THE EFFECT OF ULTRAVIOLET RADIATION ON THE OVA OF THE ASCARID ROUNDWORMS TOXOCARA CANIS AND TOXASCARIS LEONINA

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INTRODUCTION

Through the courtesy of Dr. C. G. Abbot, Secretary of the Smithsonian Institution, the opportunity was presented to study the action of ultraviolet light on the ova of the two species of ascarids commonly infesting the dog. It was desired in the experiments reported here to determine, if possible, the radiotoxic effect of ultraviolet light of different wave lengths and, as a practical measure, to correlate the possible sterilizing action of sunlight on the ova of these two species of ascarids.

The apparatus employed for the radiation of the ova, described in detail by Brackett and McAlister (1932), consisted of a quartz monochromator and a quartz mercury arc. This apparatus eliminates the disturbing effect of the large amount of heat attending all total arc exposures and permits irradiation by the different wave lengths of the mercury spectrum. The intensities of the spectral lines employed were as follows:

<table>
<thead>
<tr>
<th>Wave length (Å)</th>
<th>Intensity (ergs/sec. cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3650</td>
<td>9500</td>
</tr>
<tr>
<td>3130</td>
<td>4200</td>
</tr>
<tr>
<td>3022</td>
<td>1900</td>
</tr>
<tr>
<td>2967</td>
<td>910</td>
</tr>
<tr>
<td>2804</td>
<td>610</td>
</tr>
<tr>
<td>2652</td>
<td>570</td>
</tr>
</tbody>
</table>

In correlating the dosage employed with ultraviolet intensities in sunlight, the determinations of Coblentz and Stair (1931) have been used. These investigators gave the value of 65 microwatts per cm² (650 ergs/sec. cm²) for noonday, midsummer sun at Washington,
D. C., for wave lengths of 3130 A to the solar limit at 2970 A. From their data there have been computed an average value of 55 microwatts per cm² (550 ergs/sec. cm²) for the above-mentioned wave lengths in July sunlight between 9:00 a.m. and 3:00 p.m. at Washington, D. C. This value has been used in translating into terms of days the exposures employed in some of the tests reported here.

The ova of *Toxocara canis* are globular, subglobular, or slightly ovoid in shape and of a light brown color. Externally, the egg is covered with a thick, albuminous coating which is mammillated at regular intervals. The eggs vary in measurement from 82 to 102 μ in length and 79 to 96 μ in width. The ova of *Toxascaris leonina* are ellipsoidal to subglobular or globular in shape, with thick, double-contoured, smooth, clear shells. They measure 82 to 96 μ in length and 82 to 92 μ in width.

**REVIEW OF LITERATURE**

The effect of ultraviolet irradiation on the ova of *Ascaris equorum* (= *A. megaloecephala*) has been studied in some detail by a number of investigators. Most of these experiments were conducted from the standpoint of the cytologist with a view to determining the effect of the light on various parts of the egg or on the whole egg at different stages of development. Stevens (1909), using a total arc exposure, found that exposure of the whole egg to ultraviolet light for 6 to 8 hours did not usually kill the egg at once, but prevented further development when the eggs were in the 2- to 4-cell stage at the time of exposure. Exposure for a period too short to prevent further cleavage (½ to 3 hours) caused various irregularities in development including irregular fragmentation of the chromosomes, delay in cleavage and abnormal gastrulation. Stevens believed that ova arrested in development may be said to be paralyzed to such an extent that they are unable to initiate any further mitosis.

In his extensive experiments in exposing various parts of the egg of *A. equorum* to ultraviolet light Schlep (1923) employed the microscopic method devised by Hertel (1904) and modified and improved by Tschachotin (1912). The apparatus used gave a spectral line of 2800 A from the magnesium arc. Schlep found that irradiation of the whole egg or various parts of the egg for varying periods of time resulted in a marked lethal effect. Schlep concluded, moreover, that no part of the egg could be influenced by ultraviolet light without producing secondarily some alteration in other parts not exposed to the light.

