CHANGES IN SUSCEPTIBILITY OF DROSOPHILA EGGS TO ALPHA PARTICLES

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In a previous report (Henshaw and Henshaw, 1933) we have shown that *Drosophila melanogaster* eggs vary markedly in susceptibility to 200 kv. X-rays at different stages during early development. A correlation of the changes in radiosensitivity with the changes in development showed that they become more sensitive during cleavage and blastulation, much more resistant at or near the beginning of gastrulation, and more sensitive again as gastrulation gets under way. Having found that susceptibility to one type of radiation varies in a certain way, it is of interest to determine whether it varies in the same way when other types of radiation are used.

Alpha particles, beta particles, and gamma rays are the three recognized forms of radiation emitted by radioactive substances. The alpha and beta rays are corpuscular in nature while the gamma radiation is like X-rays of very short wave length. All of these forms of radiation, including X-rays, are capable of causing ionization in matter subjected to their influence. According to the Rutherford-Bohr theory of the structure of the atom, the alpha particle is identical with the helium atom stripped of its two planetary electrons. It therefore has an electric charge of two positive and a mass about 7000 times that of the beta particle or electron (Failla, 1927). Since it has atomic dimensions and has a velocity of .15 to .20 times that of light, it represents an enormous concentration of kinetic energy. " In fact the alpha particle is the most potent agent known to science " (Lind, 1928).

In addition to the 200 kv. X-rays used previously, we have had at our disposal 40 kv. X-rays, gamma rays, and alpha particles. Tests determining susceptibility changes have been made with each of these. The responses to 40 kv. X-rays and gamma rays were essentially the same as to 200 kv. X-rays. With alpha particles, however, the effects were very different. Experiments with alpha particles will therefore be presented in detail, after which results for all will be compared.

Alpha Radiation

The source of alpha particles was polonium, which is one of the radioactive products of radium. Polonium was extracted from the walls of old radon tubes which had been used in cancer therapy. This was done with a dilute acid solution (.2 per cent HCl, approximately). The extract solution was flooded onto one surface of a copper disc 3.5 cm. in diameter and the polonium deposited on the metal surface. The amount retained by the disc was sufficient to kill *Drosophila* eggs after several minutes exposure. That alpha particles were responsible for the killing observed and not some other form of radiation was made certain by experiments which will be described later.

Treatment of Eggs

A large collection of eggs was obtained from actively laying flies during a two-hour period.[1] This was divided into several portions, usually fifteen, each containing more than 100 eggs and often more than 200. With a soft brush the eggs were spread evenly in a single layer on small pieces of filter paper and were then ready for treatment.

The exposures were made in moist chambers, arranged by placing moist filter paper on the bottom of Petri dishes 15 cm. in diameter. The filter paper strips carrying the eggs were placed on these near the center. The copper disc was fastened in the center of the Petri dish cover so that the plated surface was directed toward the eggs and was about two centimeters above them when the dish was covered in the usual way. Eggs placed directly under the disc were killed by the radiation while those placed 5 cm. or more away were unaffected.

In order to determine the susceptibility of the material at different ages, tests were performed on different parts of the same collection of eggs at hourly intervals. The first began 15 minutes after the end of the collection period. Four samples were used for each test; the first three received 15, 30, and 45 minutes exposure, respectively; the fourth was kept as a control. Three exposures were used in order that the effect of different amounts of radiation could be considered. After treatment the samples and the control were put away in moist chambers at room temperature (22–25° C.). On the third day after irradiation, counts were made to determine the percentage hatched. This served as the criterion of effect.

