

EFFECTS OF COBALT-60 GAMMA RADIATION ON THE
SURVIVAL OF TRIBOLIUM CONFUSUM DUVAL
INFECTED WITH CYSTICERCIDS OF
RAILLIETINA CESTICILLUS MOLIN

by 1254

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INTRODUCTION

There is little doubt that the host-parasite relationship constitutes one of the most intricate and diversified of all animal associations. The ideal relationship supposedly results in a mutual tolerance, a homeostatic, dynamic equilibrium in which the parasite depends upon the host and lives at its expense. The parasite usually harms the host by depriving it of some essential component, by tissue damage, or by producing some toxic substances.

Because of the intricate association between the host and parasite, subjection of the host to a rapid environmental change could reflect on the parasite. Subjection of the host to large doses of ionizing radiation produces a momentary change in the external environment and a more pronounced change in the internal environment. The parasite must adapt itself to these changes or perish.

Studies concerning the effects of radiation on cestodes have been conducted by relatively few researchers. Tapeworms are especially suited for radiation research because they are homonomous, being comprised of a series of similar units, the proglottids. Each proglottid contains tissues sensitive to radiation such as germinal tissue. The foremost problem in radiation studies of tapeworms has been the determination or establishment of basic criteria for comparing radiation induced variations. Most studies have been based on morphological changes rather than physiological changes which are difficult to ascertain because of the necessity of examining a large

number of worms. Another problem in radiation studies is the difference in sensitivity between host and parasite. The range of radiation doses must produce effects on both the host and parasite yet not be lethal to one or the other. Usually the adult parasite is more radioresistant than the host and sublethal doses for the host produce no noticeable effects in the parasite. Therefore, most studies concerning the effects of radiation on cestodes have been conducted in vitro with the host and its parasite treated as separate entities.

The objectives of this study were, 1) to determine whether parasitized hosts are more susceptible to ionizing radiation than are non-parasitized ones and 2) to develop a radiographic technique for determining the degree of infection of Tribolium confusum with cysticercoids of Raillietina cesticillus.

REVIEW OF LITERATURE

The first studies concerning the effects of radiation on cestodes were conducted by Palais in 1933. She tried to induce abnormalities in adult Hymenolepis diminuta by exposing the eggs to X-rays and ultraviolet radiation. The strobilae were studied for morphological variations such as lateral and axial reversals of polarity, sterility of proglottids, etc. The amount of radiation employed by Palais was difficult to interpret. She was unsuccessful in showing any significant difference in the number of morphological variations between the irradiated worms and controls. The consensus

is that she employed insufficient doses of radiation to induce abnormalities. Kisner (1957) exposed rats infected with Hymenolepis diminuta to 120-720 R of gamma radiation from cobalt-60. He, too, was unsuccessful in showing any significant difference in the number of anomalies occurring in the irradiated and control tapeworms. It is now known that both Palais (1933) and Kisner (1957) employed insufficient doses of radiation to induce abnormalities in the worms. Schiller (1957, 1959) used larger doses of radiation and was able to increase the number of abnormalities. He irradiated the eggs of Hymenolepis nana with doses of from 5000 to 40,000 R. The cysticercoids developing from irradiated eggs were malformed, poorly developed, and not viable. Eggs receiving 30,000 R or more failed to develop into adult worms. Worms which developed from eggs exposed to sublethal doses of radiation showed a significantly higher number of anomalies than the unirradiated controls. He also showed that the frequency of these anomalies was directly proportional to the dose of radiation employed and that the progeny of worms previously exposed to X-irradiation showed a greater number of anomalies than normal worms.

Kuhlman (1961) exposed Tribolium confusum larvae infected with cysticercoids of Hymenolepis microstoma to 2000, 10,000, 15,000, 20,000, 25,000, 30,000, 40,000 and 50,000 R of gamma radiation, respectively. Cysticercoids which received 20,000 R or more did not develop into adult worms. Among worms that developed after receiving less than 20,000 R the number of anomalies (abnormal testes

development, abnormal cirrus pouch, sterility, and abnormal segmentation) increased as the radiation dose increased. Eggs of this cestode exposed to 10,000 and 15,000 R showed no significant difference in the number of anomalies occurring in adult worms developed from them. He attributed this lack of effect to the cestodes' being recovered from mice after they had fully matured and had shed a number of proglottids. He suggested that all adult worms be collected before terminal proglottids are lost. Job et al. (1963) studied the effects of cobalt-60 gamma radiation on the cestode Hymenolepis diminuta over several generations. They began this study in 1958 by exposing cysticercoids of this worm (in vitro) to 15,000 R and then feeding them to rats via stomach tube. Gravid proglottids containing oncospheres were fed to Tribolium confusum, allowed to mature, then again irradiated (in vitro) with 15,000 R of gamma radiation before feeding to rats. This procedure was continued through 10 generations, thus a total of 150,000 R was given. Generations of worms exposed to a total of 60,000 R and 120,000 R, respectively, were examined to determine whether morphological variations were increased or diminished by exposure to cumulative radiation. Contrary to what might be expected, they found fewer morphological variations in these two groups than they did in the controls. Actually they found a reduction in the number of abnormalities in these irradiated groups. They attributed these incongruous results to mean that a selective factor was operating each generation to counteract the disruptive effect of radiation. This factor operated as a selective screen by being

interposed between each irradiated group and its descendants, the viability of those select descendants being linked somehow with normal morphogenesis and the absence of anomalies. Even though the irradiated worms showed fewer morphological variations than the non-irradiated, they showed some deviations from the norm by having a retarded growth rate and abnormally large gravid proglottids.

Jones and Dvorak (1963b) described an abnormal cestode from the eighth generation (105,000 R) of tapeworms from Job's experiment of 1963. This worm was so different from the parent species that the authors thought it should merit separate taxonomic consideration. The worm was small and delicate, and about 80% of the oncospheres showed a preponderance of two rather than six hooks.

A preliminary investigation concerning the effects of radiation on cestode oncospheres was conducted by Jones (1963). Oncospheres from irradiated cestodes developed adults having frequently small or undeveloped eggs or eggs having oncospheres with less than six hooks or were abnormally large and hookless. The work had only limited significance since he studied only two specimens, but it did show a measurable difference presumably related to radiation.

Brannon (1961) irradiated larval Hymenolepis microstoma in Tribolium confusum in order to study the direct effects of gamma radiation on this cestode. After irradiation he dissected out the cysticercoids, induced them to excyst, then cultured them in a modified Tyrodes' solution at 36.5°C to which antibiotics had been added. In order to compare in vivo results with in vitro results he fed some

of the irradiated larvae to mice via stomach tube. Approximately 90% of these (both the irradiated and unirradiated cysticercoids) excysted and appeared to move normally. He concluded that failure to excyst could not be used as a measure of radiation damage.

Villella, Gould and Gomberg (1960) conducted a study on the effects of cobalt-60 and X-rays on the infectivity of cysticercoids of Hymenolepis diminuta. They exposed cysticercoids of this cestode in saline to various doses of cobalt-60 and X-rays of various energies (245 Kv., 120 Kv., and 80 Kv.). Doses of from 5000 to 30,000 Rep* cobalt-60 gamma radiation were applied to cysticercoids. Fifteen irradiated cysticercoids were fed by pipette to white rats. Controls for each experiment consisted of 2 or 3 rats fed no cysticercoids and of 1 to 6 rats, each fed non-irradiated cysticercoids. A dose of 12,000 Rep cobalt-60 or 12,000 R X-ray prevented most cysticercoids from developing to tapeworms and the few tapeworms that did develop were stunted or sexually sterile. Doses of 15,000 Rep cobalt-60 or 15,000 R X-ray generally prevented cysticercoids from developing into adult tapeworms.

Radiation induced chromosomal aberrations in Hymenolepis diminuta were studied by Kisner (1961). The most prominent effect was the production of extremely long chromosomes which occurred in early cleavage stages of the irradiated worms. He attributed the long chromosomes to the fusion of chromatids after chromosome breakage. Other aberrations observed were stickiness (a radiation effect whereby the chromosomes fail to separate easily at anaphase), fragmentation

*Rep - roentgen equivalent physical - that amount of any radiation which results in the deposition of 93 ergs/gram of soft tissue.

(small pieces of chromosomes), and abnormal chromosomal complements. A dose of 5000 R resulted in 45% of the irradiated worms displaying such aberrations. The only aberration encountered in the controls was an occasional aneuploidy.

