Vulval cuticular blister | - | 0 | - | 12 |
| Without eggs | - | 0 | - | 0 |

Table 12. Percentages of abnormalities in Heterakis. Expt. IIb.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 0 | - | 2 | - |
| Abnormal distribution of bursal rays | 12 | - | 14 | - |
| Abnormal eggs | - | 0 | - | 4 |
| Cervical cuticular blister | 0 | 0 | 10 | 6 |
| Coagulated alae | 3 | 7 | 0 | 14 |
| Coagulated bursa | 6 | - | 4 | - |
| Darkened intestine | 0 | 0 | 8 | 0 |
| Darkened reproductive organs | 0 | 0 | 0 | 0 |
| Reduced bursa | 0 | - | 0 | - |
| Reduced number of eggs | - | 0 | - | 2 |
| Retarded development of reproductive organs | 0 | 0 | 0 | 4 |
| Short spicule | 3 | - | 0 | - |
| Stubby tail | 6 | 4 | 10 | 4 |
| Unfertilized eggs | - | 4 | - | 2 |
| Vacuolate intestine | 0 | 0 | 0 | 0 |
| Vacuolate ovary | - | 0 | - | 0 |
| Vacuolate testes | 0 | - | 0 | - |
| Vulval cuticular blister | - | 0 | - | 0 |
| Without eggs | - | 0 | - | 10 |

Table 13. Percentages of abnormalities in Heterakis. Expt. IIc.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 6 | - | * | - |
| Abnormal distribution of bursal rays | 14 | - | * | - |
| Abnormal eggs | - | 0 | - | 33 |
| Cervical cuticular blister | 6 | 6 | - | 33 |
| Coagulated alae | 0 | 6 | * | 33 |
| Coagulated bursa | 8 | - | * | - |
| Darkened intestine | 0 | 0 | * | 0 |
| Darkened reproductive organs | 0 | 2 | * | 0 |
| Reduced bursa | 0 | - | * | - |
| Reduced number of eggs | - | 8 | - | 33 |
| Retarded development of reproductive organs | 0 | 0 | * | 66 |
| Short spicule | 4 | - | * | - |
| Stubby tail | 6 | 6 | * | 0 |
| Unfertilized eggs | - | 0 | - | 100 |
| Vacuolate intestine | 0 | 0 | 0 | 0 |
| Vacuolate ovary | - | 0 | - | 0 |
| Vacuolate testes | 4 | - | * | - |
| Vulval cuticular blister | - | 2 | - | 0 |
| Without eggs | - | 0 | - | 0 |

* No male worms recovered

Table 14. Percentages of abnormalities in Heterakis. Expt. IIIa.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 2 | - | 4 | - |
| Abnormal distribution of bursal rays | 16 | - | 18 | - |
| Abnormal eggs | - | 0 | - | 4 |
| Cervical cuticular blister | 4 | 2 | 8 | 2 |
| Coagulated alae | 2 | 0 | 2 | 8 |
| Coagulated bursa | 0 | - | 2 | - |
| Darkened intestine | 2 | 0 | 0 | 0 |
| Darkened reproductive organs | 0 | 0 | 0 | 4 |
| Reduced bursa | 0 | - | 4 | - |
| Reduced number of eggs | - | 0 | - | 2 |
| Retarded development of reproductive organs | 0 | 0 | 0 | 0 |
| Short spicule | 0 | - | 0 | - |
| Stubby tail | 4 | 2 | 0 | 2 |
| Unfertilized eggs | - | 0 | - | 0 |
| Vacuolate intestine | 0 | 0 | 0 | 0 |
| Vacuolate ovary | - | 6 | - | 4 |
| Vacuolate testes | 0 | - | 2 | - |
| Vulval cuticular blister | - | 0 | - | 0 |
| Without eggs | - | 0 | - | 0 |

