STUDIES ON TRICHINELLA SPIRALIS *

I. Concerning the Time and Site of Insemination of Females of Trichinella spiralis

II. Time of Initial Recovery of Larvae of Trichinella spiralis from Blood of Experimental Animals

III. Effect on the Intestinal Phase of Trichinosis of Feeding Massive Numbers of Irradiated Trichina Larvae

IV. Effect of Feeding Irradiated Trichinella Larvae on Production of Immunity to Re-Infection

V. Tests for a Strain of Trichina Larvae Resistant to Radiation

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I. CONCERNING THE TIME AND SITE OF INSEMINATION OF FEMALES OF TRICHINELLA SPIRALIS

No definite information is available as to the site, in the intestine of the host, of insemination of the adult female *Trichinella spiralis*, i.e., whether it occurs while the adult worms lie free in the intestinal lumen or while either the male or the female, or both, are partially or wholly imbedded in the mucosa. Hemmert-Halswick and Bugge[1] stated that copulation occurs deep in the mucosa of the small intestine, but they were not able to observe it. They fed trichinous meat to rats and found that at 38 hours the seminal vesicle of the male worm was filled with sperms while the vagina of the female contained none, but that at 44 hours the seminal vesicle was empty while the anterior portion of the vagina in the majority of females contained a large quantity of sperms.

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They concluded, therefore, that copulation took place approximately 40 hours after feeding trichinous meat. Gursch[2] found that from 4 to 22 hours after experimental infection of rats, the trichinae were located principally in the mucosa, but that at 24 hours most of them had emerged into the lumen. He believed that this emergence might be for the purpose of copulation.

Method

In order to determine the time and site of insemination of female intestinal trichinae, white rats were tube-fed with excysted trichina larvae and then sacrificed at various intervals from 24 to 96 hours. Immediately after the animal was killed, portions of the small intestine were removed and quickly plunged into hot formalin in order to fix the worms *in situ*. Microscopic sections were made and, from selected blocks, serial sections were cut. From other portions of the intestine, adult male and female worms were removed and examined, both in the living state and after rapid fixation.

Direct observation of the adult trichinae in the small intestine of rats also was attempted between 33 and 36 hours after infection. Four animals, ranging in weight from 320 to 370 gm., were each infected with 10,000 larvae. The animals were narcotized by intramuscular injections of nembutal (0.4 ml. per 100 gm. of body weight) and an incision was made in the abdominal wall. A loop of small intestine 6 inches below the gastric pylorus was delivered, and an incision made along the antimesenteric border, bleeding being controlled by topical application of Thrombin (Parke, Davis & Co.). The animal was placed on a tray, the incised portion of the intestine resting on a piece of lucite. The exposed mucosa was examined under a dissecting microscope at a magnification of $\times$ 30.

Findings

Examination of adult trichinae removed from the intestine of infected rats revealed that insemination had taken place in a large number of female worms 33 hours after the rats were fed excysted larvae, in a few at 32 hours, and in a single worm at 30 hours. In microscopic sections of the small intestine of the infected rats, both the female and the male worms were found partially or deeply imbedded in the mucosa at 33 hours (Figs. 1 and 2). Figure 1 shows an adult male that has burrowed throughout most of its length into a villus, the posterior end (right upper portion) apparently lying outside of the villus. Figure 2 shows the anterior portion of an adult female lying

within the mucosa; the vulval opening may be seen in the left lower portion of the field. Insemination has occurred, and sperms may be seen in the forepart of the uterus. The more caudad portion of the worm is not shown in the figure; in microscopic sections of the intestine, this portion of the gravid female is often exposed in the lumen. Figure 3 shows the full length of the uterine portion of a female lying between two villi, while Figure 4 shows this portion of another inseminated female imbedded in the mucosa. Both of these sections (Figs. 3 and 4) were taken from a rat 33 hours after infection. In Figure 5 the posterior portion of a male (right) and the anterior portion of a female (left) are seen lying between two villi. The more cephalad portion of the male worm has penetrated the mucosa of a villus while the anterior portion of the female appears to be directed into the mucosa of an adjacent villus. The posterior end of the male lies in proximity to the vulval opening (not shown) of the female.

Direct observation of the mucosa of approximately 2 inches of intestine was made in each of 4 rats, for about 45 minutes, but no worms were seen in the lumen or attached to the edematous mucosa. During peristaltic movements it appeared that some fluid was expressed from the mucosa. A drop of the fluid was removed periodically from each rat by means of a capillary tube and examined microscopically. A few worms were seen in fluid from one rat infected 36 hours before. Following the direct observation of the mucosa, portions of the intestine from each of the 4 rats were placed in warm (37° C.) saline solution. When examined within 20 to 30 minutes, large numbers of extremely active worms appeared on the surface of the mucosa and were freed from the mucosa after several minutes. Microscopic examination showed that insemination had taken place in some females. Two of 10 females examined at 33 hours after infection showed a small quantity of sperm in the seminal receptacle. The seminal vesicles and vasa deferentia of 10 males examined showed large quantities of sperm. Each of 6 females examined at 36 hours had been inseminated. To date, we have not observed copulation or actual insemination in living worms or in microscopic sections.

Comment

Figures 1, 2, and 5 lend support to the belief that the male adult is imbedded throughout most of its extent in the mucosa, while a portion of the female cephalad to the vulva is also imbedded in a villus; and that the posterior end of the male and the vulval portion of the female lie between adjacent villi or in the lumen of the intestine.

Inasmuch as increasing numbers of sperms in the seminal receptacle of the females were seen at progressive intervals from 33 hours to 6 days, it seems likely that insemination occurs more than once in the same female worm. At 6 days female worms were seen with seminal receptacle distended with sperms while the forepart of the uterus contained embryos and sperms, indicating one or more inseminations; in addition, a large quantity of sperms in the vagina indicated a subsequent, very recent insemination.

Summary

White rats were fed excysted trichina larvae and were sacrificed at various intervals, from 24 to 96 hours after infection. The partially or fully developed adult forms of *T. spiralis* were removed from the small intestine and examined in the living and fixed states, and in addition microscopic sections of the small intestine of the rat were studied for evidence of the time and site of insemination. The findings indicate that insemination occurs as early as 30 hours after feeding. Our evidence suggests that during insemination a large portion of the adult male (except the posterior end) and the anterior end of the female adult are imbedded in the mucosa of villi, the cloacal portion of the male and the vulval portion of the female lying exposed between adjacent villi. Insemination of females apparently may occur more than once.

II. TIME OF INITIAL RECOVERY OF LARVAE OF TRICHINELLA SPIRALIS FROM BLOOD OF EXPERIMENTAL ANIMALS

In the symptomatology of trichinosis, it is of interest to know how early the larvae begin to invade the muscles of the host. Edney, Arbogast, and Stepp[3] (1953) stated that adult intestinal trichinae begin to produce larvae 6 to 7 days after the infection is established, i.e., after the host ingests infective larvae. However, Dunlap and Weller,[4] in 1933, found trichina embryos in focal myocardial lesions as early as 5 days after feeding and Hemmert-Halswick and Bugge[1] (1934) stated that young trichinae are born continually from the fifth day on. In all of these studies the experimental animal was the white rat. The purpose of the present study was to determine the earliest time at which young larvae of the second generation (first stage Trichinella larvae) may be recovered from the blood of experimentally infected animals of several different species. This information may be useful in indicating the approximate time when the newborn larvae begin to invade the musculature of the host.

Method

White rats, ranging in weight from 170 to 200 gm., were each fed approximately 5,000 or 6,000 excysted larvae of *T. spiralis* by stomach tube. At various periods between 96 to 120 hours thereafter, each animal was lightly anesthetized with ether. Five-tenths to 1 ml. of heparin (1:1,000 solution) was injected into the heart and a few minutes later the animal was more deeply anesthetized. After the thorax was opened, the supravalvular portion of the aorta was incised, permitting the blood to escape from the heart into the thoracic cavity. In this manner from 3 to 11 ml. of fluid blood (average, 7 ml.) was obtained from each animal. The blood was collected in test tubes and was hemolyzed by addition of four volumes of 3 per cent acetic acid. After standing for 10 minutes, the mixture was centrifuged at 2,000 r.p.m. for about 15 minutes. The supernatant was discarded and the sediment again was treated with acetic acid and centrifuged. Thin smears of the sediment were made on glass slides and allowed to dry. From 25 to 50 slides were required for examination of all of the sediment from each specimen of blood. The smears were then stained with Wright's stain and examined under a low-power lens for young larvae. This experiment was carried out by each of two observers working independently in different laboratories.