Tan and Jones (1966) exposed 7-day-old specimens of Hymenolepis microstoma in vitro to 2500, 5000, and 7500 R X-rays, respectively. These were transplanted into mice as were non-irradiated controls. The hosts were sacrificed at intervals of 2, 4, 6, 8, and 32 days, respectively, and worms removed for morphological studies. These studies were performed only on the 8-day and 32-day post-transplantation groups and suggested that the neck region of tapeworms is highly radioresistant, while newly forming proglottids and the existing strobilae are more radiosensitive. They reported that this radiosensitivity where proglottids are developing is evidenced by organ abnormality, e.g., number and position of testes, fusion of cirrus pouches, incomplete segmentation and sterility. They found that the damage, or abnormalities, was directly proportional to dose, and apparently complete recovery occurs by the thirty-second day after initial exposure.

Tan and Jones (1967) exposed 7-day-old specimens of Hymenolepis microstoma in vitro to 40,000 and 20,000 R, X-rays and then implanted these worms into mice. At intervals of 8 and 20 days post-implantation they fed the infected mice (infected with previously irradiated worms) with 5 cysticercoids to the group which had received worms irradiated with 40,000 R and 10 cysticercoids to the mice which had received

worms exposed to 20,000 R. Worms exposed in vitro to 40,000 R did not grow very well and within 18 days were all dead. But secondary infection rates were the same as those in the controls. Different results were obtained from the worms exposed to 20,000 R. Although these worms also passed out of the host, secondary infection rates were below 20% as compared with 80% in the controls. They also state that the growth of these challenge worms was greatly inhibited. They concluded that acquired resistance to Hymenolepis microstoma depends upon metabolic activity of the immunizing worms. Thus 40,000 R radiation apparently had little effect.

In 1968 Tan and Jones exposed 7-day-old worms (H. microstoma) in vitro to 20,000 R X-rays and then implanted 10 worms per host into the duodenum of mice. Mice that received these lethally irradiated worms were challenged with cysticercoids 8, 50, 100, and 150 days after inoculation. The challenge worms were removed after 10 days, counted, and measured. Worms in immunized mice were significantly fewer and shorter than in controls. This inhibitory response arose between 8 and 50 days after immunization, and began to decline between 100 and 150 days.

Fairbairn et al. (1961) conducted in vitro metabolic studies on irradiated and normal strains of Hymenolepis diminuta. The irradiated strain was described by Schiller (1961) and morphologic anomalies had persisted for seven generations. The metabolic characteristics of this strain were compared with those of the normal strain from which it was derived. They were unable to find any biochemical differences between normal and irradiated strains of this species.

In the following review of literature pertinent studies are cited concerning the effects of radiation on the intermediate host, Tribolium confusum.

Banham and Crook (1966) studied the susceptibility of Tribolium confusum to gamma radiation. They studied all developmental stages, viz. egg, larvae, pupae, and adult. Radiation was from a cobalt-60 source at a dose rate of 4000 Rads/hr. (1.11 Rads/sec.). They found that exposure of the eggs to doses of 1200 and 4500 Rads induced 50% and 99.9% reduction, respectively, in the numbers of emerging adults. In order to attain the same percentages of reduction in numbers of larvae and pupae reaching adulthood, doses of 3400 and 5300 Rads and 7500 and 14,500 Rads were needed, respectively. It required 9400 Rads to kill 50% of the adults and 12,800 to kill 99.9% of them. The doses required to kill 50% and 99.9% of the adults were somewhat higher than the doses required to produce the same effects in the present study.

Jefferies and Banham (1966) tested the effect of dose rate on the response of Tribolium confusum, Oryzaephilus surinamensis, and Sitophilus granarius to cobalt-60 gamma radiation. A dose rate within the range 1500 to 4700 Rads/hr. modified the lethal response of the three insect pests of stored products to gamma irradiation. They found that the higher dose rates induced greater mortality and provided a lower LD₅₀.

MATERIALS AND METHODS

Invertebrate Host, Tribolium confusum DuVal

The selection of this invertebrate host was based on the following criteria: (1) the beetles are easy to obtain, (2) easy to rear, (3) require an uncomplicated diet, (4) have a high rate of reproduction, (5) short life cycle, (6) long life span, (7) susceptible to cysticercoids of Raillietina cesticillus, (8) extensive studies have been done on them concerning the effects of radiation, and (9) stock cultures are easily maintained in the laboratory.

The beetles were maintained in an insect rearing room under constant environmental conditions at a temperature of 30°C and a relative humidity of 65-70%. They were fed preconditioned wheat shorts (sifted through an 80-mesh screen) to which was added powdered Brewer's yeast (1.5 grams of yeast per 50 grams of feed).

The beetles selected for the experiments were from 3-5 months old. Selection of this age group was based on the fact that Raychaudhuri and Butz (1965) reported that 5-23 week-old beetles were considered middle aged, thus individuals were selected in an effort to maintain as many parameters as constant as possible.

Life Cycle

This beetle was described by Jacquelin DuVal in 1858. Life cycle studies have been published by several workers (Chapman, 1918, Brindley, 1930, Holdaway, 1932, Good, 1933, and Park, 1934). The adult T. confusum is a shiny, brownish-red organism measuring 3.47 mm.

in length by 1.07 mm. in width (Good, 1933). The female lays about 450 eggs in her lifetime which hatch into small larvae that undergo several moults over a 23-day period before pupation (Good, 1933). The pupal period is approximately 9 days. The total length of the life cycle from the egg to adult under laboratory conditions of 30°C and 70% relative humidity is about 31 days (Good, 1933). Park (1934) reported that the majority of individuals which successfully emerge and meet with no accidents live at least six months.

Tapeworm, Raillietina cesticillus (Molin, 1858)

This tapeworm of chickens was selected because (1) previous work on other aspects using this organism has been conducted at Kansas State University and stock infections were available, (2) the life cycle of this organism is well known and can easily be maintained in laboratory animals, (3) a large amount of research concerning the effects of radiation on other closely related cyclophyllidean cestodes has been reported in the literature, and (4) the size of the adult and cyst stages of this parasite permit morphological investigation.

Life Cycle

This tapeworm is a common parasite of chickens but has been reported from turkeys and guinea fowls. Its length is between 70 and 90 mm., sometimes reaching 130 mm. Maximum width is 1.5 to 90 mm. with the anterior proglottids 3 to 6 times as broad as long with the following ones increasing in size until the length exceeds the width (Ackert, 1918). It is cosmopolitan in distribution.

Various beetles are the only organisms now recognized as intermediate hosts for this tapeworm. In a preliminary note, Ackert (1918) incriminated the housefly (Musca domestica) as an intermediate host after having fed several hundred houseflies to chickens reared in confinement. He did not demonstrate the cysticercoïd stage in the housefly. Later experiments (Guberlet, 1916 a and b; Joyeux, 1920; Zimmerman, 1930; Wetzel, 1933; Reid, Ackert and Case, 1938; and unpublished, Enigk, 1959) were all unsuccessful in incriminating this dipteran as an intermediate host.

Ten families of beetles representing over 100 species may act as intermediate hosts for this cestode (Reid, 1962). The cysticercoïds normally occupy the hemocoel of the abdominal region but in heavy infections are commonly found in the thoracic region. The beetles become infected by ingesting gravid proglottids containing eggs. The oncospheres hatch, penetrate the gut wall, and develop into infective cysticercoïds. A detailed description of penetration of the gut wall by the oncospheres and development of the cysticercoïds in the intermediate host is given by Schiller, 1959; Reid, 1946, 1948; Silvermann and Maneely, 1955; and Ogren, 1955.

Depending on temperature the minimum time for development of the oncospheres into infective cysticercoïds is 14 days (Reid, 1962). When the infected beetles are ingested by chickens, the cysticercoïds are liberated from the hemocoel, attach to the small intestine, and develop. Reid, Ackert and Case (1938) found that the minimal time required for the production of gravid proglottids from cysticercoïds

in White Leghorn chicks was 13 days. The total length of the life cycle of R. cesticillus from egg to egg under laboratory conditions is 27 days (Reid, 1962).

Laboratory Maintenance

Stock infections of R. cesticillus to infect beetles were maintained in chickens in the laboratory. Gravid proglottids were removed from chicken feces with a spatula and placed in 0.85% saline. After a large number of proglottids had been collected they were further separated from adhering fecal debris by a flotation method developed in our laboratory. The saline containing the proglottids was poured onto an 80 mesh screen and washed with tap water. The contents were then added to a 1000 ml test tube. Ninety ml of NaNO_3 (specific gravity 1.43) was poured into the tube and tap water was added until the total volume was 1000 ml. A hand was placed over the mouth of the tube and it was shaken and inverted vigorously several times in order to facilitate mixing. The tube was placed in a rack and not disturbed. The proglottids floated to the top of the tube, whereas, most debris remained in the middle and bottom portions. The proglottids were poured off into a beaker of water and rinsed with fresh water three times in order to remove excess NaNO_3 . Using a pipette the clean proglottids were added to a vial containing 0.85% physiological saline and stored at 4°C until needed.