Table 15. Percentages of abnormalities in Heterakis. Expt. IIIb.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 2 | - | 20 | - |
| Abnormal distribution of bursal rays | 8 | - | 30 | - |
| Abnormal eggs | - | 0 | - | 0 |
| Cervical cuticular blister | 0 | 4 | 0 | 0 |
| Coagulated alae | 0 | 0 | 0 | 3 |
| Coagulated bursa | 4 | - | 10 | - |
| Darkened intestine | 0 | 0 | 0 | 3 |
| Darkened reproductive organs | 0 | 0 | 10 | 0 |
| Reduced bursa | 0 | - | 0 | - |
| Reduced number of eggs | - | 0 | - | 6 |
| Retarded development of reproductive organs | 2 | 2 | 0 | 6 |
| Short spicule | 2 | - | 10 | - |
| Stubby tail | 4 | 14 | 30 | 9 |
| Unfertilized eggs | - | 0 | - | 6 |
| Vacuolate intestine | 8 | 4 | 10 | 12 |
| Vacuolate ovary | - | 0 | - | 2 |
| Vacuolate testes | 6 | - | 12 | - |
| Vulval cuticular blister | - | 2 | - | 0 |
| Without eggs | - | 0 | - | 0 |

Table 16. Percentages of abnormalities in Heterakis. Expt. IIIc.

| Abnormalities | Control | | Irradiated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Abnormal bursa | 0 | - | * | - |
| Abnormal distribution of bursal rays | 20 | - | * | - |
| Abnormal eggs | - | 0 | - | * |
| Cervical cuticular blister | 6 | 6 | * | * |
| Coagulated alae | 6 | 0 | * | * |
| Coagulated bursa | 2 | - | * | - |
| Darkened intestine | 4 | 0 | * | * |
| Darkened reproductive organs | 0 | 0 | * | * |
| Reduced bursa | 0 | - | * | - |
| Reduced number of eggs | - | 6 | - | * |
| Retarded development of reproductive organs | 0 | 0 | * | * |
| Short spicule | 4 | - | * | - |
| Stubby tail | 0 | 8 | * | * |
| Unfertilized eggs | - | 0 | - | * |
| Vacuolate intestine | 0 | 0 | * | * |
| Vacuolate ovary | - | 8 | - | * |
| Vacuolate testes | 4 | - | * | - |
| Vulval cuticular blister | - | 4 | - | * |
| Without eggs | - | 0 | - | * |

* No male or female worms recovered

# DISCUSSION

Effects of X-irradiation on embryogenesis. The development of control groups remained relatively constant throughout all experiments. Irradiated groups, however, showed progressive reduction in embryological development in all generations indicating a retardation not in rate but in development itself. Thus, it appears that the effects of irradiation on developing eggs of successive generations were cumulative and expressed themselves in the development of radio-sensitivity and not radio-resistance.

Effect of X-irradiation on the transmission of Histomonas. The lower incidence of histomoniasis among birds receiving irradiated eggs as compared with control groups suggested that irradiation had a detrimental effect on Histomonas. However, Ostlind (1966) found Histomonas to be far more resistant to X-irradiation than Heterakis. The dosages used in the present experiments did not approach the lethal level for Histomonas, therefore, it would follow that the decrease in the incidence of histomoniasis was not due to the effects of irradiation.

Lund and Burtner (1957) estimated that 1 out of every 200 heterakid eggs contained Histomonas. Considering this as accurate, theoretically not more than 50 per cent of the birds given 100±10 eggs should develop blackhead. This was generally found to be true in the present study, but only for the first generation in each experiment. Subsequent generations showed an incidence much lower than 50 per cent. Ostlind (1966)

concluded that total egg dose, such as used by Lund and Burtner (1957), should not be used to calculate the number of birds containing histomonads. Calculations should be based on the average number of worms surviving in blackhead free birds because there is always a natural 50 to 80 per cent loss of potential eggs and/or worms. Some eggs may never hatch, hatch too soon, larvae may be destroyed in the digestive tract, may never reach the cecum or may not remain in the cecum long enough to transmit the disease. The present study showed a positive correlation between total worm burden and total incidence of histomoniasis. Thus, the reduction of the total incidence of histomoniasis was related to the reduction of the total worm burden in each generation and not to the effects of X-irradiation per se.

Effect of X-irradiation on the prepatant period. The prepatant period was longer in irradiated groups than in control groups in all three experiments. It appeared that X-irradiation of successive generations caused an increase in the prepatant period which is in agreement with Ostlind (1966). However, the method (Willis flotation,1921) used to determine the prepatant period may have conditioned the results. If eggs were present in sufficient numbers, in the fecal sample, they could be detected. It should be noted, however, that the worm burdens in second and third generations in irradiated groups decreased markedly. A decreased worm burden leads to a decreased number of eggs voided in the feces. Eggs may actually have been present in the fecal material but not present in sufficient numbers to

be detected, thus giving the impression that patency had not been attained.