Ruppert (1924) also employed the apparatus of Tschachotin in irradiating the ova of *A. equorum*. In Ruppert’s experiments short
exposures of the whole egg resulted in marked abnormal embryonal development, which appeared most frequently in the gastrula stage. Longer exposures gave a marked lethal effect. Ruppert concluded that there is a rhythmic alteration in the lethal effect of ultraviolet light, depending on the stage of development of the egg at the time of exposure.

Seide (1925), in irradiating ova of *A. equorum*, used the method of Tschachotin as well as total arc exposures at wave lengths between 4050 and 2530 A of the mercury spectrum. In his experiments there was no apparent lethal effect on the eggs and no apparent lag in development.

Nolf (1932) observed that a very small total arc exposure at wave lengths between 2800 and 3150 A or between 1800 and 3150 A was sufficient to prevent a large percentage of the ova of *A. lumbricoides* from reaching embryonation. A slightly greater exposure was completely lethal to the eggs.

Although results obtained by the above-named investigators are of general interest, their findings offer no means of comparison with results of experiments reported in this paper, in which measured intensities of single wave lengths of ultraviolet light were employed. The intensity of the 2800 A spectral line from the magnesium arc was not reported by those using this method, making comparison impossible, and it is equally impossible to compare results with those of workers who employed total arc exposures.

**EXPERIMENTS**

**SERIES A**

Preliminary tests were made by exposing *Toxocara* and *Toxascaris* eggs to six wave lengths of ultraviolet light in order to gain some idea as to lethal effect, if any, of these various wave lengths. The ova of both species of ascarids were mixed and placed on a glass slide and allowed to dry at room temperature. The slides were exposed to ultraviolet light for a length of time sufficient to provide an equivalent dosage (the product of time and intensity) at the various wave lengths. As the longest period of exposure was 20 minutes, each slide was dried for 20 minutes, including the time of exposure to the light, in order to provide equal conditions for the test. Each slide was then exposed to the arc for the time stated in Table 1. The dosage was 684,000 ergs/cm² or approximately equivalent for the 3022 A slide to 18 minutes exposure to noodays, midsummer sun at Washington, D. C.
The exposed area on each slide was marked off with a diamond point; this area was equivalent to the dimensions of the light ray discharged from the aperture, or 5 by 30 mm. Eggs on the same slide outside of the irradiated area served as controls; the control eggs were therefore subjected to identically the same conditions during development as were the irradiated eggs. After exposure, each slide was immediately placed in a Petri dish and covered with a 1 per cent solution of formalin, in which the eggs were permitted to develop. For the most part the eggs adhered to the slide. The culture was allowed to develop for a period of 8 to 9 days at temperatures ranging from 26° to 28° C. At the end of this time, counts were made to determine the

Table 1.—Results of Exposure of Ova of Toxocara canis and Toxascaris leonina to Ultraviolet Light

Series A—Exposed on May 23, 1934
Dosage—684,000 ergs/cm²

<table>
<thead>
<tr>
<th>No. of slide</th>
<th>Wave length</th>
<th>Duration of exposure</th>
<th>Date of count</th>
<th>Percentage embryonated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minutes</td>
<td>Seconds</td>
<td>1934</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>2652</td>
<td>20</td>
<td>May 31</td>
</tr>
<tr>
<td>2</td>
<td>2804</td>
<td>18</td>
<td>46</td>
<td>May 31</td>
</tr>
<tr>
<td>3</td>
<td>2967</td>
<td>12</td>
<td>30</td>
<td>June 1</td>
</tr>
<tr>
<td>4</td>
<td>3022</td>
<td>6</td>
<td>.</td>
<td>June 1</td>
</tr>
<tr>
<td>5</td>
<td>3130</td>
<td>2</td>
<td>44</td>
<td>June 1</td>
</tr>
<tr>
<td>6</td>
<td>3650</td>
<td>1</td>
<td>12</td>
<td>June 1</td>
</tr>
</tbody>
</table>

The percentage of embryonation in the eggs of the two species, both on the control part of the slide and on the irradiated part of the slide. For each count, 200 ova were taken. Table 1 summarizes the results obtained from the irradiation of ova in series A.