Infection of the Invertebrate Host

Beetles infected with cysticercoids were obtained in the following manner. The beetles were screened from their culture medium and placed in an insect rearing room where they were starved for three days. Fresh proglottids were removed from vials with a pipette and transferred to round-bottomed glass dishes (2 cm. in diameter). Excess saline was removed with a pipette. Proglottids were evenly dispersed around the dish in groups of 3 or 4 and sprinkled with a little feed in order to absorb excess saline and help attract the beetles.

Approximately 100 starved beetles were added to each dish. The dishes containing the proglottids and starved beetles were returned to the insect rearing room. The beetles consumed the proglottids and feed within 12 hours. After the first feeding the beetles were again starved for two or three days and the procedure repeated. In some instances involving large populations of beetles they were offered a third feeding. After the final feeding the beetles were placed in pre-conditioned wheat shorts and returned to the insect rearing room to permit the development of cysticercoids in 12 to 14 days.

Degree of Infection with Cysticercoids

After allowing a minimum of 16 days (after the initial feeding) for the cysticercoids to mature, the infected beetles were removed from feed by screening. Using forceps the beetles were grasped at the thorax and crushed. They were then placed in a drop of saline on a slide and examined with a zoom-binocular microscope.

The head was severed at the cephalothoracic junction with the aid of two teasing needles. Then the elytra were removed and the alimentary tract was removed by placing one needle into the area of the anus and pulling. The cysticercoids which were free in the hemocoel were freed onto the slide, were isolated from the alimentary debris and segregated into one place on the slide. Next, using the sharp-pointed dissecting needles, the alimentary tract-free abdomen and thorax were completely macerated. Cysticercoids were not damaged by this technique and if present would become visible after maceration of the tissue. Often in highly infected beetles, cysticercoids would be found adhering to the muscles of the thorax.

Twenty-five beetles were randomly selected from the population and examined in this fashion. From this the percentage infection of the entire population was estimated. For example, in one population of beetles exposed to tapeworm eggs by the aforementioned method, 100% infection was obtained. The numbers of cysticercoids per beetle ranged from 2 to 58 with an average of 17.

Transport to Radiation Source

To help maintain environmental conditions as constant as possible the beetles were transported from the insect rearing room to the radiation source by placing them in glass dishes with covers. These dishes were placed in a styrofoam container and placed in the insect rearing room several hours in order for them to attain the temperature of the rearing room. The above precautions were taken because other

workers reported that changes in environmental conditions prior to or after irradiation may be detrimental.

Radiation Unit

Gamma irradiations were administered in the Gamma-cell-220 in the Department of Nuclear Engineering at Kansas State University. It is ideal for radiation studies because it is very easy to work with and delivers a large amount of radiation in a short time.

An electrically controlled and timed chamber moves in or out of the radiation field according to the amount of radiation the operator desires. The cobalt-60 source was producing 3.27×10^5 Rads/hr. of gamma-radiation on March 16, 1965. Knowing this and the disintegration rate of cobalt-60 it was possible to calculate the dose at any given time. For gamma and X-rays, 1 Rad equals 1 Roentgen.

Irradiation of the Invertebrate Host

At the radiation source the dishes containing the beetles were removed from the styrofoam container and placed in the portable chamber of the radiation unit. Corrections were made to make sure that the dishes would be in the field of maximum flux, also time for descent and ascent of the chamber into and out of the flux field was taken into consideration. It was calculated that it took a total of 7 seconds for the chamber to descend and ascend from the flux so that corrections in exposure time could be made. Immediately after the desired dose had been delivered the beetles were returned to the

styrofoam container and returned to the laboratory where fresh feed was added to each dish.

Experiment 1 Survival of Beetles Subsequent to Irradiation

This experiment used 3 to 5-month-old beetles and a radiation dose rate of 60 R/sec. Cultures of 50 beetles per group were exposed to 0, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 17,000, 19,000, and 22,000 R of gamma radiation, respectively. Each week for 4 weeks after the initial exposures the cultures were examined and the number of surviving beetles determined.

Experiment 2 Effects of Radiation on Parasitized and Non-parasitized Beetles

The purpose of this experiment was to determine whether beetles which are parasitized with cysticercoids of R. cesticillus are more susceptible to gamma radiation than non-parasitized beetles. There were three replications of the experiment, with only slight differences existing in dose rate and age of the beetles.

A population of T. confusum of known age was divided into two groups; one group was fed cysticercoids of R. cesticillus, the other group received only feed. After 16 days of incubation the infected group and uninfected group were subdivided. The infected and uninfected groups were divided into 7 subgroups of 50 beetles each. Six subgroups from each of the infected and non-infected groups were irradiated with 4000, 5000, 6000, 7000, 8000, and 9000 R, respectively.

One subgroup from each of the subcultures was maintained as a control non-irradiated groups. After irradiation all groups were maintained under usual conditions in the rearing room. Each week for 5 weeks the cultures were examined and the percentage of surviving beetles determined. The experimental design is given in Table 1.

Experiment 3
Development of Raillietina cesticillus after
Exposure of the Cysticeroid in
Tribolium confusum to
Gamma Radiation

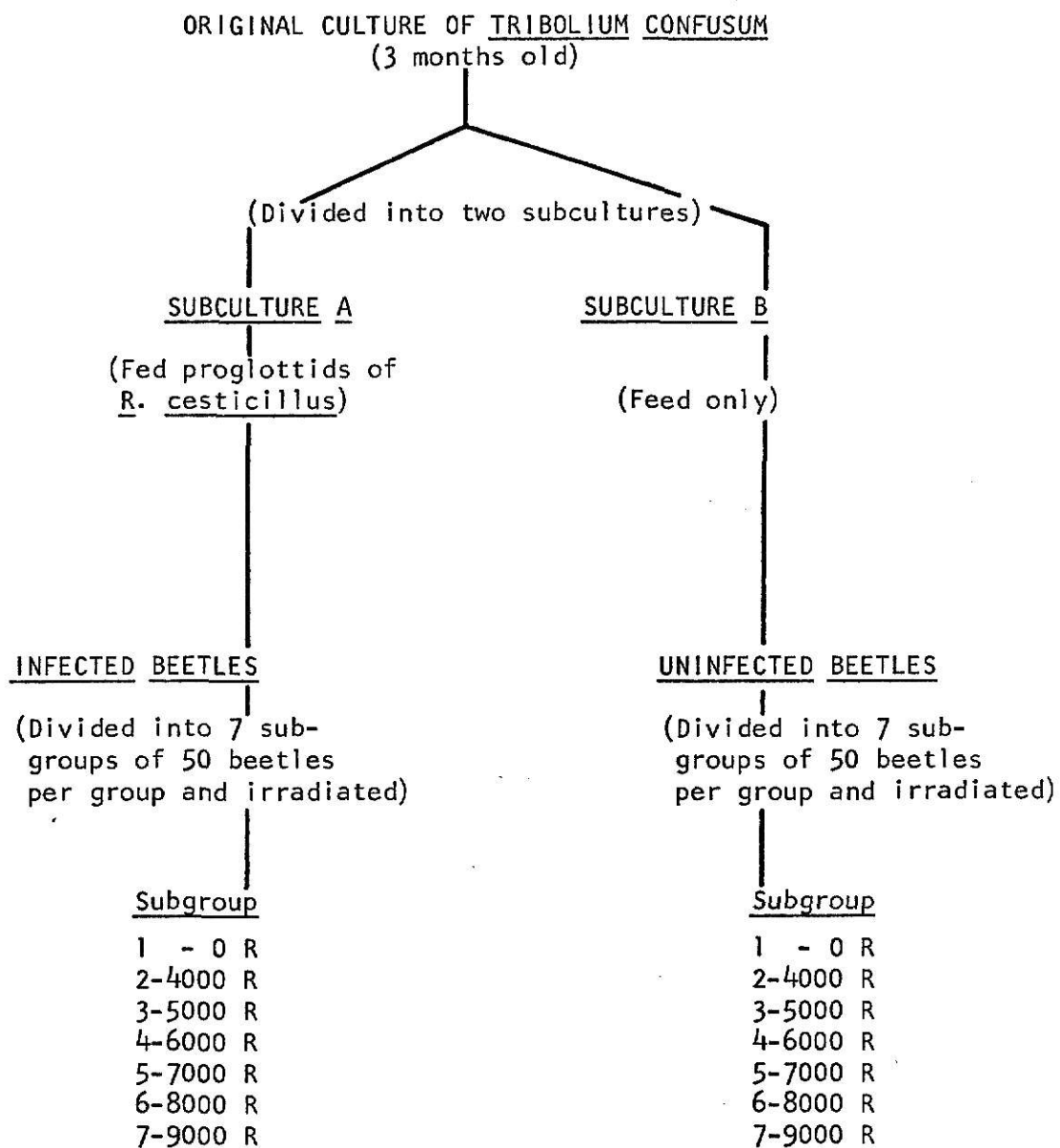
This experiment was a continuation of the previous one and conducted primarily to determine the effects of cobalt-60 gamma radiation on the development of R. cesticillus after exposure of the cysticeroids in T. confusum to 4000, 5000, 6000, 7000, 8000, and 9000 R, respectively.

