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SOME EFFECTS OF IONIZING RADIATION ON THE ORAL SUCKERS OF RANA PIPIENS EMBRYOS

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INTRODUCTION

Gastrulation is perhaps the most critical stage in the development of the embryo.[1] During this stage, cells, in addition to normal cell division, are undergoing differentiation to independent and interdependent structures. During this period of differentiation the embryo is in the period of maximum susceptibility to environmental changes. Any adverse environmental changes occurring during gastrulation should produce the maximum effect on the embryo.

It has often been shown that epithelial cells possess a high degree of sensitivity, especially those that are active secretors.[2] The question immediately arises as to the sensitivity of the epithelial cells exposed to adverse environmental conditions prior to the time of their differentiation. If the embryos were subjected to exposure to gamma radiation during gastrulation, the columnar epithelial cells of the oral suckers would be exposed to the ionizing radiation prior to the time the neural plate is formed. Since the oral suckers arise from the neural plate, the cells of the oral suckers would have been exposed to gamma radiation prior to their differentiation.

The purpose of this investigation is to study the various effects of ionizing radiation, specifically gamma radiation, on the yet undifferentiated oral suckers of the **Rana pipiens** embryo in respect to effects on total body size, size of oral sucker, and position of oral sucker.[3]

MATERIALS AND METHODS

Adult **Rana pipiens** were obtained and were kept in hibernation at approximately 4°C. until needed in the investigation. Eggs were obtained by Rugh's ovulation method via anterior pituitary injection and artificial fertilization.[4]

The zygotes were then placed in finger bowls in 5 cm of pond water. There was a maximum of thirty eggs per finger

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[1]Rugh, Roberts, **The Frog Reproduction and Development**, p. 101.
[2]Rugh, Roberts, "Histological Effects on the Embryo Following X-irradiation" **Journal of Morphology**, Vol. 85 (1949), pp 483-501.
[3]The author would like to express his gratitude to Dr. James H. Fribourgh, Little Rock University, for his suggestions and guidance throughout this investigation.
[4]Rugh, Roberts, **Experimental Embryology**, pp 102-106.

bowl. The finger bowls were kept in an air-bath and by means of an oil-mercury thermo-regulator the temperature of the water in the finger bowls was maintained at 18°C -+ .08C.

The embryos were subjected to single exposures of gamma radiation at stages 8, 9, or 10. Shumway stages were employed.[5] The gamma radiation was obtained from a Keleket-Barnes Telcobalt Unit employing 1,000 curies of Cobalt 60 as the radiation source.[6] Internal filtration prevented all radiations from reaching the target area with the exception of two gamma rays. One gamma ray had an energy of 1.17 MEV and the other had an energy of 1.33 MEV. The Telecobalt Unit had recently been calibrated with a Victoreen Roentgen Meter employing the ionization method of calibration. The total error in dosage calculation inclusive in the experiment was -+ 3 percent. The dose rate was 2,000 roentgens per ten minutes and fifty-six seconds at a distance of 35 cm from the cobalt source. The embryos were irradiated 35 cm from the source in regular size finger bowls in 5 cm of water. The eggs were floating less than 1 cm below the surface of the water. The water depth was included in the dosage calculation. One group of embryos was subjected to a single exposure of 6,000r, the second group to 12,000r and the third group to 20,000r.

The embryos were killed and fixed in Bouin's Fluid at stages 18 and 19. Those embryos which had accomplished hatching were then dehydrated, cleared, infiltrated and sectioned at 10 microns. The sectioned embryos were stained in Delafield's haematoxylin and eosin counter stain. The slides were studied microscopically.

Drawings were made of the embryo and the particular regions under study with the aid of a camera lucida. The areas of the embryo and of the structures under consideration were measured with a planimeter.

On the camera lucida drawings, lines were constructed in order to measure the area of the embryos, the area of the oral suckers, and various degrees of the oral suckers. One line was drawn in the area of the notochord paralleling the structure. A second line was drawn perpendicular to the first line and through the mid point of the area of the pharynx. The line through the pharynx was the base line for measurements of various angles of the oral suckers. Using a compass, the angles were measured from the base line anteriorly.

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[5]Ibid., pp 56-72.

[6]This unit was made available through the courtesy of Dr. Howard Barnhard of the Radiology Department, University of Arkansas Medical School.

