# THE EFFECT OF X-RAYS ON THE RICKETTSIOSTATIC ACTIVITY OF STREPTOMYCIN, AUREOMYCIN, AND PENICILLIN*

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The administration of a single dose of x-rays (500 r) to embryonate eggs has been shown to reverse the inhibitory action of a single dose of streptomycin (1 mg.) on the growth of *Rickettsia mooseri* (10). The rickettsiostatic activity of penicillin or aureomycin was not altered by x-rays. In the present investigation a more extensive study of the reaction was made with respect to the relation between dosage of x-rays and concentration of antibiotics, and also with respect to the permanence of the x-ray induced changes in the host that allow greater growth of rickettsiae.

## *Methods and Materials*

Previously described methods (11–13) were used for preparing the inoculum, injecting the rickettsial suspension into the yolk sacs of fertile eggs, irradiation of the embryonate eggs, making and staining of yolk sac smears, and determining the degree of infection.

## RESULTS

*The Effect of Graded Doses of X-Rays on the Rickettsiostatic Activity of Constant Amounts of Streptomycin, Penicillin, and Aureomycin (Tables I, II and III).*—

On the 4th day of incubation, embryonate eggs, in groups of 30, were x-irradiated with 100 r, 500 r, 800 r, or 1000 r. Rickettsiae were inoculated on the 5th day and 5 mg. of the antibiotics was injected into each group of eggs on the 7th day of incubation. The control groups received (1) rickettsiae inoculation but no other treatment, (2) rickettsiae plus 100 r or 1000 r of x-rays, or (3) rickettsiae plus 5 mg. per egg of the antibiotics.

Of the controls, the first group (rickettsiae alone) showed moderate infection (2+ and 3+) on the 7th day after inoculation; all eggs of this group were dead by the 9th day after inoculation and 4+ infections predominated at this time (*cf.* Table I). This pattern of infection was intensified in the second group (rickettsiae plus either of the four x-ray dosages used). Streptomycin

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(Table I), penicillin (Table II) and aureomycin (Table III) markedly inhibited the growth of rickettsiae in the embryos of the third group (rickettsiae plus antibiotic).

In the experimental groups, the eggs receiving 100 r of x-rays showed only a slight reversal of the rickettsiostatic action of streptomycin, but the reversal was almost complete in the eggs given 1000 r (Table I). The intermediate dosage groups showed a graded response. Thus the reversal of the rick-

TABLE I

*The Effect of Varying Doses of X-Rays on the Rickettsiostatic Activity of Streptomycin*

| Age of embryos | Control | 100 r‡ | 1000 r‡ | Streptomycin (5 mg./egg) | 100 r + streptomycin (5 mg./egg) | 500 r + streptomycin (5 mg./egg) | 800 r + streptomycin (5 mg./egg) | 1000 r + streptomycin (5 mg./egg) |
|---|---|---|---|---|---|---|---|---|
| *days* | | | | | | | | |
| 4 | | | | Embryonate eggs irradiated | | | | |
| 5 | | | | Rickettsiae inoculated | | | | |
| 7 | | | | Streptomycin injected | | | | |
| 8 | 0, 0 | | 0, 0, 0 | | | | 0, 0 | 0, 0, 0, 0 |
| 9 | 0, 0 | 0, 0, 0 | 2, 2, 3, 3 | 0, 0, 0, 0 | 0 | 0, 1, 1 | 1, 1, 2, 2 | 2, 2, 2 |
| 10 | | | | | | 0, 1, 2 | 2, 2 | 1, 2, 3, 3 |
| 11 | 0, 1, 1 | 2, 2, 3, 3 | 3, 3, 3, 3, 4, 4, 4, 4, 4 | 0, 0 | 1, 1, 2, 2 | 2, 2, 3, 3 | 3, 3, 3, 3, 3, 3, 3, 4, 4, 4, 4, 4 | 3, 3, 3, 3, 3, 4, 4, 4, 5, 5 |
| 12 | 1, 2, 2, 2, 3, 3, 3, 3 | 2, 2, 3, 3, 4, 4, 4, 4, 4, 4, 4 | 4, 4, 4, 4, 4, 5, 5, 5, 6, 6, 6, 6, 6, 6 | 0 | 2, 2, 2, 3, 3 | 4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, | 4, 4, 5, 5, 6 | 4, 4, 4, 4, 4, 4, 5, 5, 6 |
| 13 | 3, 3, 3, 4, 4, 4, 4 | 3, 4, 4, 4, 4, 4, 5, 5 | | 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 1*, 2*, 2*, 2* | 2*, 3*, 3*, 4, 4, 4, 4, 4, 4 | 4, 4, 4, 5, 5, 5, 6, 6 | 5, 5, 6, 6, 6 | 5, 6 |
| 14 | 3, 3, 4, 4, 4, 4, 4, 4, 4, 4 | 4, 4, 5, 5 | | 0*, 0*, 0*, 0*, 1*, 1*, 2*, 2*, 2*, 2* | 3, 4, 4, 4, 5 | 6, 6 | | |