A culture of infected beetles was examined as described above and the degree of infection determined. In this culture each beetle harbored on the average about 4.3 cysticeroids. The beetles were divided into 7 groups of 9 beetles each, placed in 2-cm round-bottomed dishes, and exposed to 0, 4000, 5000, 6000, 7000, 8000, and 9000 R, respectively.

Immediately after exposure the infected-irradiated beetles were placed in gelatin capsules (No. 1) and fed to chickens via stomach tube. Each chicken received approximately 39 cysticeroids. Eleven days after exposure the chickens were necropsied and the tapeworms recovered. Relaxation of the worms was accomplished by refrigerating overnight

TABLE 1

Design of Experiment 2



in tap water before they were killed in distilled water at 75°C. They were fixed in hot alcohol-formalin-acetic acid (A.F.A.) and stained with either Harris' haematoxylin or Semichon's acetocarmine.

Experiment 4 Radiographic Techniques for Determining Presence of Cysticeroids

Radiography was used as a possible means for determining the presence of cysticeroids in beetles. Five groups of beetles studied were live-infected, live-uninfected, dead-infected, dead-uninfected, and uninfected-starved. These groups were placed in a specially constructed X-ray grid and X-ray negatives were made in a General Electric X-Ray Grain Inspection Unit (rating-25 kv- at 5 ma-continuous; Line supply 100 to 130 volts, 60 cycle, single phase; line current 2.0 to 2.5 amperes; power consumption 150 watts; power factor 0.6). Photographic negatives were made on two different types of X-ray film; Kodak Industrial X-Ray Film, Blue Tinted Type R, and Kodak Type M-14, both with ultra fine grain, high contrast, for either direct exposure or use with a lead screen. The negatives were developed in Kodak Liquid X-ray Developer and Replenisher, washed in water and dried. The exposure times as well as developing times were variable and are discussed later. Enlarged photographs were used to make 35 mm. slides.

RESULTS

Experiment 1 The Effects of Co-60 Gamma Radiation on the Survival of Tribolium confusum

This experiment showed the response of Tribolium confusum to gamma radiation from a cobalt-60 source at a dose rate of 60 R/sec. (216,000 R/hr.), over a four week period. Beetles were uninfected and exposed to doses of 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 17,000, 19,000, and 22,000 R, respectively. From Table II it can be seen that one week after initial exposure of the beetles to radiation that most of them survived (Figure 1, Graph A). Two weeks post-irradiation beetles exposed to more than 5000 R began to exhibit more marked responses (Figure 1, Graph B). A statistical analysis revealed that an inverse relationship existed between per cent survival and dose ($r = 0.99$). At the end of three and four weeks post-irradiation doses at 9000 R or more were lethal (Figure 1, Graphs C and D). An LD₅₀ dose determined from the two week post-irradiation data was 6340 R (Figure 1, Graph B).

Experiment 2 Effects of Radiation on the Survival of Parasitized and Non-parasitized Beetles

The results of exposing infected and uninfected beetles to various doses of radiation are given in Tables III and IV. There were three replications of the experiment.

TABLE II

The Effects of Cobalt-60 Gamma Radiation on the
Survival of Tribolium confusum

Culture	Number of beetles	Doses (R)	Percentage of surviving beetles			
			Weeks post-irradiation			
			1	2	3	4
1	50	0	100	100	100	100
2	50	4000	100	100	96	96
3	50	5000	100	96	96	94
4	50	6000	97	78	72	72
5	50	7000	100	32	22	20
6	50	8000	100	30	18	16
7	50	9000	98	6	0	0
8	50	10,000	100	4	0	0
9	50	15,000	100	7	0	0
10	50	17,000	100	5	0	0
11	50	19,000	100	3	0	0
12	50	22,000	100	3	0	0

*Radiation dose rate = 60 R/sec.

EXPLANATION OF FIG. 1

Effects of various radiation dosages on the survival of Tribolium confusum over a four-week period, Experiment 1.

Graph A = 1 week post-radiation
Graph B = 2 weeks post-radiation
Graph C = 3 weeks post-radiation
Graph D = 4 weeks post-radiation

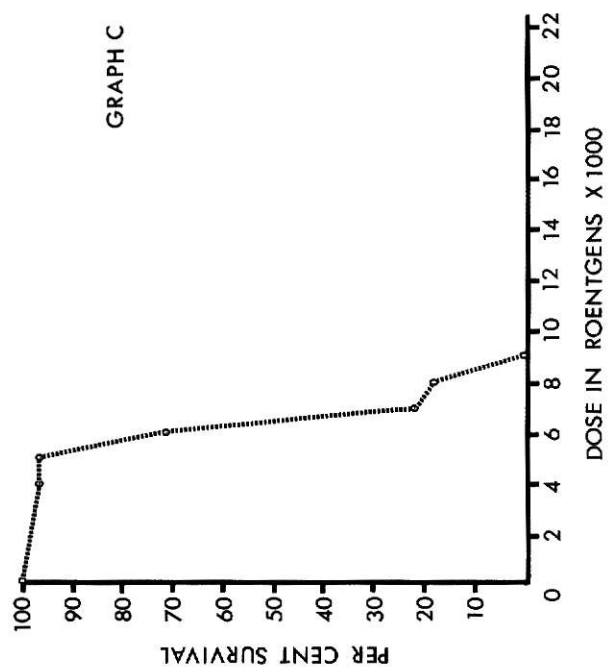
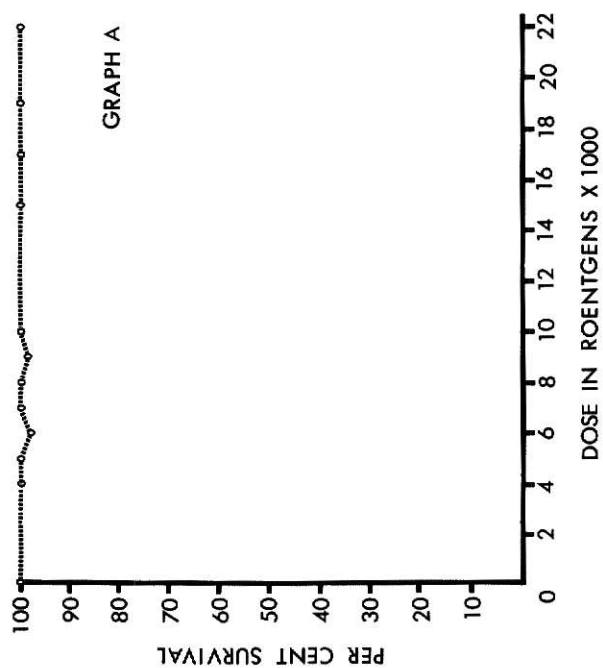
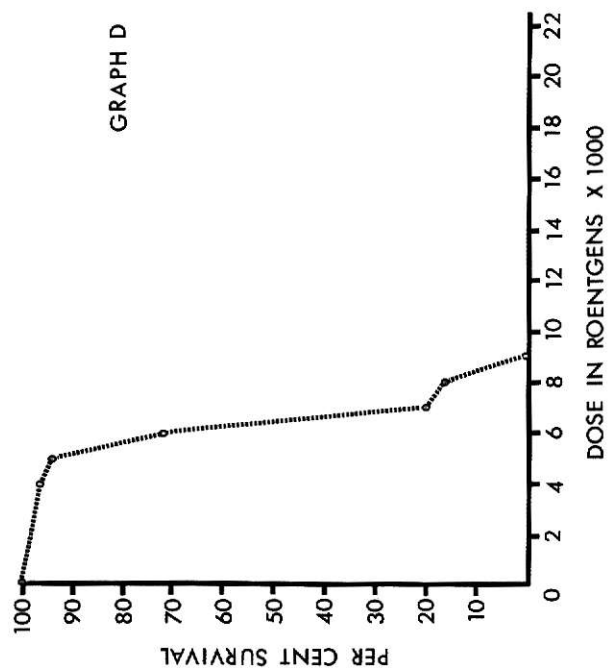
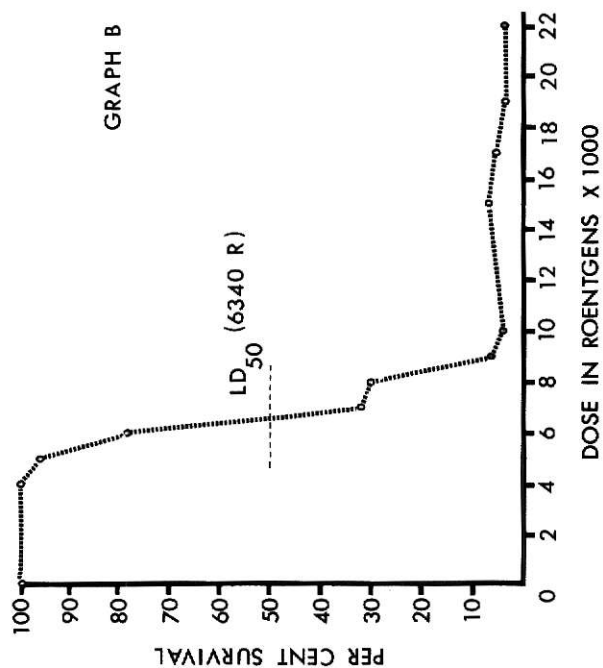


