THE EFFECTS OF IRRADIATION ON THE PARASITIC NEMATODE *NEMATOSPIROIDES DUBIUS*: AN ALTERATION IN THE LIFE CYCLE

by

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Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Department of Biological
Sciences

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YOUNGSTOWN STATE UNIVERSITY
August, 1975

ACKNOWLEDGEMENTS

This research would not have been possible if not for the efforts and assistance of many people.

Special gratitude is due my advisor, Dr. Paul Van Zandt, for all the special attention, suggestions and guidance given during the research and preparation of this Thesis.

I also wish to thank Dr. A. Chabbra, radiation phycisist at Saint Elizabeth Hospital, Youngstown, Ohio, who relinquished time and abundant assistance during this research.

I am also indebted to the following persons:
Dr. Anthony Sobota, Dr. John Yemma and Dr. Leonard Spiegel for serving on my graduate committee and who were always there for guidance.
The Radiation Therapy personnel of Carol, Karen and Peggy for their time, encouragement and extreme friendliness.
Dr. Bertwin Einfeldt who arranged for the irradiation at Saint Elizabeth Hospital, Youngstown, Ohio.
Randy Sarteschi who aided me in the statistical analysis portion of the Thesis.
Patti Brown and Justina Wilcox who took care of my cultures and animals.
A special acknowledgement is due Denise Birckbichler for the typing of this Thesis.

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INTRODUCTION

In 1942 Levin and Evans induced immunity in rats to re-infection by Trichinella spiralis by feeding the rats infective larvae irradiated at 3250 and 3750 rads (roentgen absorbed dose) of x-ray. This amount of radiation permitted the larvae to develop to adults although most of the adults were sterile. Very few larvae of the second generation were recovered from the muscles of the host due to the sterility of the adult worms. Therefore, the study concluded the origin of the mechanism of immunity against re-infection is located in the intestine.

Gould et al (1955) infected white rats with 12,000 non-irradiated encysted larvae of T. spiralis and these animals died as a result of the intestinal phase of the infection. A quantity of 12,000 encysted larvae of T. spiralis exposed to 10,000 rads of cobalt-60 were administered to white rats. It was found that these irradiated larvae caused only transient diarrhea in some of the experimental animals. The feeding of 12,000 and 24,000 larvae exposed to 18,000 rads of cobalt-60 generally caused no diarrhea and permitted the rats to gain weight. It was found that the parasites were lost rapidly from the intestinal tract if rats were fed with larvae of T. spiralis irradiated at 10,000 or 18,000 rads. This inferred that if heavily trichinosed pork were irradiated with at least 18,000 rads of cobalt-60 a person who might eat this meat would suffer little or no ill effects from irritation of the intestine

by the larvae and that the parasites would disappear rapidly from the intestinal tract.

Later, Gould et al (1957) exposed encysted larvae of T. spiralis to various doses of cobalt-60 and of x-rays. These irradiated larvae were then fed to white rats to determine the effects of radiation on the development and reproduction of these larvae. Morphologic changes were noted in female trichinellae at various intervals from 24 to 144 hours after infection with irradiated larvae that was exposed to cobalt-60 or x-rays. Trichinella larvae irradiated with 6,000 rep (roentgen equivalent physical) or more of cobalt-60 or 2,000 rads or more of x-rays produced decreases in body length when measured 3 and 6 days after irradiation. A severe stunting in growth resulted from larvae exposed to 18,000 rep of cobalt-60 or 6,000 rads of x-rays. It was found that 6 days after exposure to 2,000 rads of x-rays the gonads of both sexes showed evidence of degeneration and only a small percentage of females were inseminated. At 3,000 rads the damage was greater and only an occasional female was inseminated. It was also found in this study that the vulvas of some females were obstructed. Practically no females were found to be inseminated after 4,000 rads of x-rays. At 4,000 rads the growth of both males and females was severely stunted. Recent evidence (Larsh 1967, 1969, 1970, 1974) indicates the mechanism responsible for the expulsion of adult T. spiralis from the intestine is due to a specific delayed hypersensitivity reaction when using either non-

irradiated or irradiated larvae. After the hypersensitivity reaction occurs the resultant tissue damage then provides the stimuli for the initiation of non specific allergic inflammation which then affects the expulsion of worms due to an unsuitable biochemical environment. It was also found in these studies that humoral antibodies play a minor role in the elimination of adult worms, although antibodies are significant in overall immunity in that they produce direct damaging effects against the worms such as reproduction potential, growth interference and cellular destruction.

In 1962, Urquhart et al studied the effects of radiation on the larvae of the cattle lungworm, Dictyocaulus viviparus. These larvae were exposed to 40,000 rads of x-rays and then were administered in quantities of 1,000 to calves. Nine days post infection one calf was killed and examined. In the lung of this calf 65 larvae were obtained. A control calf infected with 1,000 non-irradiated larvae was sacrificed 9 days post infection and the lung exhibited 181 larvae. On the 13th day post infection an experimental animal was sacrificed and 23 worms were recovered from the lung in comparison to the 608 worms obtained from the control calf. A final examination at 35 days post infection revealed 231 adult D. viviparus in the control calf and no worms were recovered in the animal which recieved the irradiated larvae.

Jennings et al (1963) studied the effects of radiation on the third stage larvae of Nippostrongylus braziliensis.

The larvae were irradiated at 40,000, 80,000, 120,000, 160,000 and 180,000 rads which were then administered to white rats. Ten days post infection the animals were killed. It was found that as the x-ray dose increased the number of worms recovered from the small intestine decreased although more immature worms were found in the lungs indicating an alteration in the life cycle of this parasite which prevented migration in mice to the intestine.

Additionally, the effects of radiation on the infective larvae of Ascaris suum were studied by Dappen (1965). It was found that by irradiating A. suum larvae, that not only was the migration rate from the stomach to the liver and then to the lungs lengthened, but also the irradiation affected the number of migrating larvae. This study found that the number of migrating larvae of A. suum recovered was inversely proportional to the level of radiation received. With the utilization of irradiated larvae as the immunizing agent the amount of injury or tissue damage to the host was appreciably less.