* Embryos alive at time of examination.

‡ The effect of x-rays on the rickettsiostatic activity of streptomycin, penicillin, and aureomycin was determined at the same time; therefore, this column will not be repeated in Tables II and III.

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.

ettsiostatic action of streptomycin is dependent on the dosage of x-rays given.

The rickettsiostatic activity of penicillin or aureomycin was not reversed by the dosages of x-rays used (Tables II and III).

*The Effect of X-Rays on the Rickettsiostatic Activity of Graded Amounts of Streptomycin.*—

Three hundred embryonate eggs were divided into 10 groups of 30 eggs each. Five groups were given 500 r of x-rays on the 4th day of incubation, and all ten groups were inoculated with rickettsiae on the 5th day. One non-irradiated and one irradiated group were used as

TABLE II

*The Effect of Varying Doses of X-Rays on the Rickettsiostatic Activity of Penicillin*

| Age of embryos | Control | Pencillin (5 mg./egg) | 100 r + penicillin (5 mg./egg) | 500 r + penicillin (5 mg./egg) | 800 r + penicillin (5 mg./egg) | 1000 r + penicillin (5 mg./egg) |
|---|---|---|---|---|---|---|
| *days* | | | | | | |
| 4 | Embryonate eggs irradiated | | | | | |
| 5 | Rickettsiae inoculated | | | | | |
| 7 | Penicillin injected | | | | | |
| 8 | 0, 0 | 0 | | 0 | 0, 0, 0 | 0, 0 |
| 9 | 0, 0 | | 0, 0, 0 | | 0, 0 | 0, 0, 0, 0 |
| 10 | | 0 | | 0, 0 | 0 | 0, 0 |
| 11 | 0, 1, 1 | | | | 0, 0 | |
| 12 | 1, 2, 2, 2, 3, 3, 3, 3 | 0, 0, 0* | 0*, 0*, 0* | 0, 0, 0 | 0, 0, 0* | 0, 0, 0 |
| 13 | 3, 3, 3, 4, 4, 4, 4 | 0, 0*, 1* | 0*, 0*, 0* | 0*, 0, 1 | 0*, 1*, 1* | 0, 0*, 0* |
| 14 | 3, 3, 4, 4, 4, 4, 4, 4 | 0*, 0*, 1*, 1*, 1* | 0*, 0*, 0*, 0*, 1* | 0*, 0*, 1*, 1*, 1* | 0*, 1*, 1*, 1*, 1* | 0*, 0*, 0*, 0*, 1* |
| 15 | | 0*, 0*, 0*, 1*, 1* | 0*, 0*, 1*, 1*, 2* | 0*, 1*, 1*, 1*, 1* | 0*, 0*, 1*, 2*, 2* | 0*, 1*, 1*, 1*, 1* |
| 16 | | 0*, 0*, 0*, 0*, 1*, 1*, 1*, 1*, 1*, 2*, 2*, 2* | 0*, 0*, 0*, 0*, 1*, 1*, 1*, 2*, 2*, 2*, 3* | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 1* | 0*, 0*, 1*, 1*, 1*, 2* | 0*, 0*, 0*, 1*, 2*, 2* |

* Embryos alive at time of examination.