One measurement was made from the base line to the anterior edge of the oral suckers and another was made to the posterior edge of the oral suckers. From these two lines the total number of degrees of arc covered by the oral suckers could be measured as well as the maximum degree reached anteriorly and posteriorly and the mid-bearing or the mid-compass degree of the area of the oral suckers. These drawings and measurements were made of the irradiated embryos as well as the non-irradiated embryos.

RESULTS

An analysis of the experimental data reveals that the total area of the oral suckers decreased with increasing radiation exposure. The total area of the embryos decreased with increasing exposure to gamma radiation.

The position of the oral suckers moved anteriorly with increasing exposure to radiation as is indicated by the increasing degree of the mid-bearing. The total arc of the oral suckers in degrees covered was unaffected significantly by the radiation.

Table 1. Average relative planimeter measurement of **Rana pipiens** embryo, the oral suckers, the degrees of arc of the oral suckers, mid-bearing of the areas of the oral suckers, the maximum anterior and posterior degree of the oral suckers when exposed to 6,000r, 12,000r, and 20,000r of gamma radiation.

	Non-Irradiated	6,000r	12,000r	20,000r
Total area of embryo	6630	3068	3113	2146
Maximum anterior bearing of oral suckers	37.5°	65°	87°	109°
Maximum posterior bearing of oral suckers	9.8°	35°	57°	73°
Mid bearing of oral suckers	24°	51°	72°	92°
Degrees of arc of oral suckers	27.7°	30°	30°	36°
Area of oral suckers	117	60	62	42

The epithelial cells of the oral suckers lacked uniformity. The position and size of the nuclei were irregular as was the general shape of the cells.

One difficulty encountered is perhaps worthy of note. In obtaining **Rana pipiens** eggs it was found that seven female anterior pituitaries were required instead of the five suggested by Rugh for the season, September-January. (See Rugh, **Experimental Embryology.**)

CONCLUSIONS

One effect noted was that of the decrease in total area of the embryo with a corresponding increase in radiation dosage. This, in part, can be explained by Rugh's observations of the histological effects of ionizing radiation.[7] Rugh found that "mitosis was abruptly inhibited in all cells which had not started the process. Those cells which were in mitosis at the time of irradiation were either able to complete the process or the nuclei and chromatin were so severely damaged that the typical intermitotic "resting cell" stage could not be achieved."

Since the actual process by which mitosis is inhibited by radiation is not known it might be assumed that this is not an all-or-none process and that increasing dosages of radiation affect increasing numbers of cells. This would tend to support the Quantum Hit Theory, which states that the effect of ionization is due to absorption of quanta by a small sensitive volume in the cell, generally thought to be the chromosome or gene. Increasing dosages of radiation would make more quanta available for absorption thus increasing the effects or the extent of the effects. Also with increased radiation exposure more cells would be affected. Since the radiation was conducted on all the embryos at the same time, reduction in body size would be the expected results with increasing dosages of radiation if the assumptions listed above are correct. Another possibility is that of reduction in amount of interstitial material or reduction in the size of the cells or a combination of all three.

The actual measurements were not germane to this paper, hence no attempt was made to scale the drawings nor to convert the planimeter readings in Table 1 to the metric system. All drawings and measurements were made under the same conditions, consequently all data are relative.

The second observation was that the total area of the oral suckers decreased as the radiation increased. In this epithelial tissue, effects were noted that were similar to those observed by Rugh in the gut epithelium of irradiated Salamanders, for example, lack of uniformity of the position and size of the nuclei, irregular shaped cells which were seriously disarranged.[8] The oral sucker epithelium in the irradiated groups seemed to be fewer in number than of the oral suckers of the non-irradiated embryos. However, this is not claimed as a radiation effect at this time.

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[7]Rugh, Roberts, "Histological Effects on the Embryo Following X-irradiation," Journal of Morphology, Vol. 85 (1949), pp. 483-501.
[8]Ibid., pp. 483-501.

The third effect was that of the displacement of the oral suckers. With increased exposure to gamma radiation the location of the oral suckers was displaced anteriorly. The increased radiation exposure did not affect the number of degrees of arc occupied by the oral suckers, but only affected the displacement of the mid-bearing.

