Studies with *Brugia pahangi*. 15. Cobalt 60 irradiation of the worm

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ABSTRACT

Infective larvae of *Brugia pahangi* were irradiated at 10, 25 or 45 krads by means of a Cobalt 60 source. In cats, 10 krads caused the worms to be stunted and sterile but allowed them to become 5th stage, migrate posteriorly into the afferent lymphatic, and produce pathology. 25 krads prevented the worms from developing beyond the early fourth stage and from migrating away from the popliteal lymph node. No gross pathological reactions were evident. 45 krads produced the same effects as 25 krads but the longevity of the worms was much reduced.

There have been numerous reports on the effects of irradiation on nematode worms since the original observations on *Trichinella spiralis* (Semrad, 1937). The major stimulus for these studies has been the development of irradiated vaccines following the, now classical, success of Jarrett et al. (1959) with their vaccine against *Dictyocaulus viviparus*. As a part of a study of the relationship between *Brugia pahangi* and its vertebrate hosts we wished to infect animals with worms irradiated so that they would not develop beyond specific life-cycle stages. As a necessary preliminary to this we observed the effects of cobalt 60 irradiation on the development of *B. pahangi* in the cat and the migration of irradiated parasites within the lymphatic system (Ewert, 1971).

MATERIALS AND METHODS

Infective larvae of *B. pahangi* were produced in *Aedes aegypti* by the methods of Denham et al. (1972). Larvae were collected in a Baermann apparatus and counted into lots of 100 actively moving, undamaged worms which were aspirated in approximately 0.5 ml of medium 199 into 1 ml syringes. A bubble of air was introduced below the liquid in the syringe, to ensure that the worms were not shielded by the needle, and the syringes irradiated to the required level in a Vickers Armstrong cobalt unit with an output of 2 krads per minute. Larvae were injected subcutaneously into the volar surface of the feet of cats. The syringes were flushed out with medium 199 and the larvae which had not been injected counted; thus we knew precisely how many larvae had been inoculated into each site. One leg of each cat was always inoculated with normal, non-irradiated, worms from the same batch. In experiments 1 and 2 the contralateral limb was inoculated with larvae irradiated at either 25 or 45 krads. In experiment 3 all legs were inoculated with either normal or irradiated larvae. This allowed the effects of 3 levels of irradiation to be evaluated in a single host. The cats were killed and autopsied at various times after infection (Denham et al., 1972): particular note was made of any abnormalities in the lymphatics and of the sites from which worms were recovered.

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The sex and degree of motility of the worms recovered was recorded and they were transferred to a solution of 35% ethyl alcohol and 2.5% glycerine in distilled water. The alcohol and water evaporated leaving the worms in pure glycerine. Outlines of the worms were drawn with the aid of a camera lucida and their lengths determined by measuring this line. Detailed morphological observations were made and drawn with the aid of the camera lucida.

RESULTS

Experiment 1. The purpose of this experiment was to evaluate the effects of irradiation with 25 krads on *B. pahangi*. Three cats were inoculated in the LHL (left hind leg) with infective larvae irradiated with 25 krads and in the RHL (right hind leg) with non-irradiated worms. The cats were killed and autopsied 7, 12 and 18 days after infection.

Table 1 shows the number of worms recovered and their mean lengths. In comparison with normal worms fewer irradiated worms were recovered on days 7, 12 and 18. On days 12 and 18, male and female worms that had been irradiated were significantly smaller than untreated worms. All the worms were active in 199 medium.

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Cat limb</th>
<th>Level of irradiation (krads)</th>
<th>day after infection</th>
<th>% of inoculated worms recovered</th>
<th>mean length (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P58</td>
<td>LHL</td>
<td>0</td>
<td>7</td>
<td>45.8</td>
<td>2.25* (0.06)</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>25</td>
<td>7</td>
<td>18.0</td>
<td>1.95* (0.06)</td>
</tr>
<tr>
<td>P60</td>
<td>LHL</td>
<td>0</td>
<td>12</td>
<td>46.0</td>
<td>4.32 (0.3)</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>25</td>
<td>12</td>
<td>45.0</td>
<td>3.89 (0.1)</td>
</tr>
<tr>
<td>P59</td>
<td>LHL</td>
<td>0</td>
<td>18</td>
<td>47.0</td>
<td>5.56 (0.01)</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>25</td>
<td>18</td>
<td>21.0</td>
<td>3.67 (0.06)</td>
</tr>
</tbody>
</table>

*larvae could not be sexed

Experiment 2. The purpose of this experiment was to determine the effect of irradiation with 45 krads on *B. pahangi*. Three cats were infected in the LHL with parasites exposed to 45 krads and in their RHL with non-irradiated worms. The cats were autopsied on days 7, 12 and 18. Table 2 shows the results obtained. There was no difference in the percentage recoveries on day 7, but on subsequent occasions fewer irradiated worms were recovered. Irradiated worms retrieved 18 days after infection could not be sexed, as their reproductive system was deformed (see later). There was no significant difference in the lengths of normal and irradiated worms on day 7 but on days 12 and 18 post-infection the irradiated worms were much shorter. All the worms were active in medium 199.