Results

The data obtained are shown in the accompanying table. Since age in development is the point in question, age is given relative to the time

[1] For culture of flies and the collection of eggs, see the earlier report—Henshaw and Henshaw.

of fertilization rather than the time of collection. Since the eggs are fertilized individually as they pass through the vagina of the female fly and are deposited at any time during the collection period, their age is

TABLE I

Data on Changes in Susceptibility of Drosophila Eggs to Alpha Particles

	Exp. No	Age in Hours							
		1¼	2¼	3¼	4¼	5¼	6¼	7¼	8¼
		Percentage Eggs Hatching							
Controls	1	86	93	96	94	87			
	2	91	92	88	89				
	3	90	94	96	91	95		93	
	4	97	93	91	96	92	93		93
	5	93	99	93	89	98			
	6	98	97	99	99	98			
Average		92	93	94	93	94	93	93	93
15 min. exposure	1	90	42	11	37	74			
	2	84	35	5	30				
	3	88	42	33	41	50		82	
	4	88	23	13	34	52	74		
	5	89	72	28	66	89			
	6	83	21	4	19	49			
Average		87	39	16	38	52	74	82	
30 min. exposure	1	81	22	5	15	32			
	2	81	22	4	10				
	3	85	18	20	22	19		83	
	4	71	10	5	8	28	44		81
	5	46	35	29	22	84			
	6	71	10	6	6	17			
Average		76	19	11	14	36	44	83	81
45 min. exposure	1	86	1	3	11	21			
	2	71	9	1	4				
	3	68	11	18	19	14		60	
	4	56	12	11	5	9	35		61
	5	49	23	26	18	79			
	6	45	8	10	6	29			
Average		63	11	11	11	30	35	60	61

known to vary as much as two hours. It is necessary, therefore, to speak of an average age for the eggs in a sample. As pointed out in the earlier report, this is approximately the middle of the laying period. Thus if age is reckoned from the average time of fertilization, the eggs are one hour old at the end of collection.

As seen from Table I, a control sample was kept for every age group irradiated. Among these the fertility varied from 87 to 98 per cent. The average, therefore, was 92 per cent; this was taken as 100 per cent and other values were adjusted accordingly. The average values were thus treated and plotted in Fig. 1 (solid line curves).

The abscissa (Fig. 1) shows age in hours and stage in development, while the ordinate shows the percentage of eggs hatching. The curves, therefore, show the percentage of eggs hatching at different ages after receiving 15, 30, and 45 minutes exposure to alpha particles. It will be seen that the eggs become increasingly sensitive during the first two or three hours after fertilization, after which time they become more resistant again. The point of maximum sensitivity (minimum resistance), therefore, is reached between 2 and 4 hours after fertilization.

The dash line curves show data obtained previously for 200 kv. X-rays. The shape and position of such curves vary depending upon the dosage of radiation administered. Accordingly, those for 2.5 (*A*) and 5 (*B*) minutes exposure (234 rœntgens per minute) were selected to show the most prominent changes. In the previous experiment a series of different dosages were used, but without exception there was a distinct increase in resistance between 2 and 4 hours after fertilization. The subsequent fall in resistance shown by curve *B* appeared only when larger dosages were given. By comparing directly the curves for the two types of radiation it is seen that, in general, they vary just opposite. While the eggs become more resistant to 200 kv. X-rays, they become more susceptible to alpha particles; the point of greatest resistance to 200 kv. X-rays and the point of greatest susceptibility to alpha particles being reached at about the same time.

Less complete data for 40 kv. X-rays (soft X-rays) and gamma rays are shown in Fig. 2. Only one dosage was used in each case. The curves for these radiations are similar to the one for 2.5 minutes exposure to 200 kv. X-rays. Presumably, therefore, if dosages had been varied for these radiations as for 200 kv. X-rays, similar variations due to dosage would have been observed. Whether this is actually the case can be determined only by further experiment, but the evidence is sufficient to show clearly that the organisms become more resistant to 40 kv. X-rays, 200 kv. X-rays, and gamma rays when they become more susceptible to alpha particles.

