Journal of the Minnesota Academy of Science

Volume 25
Number 1 *Vol. 25/26*

Article 26

1957

The Effects of Low Temperature Postirradiation Treatment on the Survival of Horse Ascaris Eggs

L. George
St. Mary's College

BROTHER L. GEORGE, F.S.C.
Saint Mary's College, Winona

The Effects of Low Temperature Postirradiation Treatment on the Survival of Horse Ascaris Eggs[1]

INTRODUCTION

Some information has been presented on the effect of low temperature after the irradiation of Ascaris eggs. Evelyn Cook (1939) reported a definite relationship between the survival of the Horse Ascaris, *Parascaris equorum*, and the length of exposure to low temperature.

A work similar to Cook's was performed by C. S. Bachofer and George Pahl (1955) with Pig Ascaris, *Ascaris lumbricoides suum*. This resulted in a disagreement with Cook's work. It was the purpose of the present study to repeat Cook's work as closely as possible in order to learn if it contained any discrepancies.

The present work deviates only slightly from E. Cook's work. The eggs were incubated at a constant 30°C instead of 25°C. As a result, 50 percent cleavage occurred sooner. 50 percent cleavage is the time necessary for 50 percent of the eggs to reach the two cell stage.

EXPERIMENTAL MATERIAL & PROCEDURE

The horse nematode, *Parascaris equorum* is a parasitic roundworm found in the lumen of the intestine. Living adult female specimens were secured from the Hill Packing Company of Topeka, Kansas. The worms were transported from the packing house to the college in a thermos jug which contained some Kronecker's solution. This solution aided in keeping the animals alive and controlling bacterial growth.

After receiving the worms the uterine eggs were removed as soon as possible. A line one inch long was drawn on a piece of paper and was covered with a glass plate. This is the length of the uteri contain-

[1]This study was supported in part by a grant from the Minnesota Section of the American Cancer Society, Inc.

ing the highest percentage of fertilized eggs. The worms are grasped at approximately the middle and cut at a point anterior to the vulva.

A properly executed cut will enable the vagina and uteri to be exposed easily. However, if an improper cut has been made, gentle pressure will expose the organs. With the help of a pair of forceps the uteri were then extracted from the body cavity, laid out on the glass plate and cut to the previously mentioned length. The uteri were kept in a beaker containing distilled water until all the other uteri had been removed.

Next came the removal of eggs from the uteri. This was accomplished by grasping the vagina with the forceps and pushing the eggs away from the vagina towards the cut end by means of a glass rod. These were kept, as were the uteri, in a beaker of distilled water until all of the eggs had been extracted.

The decoating process followed. A 25 percent solution of Hylex was added to the beaker, in an amount which equaled one-third of the volume of the eggs and water.

The eggs were frequently agitated for a period of fifteen minutes. This is the length of time necessary to dissolve the outer membrane. The eggs were removed after the required time by centrifugation and were washed until the Hylex had been removed. The eggs were then transferred to an Erylenmeyer flask and procedures were begun for determining their concentration.

The figure for the concentration of the eggs was placed on the outside of the flask along with the date that the worms were received. Then the eggs were placed in the refrigerator (5°C), which kept them in a dormant state until they were to be used for the experiment. In this case the concentration of the eggs was 30,000 per cubic centimeter.

For irradiation a portable X-ray machine was used. It was a Picker X-ray unit, similar to those used for field therapy by the United States Army during World War II. The machine was operated at ninety kilovolts (90KV) and four milliamperes (4 ma) throughout the experiment.

Before the main experiment was performed a pilot run was used to determine the approximate time that 50 percent cleavage could be expected and also the duration of the radiation required in order to produce the desired percentage of survival.

From the above results it was found that a four minute exposure to the X-rays produced the desired 2 percent survival. Actually four controls were used, two with no exposure to the X-rays and the other two with a 2-minute exposure to the rays.

Through mathematical computations it was determined to use 20 cc. of the known concentration; in other words approximately 600,000 eggs were used in each radiation. These eggs were concentrated in one milliliter of distilled water. After radiation the eggs were diluted to 100 cc. in Ascaris saline and placed in flasks.

These numbered flasks were immedately put in the refrigerator at 5°C. in order to check any further development. A one milliliter sample was taken from each of the six flasks respectively, placed in correspondingly numbered vials, and put in the incubator at 30°C.

For finding the percent survival one week later, four aliquots of 200 eggs were taken from each vial and counted. The number of eggs that reached motility in each 200 eggs counted was divided by two to give percent survival.

Each week six one milliliter samples were taken from the refrigerator and placed in small vials. This process was continued for a period of 8 weeks.

RESULTS AND CONCLUSION

The percentage of survival (completion of embryogenesis) showed a consistent pattern for each of the irradiation doses over the entire eight week period (Table I). There was complete disagreement with

TABLE I.—*Percent Survival of X-irradiated Eggs of Ascaris After Extended Periods of Low Temperature Treatment After Exposure.*

	MINUTES OF EXPOSURE		
Weeks at 5° C	Control	2	4
0	93.0	19.7	3.0
½	92.5	17.5	2.5
1	92.0	17.7	2.2
2	88.0	17.0	3.0
3	91.0	15.5	3.4
4	93.0	17.0	2.5
5	92.7	16.4	3.0
6	90.1	15.0	2.4
8	88.7	16.7	4.2

the findings of Cook (Table II). While Cook observed a phenomenal recovery from 2% to 45% in a period of 8 weeks, the present study showed no change in survival.

The only conclusion that can be made as a result of the present study is that a thorough analysis of all precedures must be made in order to determine the source of the discrepancies that have been found.

TABLE II.—*Comparison of Percent Survivals of Present Experiment* (1957) *with Those of Cook* (1939).

Weeks at 5° C	1939	1957
0	2	3.0
½	3	2.5
1	4	2.2
2	6	3.0
3	10	3.4
4	15	2.5
5	24	3.0
6	38	2.4
8	45	4.2

LITERATURE CITED

E. V. COOK. 1939. Influence of low temperature on recovery from roentgen rays. *Radiology* 32: 289-293.

C. S. BACHOFER and GEORGE PAHL. 1955. Influence of extended temperature on recovery of x-irradiated ascaris eggs. *Radiation Research* 2: 50-63.