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Published version. *Journal of Experimental Medicine*, Vol. 111, No. 6 (June 1960): 841-849. DOI. © 1960 Rockefeller University Press. Used with permission.

THE EFFECTS OF X-RAYS AND BETA RAYS (TRITIUM) ON THE GROWTH OF RICKETTSIA MOOSERI AND RICKETTSIA AKARI IN EMBRYONATE EGGS*

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(Received for publication, January 26, 1960)

The growth of *Rickettsia mooseri* has been shown to be accelerated and quantitatively greater in cells and organisms irradiated with x-rays (5, 8–11, 13–15). The effects of other types of radiations on this host-parasite relationship have not been reported. The present experiments were designed to investigate the changes in the growth patterns of *Rickettsia mooseri* and *Rickettsia akari* in embryonate eggs injected with a radiocompound as an internal source of continuous radiation. Alterations in the growth of *R. akari* in embryonate eggs given x-rays were investigated also.

The radiocompound used was tritiated water. As an experimental source of ionizing radiation, tritium-labeled water possesses unique advantages. It affords a uniform radiation field which can be introduced into any aqueous reaction system, and the short range of its beta particle simplifies calculations of dosimetry (12).

Materials and Methods

The techniques used for preparation of the inoculum, injection of the rickettsial suspension into the yolk sacs of fertile eggs, irradiation of embryonate eggs, introduction of chemical compounds into the yolk sac, preparation and staining of smears of the yolk sac membrane, and determination of the degree of infection present, were those described in previous papers (6, 7, 9).

Measurement of the tritium oxide solution used for injection was made with a fast coincidence liquid scintillation counter (tri-carb counter, model 314, Packard Instrument

* The data reported in this paper were secured in the course of an investigation sponsored by the Atomic Energy Commission, Contract No. AT (11-1)-148, St. Louis University and Contract No. AT (11-1)-596, Marquette University School of Medicine. Part of the work was performed at Argonne National Laboratory under the auspices of the United States Atomic Energy Commission.

‡ This investigation was supported, in part, by a Senior Research Fellowship (SF-89) from the Public Health Service.

Co., La Grange, Illinois). The solution was prepared for assay by dissolving 0.1 ml. of the tritiated water in 15 ml. of absolute alcohol and then adding to this 35 ml. of liquid scintillator (6 mg. of 2,5-diphenyloxazole per ml. of toluene).

Appropriate dilutions of the calibrated tritium oxide solution were prepared with distilled water so that 0.2 ml. of the final solution contained the total radioactivity for each egg. Sterilization of the solution was achieved by placing it in a rubber-capped vial, inserting a hollow needle through the cap, and immersing the bottle in boiling water for 1 hour.

TABLE I

The Effect of Varying Amounts of Tritium Oxide on the Growth of Rickettsia mooseri

Age of embryos	Control	Tritium oxide (180 mc./egg)	Tritium oxide (90 mc./egg)	Tritium oxide (45 mc./egg)	Tritium oxide (22.5 mc./egg)	Tritium oxide (11.2 mc./egg)
days						
5			*R. mooseri* inoculated			
6			Tritium oxide injected			
7	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
8		2, 3, 3, 3	2, 2, 2	1, 1, 2, 2	0, 1, 1	0
9			1, 2, 3, 3	1, 2, 2		0, 1
10	0, 0	3, 3, 3, 3, 3, 4, 4	2, 3, 3, 3, 3, 4	2, 2, 3, 3	0, 1, 1, 1, 2, 2, 2, 2	0, 0, 0
11	0, 1, 1	5, 5, 6, 6	4, 4, 4, 4, 4	3, 3, 3, 4, 4, 4	2, 2, 3, 3, 3	1, 2, 2
12		3, 4, 4, 4, 4, 5, 6, 6, 6	4, 4, 4, 4, 4, 4, 5, 5	4, 4, 4, 4, 4, 5, 5, 5	2, 3, 3, 3, 4,	1, 1
13	1, 1, 2, 2, 3				3, 3, 4, 4	2, 2, 3
14	3, 3, 4, 4, 4, 4, 4					2, 2, 3, 4, 4
15	2, 2, 3, 3, 3, 4, 4, 4, 4, 4, 4					3, 3, 3, 3, 4, 4, 4, 4, 4, 5, 5, 5

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1,000 to 5,000; 5, 5,000 to 10,000.

During incubation there is thought to be a steady exchange of water of the embryonate egg with the water of the surrounding air. Thus, it was conceivable that the injected tritium oxide was lost during incubation. Because it was necessary to know concentrations of tritium during the entire time of exposure in order to estimate radiation dosages, water exchange between developing embryonate eggs and the surrounding air was studied (to be published). The conclusions of that investigation pertinent to this report were as follows: (*a*) very little tritium was lost from the egg during embryonic development, and (*b*) almost all of the tritium introduced as the oxide was recoverable as water, indicating very little exchange.

