THE EFFECT OF X-RADIATION ON THE MULTIPLICATION OF RICKETTSIA MOOSERI IN EMBRYONATE EGGS*

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Single doses of x-radiation are known to cause profound alterations in cellular metabolism, particularly with respect to nucleic acid synthesis (1). The purpose of the experiments to be reported here was to determine as precisely as possible the effect of radiation on the host-parasite relationship in experimental rickettsial infection. Zinsser and Castaneda showed in 1928 that x-radiation caused the accumulation of unusually large numbers of rickettsiae in the peritoneal cavity of the rat after intraperitoneal inoculation of *Rickettsia mooseri* (2). Radiation at the dosages used (600 r) caused profound bone marrow damage as well as severe illness and lowered body temperature, and it was not clear whether the effect observed was the result of such systemic factors or of specific changes caused by radiation in the peritoneal lining cells within which the rickettsiae multiplied. By the use of embryonate eggs, these variables can be largely eliminated, since antibody formation does not occur (3), and the temperature remains constant.

Material and Methods

The methods for injecting rickettsiae and drugs into the yolk sacs of embryonate eggs, and for estimating the degree of infection at various time intervals after injection have been described in detail in previous papers (4, 5).

In the first experiment, eggs of the White Rock variety were divided into 18 groups of 25 to 40 each. All eggs were injected with rickettsiae after 5 days of incubation of 37.5°C. Thereafter the temperature of incubation was 37.5°C., unless otherwise stated. The groups were as follows: (1) control, kept at 37.5°C.; (2) control, incubated at 40°C. after injection; (3) 6.6 mg. PABA injected into yolk sac 48 hours after rickettsial injection; (4, 5, 6, 7, and 8) given, respectively, 100, 250, 500, 1000, and 1500 roentgens of x-radiation 24 hours before rickettsial injection; (9, 10, 11, and 12) given 100, 250, 500, and 1000 r 48 hours after rickettsial injection; (13, 14, and 15) given 100, 250, and 500 r 48 hours after rickettsial injection, and also PABA as in group 3; (16, 17, and 18) given 100, 250, and 500 r at 48 hours and incubated at 40°C.

Eggs were candled daily, and smears were made from the yolk sacs of those showing death

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of the embryo. On the 8th day after rickettsial injection, the experiment was 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 determined by counting the number of rickettsiae per oil immersion field. Results were recorded as fol-

TABLE I

Effect of Radiation 24 Hours before Injection on Rickettsial Growth in Eggs

Time after¶ Injection	Control	100 r	250 r	500 r	1000 r	1500 r
days						
1		0, 0, 0+	0, 0, 0, 0 0, 0, 0	0, 0, 0, 0 0, 0, 0, 0	0, 0, 0, 0, 0 0, 0, 0, 0	0, 0, 0, 0 0, 0, 0
2			0	0, 0		0, 0
3	0, 0, 0, 0 0, 0	+2, 2	0	0, 1	1	1, 1, 2, 2
4	0, 0, 0	3		0, 1	1, 2, 3, 3, 3	2, 2, 2
5	0, 0	3, 3, 4, 4	3, 3, 3, 4 3	2, 3, 4, 5 5, 5, 5	3, 4, 4, 4, 4 4, 5, 4	3, 3, 3, 3 4, 4, 5
6	3	5, 6, 6	4, 4, 4, 4 5	4, 4, 5, 5 6	3, 4, 4, 5, 5 5, 6, 6	4, 4, 5, 5 5, 5
7	3, 4	3, 5, 5, 6	4, 5, 5, 5 5, 6, 6	4, 5, 5, 6	5, 6, 6	4, 5
8	3*, 3*, 4* 4, 4, 4, 4 4, 4, 5	5		5		