Examination also was made of blood drawn from the hearts of 5 infected rabbits. Each of 4 rabbits was fed approximately 30,000 excysted trichina larvae by stomach tube and one rabbit was fed approximately 35,000 larvae. Samples of 6 ml. of blood were withdrawn from the heart at various intervals and placed in test tubes containing dried anticoagulant (ammonium and potassium oxalate).

Four dogs (two kennel-raised beagles and two mongrels), all over 1 year of age, weighing from 22 to 27 lb. (10 to 12.3 kg.), were fed 60,000, 300,000, 150,000, and 300,000 isolated larvae, respectively, mixed with ground meat. These large doses were given because of the great resistance of dogs to trichinous infection (Matoff[5]). Five to 10 ml. specimens of venous blood were taken from each dog at each of the following intervals: 96, 110, 114, 117, 120, 144, and 168 hours. All of the dogs tolerated the infections unusually well, although 2 dogs showed transient diarrhea during the first day or two of infection.

Four monkeys (*Macacca rhesus*),* ranging in weight from 3.31 to 3.60 kg., were each fed 5,000 trichina larvae by means of a catheter passed transnasally. This dose was intermediate between a lethal dose

* These animals were made available through the courtesy of Dr. Gordon C. Brown, Associate Professor of Epidemiology, School of Public Health, University of Michigan.

(5 larvae per gm. of body weight, according to McCoy[6]) and one that produced edema, fever, and eosinophilia in monkeys (one larva per gm. of body weight, according to Welt[7]). Specimens of 5 to 10 ml. of blood were taken from the heart at the same intervals after infection, as from the dogs. One animal died on the seventh day, the other 3 were sacrificed after 11, 14, and 18 days, respectively, and the small and large bowels were examined for adult trichinae.

Altogether, approximately 3,500 smears were examined.

Results

Rats. Table I indicates that no larvae were recovered up to 110½ hours after feeding rats excysted larvae; beginning at 114 hours and up to 144 hours, larvae were found in the blood of each of 11 rats. The average number of larvae recovered per ml. of blood at the various intervals was as follows: 114 to 116 hours, 1.2; 118 to 120 hours, 1.8; 124 hours, 9; 144 hours, 18 (Text-fig. 1).

TABLE I

Number of Trichina Larvae Recovered from Blood of White Rats at Various Periods after Infection with Excysted Trichinellae

Rat no.*	Ml. of blood collected	Hours after feeding	Number of larvae recovered from blood
	Results of observer A		
1, 2	7.5, 8.5	52	0, 0
3, 4	7.5, 8	76	0, 0
5, 6	7.5, 7.5	100	0, 0
7, 8	6.5, 10	110½	0, 0
9, 10	7, 8	116	2, 18
11, 12	9.5, 11	120	14, 19
13, 14	6, 6	124	41, 66
15, 16	10, 10.5	144	150, 216
	Results of observer B		
17	5	96	0
18	5	102	0
19–22	6 (av.)	106	0
23–26	7.5 (av.)	110	0
27	4.5	114	5
28	3	116	2
29	6	118	26

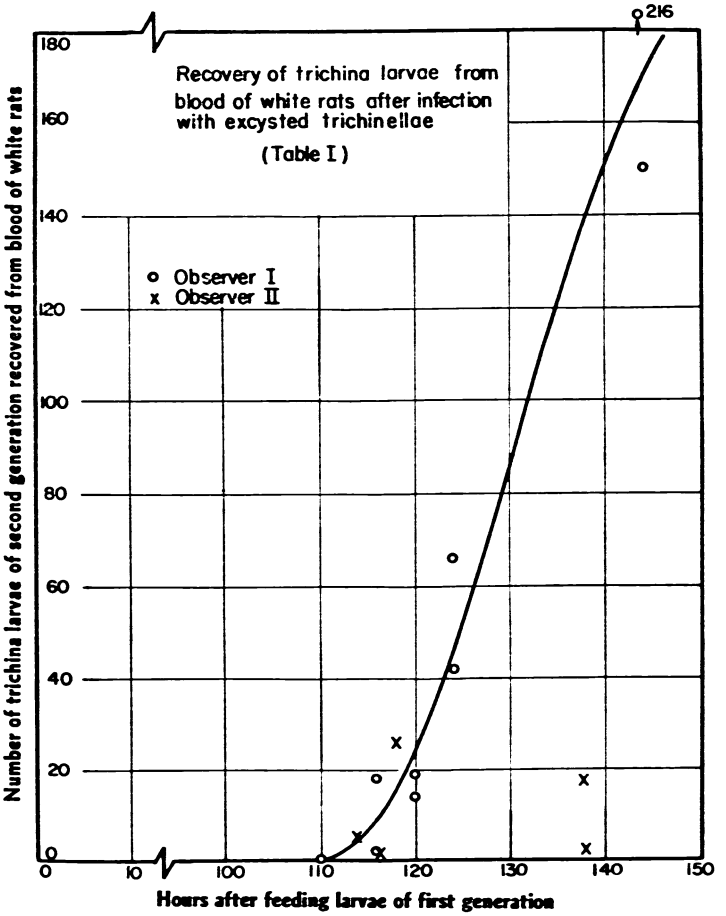
* Rats 1 to 6 and 13 to 29 were each fed 5,000 excysted larvae; rats 7 to 12 were each fed 6,000 larvae.

TABLE II

Larvae Recovered from 6 ml. Blood of Rabbits after Various Intervals Following Infection

Rabbit no.*	Number of larvae recovered from blood at designated time after infection							
	96 hrs.	108 hrs.	110 hrs	114 hrs.	116 hrs.	120 hrs.	132 hrs.	144 hrs.
64	0	0				18	16	25
67	0	0				6	16	18
70	0	0				1	2	6
74	0	0				0	1	5
80			0	2	2	5		

* Rabbits 64, 67, 70, and 74 were each fed 30,000 excysted larvae; rabbit 80 was fed 35,000 larvae. Rabbit 67 (weight, 1,300 gm.) died 30 days after infection; one adult trichina was found in the intestine and 1,173,500 larvae were recovered from the muscles.



Text-figure 1. Recovery of trichina larvae from the blood of white rats after infection with excysted trichinellae (Table I).

Rabbits. Table II indicates that no larvae were recovered from the blood of 4 rabbits at 108 hours. In a fifth rabbit none were seen at 110 hours but 2 were found at 114 hours. In 6 ml. specimens of blood from these animals the average number of larvae found at 120 hours was 6; at 132 hours, 9; and at 144 hours, 13.5.

Dogs. Only one larva was found in all the smears of blood from the

TABLE III

Larvae Recovered from Muscles and from Blood of 4 Dogs Infected with Excysted Trichinellae

Dog no.	No. of larvae fed	Day of death	Weight at death	Larvae recovered from muscles			Larvae recovered from blood at 96, 110, 114, 117, 120, 144, 168 hrs.	Remarks
				Gm. of muscle	Muscle	No. of larvae		
1	60,000	30	24 lb., 10.9 kg.	38 52 68	Tongue Diaphragm Thigh	45 42 30	None	No adult trichinae in small or large bowel
2	300,000	92	27 lb., 12.3 kg.	42 46 76	Tongue Diaphragm Thigh	750 332 444	None	Biopsy (50 sections) and digestion of 2 gm. of gluteus maximus at 15 days: no evidence of old trichinosis
3	150,000		22 lb., 10 kg.		Not examined		None	
4	300,000	31	25 lb., 11.4 kg.	34 28 119	Tongue Diaphragm Thigh	624 180 1297	1 larva at 120 hours	No adult trichinae found in small or large intestine

4 dogs. This larva was seen in a smear of blood obtained at 120 hours. The numbers of larvae recovered from several muscles of these dogs are listed in Table III.

Monkeys. One larva was recovered from one animal at 120 hours and 1 to 9 larvae were recovered from all 4 animals at 144 hours (Table IV).

Comment

The findings in the rats and rabbits are in good agreement.

The negative results in 3 of the 4 dogs are to be explained by the great natural resistance of adult dogs to trichinous infection. This is borne out by recovery of relatively few larvae on digestion of the skeletal muscles of the dogs. If it is assumed that the weight of the skeletal musculature of the dog is approximately 40 per cent

of the total weight of the body, the calculated numbers of larvae in the muscles of the dogs would be: dog 1, 3,000; dog 2, 44,000; and dog 4, 52,000. In each instance, the calculated number of larvae in the muscles is considerably less than the number fed to the animal. On the other hand, in rats, guinea-pigs, and rabbits, the number of larvae recoverable from the muscles is from 100 to 1,000 times the number fed.