The problem, therefore, is to account for the difference in response to alpha particles. Since the response varies for different radiations

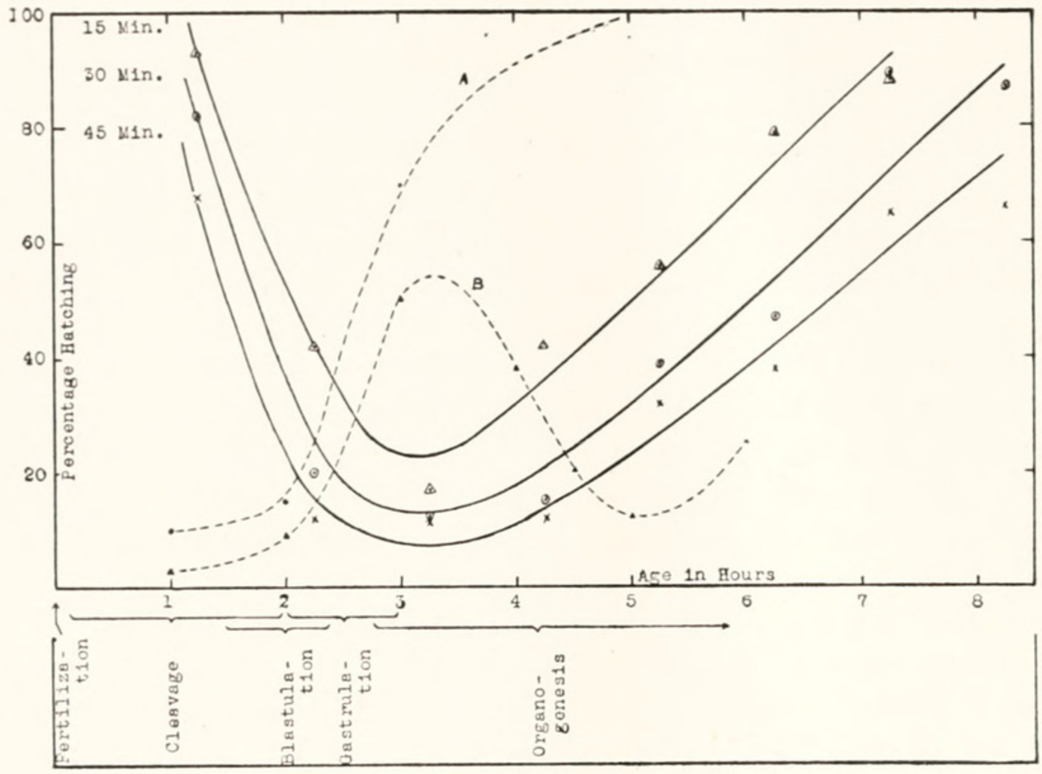

FIG. 1

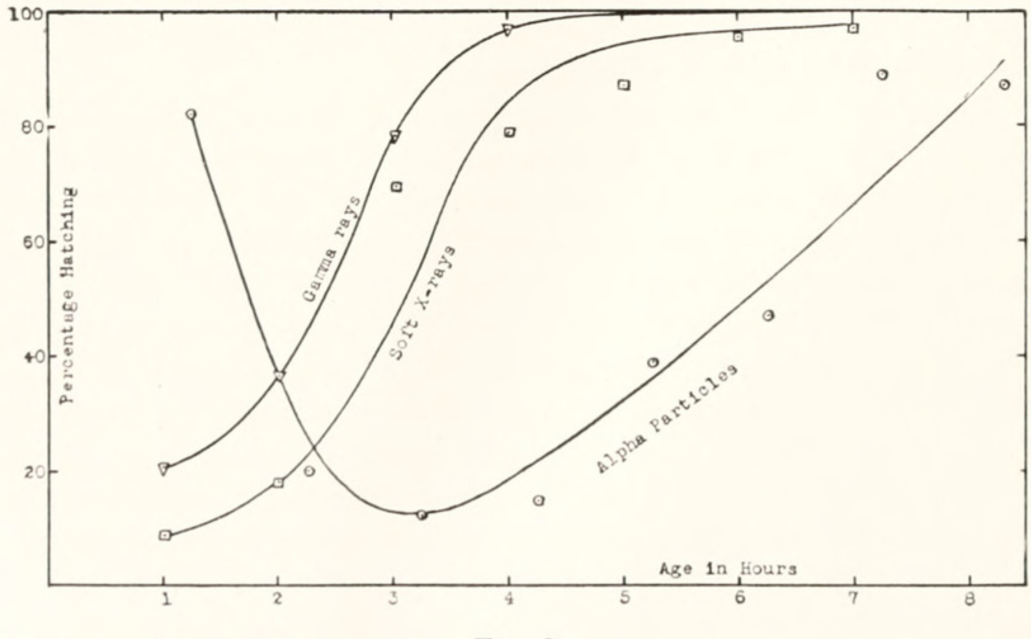

FIG. 2

FIG. 1. Curves showing changes in susceptibility to alpha particles (solid line curves) and 200 kv. X-rays (dash line curves). The index at the base of the figure shows the stage in development at different ages.