Each figure or symbol represents an individual egg.
0, No rickettsiae recognized.
+, Less than one organism per oil immersion field.
1, 1–10 organisms per oil immersion field.
2, 10–100 organisms per oil immersion field.
3, 100–1000 organisms per oil immersion field.
4, 1000–5000 organisms per oil immersion field.
5, 5000–10,000 organisms per oil immersion field.
6, 10,000–15,000 or more organisms per oil immersion field.
* Embryo alive at time of examination.

lows: 0, no rickettsiae seen; +, less than 1 per field; 1, *1–10* per field; 2, *10–100*; 3, *100–1000*; 4, *1000–5000*; 5, *5000–10,000*; 6, *10,000 to 15,000 or more*. Several fields were studied in each smear and in heavily infected eggs organisms were counted in only a portion of each field.

Although the figures represent only approximations, due to variations in thickness of the smears and other factors, it is believed that in the aggregate they reflect fairly accurately the degree of infection present in each group of eggs.

TABLE II

Effect of Radiation 48 Hours after Injection on Rickettsial Growth in Eggs

Time after injection	Control	100 r	250 r	500 r	1000 r
days					
3	0, 0, 0, 0 0	0, 0, 0, 0	1, 1	2, 1, 1, 1 1	2, 1, 1
4	0, 0, 0		0	2	2, 1
5	0, 0	1	3, 2	3, 3, 4, 4 4, 4, 3, 3	3, 3, 3, 4 4, 4, 4
6	3	4	4, 4, 5, 6 4, 4, 6	4, 4, 5, 5 5, 5, 5, 5 6, 6, 6	4, 4, 4, 4 5, 5, 5, 5 5, 5, 6
7	3, 4	2, 4	4, 5, 5, 5, 5 6, 6, 6, 6	4, 5, 6	4, 6, 6, 6 6, 6
8	3*, 3*, 3* 4, 4, 4, 4 4, 4, 5	3*, 3*, 3* 4*, 4*, 4* 4*, 4*, 4 4, 4, 4, 5 5, 5, 5	4, 4, 4, 4 5, 5, 5, 5 5		4

For explanation of symbols see Table I.

TABLE III

Prevention by Radiation of Rickettsiostasis Due to Incubation at 40°C.

Time after injection	37.5° control	40° control	40° 100 r	40° 250 r	40° 500 r
days					
2			Radiated	Radiated	Radiated
3	0, 0, 0, 0 0, 0	0, 0, 0, 0 0	0, 0, 0, 0 0, 0, 0, 0 0, 0, 0, 0	0, 0, 0, 0 0, 0, 0	0, 0, 0, 0 0, 0, 0, 0 0
4	0, 0, 0, 0		0, 0	0, 0, 0, 0	0, 0
5	0, 0	0, 0	0, 0, 0		
6	3	0	0, 0	1, 1, 3	2, 2, 3, 3, 3 3, 3, 4, 5
7	3, 4			2, 3, 4	2, 2, 3, 4, 4
8	3*, 3*, 4* 4, 4, 4, 4 4, 4, 5	0*, 0*, 0* 0*, 0*, +* +*, 1*, 1* 1*, 1*	+*, 1*, 1* 2*, 2*, 3* 3, 3, 4* 3*, 3, 3, 3	1*, 2*, 2* 2*, 2*, 2 2, 2, 3	3, 3, 4, 5 5

For explanation of symbols see Table I.

TABLE IV

Prevention by Radiation of Rickettsiostasis Due to Streptomycin

Time after injection	Control	Penicillin, 2000 units	Streptomycin, 1 mg.	Aureomycin, 1 mg.	Penicillin plus radiation	Streptomycin plus radiation	Aureomycin plus radiation
days							
3	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0	0, 0, 0, 0, 0, 1	0, 0, 0, 0
4	0, 0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0	0, 0	2, 2	0, 0, 0
5	3		0	0	0	3	
6	3, 4, 4	0, 0	0, 0, 1			4, 4	0
7	3, 3, 3, 3, 3 3, 4	0	0, 0		0, 0	4, 4	+0
8	3, 4, 4, 4, 4	0	0, 1	0		3, 3, 4, 4, 4, 4	0
9		0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 1 1, 2	0*, 0*, 0*, 0*, 0* 0*, 0*, 1*, 1* 1*, 1*	0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0* 0*, 0*	0*, 0*, 0*, 0* 0*, 1*, 1*, 1* 1*	4, 4, 4, 4, 4 4*, 4*, 4* 5*, 5, 5	0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0* 1*, 1*, 1*

For explanation of symbols see Table I.