TABLE III

*The Effect of Varying Doses of X-Rays on the Rickettsiostatic Activity of Aureomycin*

| Age of embryos | Control | Aureomycin (5 mg./egg) | 100 r + aureomycin (5 mg./egg) | 500 r + aureomycin (5 mg./egg) | 800 r + aureomycin (5 mg./egg) | 1000 r + aureomycin (5 mg./egg) |
|---|---|---|---|---|---|---|
| *days* | | | | | | |
| 4 | Embryonate eggs irradiated | | | | | |
| 5 | Rickettsiae inoculated | | | | | |
| 7 | Aureomycin injected | | | | | |
| 8 | 0, 0 | 0, 0, 0 | 0 | 0, 0 | 0, 0, 0, 0 | 0, 0 |
| 9 | 0, 0 | | | 0 | 0, 0, 0 | 0, 0, 0 |
| 10 | | 0 | 0, 0 | | | |
| 11 | 0, 1, 1 | 0, 0 | 0, 1 | | 1, 1 | 0, 0, 1, 1 |
| 12 | 1, 2, 2, 2, 3, 3, 3, 3 | 0, 0, 1 | 0, 0, 0 | 1, 1, 1 | 0, 0, 1 | 0, 0, 0 |
| 13 | 3, 3, 3, 4, 4, 4, 4 | 0*, 1*, 1* | 0*, 0*, 0* | 0*, 0*, 1* | 0*, 0*, 2* | 0*, 1*, 2* |
| 14 | 3, 3, 4, 4, 4, 4, 4, 4 | 0*, 0*, 1*, 1*, 2* | 1*, 1*, 1*, 2*, 2* | 0*, 0*, 0*, 1*, 1* | 0*, 0*, 1*, 2*, 2* | 0*, 0*, 0*, 2*, 3* |
| 15 | | 0*, 0*, 1*, 2*, 2* | 0*, 0*, 0*, 1*, 3* | 0*, 1*, 1*, 1*, 2* | 0*, 0*, 0*, 0*, 1 | 0*, 1*, 1*, 2*, 2* |
| 16 | | 0*, 1*, 1*, 1*, 1*, 2*, 2*, 3* | 0*, 0*, 1*, 1*, 1*, 1*, 1*, 1*, 1* | 1*, 1*, 1*, 1*, 1*, 1*, 2*, 2*, 2, 3, 3 | 0*, 1*, 1*, 3*, 3 | 1*, 2*, 2*, 2*, 3* |

* Embryo alive at time of examination.

controls. The remaining non-irradiated and irradiated groups were paired, and, on the 7th day, treated with 2 mg., 4 mg., 8 mg., or 16 mg. of streptomycin per egg, respectively.

As shown in Table IV, the majority of the embryos in the non-irradiated control group died between the 8th and 10th day after the inoculation of rickettsiae, with stained smears of the yolk sac membranes showing 3+ and

## TABLE IV

*The Effect of X-Rays on the Rickettsiostatic Activity of Varying Amounts of Streptomycin*

| Age of embryos | Control | 500 r | Streptomycin (2 mg./egg) | 500 r + strepto-mycin (2 mg./egg) | Streptomycin (4 mg./egg) | 500 r + streptomycin (4 mg./egg) | Streptomycin (8 mg./egg) | 500 r + strepto-mycin (8 mg./egg) | Streptomycin (16 mg./egg) | 500 r + streptomycin (16 mg./egg) |
|---|---|---|---|---|---|---|---|---|---|---|
| *days* | | | | | | | | | | |
| 4 | | Irradiated | | Irradiated | | Irradiated | | Irradiated | | Irradiated |
| 5 | | | | | Rickettsiae inoculated | | | | | |
| 7 | | | | | Streptomycin injected | | | | | |
| 9 | 0, 0 | 0 | | 0, 0, 0 | 0 | 0, 0 | 0, 0 | 0, 0, 0 | 0, 0 | 0, 0, 0, 0 |
| 10 | 0, 0 | 1, 1, 1 | | 1, 1, 2 | 0 | 0, 2, 2 | | 0, 0 | 0 | 0, 0 |
| 11 | 0, 1, 1, 2 | 1, 2, 2, 3 | 0, 0, 0, 1 | 1, 1 | | 2, 2, 3, 3, 3 | 0 | 0, 1, 1 | | |
| 12 | 1, 1, 2, 2 | 2, 2, 3, 3, 3, 3, 3, 4, 4, 4, 4 | | 2, 2, 3, 3, 3, 3 | 0, 1 | 2, 2, 2, 3 | 0 | 0, 1, 1, 2 | | 0, 1, 1 |
| 13 | 2, 2, 3, 3, 3, 3, 3 | 3, 3, 4, 4, 4, 4, 5, 5, 5, 5, 5, 6, 6 | 0*, 0*, 0* | 2, 3, 3, 4, 4, 4, 4 | 0*, 1*, 1* | 2*, 3, 3, 3, 3, 4, 4 | 0, 0, 0 | 1, 2, 2 | 0, 0, 0 | 0, 0, 1 |
| 14 | 3, 4, 4, 4, 4, 4, 4 | | 0*, 0*, 2* | 3, 3, 4, 4, 4, 4, 5, 5, 5 | 0*, 0*, 1* | 2*, 2*, 2, 3, 3, 3, 3 | 0*, 1*, 1* | 2*, 3*, 3* | 0*, 0*, 0* | 0*, 0*, 2* |
| 15 | 4, 4, 4, 4 | | 0*, 0*, 1*, 1*, 1* | | 0*, 0*, 0*, 2*, 2* | 3, 4 | 0*, 0*, 0*, 1*, 2* | 2*, 2*, 2, 3, 3 | 0*, 0*, 0*, 0*, 1* | 0*, 0*, 1*, 2*, 2* |
| 16 | | | 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 2*, 2*, 2*, 2*, 2*, 2, 3 | | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 1*, 2*, 2* | | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1* | 1*, 2*, 3*, 3, 3, 3 | 0*, 0*, 0*, 0*, 0* 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0* | 0*, 1*, 1*, 1*, 2* 3*, 3*, 4, 4, 4 |