TABLE III

Effects of radiation on parasitized and non-parasitized beetles
Dose rate 67.9 R/sec, beetles 3 months old

Dose of Radiation (R)	Average percentage of surviving beetles in two experiments									
	Weeks post-irradiation									
	1		2		3		4		5	
	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec
0	100	100	100	99	100	98	99	97	98	98
4000	100	100	99	99	97	96	97	95	97	95
5000	100	99	96	96	91	96	90	95	89	94
6000	97	99	70	76	67	71	67	71	66	71
7000	98	99	26	31	26	23	26	22	25	22
8000	96	99	6	17	4	9	4	8	4	8
9000	96	98	3	6	0	0	0	0	0	0

TABLE IV

Effects of radiation on parasitized and non-parasitized beetles
Dose rate 67.3 R/sec, beetles 5 months old

Dose of Radiation (R)	Average percentage of surviving beetles in two experiments									
	Weeks post-irradiation									
	1		2		3		4		5	
	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec	Infec	Uninfec
0	100	100	100	100	99	99	97	98	97	98
4000	100	100	100	99	100	98	100	97	97	98
5000	100	100	93	98	90	98	87	98	86	98
6000	97	100	66	77	64	75	64	75	63	75
7000	97	100	10	14	6	14	3	14	3	14
8000	92	96	3	14	2	9	1	9	1	9
9000	93	97	2	5	0	0	0	0	0	0

Examination of the tables indicates that there was a delayed response to radiation. The most pronounced reduction in the percentage of surviving beetles was between the 1st and 2nd weeks and then stabilized at three, four and five weeks post-irradiation (Fig. 3). In most cases, particularly at the higher dosage levels, the infected beetles were more susceptible as indicated by their lower percentage of survival (Tables III and IV). As in experiment 1, at the end of three weeks post-irradiation doses of 9000 R or more were lethal.

Results of statistical analysis of the combined data from this experiment (Tables III and IV) at each week of the five-week experimentation period are shown in the analysis of variance Tables V-IX. Examination of the statistical data in Tables V and VI for the first and second week post-irradiation indicates that survival of the infected beetles was significantly less than the survival of the uninfected beetles. The level of irradiation had significantly conditioned the survival of the beetles in that as dosage increased survival rate decreased. At three, four and five weeks post-irradiation the survival of the infected beetles was still significantly less than that among the uninfected beetles. Again the dosage of irradiation conditioned the survival rate in that there was an inverse linear relationship between survival and dosage.

The percentage of surviving beetles in the infected-irradiated cultures was usually less than that of the uninfected-irradiated cultures (Fig. 2). Whereas the infected beetles were shown to be more susceptible to radiation than the uninfected, the statistical analysis showed a parallel decline in percentage survival between the two groups (Tables V-IX, Fig. 2).

TABLE V
Analysis of Variance Table for One Week Post-Irradiation
Experiment 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	F statistic
Replications	3	23.68750	7.89583	2.71760
Infected	1	31.56250	31.56250	10.86321 *
Irradiated	6	157.81250	26.30208	9.05268 *
Infected X Irradiated	6	31.93750	5.32292	1.83205
Experimental error	39	113.31250	2.90545	

* $P < 0.005$

TABLE VI
Analysis of Variance Table for Two Weeks Post-Irradiation
Experiment 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	F statistic
Replications	3	241.31250	80.43750	2.94370
Infected	1	232.06250	232.06250	8.49258 *
Irradiated	6	93716.75000	15619.45703	571.61108 *
Infected X Irradiated	6	226.43750	37.73958	1.38112
Experimental error	39	1065.68750	27.32532	

* $P < 0.005$

TABLE VII
Analysis of Variance Table for Three Weeks Post-Irradiation
Experiment 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	F statistic
Replications	3	103.56250	34.52083	1.67987
Infected	1	108.62500	108.62500	5.28597 *
Irradiated	6	98331.00000	16388.50000	797.50659 *
Infected X Irradiated	6	179.87500	29.97916	1.45886
Experimental error	39	801.43750	20.54967	

* $P < 0.005$

TABLE VIII
Analysis of Variance Table for Four Weeks Post-Irradiation
Experiment 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	F statistic
Replications	3	93.06250	31.02983	1.37645
Infected	1	151.12500	151.12500	6.70568*
Irradiated	6	9789.87500	16314.97656	723.92432 *
Infected X Irradiated	6	193.87500	32.31250	1.43376
Experimental error	39	878.93750	22.53685	

* $P < 0.005$

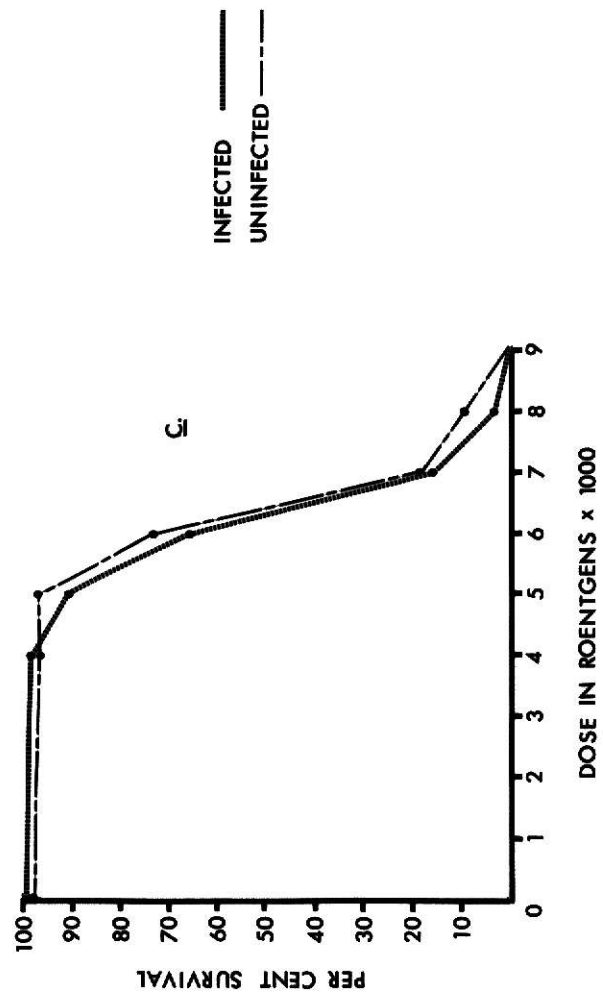
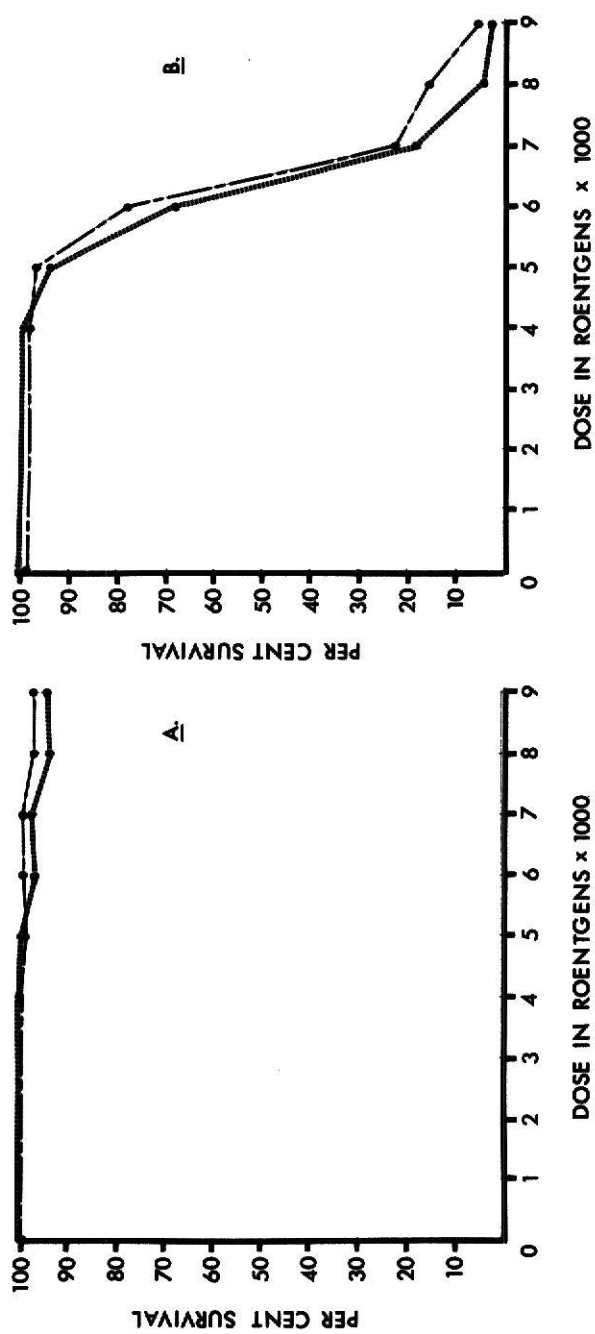
TABLE IX
Analysis of Variance Table for Five Weeks Post-Irradiation
Experiment 2