Van Zandt (1961) studied the immunity relationships in white mice given infections with third stage larvae of Nematospiroides dubius. This study found that after one stimulating infection of N. dubius larvae, white mice failed to develop acquired immunity. This was determined by measured adult counts 15 days post infection. After two stimulating infections given at 21 day intervals, Van Zandt found that the animals failed to exhibit immunity

although smaller numbers of worms were recovered. After 3 stimulating infections however, the mice demonstrated acquired immunity.

As far as it is known, there is no published work on the effects of x-radiation with regard to the alteration of the life cycle of N. dubius and stimulation of immunity utilizing irradiated larvae. Thus, this study investigated (1) the effects of varying levels of radiation to find a dosage that would permit the infective larvae of N. dubius to penetrate the intestinal mucosa, encyst and proceed no further in the life cycle and (2) to determine whether or not irradiated larvae would cause active immunization.

CLASSIFICATION OF THE GENUS NEMATOSPIROIDES

Maybelle Chitwood, 1967.

Kingdom Animal

Phylum Nematoda

Class Sercernentea (Phasmidia)

Order Strongylida

Suborder Trichostrongylina

Superfamily Heligmosomatoidea

Family Heligmosomatidae

Subfamily Heligmosomatinae

Genus Nematospiroides

Species dubius

DESCRIPTION OF NEMATOSPIROIDES DUBIUS

Baylis (1926) first described N.dubius from the wood mouse, Apodemus sylvaticus. Female specimens measure 18 to 21 millimeters while males measure 8 to 10 millimeters. The diameter of the f male body is approximately 0.12 millimeters and in males the diameter is approximately 0.11 millimeters. The cuticle near the head region is slightly expanded in both sexes. The diameter of this particular region ranges from 0.036 millimeters in males to 0.043 millimeters in the females. Very fine striations which are accentuated at the anterior and vulval regions are characteristic of the cuticle. Throughout the length of the body are 30 to 50 longitudinal ridges. There exists

a very short buccal capsule. The esophagus measures 0.40 millimeters in length in males and 0.47 millimeters in females. A large bursa divided into two unequal lobes is at the caudal end of the male. A bursal membrane encloses a space which is occupied by a balloon-like, cloacal swelling located posteriorly. This swelling is spherical in shape and contains granular material. The male posesses copulatory spicules which are slender and filiform. The spicules appear fused but are not. The caudal end of the female is narrowed behind the vulva and from here the body tapers to a blunt tail posessing a slender, sharp terminal spike measuring 0.01 to 0.013 millimeters in length. The genital tube of the female is single and extends anteriorly from the vulva.

LIFE CYCLE OF NEMATOSPIROIDES DUBIUS

The eggs of Nematospiroides dubius, measuring 70 to 84 microns long by 37 to 53 microns wide, are expelled with the feces of the host mouse in the 8 to 16 cell stage. and become fully embryonated 8 to 10 hours before hatching. In an environment of 23 to 28 degrees Centrigrade, hatching of the larvae occurs in approximately 26 hours after passing from the host in moist fecal material. The first stage larvae measure 300 to 600 microns in length and rudimentary lips are visible around the buccal orafice. Ecdysis begins 48 hours after hatching and the cuticle can be seen loosening from both ends of the first stage larvae. Ecdysis is not

complete outside the host and the first stage cuticle is retained as a sheath of the infective larvae (Ehrenford,1954). The free living larval stages require 4 to 6 days to become the third stage infective larvae. At this time they migrate to the surface of the fecal mass. The larvae enter the host through ingestion. Upon ingestion the larvae lose their sheaths and 24 hours later are found in the intestine closely associated with the mucosa. At this point they measure 376 to 540 microns. The third stage larvae molt while in the lumen of the intestine then migrate through the mucosa and come to lie in loose cysts in the circular and longitudinal muscles of the intestinal wall. Cross (1960) stated that the muscle layers of the small intestine are thickened at this time due to degeneration of the leucocytic infiltration. At this point the larvae are grossly visible from the serous surface. The final molt occurs between the 6th and 9th days. This is shortly before the worms re-enter the lumen of the intestine. Copulation and oviposition occur in the lumen and the eggs may be found in the feces of the host for a period of 7 to 8 months following infection. Ehrenford (1954) stated that variations in length of prepatent and patent periods are influenced by both host strain and age.

MATERIALS AND METHODS

Culturing of Nematospiroides dubius larvae

The mice used throughout the research were of Swiss-white breed and obtained from Youngstown State University, Youngstown, Ohio. The mice were infected with third stage larvae obtained by collection of fecal pellets which were then mixed with enough distilled water to form an emulsion. The emulsion was then passed through several layers of cheesecloth with the aid of additional distilled water. The filtrate was collected. This process enabled the filtration of Nematospiroides dubius eggs from the fecal pellets. The filtrate was then centrifuged. After centrifugation the sediment was resuspended and washed three times with decantation of the supernatent after every washing. After the third washing distilled water was added to reform a liquid emulsion. This emulsion was then poured into a culture dish containing five layers of dampened (Wattman #40) filter paper. The covered culture dish was then placed in a 23 degree Centrigrade incubator for five days with daily aeration periods of 7 to 8 minutes. Harvesting of the third stage infective larvae was accomplished by gently spraying distilled water over the slanted filter paper and collecting the larvae through sedimentation.

Irradiation Procedure

The harvested larvae were divided into two groups, one which

was to be irradiated and one which remained unirradiated. The larvae to be irradiated were placed in a one inch Stentor dish. The dish was filled to an approximate height of one centimeter to insure uniform irradiation. Placement of the Stentor dish from the focal point of the Picker Vanguard X-ray Unit varied with the radiation dosages. All calculations were made prior to irradiation. The dosage time was divided into half to permit agitation of the larvae to keep the larvae in suspension. Transportation to and from Saint Elizabeth Hospital, Youngstown, Ohio was accomplished by carrying the larvae in glass, conical, centrifuge tubes which were placed in beakers containing ice chips.