Because monkeys do not tolerate a heavy infection, they were given a light dose. Like the dogs, the monkeys may be regarded as having had relatively few first stage larvae circulating in the blood stream at the time the specimens of blood were obtained. Furthermore, the specimens of blood collected from the dogs and monkeys represented a small portion of the total blood volume while the specimens obtained from the rats represented the entire volume of blood that could be collected. The experiment on the dogs and monkeys would need to be extended in order to determine whether the 6-hour delay in earliest recovery of larvae (120 hours) from the blood of the dogs and the monkeys, compared to that from the blood of white rats and rabbits (114 hours), represents a slower development of reproduction in the adult female trichinae within the larger animals, or is merely a reflection of a low concentration of the first stage larvae in the blood of the dogs and monkeys tested.

TABLE IV

Number of Trichina Larvae Recovered from Blood of Rhesus Monkeys at Various Periods after Infection

Monkey no	Day killed or died (d)	Adult trichinae recovered		Larvae recovered from blood after designated time						
		Small intestine	Large intestine	96 hrs.	110 hrs.	114 hrs.	117 hrs.	120 hrs.	144 hrs.	168 hrs.
1	d 7	119	0	0	0	0	0	0	2	1
2	11	467	9 females	0	0	0	0	0	9	6
3	14	1348	1 male, 5 females	0	0	0	0	1	2	2
4	19	341	2 females	0	0	0	0	0	1	5

Summary

Twenty-nine adult white rats, 5 rabbits, 4 dogs, and 4 rhesus monkeys were infected with counted numbers of excysted Trichinella larvae. At various intervals ranging from 96 to 168 hours after infection, specimens of venous or cardiac blood were

examined for first stage larvae. In the rat and rabbit, first stage Trichinella larvae were recovered earliest at 114 hours after negative results at 110 hours; in the dog and monkey, larvae were recovered earliest at 120 hours after negative results at 117 hours.

Conclusion

First stage larvae of *T. spiralis* are present in the blood of white rats, rabbits, dogs, and rhesus monkeys in the latter part of the fifth day after infection of these animals with excysted larvae.

III. EFFECT ON THE INTESTINAL PHASE OF TRICHINOSIS OF FEEDING MASSIVE NUMBERS OF IRRADIATED TRICHINA LARVAE

In previous experiments[8] it was shown that a dose of 18,000 r. of cobalt-60 applied to trichinous muscle prevented over 99 per cent of larvae of *T. spiralis* from developing to adult worms, when the irradiated larvae were fed to white rats. This dose, however, does not kill the larvae and one may ask if a sufficiently large number of muscle larvae present in pork exposed to this dose of cobalt-60, upon being ingested with the meat, would produce irritation of the intestinal tract (enteritis) and ill effects in the host; or, specifically, if a person were to eat raw or undercooked trichinous pork that had been subjected in all parts to a dose of at least 18,000 r. cobalt-60, would it be likely that he would suffer ill or serious effects as a result of intestinal irritation by the ingested larvae? An indirect answer to this question was sought in the present experiments on the effects of feeding rats irradiated larvae in massive numbers equal to or greater than the number of non-irradiated larvae that cause fatal infection.

This investigation is in line with a recommendation included in the report of the Second National Conference on Trichinosis[9] that a study be conducted on the effect of gamma irradiation of trichinous meat on the enteric phase of trichinosis. The results of such a study should be of interest in connection with the proposal[10] that all raw pork be exposed to gamma radiation as a practical means of controlling trichinosis in man and pig.

Experiment 1. Effect of Feeding 10,000 Non-irradiated Larvae Upon Survival of Rats

McCoy[11,12] found that the minimal lethal dose of trichina larvae that usually kills white rats is about 40 per gm. of body weight. A preliminary experiment was carried out to test the effect of feeding

10,000 non-irradiated larvae upon survival of 6 white rats weighing 125 to 150 gm. (67 to 80 larvae per gm. of body weight) for 30 days.

Results. Three of the animals died within 13 days and all showed evidence of having had diarrhea. Three survived the test period of 30 days, and 2 of these showed evidence of diarrhea. In one animal that died after 4 days, 10,000 adult worms were recovered from the small bowel; in one that died after 6 days, 6,400 adult worms were found in the small intestine, and in one rat that died after 13 days, 5,000 adult worms were recovered. The remaining 3 rats were sacrificed on the 30th day. On examination of the contents of the small intestines, 0, 1,800, and 3 adult trichinae, respectively, were found; and on digestion of eviscerated, skinned carcasses, 73,920, 128,520, and 75,600 muscle larvae, respectively, were recovered.

Summary. Feeding of 10,000 non-irradiated trichina larvae to 6 rats (weight, 125 to 150 gm.) resulted in diarrhea in 5 rats and in death of 3 of the 6 rats during the intestinal stage.

Experiment 2. Effect of Feeding Rats with 12,000 Larvae Exposed to 10,000 r. Cobalt-60

In a second experiment, trichina larvae were obtained by digestion of the ground muscles of a white rat that had been infected 3 months previously. A portion of the yield of larvae was exposed ***in vitro*** in tap water in a lusteroid tube to 10,000 r. cobalt-60 from a kilocurie source (radiation rate, 1,333 r. per minute). The remaining larvae were not irradiated. This dose of cobalt-60 (gamma rays) applied to trichina larvae ***in vitro*** produces sexual sterilization of most of the developing adult worms.[8] Inasmuch as the rats available for this experiment weighed more than those in experiment 1, it was decided to increase the number of larvae to be fed to each rat to 12,000. In one group of 12 rats (weight, 200 to 250 gm.), each was fed 12,000 non-irradiated larvae (48 to 60 larvae per gm. of body weight), presumably a lethal infective dose; and in another group of 12 rats of the same weight, each was fed 12,000 larvae that had been irradiated with 10,000 r. cobalt-60.

Results. All 12 rats fed non-irradiated larvae (Table V) died within 24 days (4 at 2 days, 1 at 12 days, 2 at 14 days, 2 at 15 days, 1 at 16 days, 1 at 18 days, and 1 at 24 days; average length of life, 11 days). The average weight of these rats at the time of feeding was 238 gm. and at the time of death, 140 gm. The average loss of weight, therefore, was 98 gm., representing 41 per cent of the average original weight. In all rats the loss of weight was greatest during the first week

TABLE V

Effect on Intestinal Phase of Trichinosis of Feeding Rats Massive Numbers (12,000 or 24,000) of Trichina Larvae Exposed to 10,000 r. or 18,000 r. Cobalt-60

Exp. no.	No. of larvae fed	Dose Co^{60} to larvae	No. of rats	Day rats died (d) or were killed (k)	No. of rats with diarrhea	Av. % loss (−) or gain (+) in weight after 1 wk.	No. of adult trichinae recovered from intestine of rats	No. of larvae recovered on digestion of muscles (after 17th day)
2	12,000	0	12 control: 4	d 2	10		4,000–7,020, av. 5,246	—
			7	d 12–18			4,136–10,000, av. 6,925	4,600 (18 da.)
			1	d 24			3,250	197,000
	12,000	10,000 r.	12 test	k 31	6		11 rats, 0; 1 rat, 2	0–620, av. 226
3	12,000	0	3 control	d 11, 12, 21	3	−27	170, 7,360, 998, av. 2,843	—, —, 187,000
	12,000	18,000 r.	10 test: 2	d 7, 7	0	+ 9	0	—, —
			8	k 30		+ 6	0	6 rats, 0; 1 rat, 12; 1 rat, 45
4	24,000	0	3 control	d 5, 6, 13	3	−21	7,000, 7,650, 3,400, av. 6,017	—, —, —
	24,000	18,000 r.	10 test: 1	d 4	1	−16 (4 da.)	0	—
			1	k 8	0	− 3	3	—
			8	k 43	0	+14	0	8 rats, 0
5	12,000	0	15 control: 12	d 2–6	9		1,470–12,000, av. 7,255	—
			1	k 12			5,160	—
			1	d 15			6,176	—
			1	d 25			350	54,000
	12,000	10,000 r.	15 test: 3	k 6	4		700–3,129, av. 2,273	—
			1	d 12			1,080	—
			2	k 12			1,050, 2,600 (av. 860)	—
			3	k 18			1, 44, 382	0, 0, 0
			1	d 19			594	6
			3	k 24			0, 0, 5	136, 506, 980 (av. 588, 1,259 694)
			2	k 33			0, 0	

(average loss, 26 per cent) but continued in successive weeks until death. Necropsy revealed adult worms in the small intestine of all rats, the number of adult trichinae recovered ranging from 3,250 to 10,000 (average number recovered, 6,060). No muscle larvae were recovered in any of the rats dying on the 16th day or earlier, inasmuch as muscle larvae do not resist digestion until the 18th day or later. In the rat that died on the 18th day, 4,600 muscle larvae were recovered and in the animal that died on the 24th day, approximately 197,000 larvae were recovered from the muscles. All but 3 rats showed evidence of diarrhea within 48 hours; one of these 3 had diarrhea at 6 days, and in 2 no indication of diarrhea was observed.