From an examination of the data it is apparent that the relatively short exposure used in this series had little or no effect on the development of the ova in the majority of cases. However, in two instances the ultraviolet light appears to have exerted a definite toxic effect on the ova of Toxascaris. On slide 1 (wave length 2652 A) only 6.5 per cent of the irradiated ova became embryonated, whereas 25 per cent of the control ova became embryonated. On slide 2 (wave length 2804 A), 6 per cent of the irradiated ova were embryonated at the time the count was made, whereas 29.5 per cent of the control ova were
embryonated. In each of these instances it is apparent that the ultra-violet light prevented development in a relatively large proportion of the *Toxascaris* eggs. In other cases the differences in embryonation were well within the limits of experimental error in the counting technic.

**SERIES B**

In view of the fact that wave lengths from 2652 to 2967 A are below the limits of the solar spectrum, there appeared to be little information of practical value to be derived from the further irradiation of ascarid eggs at these wave lengths even though *Toxascaris* ova were considerably affected by irradiation at wave lengths of 2652 and 2804 A. For this reason further experiments were confined to irradiation of the ova at wave lengths within the range of the solar spectrum with a view to ascertaining the relative lethal effect of sunlight, exclusive of heat and desiccation, on the ova of these two species of ascarids. This point has practical application in the control of ascariasis.

In series B the ova were exposed to a dosage approximately equal to 40 times that used in series A. The dose was equivalent to 27,400,000 ergs/cm². This exposure for dish 1 (wave length 3022 A) was approximately equivalent to 12 hours of noonday, midsummer sun at Washington, D. C.

For this test, a mixed culture of *Toxocara* and *Toxascaris* ova was dried in the bottom of 50-mm culture dishes for 10 minutes. After the eggs had dried on the bottom of the culture dish, water was added to the dish to a depth of 2 mm. This prevented drying of the culture during the period of irradiation. An area equivalent to the light aperture, or 5 by 30 mm, was marked off on the bottom of each culture dish with a diamond point and the eggs within that area were exposed to the ultraviolet light. Eggs without this area were not exposed and were used as controls. After exposure of the eggs, formalin was added to the culture dishes to provide a concentration of 1 per cent in order to prevent bacterial growth in the cultures. Temperatures during development of the cultures ranged between 29° and 30° C. Counts were made nine days after irradiation; 200 ova were taken in each count. The results of the experiment are recorded in table 2.

In only one case was there any apparent toxic effect from the ultraviolet irradiation in series B. In dish 1 (wave length 3022 A) there resulted a marked lethal effect on the ova of both *Toxocara canis* and *Toxascaris leonina*, although the effect was most marked on the ova of the latter species. Of the ova exposed at this wave length, 24.5 per cent of the irradiated *Toxocara* eggs developed to embryonation, where-
as 58.5 per cent of the control eggs became embryonated. Only 8 per cent of the irradiated *Toxascaris* eggs developed, whereas 29 per cent of the control eggs became embryonated. A slight toxic effect may have been exerted on the ova exposed in dish 2 (wave length 3130 Å), although reference to the series C experiment would seem to indicate that the differences noted above are probably due to chance variation in the counts.

Table 2.—Results of Exposure of Ova of *Toxocara canis* and *Toxascaris leonina* to Ultraviolet Light

Series B—Exposed on June 5, 1934

Dosage—27,400,000 ergs/cm²

<table>
<thead>
<tr>
<th>No. of dish</th>
<th>Wave length</th>
<th>Duration of exposure</th>
<th>Date of count</th>
<th>Percentage embryonated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hours</td>
<td>Minutes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>3022</td>
<td>4</td>
<td>1934 June 14</td>
</tr>
<tr>
<td>2</td>
<td>3130</td>
<td>1</td>
<td>57a</td>
<td>June 14</td>
</tr>
<tr>
<td>3</td>
<td>3650</td>
<td>..</td>
<td>48</td>
<td>June 14</td>
</tr>
</tbody>
</table>

*a 7 percent overdose.

SERIES C

In series C irradiation of *Toxocara* and *Toxascaris* ova with ultraviolet light was prolonged to the time indicated in table 3. The dosage used was approximately five times that employed in series B, and amounted to 137,000,000 ergs/cm². For dish 1 (wave length 3022 Å) the exposure utilized was approximately equivalent to 60 hours of noonday, midsummer sun at Washington, D. C., or 12 days of July sunlight.