RESULTS

Modifications in the patterns of growth of *R. mooseri* and *R. akari* in embryonate eggs following the injection of graded doses of tritium per egg are

shown in Tables I and II. Stained smears of the yolk sac membranes of control group inoculated with *R. mooseri* showed moderate infections 8 days after inoculation. All embryos were dead on the 10th day after inoculation with moderate (2+ and 3+) to heavy (4+) infections. The growth of *R. mooseri* was accelerated and quantitatively increased by the injection of 180, 90, and 45 mc./egg of tritium. The patterns of infection of eggs of the three groups

TABLE II

The Effect of Varying Amounts of Tritium Oxide on the Growth of Rickettsia akari

Age of embryos	Control	Tritium oxide (180 mc./egg)	Tritium oxide (90 mc./egg)	Tritium oxide (45 mc./egg)	Tritium oxide (22.5 mc./egg)	Tritium oxide (11.2 mc./egg)
days						
5			*R. akari* inoculated			
6			Tritium oxide injected			
7	0, 0	0, 0, 0		0, 0	0, 0, 0, 0	0
9	0, 0, 1	0, 0	0, 0, 0			0, 0
10	0, 1, 1					0, 0, 0, 0, 1
11	1, 2, 2, 3, 3, 3, 4, 4		0, 0	0		0
12	1, 2, 2, 2, 3, 3, 3, 3, 3, 3, 4, 4, 4, 4				0, 1, 1, 2	0, 1, 2, 3, 3
13		0* 0* 0* 0* 0*	0* 0* 0* 0* 0*	0* 0* 0* 0* 0*	1, 1, 1, 1, 2, 3, 3, 2, 2, 3	1, 2, 2, 2, 2, 3, 3, 3, 4
14		0* 0* 0* 0* 0* 0* 0* 0* 0* 0* 0* 0* 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* 1* 2*	2* 2* 2* 2* 2* 2* 3* 3* 3* 3* 4* 4*	2* 2* 3* 3* 3* 3* 3* 3* 4*

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1000 to 5000; 5, 5000 to 10,000.

* Asterisks indicate embryos were alive at the time of examination.

were similar. The eggs of the group injected with 22.5 mc./egg of the radiocompound showed a slight increase in the rate of growth of the organisms; the infections in the eggs of the group given 11.2 mc./egg did not differ significantly from those of the control group.

The embryonate eggs of the control group inoculated with *R. akari* had light infections 5 days after the injection of organisms. The majority of the embryos died 7 days after inoculation and stained smears showed moderate to heavy infections. The growth of *R. akari* was markedly inhibited in those groups injected with 180, 90, and 45 mc./egg of tritium and partially inhibited in the groups given 22.5 and 11.2 mc./egg.

The modifications in the growth patterns of *R. mooseri* and *R. akari* in eggs injected with 45 mc. of tritium oxide prior to the inoculation of rickettsiae (Table III) were similar to those observed when the radiocompound was injected 24 hours after inoculation of rickettsiae (Tables I and II).

The growth of *R. akari* in embryonate eggs given x-radiation (500 r) 24 hours prior to inoculation did not differ significantly from the controls; when x-irradiation was given 24 hours after inoculation, growth was retarded and quantitatively less (Table IV).

TABLE III

The Growth Patterns of R. mooseri and R. akari Following the Injection of 45 mc. of Tritium Oxide Prior to the Inoculation of Rickettsiae

Age of embryos	Control		Tritium oxide	
	R. mooseri	*R. akari*	*R. mooseri*	*R. akari*
days				
4			Tritium oxide injected	
5			Rickettsiae inoculated	
6				
7	0, 0	0	0, 0, 0, 0, 0	0, 0, 0
8		0, 0		0
9			1, 1, 2, 2	
10	0, 0	0, 0, 1, 1, 2, 2	1, 2, 2	
11	0, 0, 1	1, 2, 2, 2, 3, 3, 3, 4, 4	2, 2, 3, 3	0* 0* 0* 0* 0*
12		2, 2, 2, 2, 3, 3, 3, 4, 4, 4	3, 3, 3, 4, 4, 4	0* 0* 0* 0* 0*
13	1, 1, 2, 2, 3	3, 4, 4, 4	4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5	0* 0* 0* 0* 0*
14	3, 3, 4, 4, 4, 4, 4			0* 0* 1* 1* 2*
15	3, 3, 4, 4, 4, 4, 4, 4, 4, 4, 4			0* 0* 1* 1* 2* 2* 2* 2* 3*

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1000 to 5000; 5, 5000 to 10,000.