All of the 12 rats that were fed irradiated larvae survived the test period of 31 days. Evidence of diarrhea, however, was noted in 6 rats (4 at 3 days, 1 at 5 days, and 1 at 7 days), but all of these soon recovered and by the eighth day appeared well. The average weight of the rats was 223 gm. at the time of feeding, 171 gm. 1 week later, 178 gm. 2 weeks later, and 230 gm. at the time of sacrifice 31 days after feeding. All 12 rats lost weight during the first week, the average loss being 52 gm. (23 per cent). The animals began to regain weight during the second week, and at 31 days had recovered their original weight and appeared active and healthy. No gross pathologic changes were noted at necropsy. Adult trichinae were recovered from the small intestine of only one of the 12 rats and in this rat only 2 adult worms were found (Table V). Trichina larvae of the second generation were recovered from the skeletal muscles of 10 of the 12 rats, the number of muscle larvae ranging from 0 to 620. The average number of the larvae recovered from the muscles in the 12 rats was 226.

Summary. Twelve control rats all died following an infective dose of 12,000 non-irradiated larvae (48 to 60 larvae per gm. of body weight). All 12 animals developed severe dehydration and marked loss of weight, and in 10 evidence of diarrhea was observed.

Twelve test rats, fed the same number of larvae that had been irradiated with 10,000 r. cobalt-60, survived a 31-day test period. These rats developed, at most, only mild irritation of the intestinal tract (transient diarrhea in 6 of 12 rats) and only slight loss of weight during the first week of infection.

Exposure of trichina larvae *in vitro* to 10,000 r. cobalt-60 prevented reproduction in most of the developing adult trichinae. In 12 rats, on the average, only 226 muscle larvae of the second generation were recovered, compared to an expected recovery ranging from 100,000 to 500,000 in rats fed the same number of non-irradiated larvae.

Experiment 3. Effect of Feeding Rats 12,000 Larvae Exposed to 18,000 r. Cobalt-60

Experiment 2 was repeated but the amount of irradiation applied to the larvae was increased to 18,000 r. Ten test rats (average weight, 171 gm.) were each fed 12,000 larvae (average, 70 larvae per gm.) that had been exposed to 18,000 r. cobalt-60, and 3 control rats (weighing 173, 172, and 182 gm.; average, 176 gm.) were fed the same number of non-irradiated larvae. During the first week the control rats showed evidence of diarrhea, appeared sluggish, did not respond readily to mechanical stimulation, and lost weight to 110, 102, and 172 gm., respectively (average, 128 gm.; average loss of weight, 27 per cent). One of the 3 control rats died on the 11th day, one on the 12th day, and the third on the 21st day; their intestinal contents had 170, 7,360, and 998 adult worms, respectively.

None of the 10 test animals showed any evidence of diarrhea, sluggishness, or lack of reactivity to mechanical stimuli, and none showed any loss of weight. Actually, after 1 week, the animals had an average gain in weight of 7 per cent. Two of the animals died after 7 days from causes apparently unrelated to trichinous infection. At the time of death, neither of the 2 that died at 7 days and none of the 8 that were sacrificed at 30 days revealed any adult worms in the intestinal tract. In 2 of the 8 that survived for 30 days, a few (12 and 45) larvae were recovered on digestion of their muscles. On the other hand, the control rat that died 21 days after being fed non-irradiated larvae yielded 187,000 larvae on digestion of its muscles.

Summary. No evidence of diarrhea was observed in any of 10 rats fed 12,000 trichina larvae (average, 68 larvae per gm. of body weight) exposed to 18,000 r. cobalt-60, a dose of irradiation that prevents the vast majority of larvae from maturing to adult forms. Despite the large number of larvae fed, the irradiated larvae and the adult forms that developed from them did not induce diarrhea and were quickly lost from the intestinal tract of the host.

Experiment 4. Effect of Feeding Rats 24,000 Larvae Exposed to 18,000 r. Cobalt-60

Experiment 3 was then repeated, feeding 24,000 instead of 12,000 larvae. Three control rats were fed non-irradiated larvae and 10 test rats were fed larvae exposed to 18,000 r. cobalt-60. All 3 control rats (average weight, 151 gm.; average number of larvae fed, 159 per gm.) developed severe diarrhea and died from intestinal effects of the infection. One died on the 5th day, one on the 6th day, and one on the

13th day. The average weight at death was 109 gm. The number of adult trichinae recovered from the small intestines of these rats was 7,000, 7,650, and 3,400, respectively.

One of the test rats, apparently in good condition, was sacrificed on the eighth day in order to examine the small intestine for adult trichinae. Three adult worms were recovered, 2 of which were females and one male; one of the females contained embryos and one showed no sperms in the receptaculum seminis.

Of the remaining 9 test rats (average weight, 147 gm.; average number of larvae fed, 163 per gm.), only one developed diarrhea and this animal died on the fourth day. No adult worms, however, were found in the intestinal tract of this rat. The other 8 test rats gained steadily in weight, their average initial weight being 144 gm. before infection, 164 gm. 1 week after infection, and 251 gm. 43 days after infection. No adult trichinae were recovered from the small intestine of any of these 8 rats.

Comments. As would be expected from the previous findings in experiment 3 in which the 3 control rats died (11 days, 12 days, and 21 days, respectively; average, 15 days) after being fed 12,000 non-irradiated larvae, in the present experiment the 3 control rats, fed 24,000 non-irradiated larvae, died sooner (5 days, 6 days, and 13 days, respectively; average, 8 days).

The cause of death in one test rat that died on the fourth day after being fed non-irradiated larvae is not clear; this rat developed diarrhea and lost 27 gm. in weight from the time of infection until death, but a thorough examination of the intestinal contents at the time of death, 4 days after infection, did not reveal the presence of any adult trichinae. In the test rat sacrificed at 8 days only 3 adult worms were recovered, compared to the average of 6,016 adults recovered from the 3 control rats that died on the 5th, 6th, and 13th days.

Summary. Nine of 10 rats, each fed 24,000 larvae exposed to 18,000 r. cobalt-60, showed no evidence of diarrhea and their weight increased steadily. In all 10 test rats the trichinae disappeared rapidly from the small intestine.

Experiment 5. Do Irradiated Larvae Disappear from the Intestine of Rats More Rapidly than Non-irradiated Larvae?

To answer this question, experiment 2 was repeated employing 30 rats, each of 15 test animals being fed 12,000 larvae irradiated with 10,000 r. cobalt-60 and each of 15 control animals being fed 12,000 non-irradiated larvae. The rats ranged in weight from 200 to 250 gm.;

the number of larvae fed was 48 to 60 per gm. of body weight. It was planned to sacrifice 3 rats of each group at intervals of 6 days. At death the intestinal contents of the rats were examined for recovery of adult worms. All animals were weighed and their appearance noted at the beginning of the experiment and at the time of death. At necropsy, tissue was taken from the tongue, heart, lungs, diaphragm, liver, spleen, small intestine, and skeletal muscles for microscopic section for evidence of trichinous infection or other disease. From rats surviving for 18 days or more, the muscles were macerated and digested for the presence and number of muscle larvae.

Results. Of the 15 rats fed non-irradiated larvae, all but one died within 25 days (3 at 2 days, 6 at 3 days, 1 at 5 days, 2 at 6 days, 1 at 15 days, and 1 at 25 days; average number of days of survival, 6); the remaining animal was sacrificed at 12 days. Among 12 rats that died within 12 days, the average number of adult worms recovered was 7,255 (Table V). In the rat that survived to the 25th day, only 350 adult worms were recovered from the intestine and 54,000 larvae were recovered on digestion of the muscles.

Of the 15 rats that were fed irradiated larvae, 2 died (1 at 12 days and 1 at 19 days). The others were sacrificed at various intervals (3 at 6 days, 2 at 12 days, 3 at 18 days, 3 at 24 days, and 2 at 33 days). From the 6 rats that were examined at 6 or 12 days, 1,925 adult worms, on the average, were recovered from the small intestine; of 4 rats examined at 18 or 19 days, the average of the numbers of adult worms found was 155; of the 5 rats examined at 24 or 33 days, 4 had no adult worms and 1 had 5 adults. The average number of trichina larvae recovered on digestion of the muscles of these 5 rats was 694.