Standardization and Infection Procedures

After each irradiation the larvae were taken back to Youngstown State University and standardized. The normal and irradiated larval suspensions were diluted with distilled water to a suitable predetermined volume. Dilutions resulted in the obtainment of 15 consecutive readings of 12 to 13 larvae per 0.05 cubic centimeters (cc). Then 0.2 cc of the larval suspension was given orally to each of the mice with a blunt, one and one-half inch, 18 guage hypodermic needle and syringe.

Autopsy Procedure

The infected animals were killed on the 7th and 14th days post infection by cervical dislocation. All of the small intestine was removed, placed on a glass plate, slit and opened. Another glass plate was placed on top of the opened intestine and then compressed. The intestine between the glass plates was placed under a dissecting microscope and examined for the presence of cysts and/or adults of *N. dubius*.

The mice used in this study were of the white Swiss strain that have been maintained in the Department of Biological Sciences for many years.

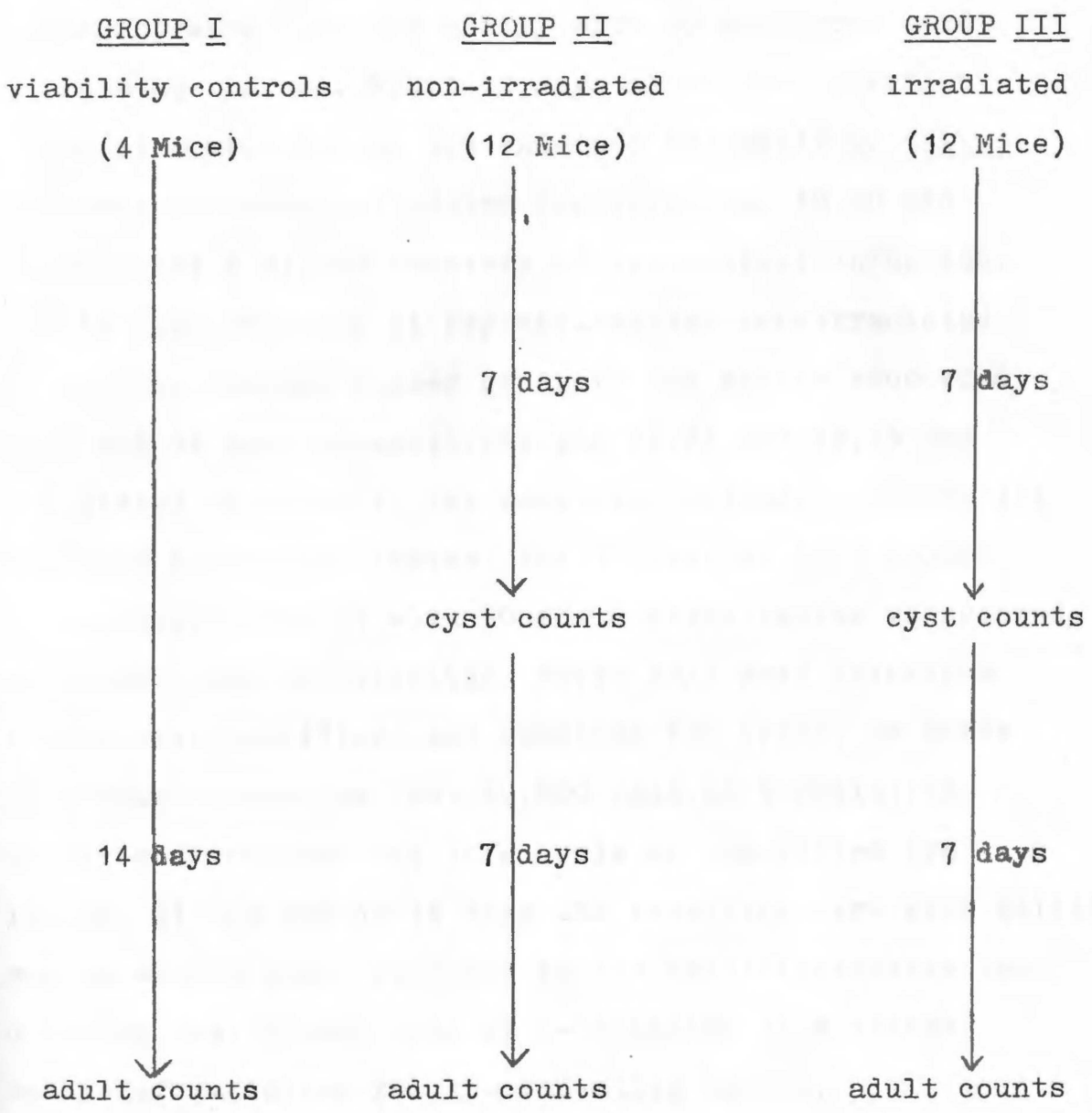
RESULTS AND DISCUSSION

Experimental Design

An experimental design was used throughout the research to insure uniformity and consistency. The design consisted of 9 experimental units and each unit represented the varying amounts of radiation employed in this project. Thus, the dosage used for Unit I was 50,000 rads, Unit II 40,000 rads, Unit III 30,000 rads, Unit IV 20,000 rads, Unit V 15,000 rads, for Unit VI 16,000 rads, Unit VII 17,000 rads, Unit VIII 18,000 rads and for Unit IX 19,000 rads of x-radiation was used. These dosages are similar to those of Staut (1971). Each unit required 28 Swiss white mice which were divided into 3 groups. The 4 mice of Group I represented the viability controls. These mice we re infected with third stage infective larvae and sacrificed 14 days post infection for adult counts. The larvae for infection were considered viable if 80% or more of the original infection was recovered as determined by adult N. dubius removed and counted from the small intestines. Group II consisted of 12 mice and represented the non irradiated controls, while the 12 mice of Group III represented the irradiated larvae group. In Groups II and III va rious mice were sacrificed at 7 days post infection for cyst counts and at 14 days post infection for adult counts. Figure I shows the experimental design employed.

FIGURE I

The experimental design used for Units I, II, III, IV and V.