* Embryos alive at time of examination.

4+ infections. The majority of the embryos of the irradiated control group died earlier than those in the non-irradiated group (7th and 8th day post-inoculation) and showed heavier infections (4+ and 5+). The response in the experimental groups was graded, ranging from partial inhibition of rickettsial growth in the group given 2 mg. of streptomycin per egg to complete inhibition in the group given 16 mg. per egg (Table IV). The response, therefore, varied inversely with the amount of compound injected; the rickettsiostatic activity of 2 mg. of streptomycin was completely reversed and the activity of 16 mg. only partially reversed.

TABLE V

*The Effect on the Rickettsiostatic Activity of Streptomycin Following Irradiation at Time Intervals after the Injection of Streptomycin*

| Age of embryos | Control | Streptomycin | 500 r | 500 r + streptomycin (4 mg./egg) | 500 r | 500 r + streptomycin (4 mg./egg) | 500 r | 500 r + streptomycin (4 mg./egg) |
|---|---|---|---|---|---|---|---|---|
| *days* | | | | | | | | |
| 5 | Rickettsiae inoculated | | | | | | | |
| 7 | Streptomycin injected | | | | | | | |
| 8 | | | Irradiated | | | | | |
| 9 | 0 | 0,0 | 0,0 | | | | | |
| 10 | 0,0 | 0,0 | 1,1,2,2 | 0,1 | Irradiated | | | |
| 11 | 0,0,1 | | 1,2,2,2,3,3 | | 1,2,2 | 1,1 | | |
| 12 | 1,1,1 | 0 | 2,2,3,3,3,4,4 | 1,1,2 | 1,1,2,3 | 2,2,3 | Irradiated | |
| 13 | 2,2,2,3,3, 3,3,3, 4,4 | 0,0,0,0,0 | 3,3,4,4,4,4, 4,4,4,5,5 | 1,1,2,2,2,3, 3,3,4 | 3,3,3,3,3,4, 4,4,4,4,4, 4,4 | 1,1,2,2,2,3, 3,3,3,3,4, 4 | 4,4,4,4,4,4, 4,4,4,5,5, 5,5,6 | 1,1,1,2,2 |
| 14 | 3,3,4,4,4, 4,4,4, 4,4,4 | 0,*0,*0,*1,*1* | | 2,2,3,3,3,4, 4,4,4 | 4,4,5,5,5,5, 5,5,5 | 2,3,3,3,3,3,3 | 4,4,4,5,5,5, 5,6,6,6,6, 6,6,6 | 2,2,2,3,3 |
| 15 | | 0,*0,*0,*1,*2* | | 3,3,3,4,4,4 | | 3,3,4,4,4,4 | | 2,2,3,3 |
| 16 | | 0,*1,*2,*2,*2 | | 3,4 | | | | 3,3,4,4,4,4, 4,5,5,5 |
| 17 | | 0,*0,*0,*2,*3* | | | | | | 3,4,4,4,4,4 |

* Embryos alive at time of examination.