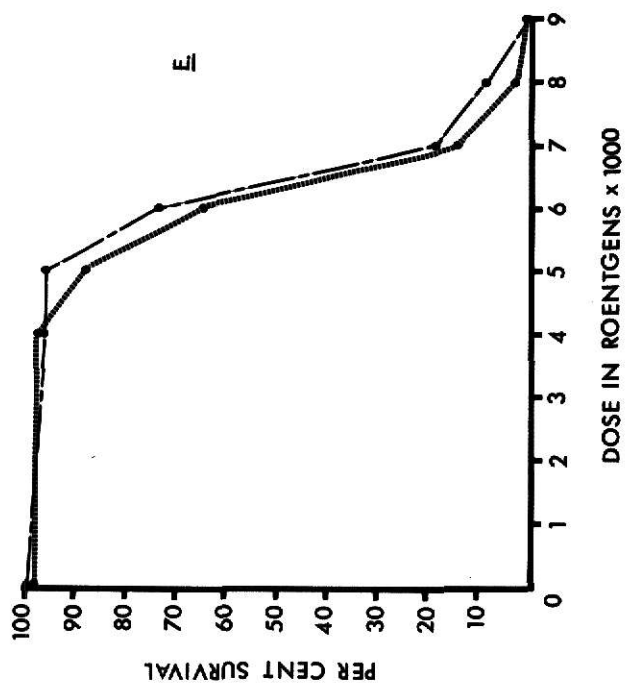
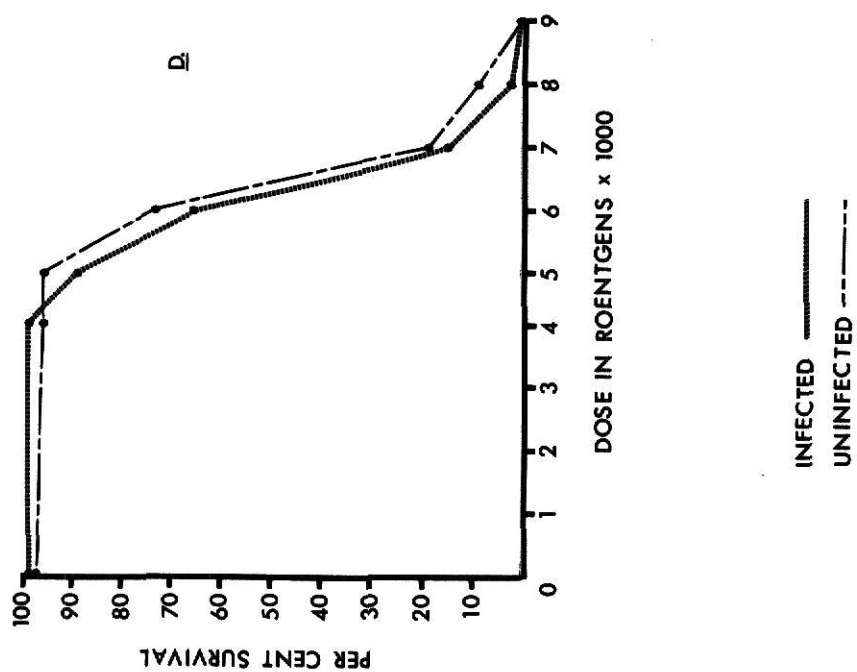
Source of variance	Degrees of freedom	Sum of squares	Mean square	F statistic
Replications	3	91.62500	30.54166	1.43444
Infected	1	208.25000	208.25000	9.78083 *
Irradiated	6	96769.43750	16128.23828	757.49097 *
Infected X Irradiated	6	185.75000	30.95833	1.45401
Experimental error	39	830.37500	21.29166	

* $P < 0.005$

EXPLANATION OF FIGURE 2

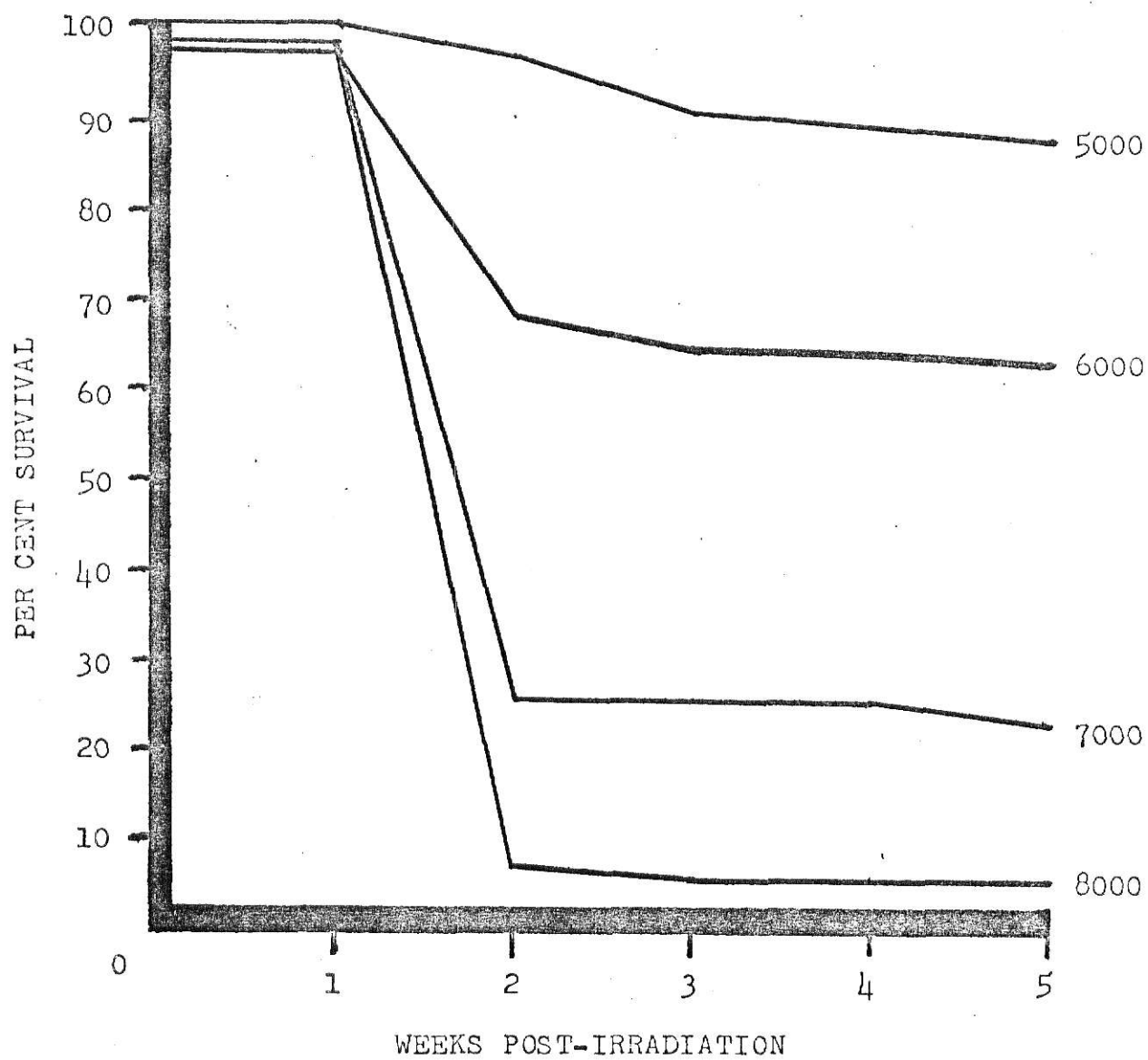
Survival of beetles related to irradiation dosage and time. Post-irradiation survival at 1 week (Graph A), 2 weeks (Graph B), 3 weeks (Graph C), 4 weeks (Graph D) and 5 weeks (Graph E).