UNIT I - 50,000 RADS

Twenty-eight mice were divided into 3 groups as previously described. The mice of Group I (viability controls) were infected orally with 50 non-irradiated third stage larvae. Fourteen days after the infection these mice were killed and examined for adult N. dubius. The average number of adults recovered was 48.50 and represented a 97.00% recovery of the initial infection. The 12 mice of Group II represented the non-irradiated group. The average number of cysts and adults recovered at 7 and 14 days respectively was 26.25 and 46.75 and are listed in Table I. The remaining animals in Group III employed irradiated larvae. The 12 mice in this group were orally infected with 50 third stage larvae irradiated at 50,000 rads as described. Seven days post infection 4 mice were sacrificed and examined for cysts. No cysts were found inferring that 50,000 rads of x-radiation either had retarded the life cycle or had killed the larvae. At the end of 14 days the remaining mice were killed and no adults could be found in the small intestines indicating that 50,000 rads of x-radiation is a lethal dosage of radiation for the infective larvae.

UNIT II - 40,000 RADS

Group I consisted of 4 mice and represented the viability controls. Adult counts 14 days post infection for

TABLE I

The effects of 50,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I viability controls	GROUP II non-irradiated		GROUP III irradiated	
adults (14 days)	cysts (7 days)	adults (14 days)	cysts (7 days)	adults (14 days)
49	28	49	0	0
49	30	51	0	0
48	16	48	0	0
48	31	44	0	0
		47		0
		46		0
		44		0
		45		0
MEANS				
$\bar{X}$=48.50	$\bar{X}$=26.25	$\bar{X}$=46.75	$\bar{X}$=0	$\bar{X}$=0
PERCENT OF RECOVERY				
97.00%	52.50%	93.50%	0%	0%

TABLE II

The effects of 40,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I viability controls	GROUP II non-irradiated		GROUP III irradiated	
adults (14 days)	cysts (7 days)	adults (14 days)	cysts (7 days)	adults (14 days)
48	31	49	0	0
48	30	52	0	0
47	24	49	0	0
45	20	48	0	0
		50		0
		44		0
		46		0
		45		1
MEANS				
$\bar{X}$=47.00	$\bar{X}$=26.25	$\bar{X}$=47.85	$\bar{X}$=0	$\bar{X}$=.125
PERCENT OF RECOVERY				
94.00%	52.50%	95.70%	0%	.25%

this group averaged 47.00 and represented a 94.00% recovery of the original infection. Four mice of Group II(non-irradiated controls) had cyst counts of 31, 30, 24 and 20 ($\bar{X}$=26.25) at 7 days as seen in Table II. The remaining 8 mice were sacrificed 14 days post infection and had an average adult count of 47.85 (Table II). Cyst and adult counts in this group are comparable with what is obtained when using non-irradiated larvae. In Group III using larvae irradiated at 40,000 rads, the mice were sacrificed at 7 days post infection and exhibited no cysts while at 14 days post infection one adult worm was recovered from one of the 8 remaining mice. This count was attributed to the infective larvae escaping irradiation. Irradiation of the third stage larvae at 40,000 rads proved to be a lethal amount of radiation to permit growth and development.

UNIT III - 30,000 RADS

The 4 viability controls of Group I had a 93.75% recovery which was calculated on adult counts of 48, 45, 48 and 47 with an average of 47.00 which was obtained 14 days post infection. This percent recovery of the original infection indicated the third stage larvae of *N. dubius* used for this infection were viable. The 12 experimental animals of Group II were infected with 50 non-irradiated third stage larvae of *N. dubius*. At 7 days post infection

TABLE III

The effects of 30,000 rads of x-radiation on the growth and development of the infective larvae of *Nematospiroides dubius*

GROUP I viability controls	GROUP II non-irradiated		GROUP III irradiated	
adults (14 days)	cysts (7days)	adults (14 days)	cysts (7 days)	adults (14 days)
48	32	47	0	1
48	30	41	0	1
47	28	46	2	0
45	14	48	1	1
		43		0
		49		0
		42		0
		54		1
MEANS				
$\bar{X}$=47.00	$\bar{X}$=26.00	$\bar{X}$=46.25	$\bar{X}$=.75	$\bar{X}$=.50
PERCENT OF RECOVERY				
94.00%	52.00%	92.50%	1.50%	1.00%

the average number of cysts recovered from 4 animals was 26.00 (Table III). The remaining 8 mice were killed and examined for adults 14 days post infection. An average adult count of 46.25 was obtained from this group. Each of the 12 mice of Group III were orally infected with 50 infective larvae irradiated at 30,000 rads. At 7 days post infection 4 mice of this group exhibited an average cyst count of .75. The remaining animals were killed and examined for the adults of N. dubius 14 days post infection. The adult counts averaged .50 and are shown in Table III. Results from Unit III indicate that 30,000 rads of x-radiation is a lethal dosage to permit the infective larvae of N. dubius to develope.

UNIT IV - 20,000 RADS

The viability controls consisting of 4 mice represented by Group I exhibited adult counts of 48, 42, 46 and 51 with an average of 46.75 at 14 days post infection. This results in a 93.50% recovery of the initial infection of 50 non-irradiated infective larvae of N. dubius and demonstrates a viable culture. In Group II each of the 12 experimental animals was administered 50 non-irradiated third stage larvae of N. dubius. At 7 days post infection 4 mice were sacrificed from this group and examined for cysts. The average cyst count was 27.00 (Table IV). On the other hand, adult counts obtained from the remaining

TABLE IV

The effects of 20,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I viability controls	GROUP II non-irradiated		GROUP III irradiated	
adults (14 days)	cysts (7 days)	adults (14 days)	cysts (7 days)	adults (14 days)
48	28	48	4	4
42	34	48	2	2
46	16	47	4	3
51	30	51	2	3
		50		2
		44		4
		42		2
		46		3
MEANS				
$\bar{X}$=46.75	$\bar{X}$=27.00	$\bar{X}$=47.00	$\bar{X}$=3.00	$\bar{X}$=2.88
PERCENT OF RECOVERY				
93.50%	54.00%	94.00%	6.00%	5.76%

8 mice of Group II averaged 47.00. The animals of Group III were infected with larvae irradiated at 20,000 rads. These animals exhibited cyst counts of 4, 2, 4 and 2 (X=3.00) at 7 days post infection while adult counts of the remaining animals at 14 days post infection averaged 2.88 (Table IV). It can be concluded from results obtained in Units I through IV that there is an increase in growth and development of the infective larvae of N. dubius as the amount of radiation decreases although development of the third stage larvae is severely affected at 20,000 rads of x-radiation.