Effect of X-irradiation on infectivity and male to female ratios. Infectivity, the percentage of worms recovered from a known dose of eggs, was markedly affected by X-irradiation. This decreased infectivity may be due to several variations such as failure of the eggs to hatch, failure of the worms to migrate into the cecum, death or inability to withstand peristalsis or expulsion from the cecum prior to necropsy. Why controls showed a decreased infectivity, never having received irradiation, is not understood. A possible answer might be found in comparing natural and experimental infections. Birds with natural infections harbor worms of all ages because of constant re-infection, accordingly, they are infected for more than 30 days. Experimental birds given one infection harbored that infection for only 30 days. Possibly, worms in natural populations, showing ranges of ages and different maturities, produce eggs of better quality and greater viability than worms in laboratory infections. Also, the recovery of all worms after 30 days may have been a degradative selective process not conducive to maintaining normal worm burdens. Although controls showed a decreased infectivity, worms recovered were sufficient in number to ensure preservation of the race indicating that irradiation contributed substantially to the early death of the irradiated populations.

Male infectivity decreased significantly (P 0.10) in all irradiated groups. Ostlind (1966) reported a similar observation.

In Experiments IIa and b significantly (P 0.10) fewer males than females were recovered in control groups. Without necropsy data at specified times after infection, it was impossible to explain when and why males were eliminated. If males did reach the infective stage within eggs it was also unknown whether they could hatch. Ruff (1966) hatched irradiated Ascaridia galli eggs which had received enough irradiation to completely eliminate all males. In view of his results it appeared highly probable that Heterakis males were lost sometime after hatching and were not eliminated by the inability of potential male larvae to hatch. Why zygotes destined to be male worms are more susceptible to irradiation cannot be answered here.

Effect of X-irradiation on morphology. The same type of morphological abnormalities were present in both control and irradiated groups. The mechanisms involved in producing these abnormalities are not understood. Genetic or nuclear damage was highly probable because eggs were in the zygote stage when irradiated. This hypothesis however, cannot explain the occurrence of the same abnormalities in control groups. A better hypothesis might be that irradiation actually aggravated an already existing condition.

The majority of observed abnormal conditions were associated with the reproductive system and its associate structures. This follows the classical concept that the highly proliferative cells and tissues associated with the reproductive system are much more sensitive to irradiation and can be damaged or destroyed more easily than cells or tissues associated with other body

systems. Possibly, some of the abnormalities considered were actually reflections of immature or underdeveloped worms. Anomalies such as reduced bursa or retarded development of reproductive organs could be influenced by the age and size of the worm. Possibly, some of the worms examined were immature and therefore not abnormal, however, other worms in the same group, which were as large as or even larger than non-irradiated worms, showed the same type of abnormality.

Abnormalities such as abnormal distribution of bursal rays, blisters, coagulation, darkening of body organs, shortening of spicules and tail, and vacuolation were considered to be direct results of irradiation in the present study and in the study of Wichterman, (1957). Other abnormalities such as increased numbers of abnormal eggs and increased numbers of unfertilized females may have been the direct and indirect result of irradiation. Eggs were considered "abnormal" if they lacked a shell and had a globulated appearance. Direct damage to reproductive material may have occurred at the time of irradiation. Indirectly, "abnormal" eggs may have been the result of low male populations or reduced fertility among males. Lack of copulation in Ascaridia galli is thought to result in the production of "abnormal" eggs (Ruff, Train and Hansen,1967). Possibly, the same situation exists with Heterakis gallinarum.

## SUMMARY

Studies were made on the effects of ionizing irradiation (X-rays) administered to two and three successive generations of unsegmented eggs of Heterakis gallinarum. Criteria used to determine the effects of irradiation were; effect on embryological development, transmission of Histomonas meleagridis, length of the prepatant period, infectivity, sex ratios of recovered worms, growth and morphology and possible development of a radio-resistant strain.

X-irradiation of successive generations of eggs progressively retarded embryogenesis. It appears that the effects of irradiation were cumulative and expressed themselves in the development of radio-sensitivity and not radio-resistance.

The total incidence of histomoniasis appeared to decrease in successive generations of irradiated worms. However, as the incidence of histomoniasis decreased, the worm burden also decreased. The reduction of histomoniasis was associated with the reduction in numbers of worms per host in each generation and not with the effects of irradiation.