Experiment 3. The purpose of this experiment was to determine longer term effects of irradiation with 10, 25 and 45 krads. Ten cats were inoculated in the LFL with larvae irradiated with 10 krads, in the RHL with larvae irradiated with 25 krads, in the LHL with larvae irradiated with 45 krads and in the RFL with normal (i.e. unirradiated) larvae. The cats were killed between 4 and 77 days after infection. The results of this experiment are shown in Figures 1 and 2. The recovery of adult worms from cats given normal larvae was always about 40% until after day 80, when it fell to only 12%. The percentage recovery of worms from the limbs given irradiated larvae was much lower and was about 10–15% from, at the latest, day 40.

Apart from the points reported under the individual experiments, the following observations were also made.
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**TABLE 2**

Percentage recovery and mean length of 45 krads irradiated and non-irradiated *B. pahangi* recovered from infected cats in experiment 2. (RHL = Right hind leg. LHL = Left hind leg).

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Cat limb</th>
<th>Level of irradiation (krads)</th>
<th>day after infection</th>
<th>% of inoculated worms recovered</th>
<th>mean length male in mm</th>
<th>mean length female in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P61</td>
<td>RHL</td>
<td>0</td>
<td>7</td>
<td>34.0</td>
<td>1.84* (0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LHL</td>
<td>45</td>
<td>7</td>
<td>50.5</td>
<td>1.96* (0.06)</td>
<td></td>
</tr>
<tr>
<td>P62</td>
<td>RHL</td>
<td>0</td>
<td>12</td>
<td>39.8</td>
<td>4.34 (0.3)</td>
<td>5.56 (0.2)</td>
</tr>
<tr>
<td></td>
<td>LHL</td>
<td>45</td>
<td>12</td>
<td>4.1</td>
<td>1.67 (0.3)</td>
<td>3.25 (0.2)</td>
</tr>
<tr>
<td>P63</td>
<td>RHL</td>
<td>0</td>
<td>18</td>
<td>40.0</td>
<td>5.53 (0.02)</td>
<td>7.28 (0.08)</td>
</tr>
<tr>
<td></td>
<td>LHL</td>
<td>45</td>
<td>18</td>
<td>18.0</td>
<td>3.78*</td>
<td></td>
</tr>
</tbody>
</table>

*larvae could not be sexed

**Pathology seen post mortem**

Lymphatics from the limb which had been inoculated with non-irradiated worms were varicosed, enlarged and fibrosed in those cats killed 30 or more days after infection as would be expected from previous experience. The lymphatics of limbs inoculated with worms irradiated at 10 krads were also dilated. Very little change was visible in the lymphatics of limbs inoculated with worms exposed to more than 25 krads or more.

![Graph](https://www.cambridge.org/core/images/2d31e5c6602f4d2d9f5d6f7f0f643d72)

**FIG. 1.** Recovery of *Brugia pahangi* from cats inoculated with normal or irradiated larvae.
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Migration of the worms

The majority of unirradiated larvae migrated to the perinodal sinus of the popliteal lymph node within the first 24 hours and 10–14 days later migrated posteriorly into lymphatics afferent to the node where they developed into the adult stages. Larvae irradiated with 10 krads migrated in the same fashion. Larvae irradiated with 25 and 45 krads migrated to the perinodal sinus but most of them failed to return to the afferent vessel. Occasionally, larvae were found encapsulated and calcified by the host reaction.

![Graph showing length of female worms recovered from cats infected with normal or irradiated Brugia pahangi larvae.](https://www.cambridge.org/core/)

**FIG. 2.** Lengths of female worms recovered from cats infected with normal or irradiated *Brugia pahangi* larvae.

Sex ratio of worms

During autopsy of an animal with a normal *B. pahangi* infection, approximately equal numbers of male and female adult stages were recovered. After inoculation of worms irradiated with 10 and 25 krads, no significant difference in the sex ratio was noticed up to day 38, from when fewer male worms were found. On occasions, all the worms recovered were females. It was difficult to decide the sex of worms exposed to 45 krads as their genital primordia did not differentiate.

