FURTHER STUDIES ON THE EFFECTS OF X-RADIATION ON THE MULTIPLICATION OF RICKETTSIA MOOSERI IN EMBRYONATE EGGS*

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Recently we have shown that the multiplication of *Rickettsia mooseri* in embryonate eggs was accelerated and quantitatively increased by single dose x-radiation given either 24 hours before or 48 hours after inoculation of the organisms. This effect was noted at all dosage levels studied, ranging from 100 to 1500 r (4, 7). However, these studies left open several questions regarding time of inoculation of rickettsiae and irradiation of the embryonate egg. The present report describes the results of experiments designed to test further the relationship of time of inoculation of rickettsiae to time of x-irradiation.

Materials and Methods

The methods used for preparing the inoculum, infecting fertile eggs, making and staining yolk sac smears, and determining the degree of infection were those described in previous papers (5, 6).

Eggs were candled daily, and smears were made from the yolk sacs of those showing death of the embryo. Between the 8th and 13th day after rickettsial inoculation (depending on the data desired), the experiments were terminated and smears were made from the yolk sacs of all eggs, regardless of whether the embryos were alive or dead. All smears were stained by Giemsa's method, and the degree of infection was determined by counting the number of rickettsiae per oil immersion field. Results were recorded as follows: 0, no rickettsiae seen; 1+, 1–10 rickettsiae per field; 2+, 10–100; 3+, 100–1000; 4+, 1000–5000; 5+, 5000–10,000; 6+, 10,000–15,000 or more. Several fields were studied in each smear; in heavily infected eggs, organisms were counted in only a portion of each field. Although the figures represent only approximations, because of variations in thickness of the smears and other factors, it is believed that they reflect fairly accurately the degree of infection present in each group of eggs.

Irradiations were accomplished with a maxitron 250 kv. generator operated at 30 ma. through 3.0 mm. of Cu and 1.0 mm. of Al. The dose rate was 67.5 r.p.m. The x-rays had a measured half-value layer of 3.5 mm. of Cu indicating a 76 per cent exit dose through the eggs. Groups of 10 eggs were mounted on a bakelite plate in a field homogeneous within 2 per cent at a target distance of 40 cm.

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RESULTS

Irradiation of the Inoculum (Table I).

A suspension of rickettsiae was prepared in the usual manner and divided into three equal parts. Two of the portions were pipetted into Petri dishes. One sample was given 500 r of x-rays; the other 1000 r. The samples were agitated gently while being irradiated at room temperature. Groups of 30 embryonate eggs were inoculated on the 5th day of incubation with the non-irradiated and the irradiated rickettsial suspensions.

The infections in all groups followed the same pattern; namely, death of all embryos by the 9th day after rickettsial inoculation, and 4+ or 5+ (or an occasional 3+) infection in all eggs in which embryonic death occurred on or after the 7th day after inoculation. Thus irradiation of the rickettsial suspension

TABLE I

The Effect of Irradiation of the Inoculum on the Growth of Rickettsiae in the Embryonate Egg

Age of embryos	Non-irradiated inoculum	Irradiated inoculum (500 r)	Irradiated inoculum (1000 r)
5		Rickettsiae inoculated	
6	0	0, 0	
7		0, 0, 0	0
9	1, 1, 2, 3	1, 2, 2	1, 1, 1, 3
10	1, 2, 2	2, 3	1, 1, 2
11	2, 2, 3, 3, 4	2, 3, 3, 4	3, 3, 3, 3, 3, 4
12	3, 3, 4, 4, 4, 4	3, 4, 4, 4, 4, 5, 5	4, 4, 4, 4
13	4, 5, 5	3, 3, 4, 5	5, 5, 5, 5, 5
14	3, 3, 4, 4, 5, 5, 5, 5	4, 4, 5, 5, 5	3, 4, 4, 4, 5, 5

0, no rickettsiae seen; 1+, 1–10 rickettsiae per oil immersion field; 2+, 10–100; 3+, 100–1000; 4+, 1000–5000; 5+, 5000–10,000; 6+, 10,000 or more.

prior to inoculation did not change the growth pattern of these organisms in embryonate eggs.