*The Rickettsiostatic Activity of Streptomycin Following Irradiation with X-Rays at Successive Time Intervals after the Injection of Streptomycin.—*

Four hundred embryonate eggs were inoculated with rickettsiae on the 5th day of incubation. A group of 30 eggs was set aside as a control on rickettsial growth. Forty-eight hours later (7th day of incubation), streptomycin (4 mg./egg) was injected into one-half of the remaining eggs; of these, 30 served as a streptomycin control series. Paired groups of 30 rickettsiae-inoculated and streptomycin-injected eggs were given 500 r doses of x-rays on the 8th, 10th, or 12th day of incubation.

The majority of the embryos in the rickettsiae growth control series died on the 8th and 9th day after inoculation, with the stained smears of the yolk sac membranes showing 3+ and 4+ infections (Table V). The usual inhibi-

## TABLE VI

*The Effect of X-Rays on the Rickettsiostatic Activity of Streptomycin Injected at Time Intervals Following Irradiation*

| Age of embryos | Control | 500 r | Streptomycin (4 mg./egg) | 500 r + streptomycin (4 mg./egg) | Streptomycin (4 mg./egg) | 500 r + streptomycin (4 mg./egg) | Streptomycin (4 mg./egg) | 500 r + streptomycin (4 mg./egg) | Streptomycin (4 mg./egg) | 500 r + streptomycin (4 mg./egg) |
|---|---|---|---|---|---|---|---|---|---|---|
| *days* | | | | | | | | | | |
| 5 | | | | | Rickettsiae inoculated | | | | | |
| 7 | | Irradiated | Injected | Irradiated injected | | Irradiated | | Irradiated | | Irradiated |
| 8 | | | | | Injected | | | | | |
| 9 | 0, 0 | | 0, 0 | 0 | 0, 0, 0, 0 | 0, 0, 0, 0 | Injected | | | |
| 10 | 0, 0 | 1, 1, 1, 1 | 0, 0 | 0, 0 | 0 | 0, 0 | 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0 | Injected | |
| 11 | 0, 0, 1 | | | | 0 | | | | 0, 0<br>0, 0 | 0, 0, 0, 0 |
| 12 | | | 0 | 0 | 0, 0 | | 0 | | 0, 0 | 2, 2, 2, 3, 3, 3, 3, 4, 4, 4 |
| 13 | 1, 1, 2, 2, 3, 3, 3, 3 | 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5 | 0, 0, 0, 1, 1 | 2, 2, 2, 2, 2, 4, 4, 4, 4, 4, 4, 4 | 0, 0, 0, 1, 1 | 1, 1, 2, 2, 2 | 0, 0, 0, 0, 0 | 1, 2, 2, 2, 3 | 0, 0, 0, 1, 1 | 3, 3, 3, 4, 4, 4, 4, 4, 4, 4, 4 |
| 14 | 3, 3, 4, 4, 4, 4, 4, 4 | 4, 4, 5, 5, 5, 6, 6, 6, 6, 6 | 0*, 0*, 0*, 2*, 2* | 2, 2, 3, 3, 3, 4, 4, 4, 4, 4, 4 | 1*, 1*, 1*, 2*, 2* | 3, 3, 3, 3, 3, 3, 3, 4, 4, 4, 4, 5 | 0*, 0*, 0*, 0*, 0* | 3, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4 | 0*, 0*, 0*, 0*, 1* | 3, 4, 4, 4, 4, 4 |
| 15 | 2, 2, 3, 3, 3, 4, 4, 4, 4, 4 | | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 2*, 2*, 2*, 2* | 3, 3, 3, 3, 3, 4 | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1*, 1* | 3, 3, 4, 4, 4, 4, 4, 4 | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1* | 4, 4, 5 | 0*, 0*, 0*, 0*, 0*, 0*, 0*, 0*, 1*, 1*, 1* | |

* Embryos alive at time of examination.

tory action of this antibiotic was observed in the streptomycin control series, although several of these eggs were found to have low grade infections (2+). In the three groups receiving x-irradiation, the growth of rickettsiae was enhanced; the effect was greatest in the group irradiated on the 12th day of incubation (5 days after the injection of streptomycin). Thus the reversal of the rickettsiostatic action of streptomycin by x-rays can be effected for eome days after the injection of streptomycin, and the rate of growth of rickjttsiae increases with increasing time intervals between streptomycin inection and irradiation.