Morphology of the worms recovered at different times after infection

**Day 7.** In normal male worms, or those irradiated with 10 krads, a clearly defined mass of cells, the spicule primordium, could be seen but there was no organization of the spicule primordium in worms irradiated with 25 or 45 krads. The genital primordia of normal female larvae had grown posteriorly after attaching to the ventral wall of the epithelial layer of the body wall but after irradiation with 10 krads the genital primordia
had not developed. Some worms had developed but not to such an advanced stage as had non-irradiated worms. Larvae irradiated with 25 krads and 45 krads had not developed beyond the infective stage.

Day 14. The spicules were well developed in normal male worms which had moulted to the 4th larval stage. The distal portion of the spicules had grown anteriorly and was associated with the cloaca and the protractor muscles had developed. In larvae irradiated with 10 krads the spicule primordium cells had not developed beyond the stage seen in 7 day old unirradiated worms. Larvae irradiated with 25 and 45 krads showed disarray of spicule primordial cells. The caudal papillae seen in infective larvae of B. pahangi were still present in the larvae irradiated with 25 or 45 krads.

The genital primordia of normal female worms had advanced into the mid-region of the body and the atrial bulb and vaginal passage were clearly visible. The reproductive organs of female worms irradiated with 10 krads had not developed as far as those of normal worms, but attachment of the primordia to the ventral wall had occurred. In larvae irradiated with 25 and 45 krads the structure of the genital primordia remained similar to that seen in early third stage larvae.

Day 24. Normal male worms had moulted to the fifth stage and the spicule complex was fully developed. Worms irradiated with 10 krads had malformed spicules. Larvae irradiated with 25 and 45 krads still looked like third stage larvae.

In normal females the vaginal passage had joined the bifurcated uterus and the atrial bulb had formed. Females irradiated with 10 krads showed all the parts present in the normal worms but the uterus was irregular and contained large vacuoles and particles of debris. The genital primordia of larvae irradiated with 25 and 45 krads still retained the primitive structure of third stage larvae and no uterus had developed. The intestine of these larvae was irregular and degenerate.

Day 36. Normal male worms were adult. Male worms that had been irradiated with 10 krads showed some spicule organization but these appeared to be non-functional. One male worm irradiated with 25 krads had traces of spicules, but disproportionate growth of the tail had resulted in a small tight coil. No distinguishable male worms were recovered after irradiation with 45 krads.

The mid-region of normal female worms had intertwining, double uteri filled with eggs. In female worms exposed to 10 krads the female genital organs were recognizable but development of the uteri was inhibited although they were bifurcate and convoluted. This stage was, roughly, comparable to that of normal 24 day old female worms. Female worms irradiated with 25 krads had only developed as far as normal worms 14 days old. Worms exposed to 45 krads still retained the structure of early stage larvae.

Day 77. Normal female worms were fully developed, their uteri were packed with eggs and microfilariae and the vagina was patent. Some of the worms irradiated with 10 krads had reproductive systems where all the adult features were distinguishable but the atrial bulb and the fibrous muscles appeared to have collapsed and there were no normal eggs within the uterus. Only deformed eggs and some ‘debris’ could be seen in the uterus. Anteriorly, a few disorganized cells showed the inhibited formation of the atrial bulb. The features were comparable with those of normal female worms recovered on day 24. Worms exposed to 45 krads had not developed beyond the third stage.

DISCUSSION

It is clear that cobalt 60 gamma irradiation radically alters the development of B. pahangi in the cat. The comparatively low level of 10 krads produced changes but the worms managed to develop into non-fertile adults which caused gross pathological reactions in the lymphatics. 25 krads prevented the development of the larvae beyond the very early
fourth stage and they failed to migrate posteriorly into the afferent lymphatic below the popliteal node as do normal worms (Ewert, 1971). No gross pathology was initiated by these worms nor by those irradiated with 45 krads. The larvae irradiated at 45 krads were very small and did not migrate posteriorly.

Irradiation also severely reduced the longevity of the worms as can be seen from Figure 2. Whilst the recovery of normal worms remained around 40% up to 77 days the recovery of irradiated worms fell appreciably before 40 days and no worms were recovered at 77 days from the limb given worms irradiated with 45 krads. In another cat given larvae irradiated at 45 krads a few larvae were found 97 days after infection.

From the point of view of experimental vaccination it is now possible to produce infections which are pathogenic but sterile and composed of 5th stage worms (using larvae irradiated with 10 krads).

It is also possible to produce non-pathogenic infections of 3rd and early 4th stage larvae (using irradiation doses of 25 or 45 krads). As larvae irradiated with 25 krads survive much longer than those given 45 krads these should be used for this particular type of vaccination procedure.

In a future publication we will report our trials of vaccination with larvae irradiated at 10 or 25 krads.

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