UNIT V - 15,000 RADS

Twenty-eight mice were divided into 3 groups. The 4 mice of Group I (viability controls) had adult counts of 48, 45, 51 and 46 (X=47.50). This demonstrates a 95.00% recovery of the initial infection of 50 non-irradiated N. dubius larvae and indicates a viable culture. The 12 animals of Group II were infected with non-irradiated infective larvae of N. dubius and exhibited an average cyst count of 27.50 7 days post infection. At 14 days post infection 8 mice were killed and examined for adults. The average adult worm count was 46.62 and were calculated on counts listed in Table V. Group III utilized third stage larvae irradiated at 15,000 rads of x-radiation. Four mice were sacrificed and examined for cysts 7 days post infection.

TABLE V

The effects of 15,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I viability controls	GROUP II non-irradiated		GROUP III irradiated	
adults (14 days)	cysts (7 days)	adults (14 days)	cysts (7 days)	adults (14 days)
48	30	44	18	38
45	19	48	19	35
51	33	46	27	39
46	28	45	15	40
		50		37
		46		38
		47		41
		47		36
MEANS				
$\bar{X}$=47.50	$\bar{X}$=27.50	$\bar{X}$=46.62	$\bar{X}$=19.75	$\bar{X}$=38.00
PERCENT OF RECOVERY				
95.00%	55.00%	93.24%	39.50%	76.00%

Cyst counts obtained were 19, 18, 27 and 15 (X=19.75) while an average adult count of 38.00 was obtained from the remaining animals at 14 days post infection. It can be concluded that 15,000 rads of x-radiation affected the development of the larvae only slightly since the majority of worms completed the life cycle to the lumen dwelling stage. Therefore, at 15,000 rads the desired encystment and termination of development was not accomplished. From data thus gathered the desirable dosage lies between 15,000 rads where the larvae were only slightly affected and 20,000 rads which killed the larvae.

The experimental design utilized for the following 4 Units (VI, 16,000, VII, 17,000, VIII, 18,000, IX, 19,000 rads) differed from the design of earlier experiments in this study (Figure I). The new design consisted of 48 mice which were randomly divided into 4 groups. As described previously, viability controls were included in each experimental design for each infection. Adult counts from these various viability controls were within expected limits in each case and proved that the larvae used for infection were viable. The remaining groups were handled in the following manner. Group I (immune controls) consisted of 12 mice which were immunized by the established procedure of 3 oral, stimulating infections of 50 infective larvae of N. dubius with 14 day intervals between infections. Fourteen days post third stimulating infection the animals were administered pyrantel tartarate (60 mg/kg

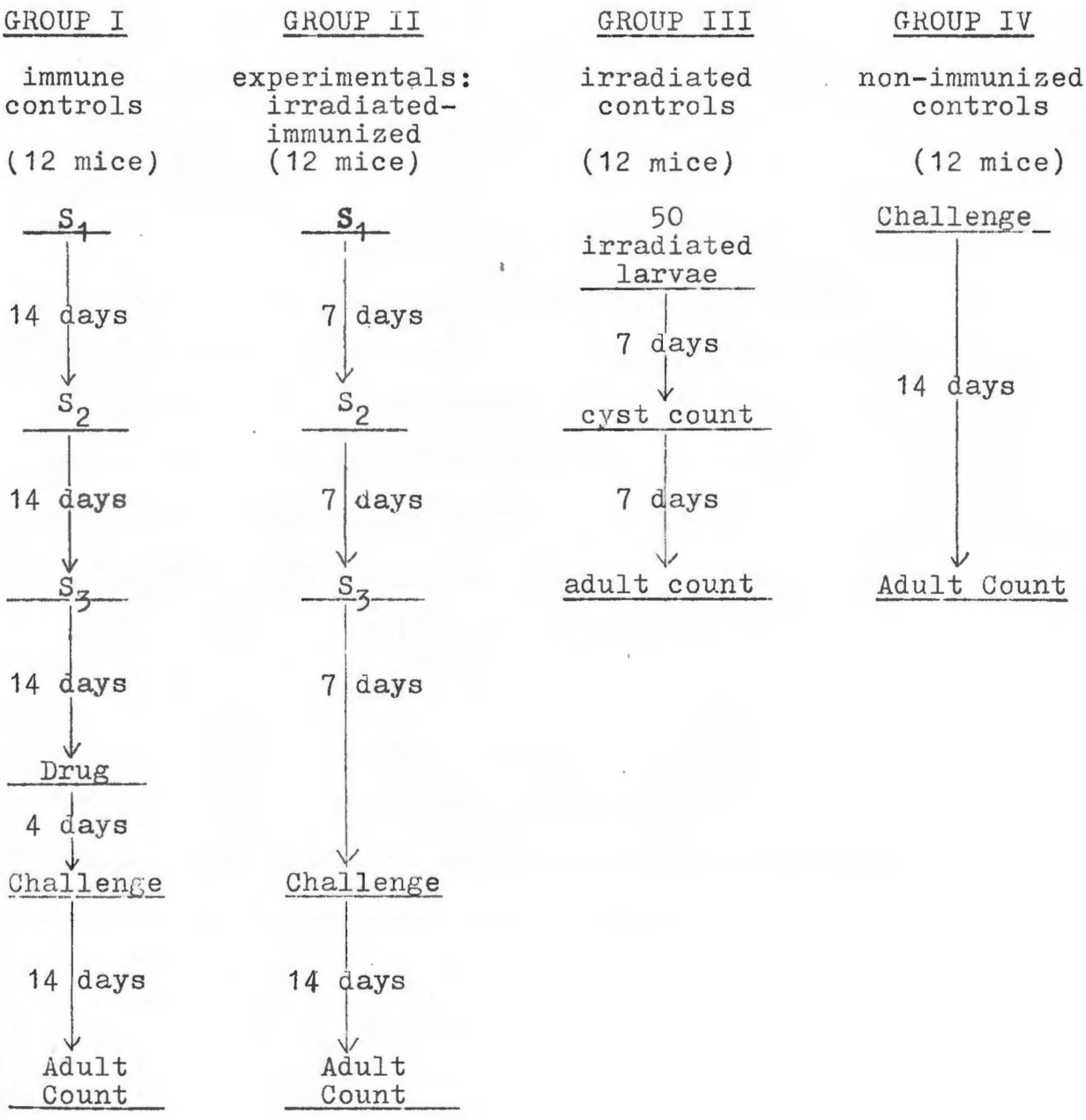
of body weight) which removes the adult parasites from the intestines. Four days after drug treatment the animals were given a challenging oral infection of 50 non-irradiated third stage larvae and sacrificed 14 days post challenge for adult counts.