Irradiation Prior to the Inoculation of Rickettsiae (Table II).

One hundred and eighty embryonate eggs were divided into 6 groups of 30 eggs each. Three groups were given 500 r of x-rays on the 4th day of incubation. An irradiated and a non-irradiated group were inoculated with rickettsiae on the 5th, 8th, and 11th days of incubation.

Rickettsial multiplication occurred earlier and reached higher peaks in all groups of eggs receiving x-radiation than in the corresponding control groups. This fact was further confirmed by counting the number of heavily infected cells, recognizable under low power by their solid bluish-purple appearance in the Giemsa-stained smears. Both methods of measurement indicated that irradiated embryonate eggs show better rickettsial growth. This may be attributed to alterations in the host which last at least 7 days. Since one finds normally

a reduced growth of rickettsiae in the control eggs inoculated on the *8th day* of incubation and a very scanty growth in eggs inoculated on the 11th day of incubation, and as irradiated eggs always showed higher titers, it appears that radiation may alter some of the changes accompanying the maturation of the host.

TABLE II

The Effect of Prior Irradiation of the Embryonate Egg on the Growth of Rickettsiae

Age of embryos	Control	500 r	Control	500 r	Control	500 r
days						
4		Irradiated		Irradiated		Irradiated
5	Rickettsiae inoculated					
8			Rickettsiae inoculated			
9	0	0	0	0		
10	0, 0	1, 1, 2, 2		0		
11	0, 1, 1	1, 1, 2, 2	0	0	Rickettsiae inoculated	
12	1, 1, 1, 1	2, 3, 3, 4, 4, 4, 4	0, 0, 0	0	0	0, 0
13	2, 2, 2, 3, 3, 3, 3, 3	3, 4, 4, 5, 5, 5, 5	1, 2, 2	3, 3, 3, 3		0, 1
14	2, 2, 3, 3, 3, 3, 3, 4, 4, 4, 4, 4	4, 4, 5, 5, 5, 5, 6, 6	1, 1, 1, 1, 2, 2, 2, 3	2, 2, 2, 2, 2, 3, 3, 3, 3	0, 0	
15			1,* 1,* 1,* 2,* 2,* 2, 2, 2, 3	4, 4, 4, 5, 5, 5, 5, 5	0, 0	
16			2,* 2,* 3,* 3, 3	4, 4, 4, 5, 5, 5	0,* 0*	2,* 2,* 3*
17					0,* 0*	2,* 3,* 3*
18					0,* 0,* 0,* 0,* 0,* 2,* 2,* 2,* 2,* 3,* 3,* 3, 3	3, 3, 3, 3, 4, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5

* Embryos alive at time of examination.

Irradiation Subsequent to the Inoculation of Rickettsiae (Table III).

Ninety embryonate eggs inoculated with rickettsiae on the 5th day of incubation were divided into three equal groups. One group was given 500 r of x-rays on the 4th day (1 day before inoculation). One non-irradiated group served as a control. When the smears of the yolk sac membranes of the eggs of the control group showed moderate numbers of rickettsiae (2+), the last group was given 500 r of x-rays (11th day of incubation).

The eggs of the control group showed 1+ and 2+ infections 6 days after the inoculation of rickettsiae and died with 3+ and 4+ infections on the 7th, 8th,

and 9th days after inoculation. The eggs of the group irradiated prior to the inoculation of rickettsiae showed 2+ and 3+ infections on the 4th day after inoculation, and all embryos were dead by the 8th day with 4+ and 5+ infections. The eggs of the group irradiated after the growth of the organisms had been established (10th day of incubation) died on the 12th and 13th days of incubation, and smears of the yolk sac membranes showed 4+, 5+, and 6+ infections to be present. This and similar experiments indicate that the greatest enhancement of the growth of rickettsiae by x-rays occurred when moderately infected cells were irradiated.