The method of exposure used in series B was found unsatisfactory from the standpoint of making microscopic counts. Owing to the long exposure in series C, it was necessary to devise a more suitable method of irradiating the eggs so that drying would be prevented. There were constructed small glass dishes 5 mm wide, 30 mm long, and 5 mm high, the first two measurements representing the size of the area over which the light was dispersed from the aperture. A mixed culture of *Toxo-
cara* and *Toxascaris* eggs was placed in the dishes and water added to a depth of approximately 3 mm. As evaporation proceeded during the course of irradiation, more water was added to prevent drying of the eggs.
A number of eggs sufficient to cover only the bottom of the dish was used in order to prevent any overlapping and shadowing of eggs from the ultraviolet light. After irradiation the eggs were transferred by means of a clean pipette to culture dishes containing a 1 per cent solution of formalin. As a control, a culture was made in 1 per cent formalin on the same date as the first irradiation exposure and was subjected to the same conditions of development as were the irradiated cultures. Temperatures during the period of development varied between 30° and 35° C., as recorded on a thermograph chart. Table 3 gives the results of this experiment. In this series 400 ova were taken for each count.

Table 3.—Results of Exposure of Ova of Toxocara canis and Toxascaris leonina to Ultraviolet Light

<table>
<thead>
<tr>
<th>No. of dish</th>
<th>Wave length</th>
<th>Duration of exposure</th>
<th>Date of exposure</th>
<th>Date of count</th>
<th>Percentage embryonated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hours Minutes</td>
<td></td>
<td></td>
<td>Toxocara</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>20 0 minutes</td>
<td>1934 July 16</td>
<td>1934 July 27</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>3130</td>
<td>9 10 minutes</td>
<td>July 20</td>
<td>July 31</td>
<td>63.5</td>
</tr>
<tr>
<td>3</td>
<td>3650</td>
<td>4 0 minutes</td>
<td>July 18</td>
<td>July 28</td>
<td>63.0</td>
</tr>
</tbody>
</table>

It will be noted that the exposure to ultraviolet light at a wave length of 3022 A was the only exposure which produced any apparent toxic effect on the eggs of either species. The counts indicated that only 20.5 per cent of the irradiated Toxocara ova reached embryonation as compared with 65.5 per cent of the eggs embryonated in the control culture, and only 1.5 per cent of the irradiated Toxascaris ova became embryonated as compared with 42 per cent of the eggs embryonated in the control culture.

In this experiment the irradiated and control cultures were examined daily under the microscope. A careful check was made on the development of the cultures in an effort to determine whether there was any lag in development in those ova which actually started to segment. No such lag in development was noted. Those ova which segmented apparently began segmentation as promptly as did nonirradiated ova in the control culture.
The radiotoxic effect on the ova of both species was exhibited in different ways. On the ova of both *Toxocara* and *Toxascaris* it was apparent in some cases that the ultraviolet light had some direct and undelayed lethal effect, inasmuch as many eggs never underwent any cleavage. On the other hand, some of the ova of both species developed in part until development was definitely arrested. In order to check on this point, the culture in dish 1 (wave length 3022 A) was recounted at the expiration of 21 days in an effort to determine whether any ova which were in intermediate stages of development at the time of the first count, 11 days after exposure, had completed their development. It was found that none of the ova which were partially developed at the time of the first count had completed their development at the time of the second count 10 days later. It would appear that such ova had been definitely and permanently arrested in development, a circumstance which agrees with the results obtained by Stevens (1909), who believed that such ova may be said to be paralyzed to such an extent that they are unable to initiate any further mitosis.