After white rats were fed 12,000 trichina larvae, the average number of adult worms recovered from the small intestine 12 to 18 days after feeding was 6,925 in animals fed non-irradiated larvae (9 control rats in experiments 1 and 2), and 860 in those fed larvae that had been irradiated with 10,000 r. cobalt-60 (6 test rats in experiment 2).

Summary. When trichina larvae irradiated with 10,000 r. cobalt-60 were fed to 15 white rats, the developing intestinal forms disappeared more rapidly from the small bowel at all intervals examined (from 6 to 33 days) than adult worms that developed from non-irradiated larvae fed to 15 control rats. A few irradiated larvae were able to develop sexually and reproduce, since small numbers of larvae (average, 694 larvae) of the second generation were recovered on digestion of the muscles of 5 experimental animals 24 to 33 days after infection.

General Summary

White rats fed 12,000 non-irradiated excysted larvae of *T. spiralis* (48 to 60 larvae per gm. of body weight) died, apparently mainly as a result of the intestinal phase of the infection. The feeding of the same number of larvae that had been exposed to 10,000 r. cobalt-60 merely caused transient diarrhea in some animals. The feeding of 12,000 larvae or of 24,000 larvae that had been exposed to 18,000 r. cobalt-60 generally (in 19 of 20 rats) caused no diarrhea and permitted the animals to gain steadily in weight.

In all rats fed irradiated larvae (10,000 r. or 18,000 r.), the parasites were lost rapidly from the intestinal tract.

It is inferred, therefore, that if a person were to eat raw or undercooked, heavily trichinosed pork that was irradiated in all parts with at least 18,000 r. cobalt-60, he would suffer little or no ill effect from irritation of the intestine by the larvae, and that the parasites would disappear rapidly from the intestinal tract.

IV. EFFECT OF FEEDING IRRADIATED TRICHINELLA LARVAE ON PRODUCTION OF IMMUNITY TO RE-INFECTION

In 1942, Levin and Evans[13] induced immunity in rats to re-infection by *T. spiralis* by feeding the rats larvae that had been irradiated with 3,250 to 3,750 r. of x-ray. This amount of x-radiation allowed the larvae to grow to maturity but most of the adult worms were sterile, so that very few larvae of the second generation were recoverable from the muscles of the host. These observers concluded that the origin of the mechanism of immunity against re-infection is located in the intestine.

The purpose of the present experiment was to determine if rats initially infected with larvae that had been irradiated with cobalt-60 would develop immunity to re-infection with non-irradiated larvae. The following doses of cobalt-60 were applied to the larvae: (1) 10,000 r., which produces sexual sterilization of most of the adult worms, and (2) 18,000 r., which prevents most of the larvae from maturing to adult forms. The findings of this experiment should be of interest in connection with the possible development of immunity to trichinosis in man as a result of eating trichinous pork irradiated with cobalt-60 (Gould, Gomberg, and Bethell[10]).

Method

Eight groups of white rats were employed ranging in weight from 150 to 175 gm. Each rat was fed 3,700 trichinae (average, 20 to 22 larvae per gm. of body weight). At the time of death of the animals, the number of intestinal trichinae was determined; in rats that lived more than 17 days after a primary infection, the number of larvae in the skeletal muscles also was determined. Each group of rats was divided into two lots and subsequent observation and examinations on each lot were carried out independently by workers in each of two different laboratories.

Group I (control) consisted of 15 rats each given a single infection with 3,700 non-irradiated larvae.

Groups II (12 rats), III (7 rats), and IV (12 rats) were re-infected with non-irradiated larvae 40 days after receiving an initial infection with (a) non-irradiated larvae, (b) larvae exposed to 10,000 r. cobalt-60, and (c) larvae exposed to 18,000 r. cobalt-60, respectively. Group II served as a control on immunity developed through an ordinary infection.

Group V (control) consisted of 12 rats each fed 3,700 non-irradiated larvae from the batch used to re-infect rats of Groups II, III, and IV.

In groups VI (17 rats), VII (15 rats), and VIII (17 rats) the same scheme was followed as in groups II, III, and IV except that rats of group VII initially received larvae exposed to 6,000 r. and those of group VIII initially received larvae exposed to 12,000 r. cobalt-60. Group VI was a control like group II, in which the animals were infected initially with non-irradiated larvae. As before, 40 days after the initial infection each rat in all three groups was re-infected with 3,700 non-irradiated larvae.

Findings and Comments

Table VI indicates the results. (Also see Text-fig. 2.)

Two rats of group I (controls with single infection) had some adults in the intestine at 15 days and one of 2 rats had a few trichinae at 20 days. These findings are in agreement with those expected, inasmuch as adult worms generally disappear by the 16th day from the small intestine of rats fed a moderate number of larvae (up to 20 larvae per gm.[14]).

Rats of group II (re-infected controls) showed relatively few or no trichinae at the various intervals examined. Thus, of 7 rats sacrificed between 4 and 10 days after infection, only 3 had any larvae (8, 4, and 52). The average number in these 7 rats was 9 compared to an average

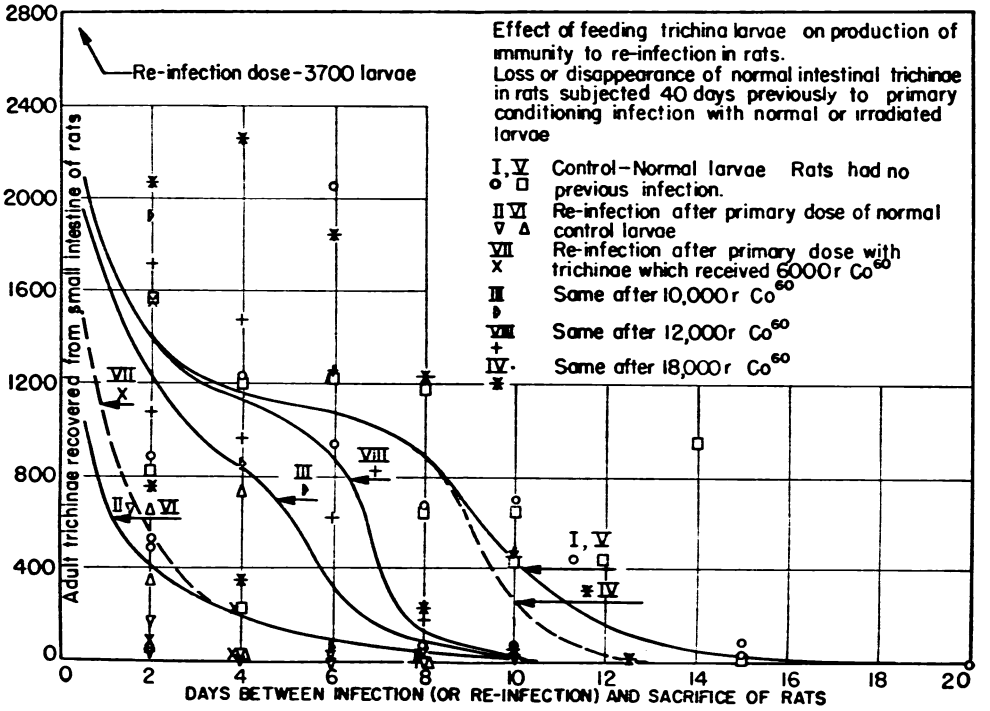
TABLE VI

Experiment 2. Effect of Feeding Rats Irradiated Trichina Larvae on Production of Immunity to Re-infection (Infective Dose 3,700 Larvae). (1) Rate of Disappearance of Intestinal Trichinae, (2) Number of Muscle Larvae Recovered from Rats, after Primary Infection and after Re-infection