Group II (experimentals: irradiated-immunized) consisted of 12 mice and were handled as Group I above, except that these animals recieved 3 stimulating oral infection at 7 day intervals with 50 larvae irradiated at the appropriate dosage of x-radiation. Seven days was chosen in that it was hoped there would be no adult worms in the intestine since at the proper dosage of radiation the infective larvae would not develop to the adult stage freeing the intestine of adult worms. If no worms were present, then the drug treatment could be eliminated and the immunization time would be reduced from 14 to 7 days for each stimulating infection. In any event, 7 days after the third infection each animal of this group (II) was challenged with an oral infection of 50 non-irradiated larvae and killed 14 days later and counts were made of the adult N. dubius recovered from the small intestine.

The remaining 2 groups (III and IV) of this experimental series served as controls. The 12 mice of Group III (irradiated controls) received a single oral infection of 50 larvae irradiated at the appropriate dosage of x-radiation. Cyst and adult counts were made at 7 and 14 days respectively as previously described. These counts would

FIGURE II

The experimental design used for Units VI, VII, VIII and IX.



indicate, therefore how many cysts containing developing larvae could be found in the intestinal tissue at 7 days and how many of these larvae had developed to the adult stage as shown by the number of adults recovered from the small intestines at 14 days post infection. Finally, Group IV (non-immunized controls) consisted of 12 mice that received only the challenging infection of 50 non-irradiated larvae each. These animals were killed 14 days after challenge and counts were made of the adult worms recovered from the intestines. Thus, comparisons of the average adult worm counts recovered after the challenging infection between Groups I and IV would indicate whether or not the animals had been immunized, while comparisons between Groups II and IV would determine whether or not the animals of Group II were immunized with irradiated larvae. On the other hand, results from Group III (irradiated controls) would indicate the efficacy of the radiation dosage. The experimental design employed in Units VI, VII, VIII and IX is indicated by Figure II.

UNIT VI - 16,000 RADS

The mice were divided into the various groups described previously. The 12 mice of Group I (immune controls) were given 3 oral infections of 50 non-irradiated *N. dubius* larvae each with 14 day intervals between infections. Fourteen days after the third stimulating infection the

TABLE VI

The effects of 16,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I	GROUP II	GROUP III	GROUP IV
immune controls	experimentals: irradiated-immunized	irradiated controls	non-immunized controls
(adults)	(adults)	(cysts)	(adults)
11	118	24	48
14	120	22	47
25	120	21	44
24	124	29	45
17	120	$\bar{X}$=24.00	50
29	119	%=48.00	41
10	111	(adults)	42
30	107	32	48
21	111	26	51
19	117	30	42
19	118	26	44
18	106	25	48
$\bar{X}$=19.75	$\bar{X}$=115.92	19	$\bar{X}$=45.83
		21	
		27	
		$\bar{X}$=25.75	
		%=51.50	

*$\bar{X}$=mean

%=percent recovery of original infection of 50 N. dubius larvae

animals were administered pyrantel tartrate to remove the adult worms from the small intestines. Four days later, the animals were killed and the average adult worm count recovered from the animals was 19.75 which is comparable to data acquired by others. Group II (experimentals: irradiated-immunized) attempted to utilize larvae irradiated at 16,000 rads to cause active immunization. These animals were given 3 stimulating infections of 50 irradiated infective larvae at 7 day intervals. Seven days post 3rd stimulating infection the animals were challenged with 50 non-irradiated larvae. These mice were sacrificed 14 days later for adult worm counts. The average adult worm count was 115.92 (Table VI). The 12 mice of Group III (irradiated controls) were infected with 50 larvae each that had been irradiated at 16,000 rads. At 7 days post infection, 4 mice were killed and examined for cysts. The average cyst count was 24.00 and is shown in Table VI. The remaining mice were killed 14 days post infection with an average adult count of 25.75 (Table VI). Finally, the 12 mice of Group IV (non-immunized controls) were given only the challenging infection. Fourteen days after the challenge the animals of this group were sacrificed and counts were made of the adult worms recovered from the intestines. The average number of worms recovered from this group was 45.83 (Table VI).

From these results it can be seen that although the

animals of Group I were immunized (average adult count=19.75) the animals of Group II had too many persisting adult worms (X=115.92) from the 3 stimulating infections to determine whether or not immunization had occured as based on the parameters of the experimental design. However, it can be seen that adult worms did develop as indicated by the average number of worms (25.75) recovered from Group III (irradiated controls). Thus, it can be concluded from data of Unit VI that 16,000 rads of x-radiation will not inhibit the development of the majority of N. dubius larvae to the adult stage.