* Asterisks indicate embryos were alive at the time of examination.

Single and divided sublethal doses of x-rays resulted in partial inhibition of the growth of *R. akari*. The results of a typical experiment are shown in Table V. The majority of the embryos of the control group were dead 6 to 7 days after the inoculation of organisms, and stained smears of their yolk sac membranes showed moderate to heavy infections. The eggs of the group given 1000 r of x-rays in a single dose were found to have a degree of infection similar to the control. The rate of growth of rickettsiae, however, was diminished in these eggs. The eggs of the group irradiated with 200 r/day of x-rays for a total of 1000 r showed a still greater decrease in the rate of growth of organisms and a decrease in the numbers of rickettsiae.

TABLE IV

The Effect of the Time of Irradiation on the Growth of R. akari

Age of embryos	Control	X-irradiated 500 r	X-irradiated 500 r
days			
4		Irradiated	
5		*Rickettsia akari* inoculated	
6			Irradiated
7	0, 0	0	0, 0, 0
8		0, 0, 0	0, 0
10	0, 1, 1	0, 0, 1, 1	0, 0, 0, 0, 1
11	1, 1, 2, 2, 3, 3, 3, 4, 4	1, 1, 2, 2, 3, 3, 3, 3, 3	0
12	2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 4, 4, 4, 4, 4, 4	1, 2, 2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, 3, 4	0, 0, 1, 2, 3, 3
13			1* 2* 2* 2* 2*
14			2* 2* 3* 3, 3
15			2* 3* 3, 4, 4, 4, 4

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1000 to 5000; 5, 5000 to 10,000.

* Asterisks indicate embryos were alive at the time of examination.

TABLE V

The Effects of Single and Divided Doses of X-Rays on the Growth of R. akari

Age of embryos	Control	X-irradiated 1000 r	X-irradiated 1000 r at 200 r/day
days			
5		Rickettsiae inoculated	
6		Irradiated	Irradiated
7	0, 0	0, 0, 0, 0	0, 0
			Irradiated
8		0, 0, 0	0, 0
			Irradiated
9	0, 1, 1		0
			Irradiated
10	1, 1, 2, 2, 3		0, 0, 0, 0
			Irradiated
11	2, 2, 3, 3, 3, 3, 3, 4, 4		
12	2, 2, 2, 3, 3, 3, 3, 4, 4, 4, 4, 4, 4, 4, 4	2, 2, 2, 2	
13		2, 2, 3, 3	0, 0, 1
14		2, 2, 2, 3, 3, 3, 3, 3, 3, 4, 4	1* 1* 1* 2* 2*
15		2, 2, 3, 3, 4, 4, 4, 4, 4, 4, 4	2* 2* 2* 2* 2*
16			1* 1* 1* 1* 2* 2* 2* 2* 2* 2* 2* 3* 3* 3* 3* 3*

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1000 to 5000; 5, 5000 to 10,000.

* Asterisks indicate embryos were alive at the time of examination.

The possible direct action of tritium on the two species of *Rickettsia* was tested.

The rickettsiae from pooled yolk sacs of heavily infected embryonate eggs were concentrated by differential centrifugation. Pellets of washed *R. mooseri* and *R. akari* were suspended in 1.8 ml. of Ringer's salt solution. Similar aggregates were dispersed into 1.8 ml. of tritiated water with a specific activity of 1 c./ml. The suspensions were kept at 4°C. for 6 hours. Following storage the rickettsiae were concentrated by centrifugation, the supernatant was removed, and the rickettsiae were resuspended in 10 ml. of Ringer's salt solution; this procedure was repeated several times. The pellets obtained after the final wash were dispersed

TABLE VI

The Effects of the Growth Patterns of Rickettsiae of the Direct Exposure of R. mooseri and R. akari to Tritium Before Inoculation

Age of embryos	Control		Tritium-treated	
	R. mooseri	*R. akari*	*R. mooseri*	*R. akari*
days				
5			Rickettsiae inoculated	
6	0	0, 0	0, 0	
8	0, 0	0		0, 0
11	0, 1, 1	0, 0, 1, 1, 2, 2, 2	0, 1, 2, 2	0, 1, 1
12	1, 2, 2	2, 2, 3, 3, 3, 4, 4, 4, 4	2, 2	1, 1, 1, 2, 3, 3, 3
13	1, 2, 3, 3, 3	3, 3, 3, 4, 4, 4, 4, 4, 4, 4, 4	1, 3, 3, 3	2, 2, 3, 3, 3, 4, 4, 4
14	3, 3, 3, 4, 4, 4, 4		2, 3, 3, 3, 4	3, 3, 3, 3, 3, 3, 3, 4, 4, 4
15	3, 3, 4, 4, 4, 5, 5, 5, 5		3, 3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, 5	