EXPLANATION OF FIGURE 3

Effects of time post-irradiation on
survival of infected beetles.



Experiment 3
Development of Raillietina cesticillus after
Exposure of their Cysticercoïds in
Tribolium confusum to
Gamma Radiation

Exposure of cysticercoïds of R. cesticillus in T. confusum to gamma radiation at doses of from 4000 to 9000 R produced variable results with reference to the development of adult worms (Table X). Statistical examination of the data from Table X indicated that no correlation existed between radiation dose and number of cysticercoïds developing to adults ($r = 0.15$). By means of staining techniques an unsuccessful attempt was made to study morphological changes.

Experiment 4
Radiographic Techniques for Determining
Presence of Cysticercoïds

It became evident from experiments 1-3 that in future studies of irradiating cysticercoïds in vivo, a method for determining the degree of infection other than dissection of beetles would be necessary. The potential of radiography for assessing the degree of cysticercoïsis in beetles was investigated. A specially constructed grid made of 1.4 cm² wire mesh taped on a layer of Saran Wrap was used in making X-ray negatives. Photographs were then made from the negatives (Fig. 4).

To test this technique as a tool in determining whether T. confusum was infected with cysticercoïds of R. cesticillus the following experiment was conducted. Twenty beetles were selected at random from an infected culture and X-rayed as described above. The negative was

TABLE X
Development of Irradiated Cysticercoids in Chickens
Experiment 3

Bird number	Number of irradiated cysticercoids fed*	Dose of radiation (R)	Adult tapeworms recovered (No.)
440	39	0	10
308	39	4000	1
772	39	5000	5
801	39	6000	10
784	39	7000	3
1048	39	8000	13
463	39	9000	2

* Probable number of cysticercoids fed
 $r = 0.15$

immediately developed and each beetle on the negative was studied under a zoom binocular dissecting microscope for the presence of cysticercoids. If the X-ray showed cysticercoids to be present they were counted for each individual beetle and the results tabulated. Next the individual beetles corresponding to the X-ray negative were dissected, and the exact number of cysticercoids counted and tabulated to see how these results compared with those obtained from the X-ray negative.

EXPLANATION OF FIGURE 4

Photograph from an X-ray negative depicting the X-ray grid used in Experiment 4. The letters at the left represent the following: IA-infected-alive; UA-uninfected-alive; ID-infected-dead; UD-uninfected-dead; and US-uninfected-starved.

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NUMEROUS
PICTURES THAT
ARE ATTACHED
TO DOCUMENTS
CROOKED.**

**THIS IS AS
RECEIVED FROM
CUSTOMER.**

FIGURE 4

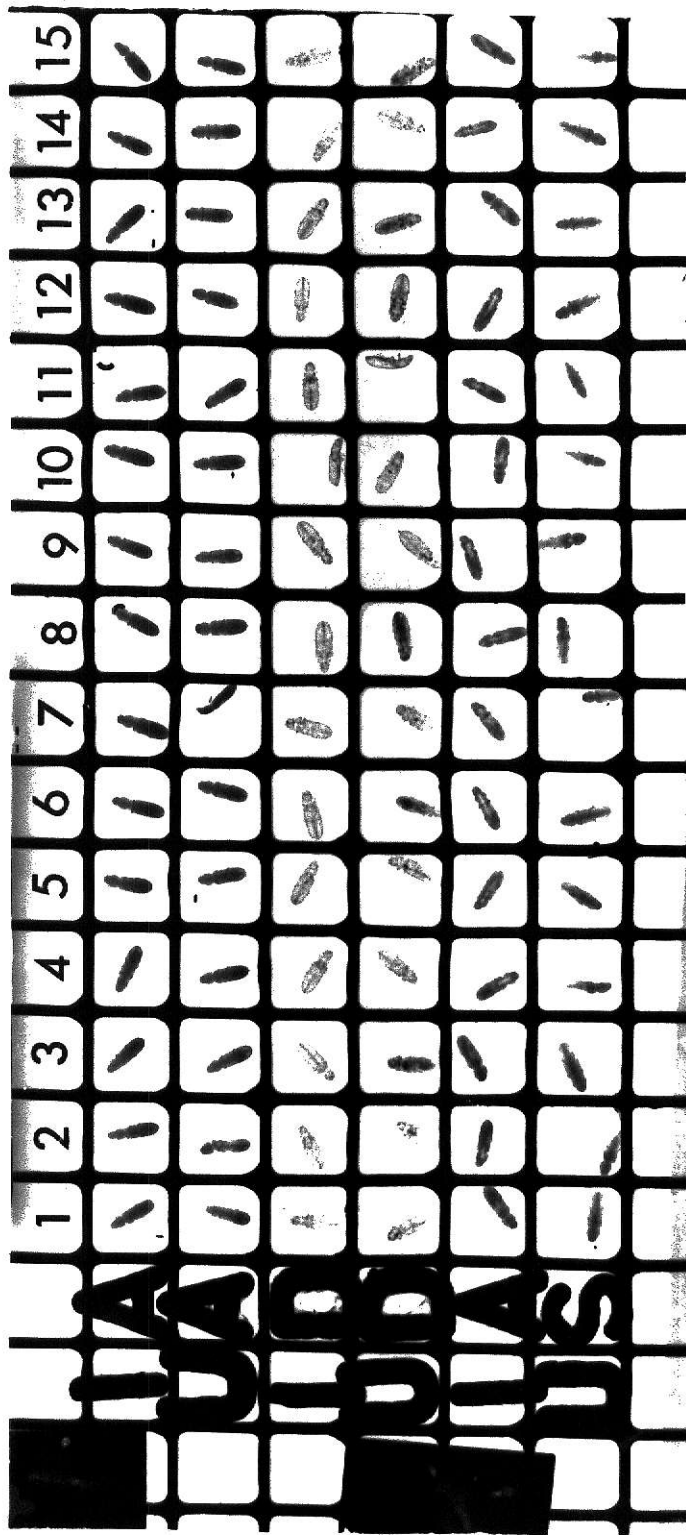


Table XI shows that radiography can only be used as a qualitative measure and is not quantitative for the degree of infection of beetles. Careful observation of Plate I, Figs. 5-8, shows the presence of oval dark bodies (see arrows) in the abdominal region of these beetles which are not present in the beetles from Figs. 1-4 (Plate I). These dark bodies represent cysticercoids of R. cesticillus.

DISCUSSION

As was reported by Jefferies and Banham (1966) radiation dose rate is one of the most important parameters in determining the response of T. confusum to radiation. The present study showed the response or susceptibility of T. confusum to gamma radiation from a cobalt-60 source at a dose rate of 60 R/sec, about 60 times the dose rate employed by Jefferies and Banham (ibid.), required 6340 and 9000 R to kill 50% and 100% of the beetles, respectively. Whereas, at a lower dose rate of 1 Rad/sec, Jefferies and Banham (ibid.) used approximately 8810 and 11,250 R to kill 50% and 100% of the beetles, respectively. Banham and Crook (1961) found that at a dose rate of 1.11 Rads/sec it required 9400 and 12,800 Rads to kill 50% and 99.9% of the adults, respectively.

The results of the second experiment indicated that beetles infected with cysticercoids of R. cesticillus were more susceptible to radiation than the uninfected beetles. Since all other parameters were held constant between the infected and uninfected beetles, it holds that the only reason the infected beetles had a lower survival rate

TABLE XI
Comparison of the Degree of Infection of
Beetles by Radiography and Dissection

Method of examination*	Number of cysticeroids																			
	Beetle number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Radiography	1	5	0	0	5	4	4	0	1	0	0	0	2	8	1	0	0	1	5	1
Dissection	3	33	0	0	15	14	10	0	1	1	2	0	5	18	3	0	1	2	5	1

* Incidence of infection of the culture of beetles as determined by radiography equal 72%,
by dissection 80%.