The radiation data were as follows: eggs were radiated without any filter other than their shells, using a 100 kv. deep therapy machine delivering 35 r per minute, at a distance from the target of 57 cm.

In the second experiment, the same general procedure was followed, and the objective was to determine whether radiation (500 r) would prevent the rickettsiostatic action of penicillin, aureomycin, and streptomycin. The radiation was given 48 hours after rickettsial injection, and the drugs were injected about 15 minutes after radiation.

RESULTS

The results of these experiments are shown in the tables. From Tables I and II, it is clear that rickettsial multiplication occurred earlier and was markedly enhanced in all groups of eggs receiving radiation, with the exception of the group given 100 r 48 hours after rickettsial injection. These facts were 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 and in sections from the radiated and non-radiated eggs.

The figures in Table III show that radiation (500 r) made it possible for rickettsiae to grow freely at 40°C., whereas only very late and scanty growth occurred at this temperature in eggs which were not radiated.

Table IV shows the prevention of the rickettsiostatic action of streptomycin by x-radiation, and the failure of x-radiation to prevent the rickettsiostatic action of penicillin or aureomycin.

In the first experiment, the multiplication of rickettsiae was almost completely inhibited in the eggs receiving PABA, and this rickettsiostatic action was not prevented by radiation. (These data are not included in the tables.)

DISCUSSION

It seems probable that the observed enhancement of rickettsial growth by radiation is a direct result of intracellular metabolic alterations caused by this agent. We have previously shown (5) that the rickettsiostatic action of increased temperature (40°C.) can be prevented by KCN, and have made the assumption that this effect is the result of decreased oxidative metabolism. Since increased temperature increases both oxidative and non-oxidative metabolism, and since radiation does not, as a rule, decrease oxidative metabolism in fertile eggs (6), it may be that radiation exerts its effect by decreasing metabolism of the non-oxidative type. Many other possible explanations might be advanced, however, and it does not appear that speculation would be profitable at present.

Radiation (7), penicillin (8, 9), and streptomycin (8, 10) are all believed to inhibit enzymes containing thiol groups. Radiation enhances rickettsial growth, while the antibiotics are inhibitory. Radiation prevents the inhibitory action of streptomycin but not that of penicillin. These observations may be explained in a variety of ways: (*a*) all these agents probably exert several separate effects, (*b*) SH enzyme inhibition by different agents may be specific for particular

enzymes, and (*c*) some agents may selectively inhibit SH enzymes of the host cell, and other agents those of the parasite. Thiol containing dehydrogenase systems of the host cell, for example, may be inhibited by radiation, and in this way rickettsiae may be favored in their competition with the host cell for intermediary metabolites.

Observations on the growth rate of intracellular parasites in radiated embryonate eggs may be of considerable theoretical interest, particularly if the effects produced can be related to specific cytological alterations. Loss of resistance caused by radiation may be of practical value in increasing the yield of vaccines, and also may make it possible to cultivate, in fertile eggs, intracellular parasites which previously have proven resistant.

SUMMARY

The multiplication of *Rickettsia mooseri* in fertile eggs is speeded up and quantitatively increased by single dose x-radiation given either 24 hours before or 48 hours after inoculation. This effect is noted at all dosage levels studied, ranging from 100 to 1500 r. The rickettsiostatic effects of high incubation temperature (40°C.) and of streptomycin are neutralized by radiation, but the rickettsiostatic actions of PABA, penicillin, and aureomycin are not altered. Possible mechanisms of action and implications of the observed effects are discussed.

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