Days after infection or re-infection that rat was sacrificed*	Group I Control rats fed non-irradiated larvae		Test rats initially fed larvae exposed to designated amount of Co^{60}; re-infected after 40 days with non-irradiated larvae						Group V — Control rats infected with same lot of larvae used to re-infect rats of groups II, III, IV		Rats initially fed larvae exposed to designated amount of Co^{60}; re-infected after 40 days with non-irradiated larvae		
			Group II — No Co^{60}		Group III — 10,000 r. Co^{60}		Group IV — 18,000 r. Co^{60}				Group VI No Co^{60}	Group VII 6,000 r. Co^{60}	Group VIII 12,000 r. Co^{60}
	Adult trichinae in intestine	Larvae recovered from muscles	Adult trichinae in intestine	Larvae recovered from muscles	Adult trichinae in intestine	Larvae recovered from muscles	Adult trichinae in intestine	Larvae recovered from muscles	Adult trichinae in intestine	Larvae recovered from muscles	Number of adult trichinae recovered from the small intestine		
2	883 525 495		17 165	181,000 32,300	1,912	15	2,060 756	– 0	1,576 816		63 357	75 1,567	1,066 1,705
4	1,226 1,177		0 8	166,000 25,800	848	11	357 2,248	18 2	208 1,187		1 733	21 230	951 1,479
6	2,100 871		0 4	164,000 47,240	42	0	939 1,844	0 0	1,211		2 1	0 0	623 1,248
8	667		52 0	101,000 640,000	9	0	220 1,240	27 3	1,185 622		100 0	0 0	17 760
10	700 72		0	156,000	0	22	545 14	0 –	649 436		0 0	0 0	186 0
12												0	
13							2	–					
14									952				
15	94 40		0 0	190,000	0	0			11		5 0	0 0	12 0
18												0	
20	0 6	18,000 23,400	0	96,000	0	496	0	28,000	1	3,000 53,000	0,0 0,0	0	0 0
25											0		0,0,17
34	0	158,000											

* Rats of groups I and V received only one infection and were sacrificed on the indicated day after infection; all other rats were infected twice and were sacrificed on the indicated day after re-infection.

of 973 in 7 rats of group I sacrificed during the same period. This finding indicates that the primary infection with non-irradiated larvae in rats of group II was effective in establishing a high degree of immunity to re-infection (see McCoy[11]). McCoy[14] found that in rats rendered immune to trichinosis, subsequent feeding of larvae resulted in rapid loss of the trichinae from the intestines 8 to 18 hours after feeding.



Text-figure 2. Effect of feeding trichina larvae on production of immunity to re-infection in rats.

Rats of group III similarly showed that some evidence of immunity was established by feeding larvae exposed to 10,000 r. cobalt-60. The number of adult worms recovered from the intestine appeared to be diminished in the rats sacrificed 6, 8, and 10 days after infection, as compared to rats of group I. It is recognized, of course, that the number of test animals is too small to permit definite conclusions to be drawn.

On the other hand, no reduction was noted in the number of intestinal trichinae recovered from rats of group IV, fed with larvae irradiated with 18,000 r. cobalt-60.

The findings in group V (controls with single infection) are in essential agreement with those in group I.

The findings in group VI (re-infected controls) generally are in agreement with those of group II, a similar control group. No adult worms were found in the intestine of any of 11 rats of group VII 6 days after re-infection, as a result of feeding them initially with larvae exposed to 6,000 r. cobalt-60. On the other hand, rats of group VIII, initially fed larvae exposed to 12,000 r. cobalt-60, showed relatively little reduction in number of adult worms 6 to 10 days after re-infection, compared to rats of group I.

Conclusion

Rats fed larvae of *T. spiralis* that have been exposed to 10,000 r. cobalt-60 (a dose of radiation that does not prevent the larvae from developing into adult forms but does result in partial or complete sexual sterilization of the adult worms), develop a definite degree of immunity to re-infection with non-irradiated larvae. If the dose of irradiation with cobalt-60 is increased to 18,000 r. (a dose that prevents most larvae from maturing to adult forms), little or no immunity results.

V. TESTS FOR A STRAIN OF TRICHINA LARVAE RESISTANT TO RADIATION

Variation in susceptibility of different trichina larvae to the effects of x-radiation was first noted by Schwartz.[15] In a previous work[16] we found that small percentages of larvae withstood the respective dose of x-ray that generally produced (1) loss of motility of larvae, (2) inhibition of maturation of larvae to adult forms, or (3) sexual sterilization of the adult worms developing from the irradiated larvae. Similar observations[8] were made by us on larvae exposed to cobalt-60. A possible explanation for the ability of a small percentage of larvae to withstand a dose of x-ray or of cobalt-60 that produces sexual sterilization in most of the developing adult trichinae is that the radiation does not hit or damage the sensitive sites in the gonadal cells of the unaffected larvae. The possibility that exposure of trichina larvae to gamma radiation might result in selective survival of those which were relatively radioresistant and that this might conceivably lead to a race of radioresistant larvae, was suggested to us by Dr. Carl V. Weller. To check this possibility, successive generations of larvae were irradiated with the same dose of cobalt-60 and then tested for their productivity.

Method

Initially, 5 groups of rats were employed, each rat being tube-fed 5,000 larvae. Group I (control) consisted of 5 rats fed non-irradiated

larvae; group II, 12 rats fed larvae irradiated with 50,000 r. cobalt-60; group III, 12 rats fed larvae irradiated with 30,000 r.; group IV,A, 12 rats fed larvae irradiated with 18,000 r.; and group V,A, 10 rats fed larvae irradiated with 10,000 r. cobalt-60.

Approximately 2 months after infection, the rats were sacrificed

TABLE VII

Tests for Strain of Trichina Larvae Resistant to Cobalt-60. Effect of Cobalt-60 Applied to Larvae in Vitro *on Reproduction in Succeeding Generations of Trichina*

Group no.	No. of rats*	Dose of Co^{60}, r.	No. of larvae recovered from muscles		Comments
			Total	Average	
I	5	0	182,000 to 600,000	345,000	
II	12	50,000	0	0	
III	12	30,000	0	0	
IV,A	12	18,000	17, 41, 1; 9 rats, each 0	5†	Progeny of these larvae fed to groups IV,C,1, 2, and 3
IV,C,1	4	0	40,000 to 177,000	108,000	
IV,C,2	6	0	224,000 to 514,000	410,000	
IV,C,3	6	18,000	3, 0, 4, 2, 2, 7	3	
V,A	10	10,000	30 to 1,105	455†	Progeny of these larvae fed to groups V,C,1 and 2
V,C,1	7*	0	76,000 to 359,000	189,000	
V,C,2	4	10,000	43, 2, 4, 1	12†	Progeny of these larvae fed to groups V,E,1 and 2
V,E,1	6	0	279,000 to 464,000	339,800	
V,E,2	5	10,000	4, 0, 0, 170, 0	35	

* All rats were each fed 5,000 larvae except those of group V,C,1 which were each fed 4,000 larvae.

† Yield of larvae was built up by passing all larvae recovered from muscles through successive rats.

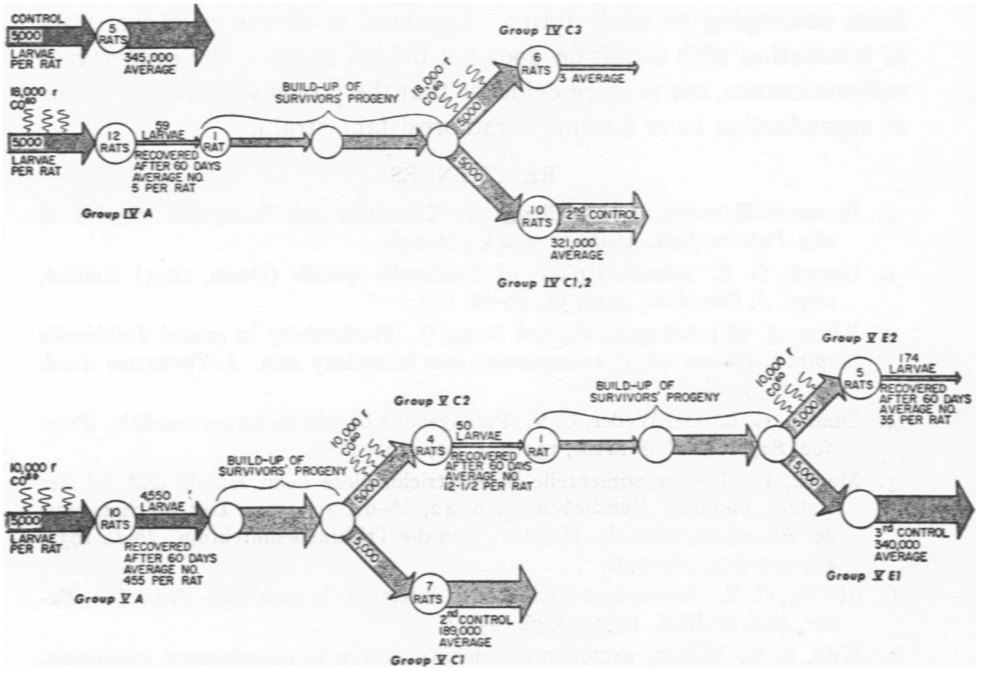
and the muscles digested for recovery of larvae. If the total number of larvae recovered from a group of rats was less than 5,000 for subsequent testing, the larvae recovered were fed to additional series of rats in order to build up their number. When a sufficient number were obtained, the larvae were exposed to various amounts of cobalt-60 and fed to uninfected rats. Upon sacrificing, the muscle larvae recovered were compared with those from the first groups of rats fed irradiated larvae.