EXPLANATION OF PLATE I

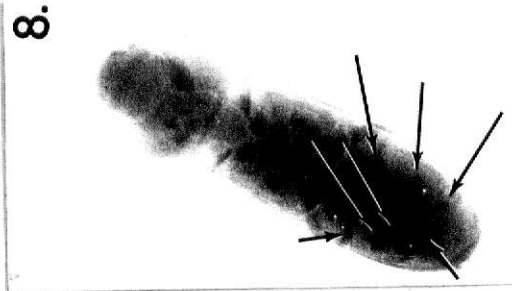
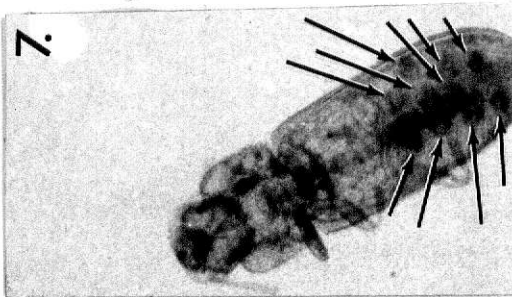
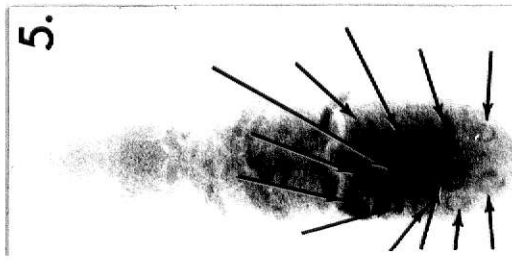
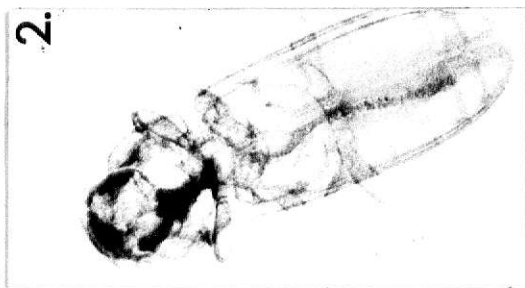
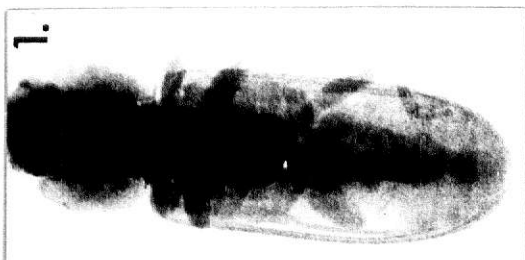
Enlargement of beetles in Fig. 4. Arrows point to cysticercoids.

1. Beetle 6 from group US, uninfected-starved.
2. Beetle 1 from group UD, uninfected-dead.
3. Beetle 6 from group UA, uninfected-alive.
4. Beetle 2 from group US, uninfected-starved.

(Note the absence of cysticercoids in the abdominal region in the beetles 1-4.)

5. Beetle 14, from group IA (5th row from top), infected-alive.
6. Beetle 11, from group IA (5th row from top), infected-alive.
7. Beetle 11, from group ID, infected-dead.
8. Beetle 15, from group IA, infected-alive.

Plate I



than the uninfected beetles was related to parasitism. What change parasitism induced in the infected beetles to render them more susceptible to radiation is unknown. The infected beetles always had a lower percentage of survival. But as was stated previously the overall change in per cent survival at each radiation dose level was the same for the infected and uninfected cultures. For example, at one week post-irradiation 90% of the infected beetles exposed to 5000 R were still alive and 95% of the uninfected beetles exposed to this dose were still alive and at two weeks post-irradiation, 80% of the infected and 85% of the uninfected were still alive. This indicates that there is an initial but not a recurring response to radiation associated with the parasitized host.

Banham and Crook (1966) observed that T. confusum exhibited a delayed response to radiation. This same phenomenon occurred in the present study. No marked reduction in the percentage of surviving beetles occurred at any of the dose levels until two weeks post-irradiation. Then the response was marked and after this time only slight changes in the survival rate were evident.

It was not possible to perform detailed morphological examination of the various regions of adult worms which developed from cysticercoids exposed in T. confusum to radiation. The major problems in examining for morphological radiation induced abnormalities was in staining the adult worms. The chromophilic affinity of the cuticle was so marked that it made it impossible to examine the internal organs for morphological variations. Also the size of the proglottids made removal of the cuticle impractical.

The reason for such a high degree of variation in the number of worms developing in chickens which received irradiated cysticercoids is unknown. Theoretically each chicken received 39 irradiated cysticercoids. One would expect that there would be an inverse relationship between degree of radiation and ability to mature. One explanation for the discrepancy may be that all the chickens did not receive 39 cysticercoids by the method used. Brannon (1961) had 90% development to adult tapeworms from irradiated cysticercoids in I. confusum. However, he dissected out the cysticercoids and fed them to chickens via stomach tube.

The radiographic technique described earlier was originally developed by M. F. Hansen and R. B. Mills, Division of Biology and Department of Entomology, respectively, Kansas State University. The work here was a continuation of their initial development with the original idea being to replace the tedious time-consuming dissection formerly used to determine the degree of infection of I. confusum with cysticercoids of R. cesticillus. Also, it was intended to elaborate on certain aspects of the technique in order to ascertain what radiographic methods produce the best results. The technique has many possibilities. Not only could it be used as a major tool in the type of research described here but it could be used in other aspects of parasitology, particularly in fields of life cycle studies. Arthropods are suspected as intermediate hosts in the life cycles of several vertebrate parasites and this technique would be an invaluable tool in helping researchers examine many kinds and large numbers of them for cystic stages

of parasites. The property of the cysticercoids which made it possible for them to be X-rayed is probably related to the chemical nature of the corpuscles because they contain calcium, magnesium, phosphorus and carbon dioxide. Which compounds are present depends on the species of cestode being investigated (von Brand et al. 1965). The more common of these corpuscular compounds are calcite (CaCO_3), dolomite ($(\text{CaMg})\text{CO}_3$), and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). The technique did not prove to be advantageous from a quantitative standpoint inasmuch as it was not possible to determine the exact number of cysticercoids in a beetle. If a beetle contains more than one cysticercoid there is a chance that the cysticercoids will be superimposed, thus on the X-ray film multiple cysticercoids may appear as one.

SUMMARY

At a radiation dose rate of 60 R/sec doses less than 4000 R had no noticeable effect on the survival of Tribolium confusum, while doses beyond 9000 R were lethal. The LD_{50} was 6340 R.

The effects of cobalt-60 gamma radiation on infected and uninfected cultures of T. confusum exposed to doses of from 4000 to 9000 R indicated that infected beetles were most susceptible to radiation.

There was no correlation between the number of tapeworms developing into adults from irradiated cysticercoids and the amount of radiation given the cysticercoids. However, possible errors in technique used to

infect chickens preclude any definite conclusions. Studies of possible morphological changes in adult tapeworms developing from irradiated cysticercoids were unsuccessful.

A qualitative radiographic technique for displaying the presence of cysticercoids of Raillietina cesticillus in T. confusum was developed.

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SURVIVAL OF TRIBOLIUM CONFUSUM DUVAL
INFECTED WITH CYSTICERCIDS OF
RAILLIETINA CESTICILLUS MOLIN

by

SAMUEL JOHN ZEAKES

B. S., The College of Emporia, Emporia, Kansas, 1966

AN ABSTRACT OF A MASTER'S THESIS

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Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Studies were conducted to determine whether parasitized Tribolium confusum Duval, infected with cysticercoids of the fowl cestode Raillietina cesticillus Molin were more susceptible to gamma-radiation from a cobalt-60 source than non-parasitized beetles. Initial studies conducted to determine the effects of radiation on uninfected cultures of T. confusum at a dose rate of 60 R/sec indicated that 4000 R had no noticeable effect on survival, while doses beyond 9000 R were lethal. The LD₅₀ was 6340 R.

The effects of cobalt-60 gamma-radiation on infected and uninfected cultures of T. confusum to doses of from 4000 to 9000 R indicated that infected beetles were most susceptible to radiation. There was an initial but not a recurring response to radiation associated with the parasitized host.

There was no correlation between the number of tapeworms developing into adults from irradiated cysticercoids and the amount of radiation given the cysticercoids. However, possible errors in technique used to infect chickens preclude any definite conclusions. Studies of possible morphological changes in adult tapeworms developing from irradiated cysticercoids were unsuccessful.

A qualitative radiographic technique for displaying the presence of cysticercoids of Raillietina cesticillus in Tribolium confusum was developed and may possibly find application in other fields of parasitology.