In control groups patency was attained earlier or at the same time than it was in irradiated groups. Abnormal eggs were recovered only from females developing from irradiated eggs. Aside from the possibility that the egg detection method may have been inadequate in very low egg counts, it was concluded that X-irradiation did increase the prepatant of development by retarding embryogenesis.

Exposure of unsegmented eggs to dosages of 74,000 r and 48,000 r X-irradiation resulted in the death of worms developing from them by the second and third generations respectively. Sex ratios of Heterakis differed significantly (P 0.10) from 1:1 among worms developing from irradiated eggs. Male worms were more susceptible to irradiation than females.

No conclusions could be made concerning the effects of irradiation on the growth of worms because too few worms were recovered from the second and third irradiated generations.

Increased numbers of morphological abnormalities occurred among adults developing from irradiated unsegmented eggs. The same types of abnormalities occurred at lower frequencies among controls. The most frequently encountered abnormalities in irradiated groups were; abnormal bursa, abnormal bursal ray distribution, "abnormal" eggs, cervical cuticular blisters, coagulation, darkened reproductive organs, shortened spicule, unfertilized eggs and vacuolate reproductive organs.

The problems associated with the production of a radio-resistant strain are many. In view of the complexity of the problem and the results of others (Alicata,1956 and Gould et al.,1955a), failure in producing a radio resistant strain of Heterakis gallinarum, was not without expectation.

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# EFFECTS OF X-IRRADIATION ON SUCCESSIVE GENERATIONS OF *Heterakis gallinarum* (Schrank, 1788)

by

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The effects of irradiation are many and varied with one of the more important being the mutational effect or change. It is known that X-rays and other ionizing radiations cause mutations at a rate greatly in excess of the spontaneous rate. Accordingly, successive generations of unsegmented eggs of Heterakis gallinarum were X-irradiated and the effects of irradiation on the developing eggs, larvae, adults and development of a radio-resistant strain were studied.

Criteria used to determine the effects of irradiation were; effects on the embryological development, transmission of Histomonas meleagridis (a protozoan symbiont found with Heterakis), length of the prepatant period, infectivity, sex ratios of recovered worms, growth and morphology.

Fixed dosages of larvated eggs, irradiated (when unsegmented) and non-irradiated (control), were fed to chicks. After thirty days all birds were sacrificed, worms were collected and examined and female worms were used as the source of new egg cultures. Eggs derived from irradiated worms (eggs) were again irradiated, incubated and fed to other chicks. This procedure was repeated until too few worms recovered (third generation) to produce adequate numbers of eggs for re-infection. Dosages of irradiation used were 74,000 r (Experiment I) and 48,000 r (Experiments II and III).

X-irradiation of successive generations of eggs progressively retarded embryogenesis. It appears that the effects of X-irradiation were cumulative and expressed themselves in the development of radio-sensitivity and not radio-resistance.

The total incidence of histomoniasis among hosts appeared to decrease with successive generations of irradiated worms. However, as the incidence of histomoniasis decreased, the worm burden also decreased. The reduction of histomoniasis was a function of the reduction in numbers of worms per host and not with the effects of irradiation per se.

In control groups patency was attained earlier or at the same time than it was in irradiated groups. Abnormal eggs (eggs without shells) were recovered only from females developing from irradiated eggs. Aside from the possibility that the egg detection method may have been inadequate in very low egg counts, it was concluded that X-irradiation did increase the prepatant period of development by retarding embryogenesis.

Exposure of unsegmented eggs to dosages of 74,000 r and 48,000 r of X-irradiation resulted in the death of worms developing from them by the second and third generations, respectively. Sex ratios of Heterakis differed significantly ($P < 0.10$) from 1:1 among worms developing from irradiated eggs. Male worms were more susceptible to irradiation than females.

No conclusions could be made concerning the effects of irradiation on the growth of worms because too few worms were recovered from the second and third generations.

Increased numbers of morphological abnormalities occurred among adults developing from irradiated eggs. The same types of abnormalities occurred at lower frequencies among controls. The most frequently encountered abnormalities in irradiated groups were; abnormal bursa, abnormal bursal ray distribution,

"abnormal" eggs, cervical cuticular blisters, coagulation, darkened reproductive organs, shortened spicule, unfertilized eggs and vacuolate reproductive organs.

The problems associated with the production of a radio-resistant strain are many. In view of the complexity of the problems and the results of other researchers, failure to produce a radio-resistant strain of Heterakis gallinarum was not without expectation.