UNIT VII - 17,000 RADS

This experimental design was similar to that of Unit VI (16,000 rads) except that the irradiated larvae used in this Unit were exposed to 17,000 rads of x-radiation. Thus, the average adult worm count recovered from the animals of Group I (immune controls) was 20.50, those of Group II (experimentals: irradiated-immunized) 85.83 and those of Group IV (non-immunized) 45.91. On the other hand, the average cyst and adult counts of the animals of Group III (irradiated controls) was 25.50 and 15.38 respectively (Table VII). It can be concluded therefore that at 17,000 rads of x-radiation the number of larvae developing to adults had decreased in comparison

TABLE VII

The effects of 17,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I	GROUP II	GROUP III	GROUP IV
immune controls	experimentals: irradiated-immunized	irradiated controls	non-immunized controls
(adults)	(adults)	(cysts)	(adults)
19	88	26	45
19	90	28	45
32	88	20	48
30	84	20	40
29	80	$\bar{X}$=23.50	48
21	80	%=47.00	47
14	89	(adults)	47
11	92	11	47
10	81	12	50
21	86	19	51
20	87	14	40
20	85	15	43
$\bar{X}$=20.50	$\bar{X}$=85.83	15	$\bar{X}$=45.91
		19	
		18	
		$\bar{X}$=15.38	
		%=30.76	

*$\bar{X}$=mean

%=percent of recovery of original infection of 50 N. dubius larvae

to counts from similar groups in Units V and VI. Although a trend is indicated, the number of larvae developing to the adult stage is still too high for comparative purposes, to consider 17,000 rads the dosage of radiation needed to immunize mice under the experimental conditions reported here

UNIT VIII - 18,000 RADS

The experimental design of this Unit was similar to that of Units VI and VII with the exception that the irradiated larvae used in this experiment were exposed to 18,000 rads of x-radiation. In this study, the average adult worm counts obtained from animals of Group I (immune controls) was 19.42, those of Group II (experimentals: irradiated-immunized) was 16.17 indicating this group had been immunized while those of Group IV (non-immunized controls) had an average of 45.83 worms. The average cyst and adult counts obtained from the animals of Group III (irradiated controls) was 27.25 and 1.25 respectively (Table VIII,(A)). In addition to the groups noted above, one other control group was included in this study. The 12 mice of this Group V (Table VIIIB) were handled as those of Group II of this Unit, except that 7 days after the 3rd stimulating infection the animals of this group (V) were treated with pyrantel tartrate to remove persisting adult worms that might remain in the intestinal lumen from previous stimulating infections. Four days post drug

TABLE VIII (A)

The effects of 18,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP I	GROUP II	GROUP III	GROUP IV
immune controls	experimentals: irradiated-immunized	irradiated controls	non-immunized controls
(adults)	(adults)	(cysts)	(adults)
21	10	24	45
20	11	23	48
27	30	28	47
10	25	34	44
16	12	$\bar{X}$=27.25	44
19	16	%=54.50	48
18	14	(adults)	49
21	17	1	46
29	10	1	46
27	24	0	46
11	12	1	46
14	13	1	41
$\bar{X}$=19.42	$\bar{X}$=16.17	4	$\bar{X}$=45.83
		2	
		0	
		$\bar{X}$=1.25	
		%=2.50	

*$\bar{X}$=mean

%=percent of recovery of the original infection of 50 N. dubius larvae

TABLE VIII (B)

The effects of 10,000 rads of x-radiation on the growth and development of the infective larvae of Nematospiroides dubius

GROUP V

experimentals: irradiated-immunized; drug treated

(adults)
10
14
26
25
9
14
17
16
21
19
18
11
X=16.67*

*X=mean

treatment, all animals of this group were given a challenging infection of 50 non-irradiated N. dubius larvae each. Fourteen days after challenge, the animals were killed and counts were made of the adult worms recovered from the small intestines. Drug treatment of this group would remove adult N. dubius from the intestines that might persist from the previous stimulating infections since it is possible that x-ray treatment did not prevent adult development of 100% of the irradiated larvae. Thus, comparisons of the average number of worms recovered at challenge between Groups II and V would indicate whether or not persisting adult worms in Group II affected the results from the experimental animals (Group II). Thus, the average number of worms recovered from the challenging infection in Group V was 16.67 (Table VIII (B)) and the difference between the means of Group II (16.17) and Group V (16.67) is not statistically significant (Table X). Thus, it can be concluded from results of Unit VIII that larvae irradiated at 18,000 rads of x-radiation penetrate the intestinal mucosa and remain at least 7 days as demonstrated by cyst counts ($\bar{X}$=27.25: Group III, Table VIII (A)), most larvae proceed no further in growth and development as demonstrated by adult counts ($\bar{X}$=1.25: Group III, Table VIII (A)) and these few larvae that are able to develop to the adult stage do not affect the number of adults recovered at challenge nor contribute in large measure,

to the immunity developed in mice by those infective larvae irradiated at 18,000 rads (X=16.67: Group II, Tables VIII (A) and X). In short, the optimum x-radiation used in this experiment is 18,000 rads and the majority of larvae thus treated are capable of stimulating immunity in mice without further growth and development to the adult stage.

UNIT IX - 19,000 RADS

This experimental design was similar to those of Units VI (16,000 rads) and VII (17,000 rads) except that the irradiated larvae used in this study were exposed to 19,000 rads of x-radiation. The results obtained in this Unit indicate the average adult worm counts recovered from the animals of Group I (immune controls) was 17.08, those of Group II (experimentals: irradiated-immunized) 44.50 and those of Group IV (non-immunized controls) 44.75. Finally, the average cyst and adult counts of the animals of Group III (irradiated controls) was 6.25 and 1.62 respectively (Table IX). In this experiment the results from cyst counts of Group III ($\overline{X}$=6.25) is lower than those of Units VI, VII and VIII indicating fewer irradiated larvae penetrated the intestinal mucosa or continued development once penetration had occured. Secondly, the large average number of worms (44.50) recovered 14 days after challenge from the experimentals (Group II) indicates the irradiated larvae did not

TABLE IX

The effects of 19,000 rads of x-radiation on the growth and development of the infective larvae of Hematospiroides dubius

GROUP I	GROUP II	GROUP III	GROUP IV
immune controls	experimentals: irradiated-immunized	irradiated controls	non-immunized controls
(adults)	(adults)	(cysts)	(adults)
24	44	5	44
11	41	5	41
19	43	8	43
18	43	8	43
11	47	$\bar{X}$=6.25	47
10	45	%=12.50	45
19	44	(adults)	44
18	41	1	41
21	40	1	40
20	52	2	52
21	48	2	48
13	46	1	46
$\bar{X}$=17.08	$\bar{X}$=44.50	4	$\bar{X}$=44.50
		1	
		1	
		$\bar{X}$=1.62	
		%=3.25	

*$\bar{X}$=mean

%=percent of recovery of original infection of 50 N. dubius larvae

TABLE X

A t test at the 5% significance level was preformed between the cysts and adults of the control and experimental groups in Units I-V and between the immune controls and irradiated-immunized controls in Units VI-IX. An extra t test was added in Unit VIII between the irradiated-immunized and irradiated-immunized (drug treated) group.