Each figure represents an individual egg. 0, no rickettsiae recognized; 1, 1 to 10 rickettsiae per oil immersion field; 2, 10 to 100; 3, 100 to 1000; 4, 1000 to 5000; 5, 5000 to 10,000.

into 18 ml. of Ringer's solution and 0.45 ml. of each suspension was inoculated into 5-day-old embryonate eggs. Based on the activity of the tritiated water, the amounts used, and the dilutions employed, each aliquot of the final suspensions of rickettsiae inoculated into each egg had been exposed to 45 mc. of tritium during storage. The activity of the final suspensions of organisms was less than 5 mc./ml.

The pattern of growth of *R. mooseri* and *R. akari* exposed to tritium oxide for 6 hours before inoculation into embryonate eggs, did not differ significantly from that of the control groups (Table VI).

DISCUSSION

Rickettsiae of the typhus group (of which *R. mooseri* is an example) and of the spotted fever group (to which *R. akari* belongs) are similar in morphology and cause similar clinical and pathologic pictures (1, 3). An outstanding

difference between the organisms of the two groups is the exclusively intracytoplasmic growth of typhus rickettsiae and the preferential intranuclear growth (under some conditions) of spotted fever rickettsiae. These two species of *Rickettsia* differ also in their response to radiation. First, a single dose of 500 r x-rays given to embryonate eggs prior to the inoculation of *R. akari* did not alter the pattern of growth of these organisms; this is in contrast to previous studies in which it was found that the stimulation of the growth of *R. mooseri* following irradiation in a similar manner persisted for 7 days (9). Second, the direction of effect of continuous radiation from tritium is opposite in the two instances; the growth of *R. mooseri* is accelerated, the embryos dying earlier with heavier infections than the controls, whereas the growth of *R. akari* is inhibited. Perhaps a significant similarity in the reactions of the two species to tritium is that the responses to continuous beta radiation, although different in direction, are observed in the same dose range.

The dependence of rickettsiae on the metabolism of the cells of their hosts has been the subject of much study (4). It seems reasonably clear that these intracellular parasites use the metabolic products and enzymes of cells for growth. The inhibition of the growth of *R. akari* following radiation is consistent with the above and the proposals of some investigators (2) that the nucleus is especially sensitive to ionizing radiations. It should be noted, however, that nothing is suggested by our results as to the nature of the changes produced. The stimulation of the growth of *R. mooseri* may be explained also in terms of damage to the nucleus, if we postulate removal by irradiation of a normally inhibiting chemical compound, force or circumstance in the nucleus that insures slower growth of *R. mooseri* in non-irradiated cells. A complication to the above, requiring further investigation, is that if nuclear damage is being expressed in the two instances, why is it that nuclear damage in one case (*R. mooseri*) from a single dose of x-rays may last as long as 7 days; while it seems to disappear almost immediately in the other (*R. akari*). An alternative kind of explanation is that nuclear damage is not involved in the acceleration observed in the case of *R. mooseri*.

While no definite explanation is possible, the result is clear. Continuous radiation accelerates the growth of *R. mooseri*, usually growing in the cytoplasm; whereas it inhibits the growth of *R. akari*, that is found in both the nucleus and cytoplasm. Since the effects are most probably on the host cells rather than on the organisms, species of organisms such as these selected for their intranuclear or intracytoplasmic growth are valuable test systems for investigations of the relative sensitivities of the nucleus and the cytoplasm, as well as giving information concerning factors governing rickettsial reproduction.

SUMMARY

The growth of *Rickettsia mooseri* was accelerated and quantitatively increased in embryonate eggs containing tritium oxide at levels of 180, 90, and

45 mc./egg during the growth period. The eggs of a group containing 22.5 mc./egg showed only a slight increase in the rate of growth of organisms; the infections in the eggs of a group given 11.2 mc./egg did not differ significantly from those of the control group. On the other hand, growth of *R. akari* was inhibited in embryonate eggs containing tritium oxide at levels of 180, 90, and 45 mc./egg, and partially inhibited in groups containing 22.5 and 11.2 mc./egg. The patterns of growth of *R. mooseri* and of *R. akari* exposed to tritium oxide for 6 hours prior to inoculation into embryonate eggs did not differ significantly from that of the control group.

Single and divided doses of x-rays to the host resulted in partial inhibition of the growth of *R. akari.*

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