As a check upon the number of larvae recovered from the muscles, microscopic sections were made of the tongue, diaphragm, and skeletal muscles of all rats at time of death or sacrifice.

Results

Reference to Table VII shows that infection of 5 control rats each fed 5,000 non-irradiated larvae resulted in an average infection of the muscles with 345,000 larvae. Doses of 50,000 and 30,000 r. cobalt-60 totally prevented reproduction, as determined by failure to recover any larvae from the muscles of any of 12 rats of group II or of group III.

Digestion of the muscles of 12 rats (group IV,A), each given an infective dose of 5,000 larvae exposed to 18,000 r. cobalt-60, resulted in a total recovery of only 59 larvae (an average of 5 larvae). These larvae were fed to rats, successively, to obtain a sufficient number for further tests. Some of these larvae were fed in the non-irradiated state



Text-figure 3. Study of possible development of radiation-resistance in succeeding generations of trichina larvae (Table VII).

to other controls (groups IV,C,1 and IV,C,2) while others were again irradiated with 18,000 r. cobalt-60 (group IV,C,3) and fed to each of 6 rats. The total yield from the ensuing infection in these 6 rats was

only 18 (average of 3 larvae). This finding did not point to the development of radioresistance.

Similar findings were obtained on feeding larvae exposed to 10,000 r. cobalt-60. Rats of group V,A fed larvae exposed to this amount of radiation yielded an average of 455 larvae on digestion of their muscles; rats of group V,C,2 fed a subsequent generation of these larvae exposed to the same amount of radiation yielded not an increased number of parasitic forms but actually a reduced number (average of 12). When the experiment was repeated by feeding the progeny of these to rats of group V,E,2, an average of only 35 larvae was obtained.

Text-figure 3 is a flow-chart summarizing these findings.

Summary and Conclusion

Larvae of *Trichinella spiralis* were exposed to cobalt-60 in a dose (10,000 r.) that causes sexual sterilization of most of the developing adult worms and in a dose (18,000 r.) that prevents most of them from developing to adult forms. Exposure of larvae to these doses of irradiation with cobalt-60 does not induce in them the property of radioresistance, nor is there evidence that the progeny of those capable of reproduction have become a radioresistant strain.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Male adult firmly anchored in a villus. Most of the anterior portion (below) and the terminal portion with its copulatory appendages (above) lie between adjacent villi. Seminal vesicle is distended with sperms. Section of small intestine of white rat 33 hours after feeding excysted larvae. × 460.

FIG. 2. Anterior portion of female adult within mucosa. Vulva (v) opens on the concave surface of the lower portion of the right arm of the loop, and sperms are shown in the forepart of the uterus (ut) in the left upper portion of the field. Remaining portion of worm, not shown in the section, apparently lies outside of villus. 33 hours. × 440.

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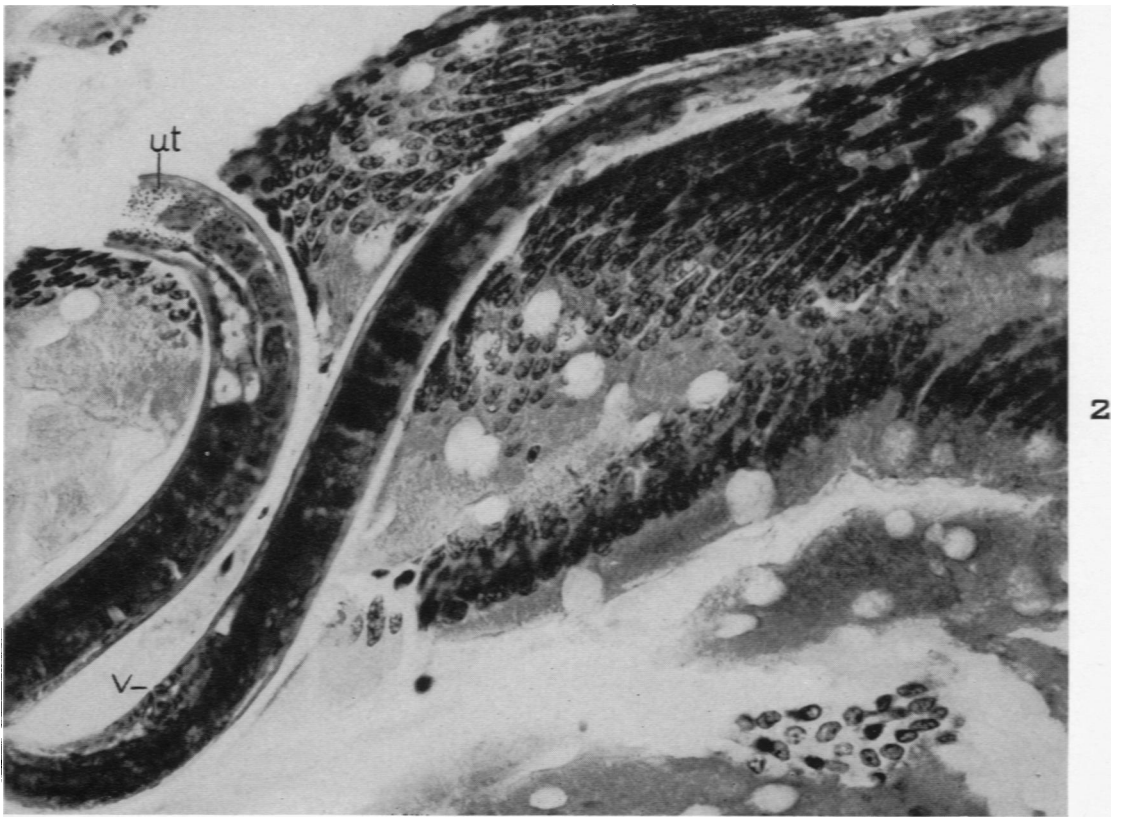

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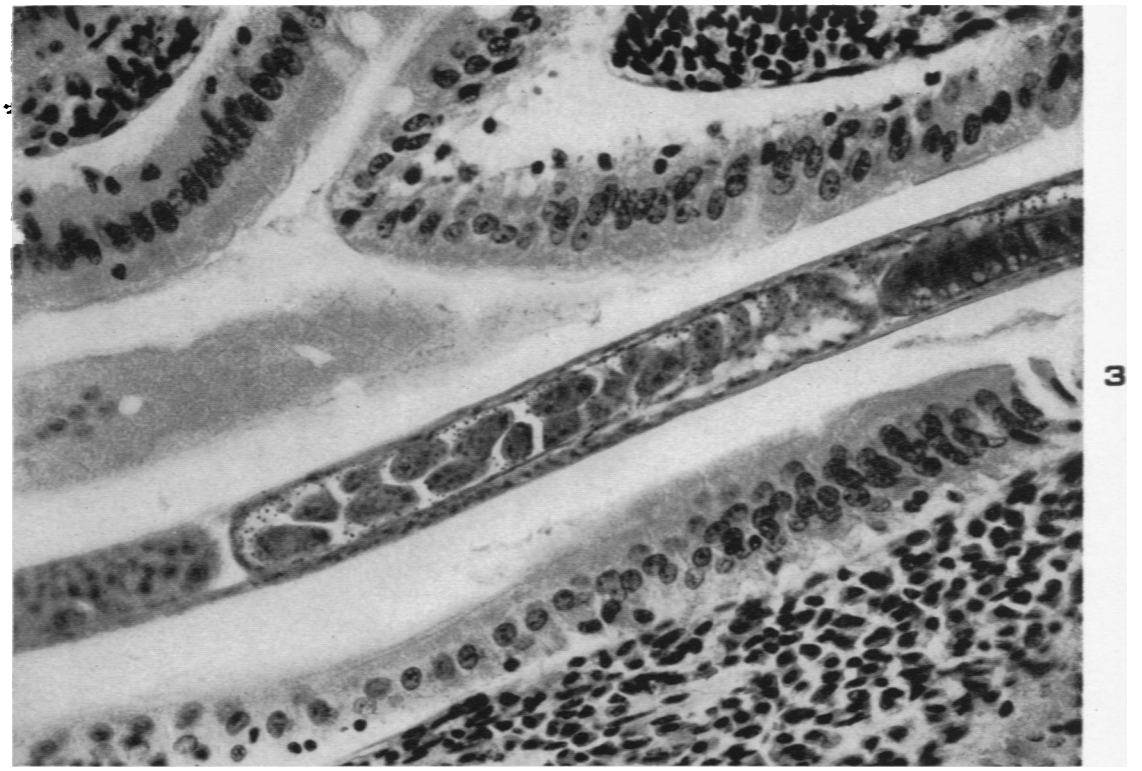
FIG. 3. Uterus of female adult, containing sperms. 33 hours. × 460.