DESIGN I

GROUP	DEGREES OF FREEDOM	TEST STATISTIC	CRITICAL VALUE	EVALUATION
50,000 r				
cysts	6	7.56	±2.447	Sig.
adults	14	53.12	2.145	Sig.
40,000 r				
cysts	6	17.74	2.447	Sig.
adults	14	49.73	2.145	Sig.
30,000 r				
cysts	6	6.14	2.447	Sig.
adults	14	49.73	2.145	Sig.
20,000 r				
cysts	6	15.09	2.447	Sig.
adults	14	40.85	2.145	Sig.
15,000 r				
cysts	6	1.96	2.447	Not Sig.
adults	14	8.99	2.145	Sig.

DESIGN II

16,000 r				
imm. cont. irr-imm	22	38.62	±2.074	Sig.
17,000 r				
imm. cont. irr-imm	22	28.15	2.074	Sig.
18,000 r				
imm. cont. irr-imm	22	1.25	2.074	Not Sig.
irr-imm irr-imm(drug)	22	.20	2.074	Not Sig.
19,000 r				
imm. cont. irr-imm	22	16.52	2.074	Sig.

immunize this group of animals. Furthermore, comparisons between the means of Group II (44.50) and Group IV (44.75) are obviously not statistically significant. This is even more apparent when comparisons of the adults obtained from the animals of Group II and the small average number of cysts (6.25) that developed in Group III are made. Thus, these results indicate there was an insufficient antigenic stimulus to immunize the experimental animals of Group II. Finally, when the average number of cysts recovered from Units VI, VII and VIII (24.00, 23.50, and 27.25 respectively) are viewed in this light it becomes even more apparent that the experimental animals of Group II were not immunized since the average worm count 14 days after challenge was 44.50 is comparable to the average worm count of 44.75 from the non-immunized controls (Group IV, Table IX). Thus, the results of this experiment indicate that 19,000 rads of x-radiation approaches the lethal dosage range for the third stage larvae of N. dubius.

CONCLUSIONS

This study obtained results similar to those of Jennings et al (1963), Dappen (1965) and Staut (1971) in that it was concluded that the number of migrating larvae is inversely proportional to the level of radiation received. This research investigated the dosage of radiation that could be administered to the infective larvae of N. dubius and the effects that could be studied under the following conditions:

1. larval penetration of the intestinal mucosa
2. encystment for at least 7 days after intestinal tissue penetration
3. no further development of the life cycle after encystment

Nine Units involving radiation levels of 50,000, 40,000, 30,000, 20,000, 19,000, 18,000, 17,000, 16,000, and 15,000 rads were used. Dosage levels of 50,000, 40,000, 30,000 and 20,000 rads were lethal to the third stage larvae as demonstrated by results obtained from the irradiated groups of Units I through IV (Tables I-IV). These high dosages killed the majority of the infective larvae and permitted little intestinal penetration or encystment. At 15,000 rads the results indicated that the development of the infective larvae was only slightly affected. This was found when the adult counts in Group III (Table V, Unit V) were comparable to the controls (Group II, Table V) which utilized non-irradiated larvae. Therefore, the desirable dosage to meet the above conditions was expected to be between 15,000 and 20,000 rads.

Thus, the final phase of this study investigated dosage levels of 16,000, 17,000, 18,000 and 19,000 rads.

At 16,000 and 17,000 rads the dosage had some effect on penetration and encystment of the larvae but did not prevent the establishment of adult N. dubius in the lumen of the small intestines of the experimental animals (Groups II and III, Tables VI and VII). At 18,000 rads however, the irradiated larvae penetrated the intestinal mucosa, encysted without marked development to the adult stage and stimulated a striking degree of immunity as indicated by the average number (16.17) of adult worms recovered from the experimentals 14 days after challenge (Group II, Table VIII (A)). On the other hand, when N. dubius infective larvae are exposed to 19,000 rads, the level of x-radiation approaches the lethal limit. This is evidenced by two observations. First, the small numbers of cysts that develop in the intestinal tissue (Group III, Table IX) and secondly, the high average number of adult worms (44.50) recovered 14 days after challenge in the experimental animals (Group II, Table IX). This latter observation indicates there were smaller numbers of larvae established in the intestinal tissue and hence there was insufficient antigenic stimulation to immunize the animals of this group against a challenging infection. Thus the use of larvae irradiated at 15,000 rads can save 25 days or a 41.66% reduction in comparison to the standard immunization procedure used by Van Zandt (1961), Urquhart (1962), Cypess and Van Zandt (1973), Cypes et al (1973) and Si-Kuang Lie et al (1974).

SUGGESTIONS FOR FURTHER RESEARCH

The major purpose of this study was to investigate and identify specific levels of radiation which would lead to encystment of the larvae with no further development to the adult stage. The findings of this study indicated that larvae irradiated at 18,000 rads penetrate the intestinal mucosa, remain for a period of time and for the most part do not develop to the adult stage. However, this study did not attempt to identify the biochemical effects of irradiation on this biological system. A brief review of related literature (Thornburn, 1972) indicates that one or more factors may be responsible for the results found in the present study. Therefore, it seems reasonable to suggest the importance of further works directed toward the biochemical effects of irradiation of the larvae of N. dubius along the following lines of investigation:

1. enzyme denaturation
2. protein denaturation
3. blockage of metabolic pathways
4. bond breakage in nucleic acids

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