FIG. 2. Curves showing the changes in susceptibility to 40 kv. X-rays (soft X-rays), gamma rays, and alpha particles.

and different ages, it is evident that both the character of the radiation and the processes of development are involved. These will be discussed in turn.

Properties of the Radiations

One of the essential differences between the radiations used is the power of penetration into matter. " As a rough working rule, it may be taken that beta particles are about 100 times as penetrating as alpha particles and that gamma rays are from 10 to 100 times as penetrating as the beta rays. . . . A thickness of .006 cm. of aluminum or mica or a sheet of ordinary writing paper is sufficient to absorb completely all the alpha particles " (Rutherford, Chadwick, and Ellis, 1930). Since, therefore, the large supply of kinetic energy possessed by the alpha particles is expended in so short a distance, much greater destruction is caused per unit length of their trajectory.

Alpha particles of polonium are completely stopped by 4 cm. of air, while beta particles require 5 mm. of aluminum or 1 mm. of lead to stop all (Lind, 1928). Gamma rays, on the other hand, will pass through 15 cm. of lead and the softer X-rays will pass through 1 mm. or more of aluminum. It is certain, therefore, that gamma rays and X-rays and some beta particles are capable of penetrating to all parts of the *Drosophila* egg. Unfortunately, microscopic preparations of eggs which have been exposed to alpha particles do not show delimited changes which mark the extent of penetration.

Alpha particles from polonium penetrate water a distance of 32 μ (Michl, 1914). Presumably, they penetrate about the same distance into protoplasm, since it has approximately the same density. Feichtinger (1931) reports that alpha particles of polonium produce visible effects in root tips to a depth of 30 μ. Zirkle (1932) described the effects of this radiation on the spores of the fern, *Pteris longifolia,* which have a diameter of about 38μ. The nucleus in this form ordinarily lies to one side of the center. When irradiated with the nucleus directed away from the source of radiation the effect was very different from that obtained when it was on the side directed toward the source. In this case, the particles must have penetrated to about half the diameter of the organism or approximately 20 μ. While carrying out some work on eggs of the sea urchin, *Arbacia punctulata,* we also have obtained some idea of the penetration of alpha particles into protoplasm.

Arbacia eggs, when placed in a hanging drop, settle to the lower surface where they can be irradiated with alpha particles. A sample of eggs exposed for 10–15 minutes while in the two-cell stage and examined at subsequent intervals will contain deformed individuals similar to those shown in Fig. 3, row *A*. If exposed while in the four- or eightcell stages, figures like those in rows *B* and *C*, respectively, will be found. Normally, the cells divide equally and simultaneously during early cleavage so that the number of cells increases 2–4–8–16, etc. It appears that during exposure some of the eggs by random distribution took a position with one cell (in the first case) shielded by the other from the radiation and that the radiation penetrated only far enough to destroy the cell nearest the surface. Since the over-all diameter of this organism in the two-cell stage is about 75 μ and since alpha particles appear to have penetrated not more than half this distance, their range in this case must have been less than 40 μ.