Since the oral suckers arise from the neural plate which extends laterally as far as the lateral neural folds and joins the anterior edge of the transverse neural folds perhaps the reason for the anterior movement was that the process of neurulation was indirectly affected through interference with the normal process of gastrulation. Radiation was carried out during gastrulation and just prior to neural plate formation. It is thought that neurulation is completely dependent upon gastrulation as far as normality is concerned.[9] If this is the case, any damage to the process of gastrulation produced by radiation would necessarily affect the process of neurulation, and might well be the answer for the anterior displacement in that the transverse neural fold occurred more posteriorly than normal thus causing the neural plate to arise more posteriorly than normal and the oral suckers more anteriorly.

The effects observed in the irradiated embryos must, at this time, be attributed **en toto** to the gamma radiation. However, the effect of the ionization of the water by the gamma rays must not be overlooked. The resulting products, for example, OH radicals, solvated electrons, protons, hydrogen atoms, molecular hydrogen and hydrogen peroxide, can not be ignored. The scope of this study does not include investigation in this area; however, it should be pointed out that Rugh observed that the effects noted in his investigations were not affected by the irradiated water.[10] Titus C. Evans concluded that the chief, if not the only, factor in the water affecting the embryos was hydrogen peroxide and the effects were definite at doses of 100,000r and questionable or ineffectual under 50,000r.[11]

It may be noted that the methods of arriving at the above conclusions did not take into consideration the half-lifes of the various ionization products. The half-lifes are extremely vari-

[9]Rollanson, G. S., "X-irradiation of Eggs of Rana Pipiens at Various Maturation Stages", **Biological Bulletin**, Vol. 95 (1949), pp. 169-178.

[10]Rugh, Roberts, "Inhibition of Growth and the Production of Oedema by X-irradiation", **Journal of Experimental Zoology**, Vol. 144 (1950) pp. 137-147.

[11]Evans, Titus C., "Effects of Hydrogen Peroxide in the Medium by Radiation on Spermatozoa of Arbacia Punctulata", **Biological Bulletin**, Vol. 93, p. 108.

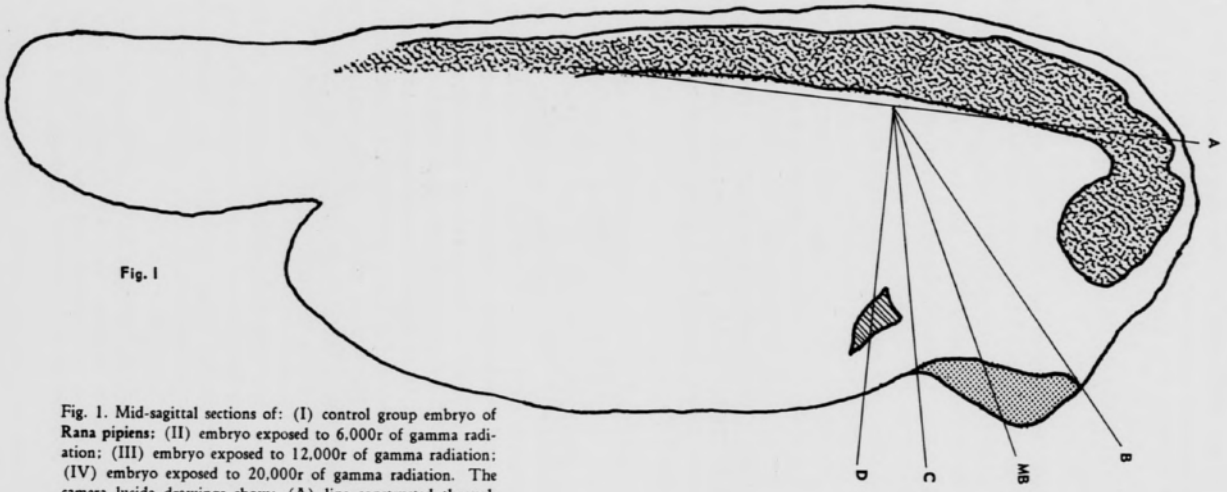

Fig. I

Fig. 1. Mid-sagittal sections of: (I) control group embryo of **Rana pipiens:** (II) embryo exposed to 6,000r of gamma radiation; (III) embryo exposed to 12,000r of gamma radiation; (IV) embryo exposed to 20,000r of gamma radiation. The camera lucida drawings show: (A) line constructed through the area of the notochord, (D) the base line through the area of the pharynx, (B) the line through the anterior extremity of the oral sucker, (MB) the mid-bearing line, and (C) the line through the posterior extremity of the oral sucker. The primitive brain is also shown.