In some of the ova arrested in the course of development it was apparent that cleavage had proceeded normally up to a certain point. On the other hand, all sorts of irregularities were noted in the developmental stages of some of these eggs. These irregularities were most frequent in *Toxocara* ova. In some the cytoplasm was apparently degenerated and was distributed in various areas within the shell. In other ova the cytoplasm contained large vacuoles. Irregularities and abnormalities in blastulation and gastrulation were marked. Some of the *Toxocara* ova contained partly formed embryos, the unformed remainders of which were composed of undifferentiated and irregularly formed masses of cells. In some of the eggs the first somatic stem cell failed to develop, whereas the first germinal stem cell divided many times. In other eggs the first germinal stem cell developed only partly. In most of the *Toxascaris* ova that showed development it would appear that segmentation proceeded normally up to the 8- to 16-cell stage, at which stage it was definitely arrested. Development in none of these ova proceeded to the blastula or gastrula stage, except in a very few eggs which actually reached embryonation. In all ova of both species which became embryonated, the larvae appear to have ensheathed normally.

In order to determine the infectivity of embryos which developed in the irradiated ova, on August 11, 1934, the three cultures irradiated in series C were each fed to a young albino rat; at the same time the control culture was fed to a rat from the same litter. The rat which received the control culture gave birth to a litter of 10 young on August
17, 1934. As it was desired to raise this litter, the animal was not killed. The three other rats were killed on August 17, 18, and 21, 1934, respectively. Ascarid larvae were recovered from the liver and lungs of each of the three animals. All of these larvae proved to be those of *Toxocara canis*. Failure to recover *Toxascaris* larvae from the rats was not unexpected, as only a very limited supply of *Toxascaris leonina* ova was available at the start of the experiment and the cultures contained only a relatively few ova of this species. Failure to recover larvae is therefore not regarded as definite evidence that the embryos within the ova were not infective.

In all of the experiments in which any radiotoxic effect was noted from ultraviolet irradiation, *Toxascaris* eggs appeared to be the most severely affected by the exposure. In series C, the lethal effect apparently resulted in a definite killing of the ova without any segmentation, or in an arrest in development during the early stages of segmentation. In no case did *Toxascaris* eggs which were arrested in development reach the blastula or gastrula stages. This is in contrast to the effect on *Toxocara* ova in which blastulation or gastrulation, even though abnormal, was reached in some cases. It would appear that the more marked radiotoxicity on the eggs of *Toxascaris* may be associated with the smooth, rather clear, nonmammillated shell of these eggs, in contradistinction to the mosaically formed, mammillated, more darkly pigmented shell of *Toxocara*. It is believed that the mosaic pattern of the shell of this species of egg would tend to diffuse the ultraviolet rays and that the deeper pigmentation of these eggs would be responsible for some absorption of the ultraviolet spectrum before the rays had had an opportunity to reach the cytoplasm or nucleus of the egg.

CORRELATION OF PRESENT RESULTS WITH PREVIOUS INVESTIGATIONS

A number of investigators have reported on the effect of exposing the ova of various species of ascarids to sunlight. Ross (1916) reported that the eggs of the human ascarid, *Ascaris lumbricoides*, developed and remained alive when kept for 6 weeks on glass slides in the direct sunlight in India; Ross was of the opinion that a relatively high humidity was not essential for the development of the eggs. Ross's observation is at variance with the results obtained by Mana-lang (1927), who found that human ascarid eggs on glass slides were all dead after 1½ hours' exposure to the direct sunlight in the Philippines, although such eggs resisted an exposure of one-half hour. However, Ohba (1926) reported that ascarid eggs in water cultures in
direct sunlight continued to develop. Brown (1927) exposed the eggs of *A. lumbricoides* to direct sunlight in Panama by placing them in sand, and after a period of 21 days of such exposure it was found that all of the ova had degenerated. Brown considered that the destruction of the ova was due to two factors, viz, high temperature and desiccation. Soil temperatures in the sand cultures were found to reach at least 123° F., which appears to be above the lethal range of heat for ova of this species. Caldwell and Caldwell (1928) exposed fecal-soil cultures of the ova of the human and pig ascarids to sunlight in Alabama, and after a period of 3 days' exposure, the ova in all cultures were disintegrated. The maximum temperature recorded in the cultures was 146° F. These investigators place great emphasis on desiccation as the chief lethal factor involved, inasmuch as cultures moistened at hourly intervals while exposed to sunlight showed little disintegration of the ova of the pig ascarid; ova of the human ascarid showed less resistance. Otto (1929) reported that eggs of the human ascarid developed and remained alive over the summer of 1928 on the surface of clay, loam, sand, and cinder-loam soils in the shade in southwestern Virginia. Many of the eggs on the first three soils in the sun died rapidly, but after 160 days about one-fourth of the eggs isolated were still alive, whereas most of those on cinders in the sun died before becoming embryonated. The high temperatures recorded on the surface of the cinders lead the author to conclude that temperature played an important part in the death of these eggs.