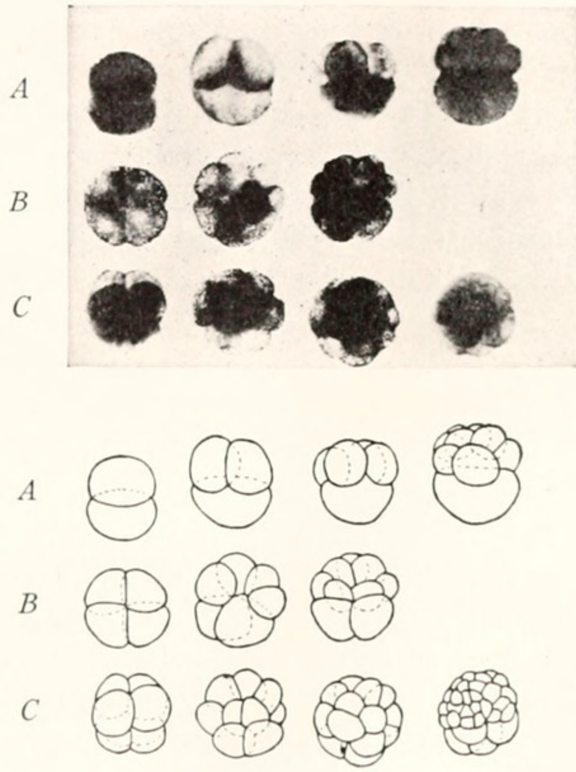

FIG. 3. Photomicrographs and diagrams showing the effects of alpha particles on *Arbacia* eggs when exposed in the 2-cell (*A*), 4-cell (*B*), and 8-cell (*C*) stages.

From the instances cited, it seems clear that the range of alpha particles in protoplasm is approximately 30 μ. Since then, the transverse diameter of the *Drosophila* egg is about 100 μ, it is improbable that the particles ever reach their center. In the case of the experiment above they were probably stopped near the surface since they passed through 2 cm. of air and the membranous chorion before reaching the living parts. Since the difference in results obtained with the different radiations may be accounted for on the basis of penetration (see below), other properties of the radiations need not be considered.

The tests performed to make certain that alpha particles and not some other radiation were responsible for the results obtained depended on differences in penetration range of the different radiations and may be given briefly at this point. An ordinary piece of writing paper was placed over the polonium-plated disc during exposure in one case, and the eggs were placed at a distance of 4 cm. from the disc in another. In both cases no eggs were killed, although the time of exposure was continuous from the end of collection to the time of hatching. Since the

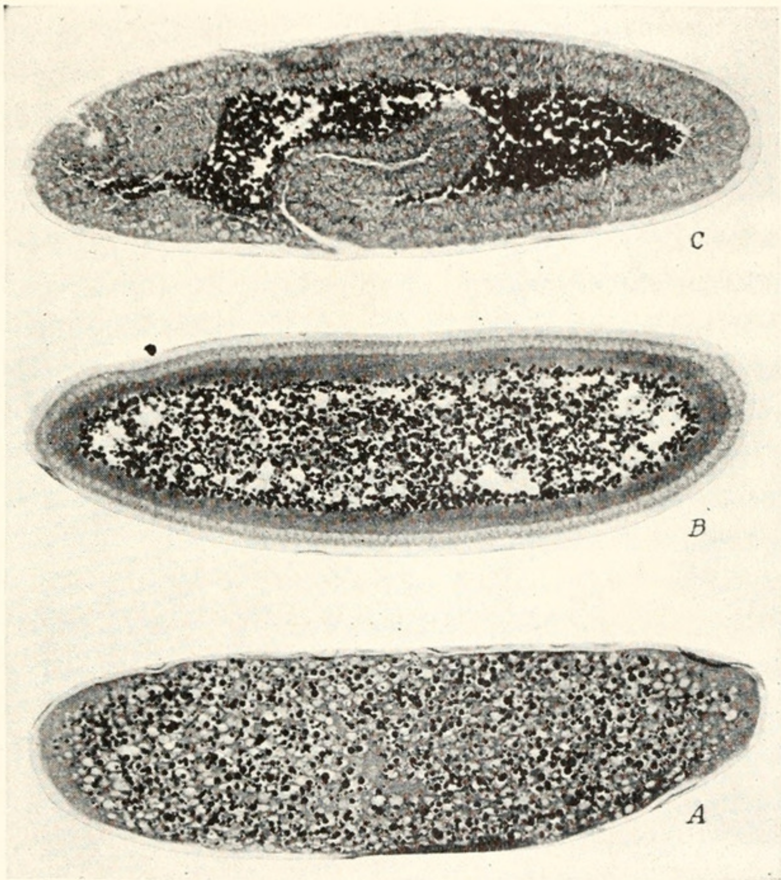

FIG. 4. Longitudinal sections of *Drosophila* eggs of different ages: (*A*) before nuclei begin to migrate to periphery, 0–1.5 hours old after fertilization (*B*) blastular stage, 1.5–2.5 hours after fertilization; and (*C*) gastrular stage, 2–3 hours after fertilization.

beta and gamma rays which might be present would have a greater range than 4 cm. of air and would not be stopped by a sheet of paper, the observed effects may safely be attributed to alpha particles.