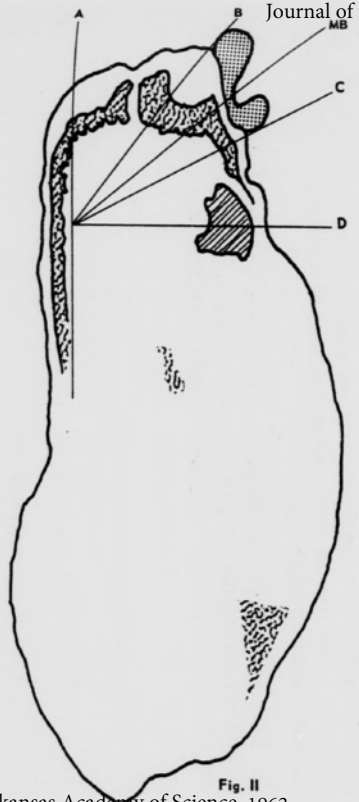

Fig. II

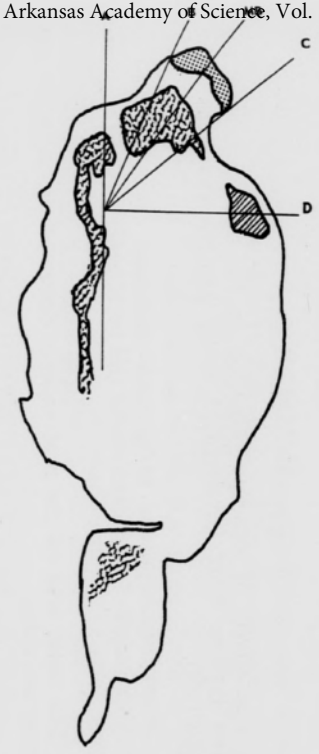

Fig. III

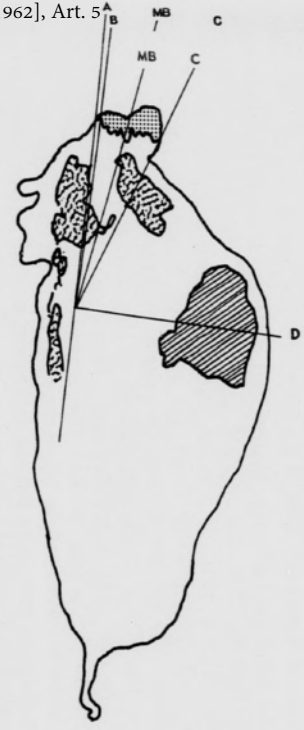

Fig. IV

able, but it is thought that the range is from 10^{-5} seconds to several minutes or hours. Investigation by Dr. Art Solomon of the BioPhysical Lab of the Harvard Medical School established that the "half-time for diffusion exchange of tritiated water into human erthrocytes was $4.2 \times 10^{-3} \pm 1.1$ milliseconds."[12] This would tend to indicate, unless red cells are unique in their rate of diffusion, that the rate of diffusion in cells of the embryos might permit infiltration of those ionization products which have half-lifes greater than 10 seconds. Since, at the time of exposure, the embryos were composed of from 59 to 96 percent water, the effect of the ionization of the media would not necessarily be the only consideration. Some of the products of ionization of the water within the embryos are extremely reactive and would have ample opportunity to react with parts of the protoplasmic structure before diffusion out of the embryos could take place.[13] Certainly extensive investigation in this area is indicated.

SUMMARY

1. The effects of ionizing radiation on the oral suckers of the Rana pipiens were investigated using gamma radiation from Cobalt 60.

2. Increasing exposure to gamma radiation from 6,000r to 20,000r resulted in a decrease in the total body area of the embryos.

3. The total area of the oral suckers decreased as radiation exposure increased.

4. Similar histoligical effects were produced in the columnar epithelium of the undifferentiated oral suckers by exposure to gamma radiation as were observed in gut epithelium of X-ray exposed salamanders.

5. Increasing exposure to gamma radiation increased the anterior displacement of the area of the oral suckers. The total degrees of arc of the oral suckers was unaffected by radiation up to 20,000r.

[12]This was established in personal communication with Dr. Solomon.

[13]The author would like to thank Dr. Daniel M. Mathews, University of Arkansas Graduate Institute of Technology, whose advice was invaluable in this investigation.