FIG. 4. Portion of adult female showing part of cell body (above) and of ovary (below) and the uterus between these structures. There is an early accumulation of sperms in the seminal receptacle. 33 hours. × 385.



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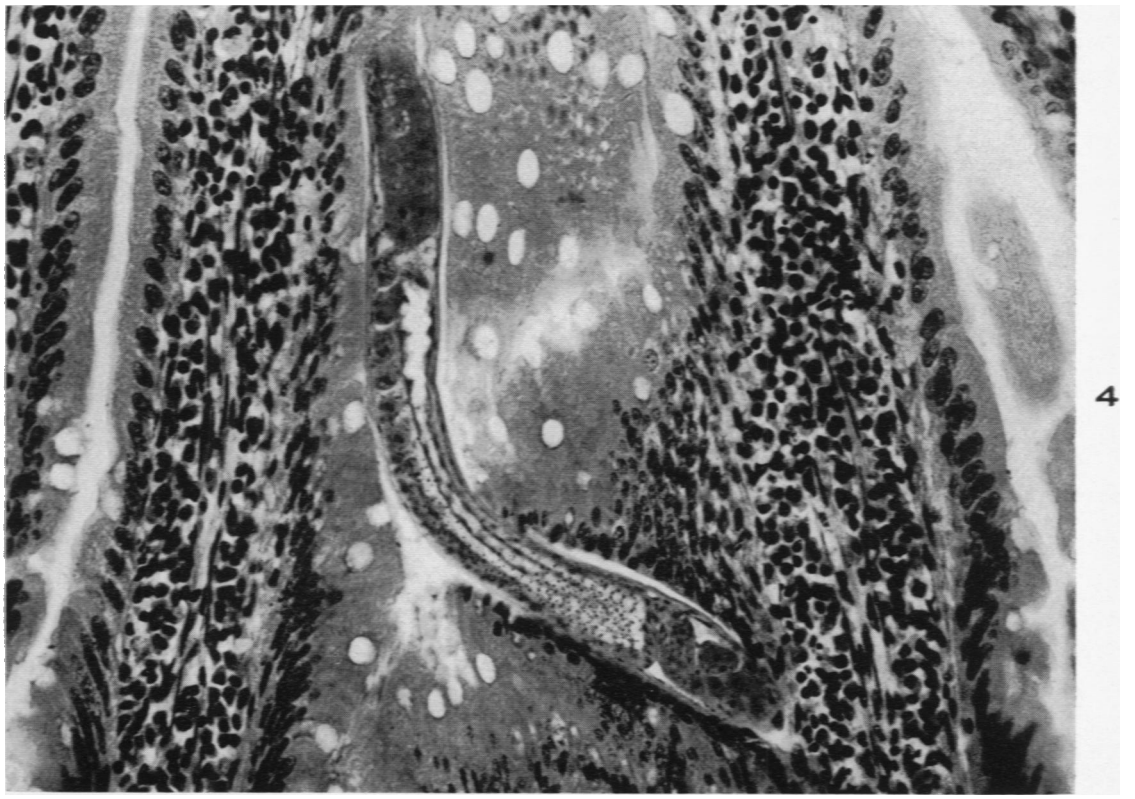

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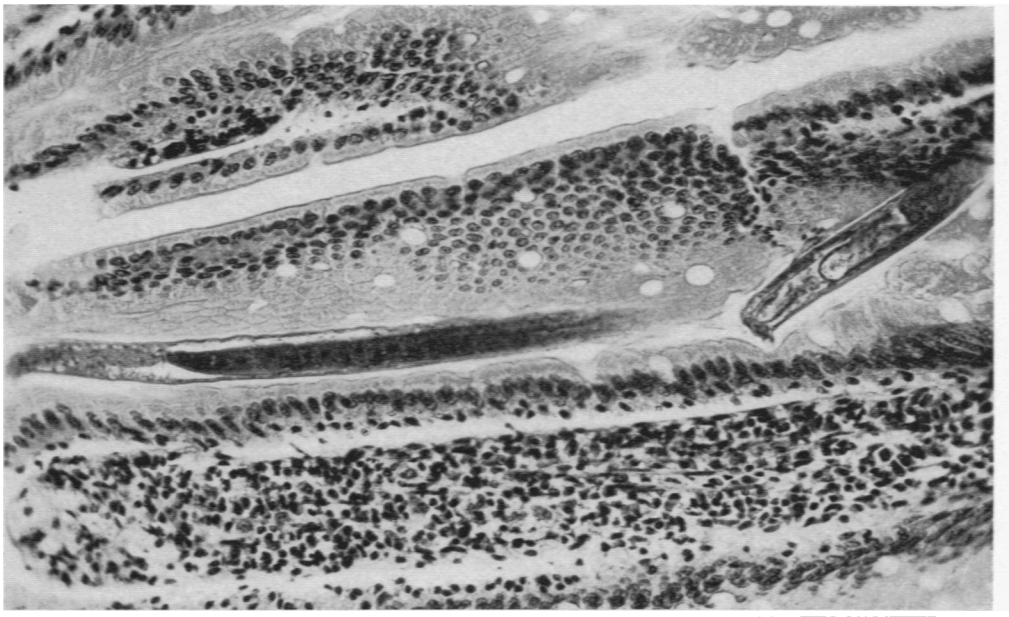
FIG. 5. At right, posterior portion of an adult male worm lies between adjacent villi. Of note are the copulatory appendages, and sperms within seminal vesicle. Remaining portion of the worm, not shown in the figure, is directed upward into a villus. At left, anterior portion of an adult female lies between adjacent villi. The vulva of the worm (not shown in the figure) apparently lies in close proximity to the copulatory appendages of the male. 33 hours. × 280.

FIG. 6. Larva of *Trichinella spiralis* recovered from the blood of a white rat 118 hours after infection. Blood was hemolyzed with 3 per cent acetic acid, and sediment was stained with Wright's blood stain. × 375.



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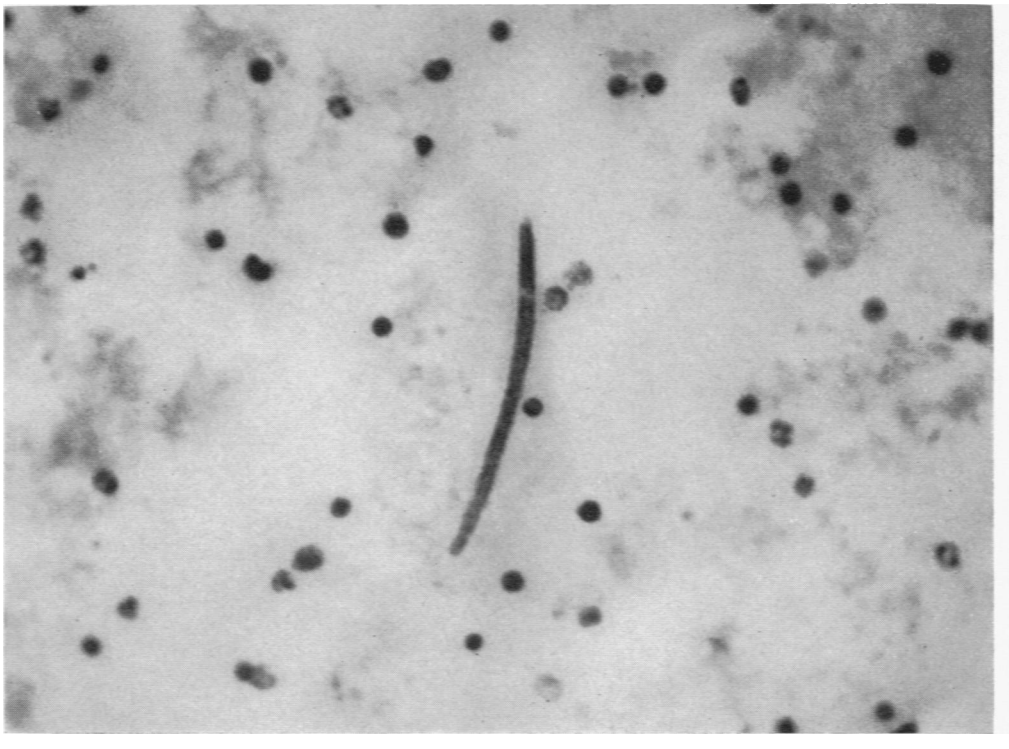

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