EARLY EMBRYOLOGY IN DROSOPHILA

Development as it pertains to the present discussion may be described briefly. The egg and sperm nuclei unite near the center of the egg and the early cleavages, nuclear only, take place synchronously in the central region at the rate of 1 in 10–12 minutes at room temperature (22–25° C.). At about the eighth or ninth cleavage (Huettner, 1923), approximately 100 minutes after fertilization, the nuclei begin to migrate to the periphery where cell membranes are formed around them and where they arrange themselves in a single layer to form the blastoderm. This thickens by continued mitosis and growth and at about 150 minutes after fertilization gastrulation begins by invagination (Figs. 1 and 4).

Correlations

By correlating the changes in sensitivity with the location of the cells at different stages, it is seen that the eggs become increasingly susceptible as the nuclei move toward the periphery, that they are the most susceptible when the largest number of cells are active at the periphery, and that they become more resistant as the actively growing regions move away from the surface toward the center again. Because of this it is believed that the changes in susceptibility to alpha particles may be accounted for by saying that (1) the amount of radiation used is sufficient to produce death only when active cells (or nuclei) are affected; that (2) at first, the cells, being in the central region of the egg, are beyond the range of the particles; that (3) they move into the range as they pass to the periphery; and that (4) the active cells move out of the range again at the time of gastrulation.

Summary

1. Changes in the susceptibility of *Drosophila* eggs to different kinds of radiation have been followed during early development.

2. It was found that the eggs become more susceptible to alpha particles during cleavage and blastulation and more resistant during organogenesis, the point of maximum sensitivity being reached at or just after the beginning of gastrulation.

3. A comparison of these findings with those obtained for 200 kv. X-rays showed that the eggs were most resistant to the X-rays when they were most susceptible to the alpha particles.

4. Further experiments with 40 kv. X-rays and gamma rays indicated that the organisms were consistent in their responses to radiation capable of penetrating uniformly to all parts.

5. It appears, therefore, that the difference in response to alpha particles was due mainly to two factors: (*a*) the short penetration range of the radiation into protoplasm, and (*b*) the movement of the active cells into and out of its range.

Acknowledgments: The writers wish to express their indebtedness to Dr. G. Failla for suggesting the use of alpha particles and for his aid and helpful suggestions during the course of the work.

REFERENCES

Failla, G., 1927. Radium in Gynecology, Chapter II. J. B. Lippincott Co., Philadelphia.

Feichtinger, N., 1931. Über die Einwirkung der Alpha und Beta Strahlen auf das Protoplasm. *Protoplasma*, **14**: 36.

Henshaw, P. S., and C. T. Henshaw, 1933. Changes in Susceptibility of Drosophila Eggs to X-rays. I. A correlation of changes in radiosensitivity with stages in development. Radiology. In press.

Heuttner, A. F., 1923. The Origin of the Germ Cells in Drosophila melanogaster. *Jour. Morph.*, **37**: 385.

Lind, S. C., 1928. The Chemical Effects of Alpha Particles and Electrons. The Chemical Catalogue Co., New York.

Michl, W., 1914. Über die Reichweite der Alpha-strahlen in Flussigkeiten. *Sitzb. Akad. Wiss. Wein.*, **123** (2a) : 1965.

Rutherford, E., J. Chadwick, and C. D. Ellis, 1930. Radiations from Radioactive Substances. Cambridge University Press.

Zirkle, R. E., 1932. Some Effects of Alpha Particles Upon Plant Cells. *Jour. Cell Compar. Physiol.*, **2**: 251.