Apparently, Owen (1930) is the only investigator to observe the effect of sunlight on the ova of *Toxocara canis*. Owen exposed the ova of this species to summer sunlight in Kentucky and Minnesota and found that such ova disintegrated before reaching the infective stage. A surface-soil temperature of 131.9° F. was obtained on the plots of soil on which the eggs were exposed. Owen was of the opinion that the failure of the eggs to develop was due to the high temperature.

Schwartz (1932) reported that ova of *Ascaris vitulorum* did not survive exposure on glass slides to 1 hour's direct sunlight in the Philippines. Eggs exposed in beakers of water also failed to survive after 1 hour of direct sunlight. Schwartz then exposed ova in vials painted with India ink to exclude light. An exposure of 1 hour in sunlight was lethal to ova in painted and unpainted vials, and Schwartz thus attributed the death of the ova to the temperature, which reached 45° C.

Ohba's negative results from exposure of ascarid ova to sunlight are difficult to interpret in the light of our irradiation tests, as the length of exposure is not stated in the English summary of Ohba's
paper. Regardless of these negative results, it is apparent from our tests that sunlight, through the ultraviolet spectrum at wave lengths of approximately 3022 A, does exert a definite radiotoxic effect on ascarid ova. However, owing to the relatively long exposures necessary for the development of this lethal effect, it appears probable from a practical standpoint that other factors, such as desiccation or high temperatures, exert a more destructive action on these ova. In humid, tropical climates, however, ultraviolet light probably does serve in some measure in preventing the development of such ascarid ova as are directly exposed to sunlight.

**SUMMARY AND CONCLUSIONS**

Irradiation of the ova of *Toxocara canis* and *Toxascaris leonina* at measured wave lengths from a quartz monochromator and quartz mercury* arc resulted in a certain degree of radiotoxicity to the ova exposed at certain wave lengths.

A dosage of 684,000 ergs/cm² at wave lengths of 2652 and 2804 A had a marked lethal effect on the ova of *Toxascaris leonina* but apparently no effect on the ova of *Toxocara canis*. A similar dosage at wave lengths of 2967, 3022, 3130, and 3650 A was without effect on the ova of either species.

A dosage of 27,400,000 ergs/cm² at a wave length of 3022 A resulted in definite radiotoxicity on the ova of both species of ascarids. Exposures to the same dosage at wave lengths of 3130 and 3650 A showed no effect.

A dosage of 137,000,000 ergs/cm² at a wave length of 3022 A showed a marked lethal effect on the ova of both species. In the case of *Toxocara*, only 20.5 per cent of the irradiated ova developed to embryonation as compared with 65.5 per cent embryonation in the control culture; only 1.5 per cent of the *Toxascaris* ova reached embryonation as against 42.0 per cent embryonation in the control culture. The dosage employed was approximately equivalent to an exposure of 60 hours noonday, midsummer sun at Washington, D. C., or 12 days of average July sunlight.

In all of these tests, the ova of *Toxascaris leonina* proved more susceptible to the action of ultraviolet light than did the ova of *Toxocara canis*. This difference is probably accounted for by the difference in structure and pigmentation of the shell; the mosaically patterned, mammillated, darkly pigmented shell of *Toxocara* ova would appear to disperse and to absorb more light than does the clear, unmammillated, lightly pigmented shell of *Toxascaris* ova.
Although the marked lethal effect of sunlight on ascarid ova, as reported by several workers, is probably due chiefly to desiccation and high temperatures, it would appear that the ultraviolet spectrum is in itself a factor under certain conditions in the destruction of such ova.

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