TABLE III

The Effect of Irradiation Subsequent to the Inoculation of R. Mooseri on the Growth of Rickettsiae in the Embryonate Egg

Age of embryos	Control	Irradiated	Irradiated
days			
4		*500 r*	
5		Rickettsiae inoculated	
8	0, 0	0	0, 0
9	0	1, 1, 2, 2, 3, 3	
10	1, 1	2, 2, 3, 3, 3	1
11	1, 1, 2, 2, 2, 3, 3	3, 3, 4	1, 1, 2, 3, 3 *500 r*
12	2, 3, 3, 4, 4	4, 4, 4, 4, 4, 4	4, 4, 4, 5, 5, 5, 5, 6, 6, 6
13	3, 3, 4, 4, 4, 4	4, 4, 4, 4, 4, 5, 5, 5	4, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 6
14	3, 3, 3, 4, 4, 4, 4		

DISCUSSION

The lack of an apparent effect of x-rays on the rickettsial suspension is not surprising, since significant effects are usually observed in suspensions of viruses and bacteria only after much higher dosages (2, 10). This observation, together with the results of the experiments in which inoculation followed irradiation by many days, makes it certain that the enhancement of the growth of rickettsia by x-rays is the result of radiation-induced changes in the cells of the host.

The initial physical and chemical changes induced in protoplasm by ionizing radiations are unknown at the present time (3, 8, 11, 12). Whatever their nature, the resulting ionized or excited atoms could give rise to chemical components which are as foreign to the cell as materials introduced by microinoculation. Subsequent biological events following irradiation are probably related to the enhancement of the growth of rickettsiae in the irradiated embryonate egg.

One of the effects of irradiation is the so called "inhibition of mitosis," which has been observed in many organisms and tissues (13). The antimitotic effects of irradiation are often followed by a period of recovery during which mitotic activity reappears. Degenerative changes of varying severity are frequently observed to follow the recovery period. The importance of the stage of development and the metabolic activity of the tissues of the host for the growth of rickettsiae and viruses has been pointed out by many investigators (1). If, in abnormal cells, degraded cellular constituents persist that are favorable to rickettsial growth, the increased growth of this organism in embryonate eggs irradiated before inoculation might be explained. The results reported here indicate that this type of change, if it exists, persists for 7 days after treatment.

It is evident from the data given in Table III that the greatest enhancement of the growth of rickettsiae by x-rays occurred when moderately infected cells were irradiated. The greatly accelerated growth probably was brought about by the presence of organisms able to capitalize immediately on the biochemical disturbances within cells produced by irradiation. A possible clue as to the nature of these biochemical alterations is furnished by recent work (9) showing that the level of plasma amino acids in the developing chick embryo increased following irradiation. In all samples of the irradiated group taurine, β-alanine, γ-amino-butyric acid, methylhistidine, cystine (as cysteic acid), hydroxyproline, ethanolamine, and phosphoethanolamine are increased significantly. The determination of the relative importance of these general radiation effects in the chick embryo (slowing of development and increased amino acid content) for the increased growth of rickettsiae awaits extended studies of the histological and biochemical phenomena.

SUMMARY AND CONCLUSIONS

The effect of x-rays on the growth of rickettsiae in the embryonate egg was investigated.

The intensifying effect of x-radiation of the host on rickettsial growth can be attributed to alterations of the cells of the host that persist at least 7 days.

The greatest enhancement of the growth of rickettsiae occurred when moderately infected cells were irradiated.

These experiments indicate that x-rays may neutralize or reverse changes in the host that are unfavorable to the growth of rickettsiae.

Explanations of the observed phenomena in terms of biochemical and biological alterations of the cells of the host are discussed.

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