*The Rickettsiostatic Activity of Streptomycin Injected at Successive Time Intervals after Irradiation (Table VI).—*

Three hundred and fifty embryonate eggs were inoculated with rickettsiae on the 5th day of incubation. Thirty eggs served as an inoculated control series. On the 7th day of incubation, 175 of the inoculated eggs were given a 500 r dose of x-rays. Thirty of these eggs were set aside as irradiated controls. Groups of 30 inoculated, non-irradiated and inoculated, irradiated eggs were paired and injected with 4 mg. of streptomycin per egg on the 7th, 8th, 9th, or 10th days of incubation, respectively.

In Table VI, it is seen that most of the embryonate eggs of the inoculated control series died between the 8th and 10th day following the inoculation of rickettsiae, showing infections ranging from moderate (1+ to 3+) on the 8th day, to heavy (4+) on the 10th day. The majority of the eggs of the irradiated series also died between the 8th and 10th day following inoculation, and stained smears of the yolk sac membranes of these eggs showed them to be more heavily infected (5+ to 6+). Streptomycin inhibited the growth of rickettsiae in all non-irradiated groups. In the irradiated groups, 4 mg. of streptomycin per egg did not alter rickettsial growth.

## DISCUSSION

It is generally accepted that the relationship between the host cell and the invading obligate parasite is a delicate and reciprocal one (9) which may be altered drastically by even small modifications in either the host cell or the parasite. For example, the changes in the metabolism of the host cells brought about by a 2.5°C. increase in incubation temperature are sufficient to inhibit the growth of rickettsiae in the embryonate egg (12) while allowing the host cells to live. The action of antibiotics is not necessarily specific toward the parasite, but is probably directed toward enzyme systems utilized by the cells of both the host and the parasite (1, 16, 21, 26, 30, 31). It is not unlikely that the systems involved are more critical for the continued survival of the parasite than they are for the host, and thus the antibiotic would be expected to modify host cell–parasite relations. The explanation for the action of x-rays might be found in these systems known to be affected by the antibiotics.

The rickettsiostatic activity of penicillin and aureomycin may be accounted for by their action on glutamate metabolism. In other microorganisms, penicillin has been found to prevent the absorption of glutamic acid from the external environment (5, 7, 8, 22, 23). Sensitivity to penicillin is associated with dependence on an external source of preformed glutamic acid, since those organisms capable of producing glutamic acid by synthesis from simpler moieties or by degradation of complex molecules are insensitive. Other investigations (24) have shown that bacteria acted on by penicillin not only lose the capacity for absorbing various materials from the external medium, but they may also, by a leaching process, lose such solutes as lipides, nucleotides, and other substances. Bovarnick and Miller (2) have shown that in rickettsiae glutamate is oxidized by the same series of reactions observed in mammalian tissue particles and, while no direct evidence concerning absorption of glutamates is at hand, it is not unlikely that rickettsiae resemble other organisms in this respect as well.

Aureomycin, in addition to interfering with protein metabolism and blocking acetate metabolism (17), also inhibits glutamate oxidation in the bacterial cells. In murine and epidemic typhus rickettsiae, Karp and Snyder (18) observed an *in vitro* decrease in respiration resulting from the inhibition of glutamate oxidation induced by aureomycin.

It is likely, then, that aureomycin and penicillin inhibit rickettsial growth, at least in part, through their action on glutamate metabolism. Therefore it is not surprising that low radiation dosages such as those tested here do not reverse the action of these two antibiotics; if anything, one would expect reinforcement of the effect.

Somewhat similar biochemical mechanisms have been proposed for the antibiotic action of streptomycin. It has been suggested that interference with tricarboxylic acid metabolism of the cell (20, 21, 27, 28, 29) may account for the suppression of rickettsial growth. However, if this be the case, the observed effect of x-rays is difficult to understand. A *reversal* by x-rays of such an action of streptomycin implies that x-rays stimulate the carbohydrate cycle to a level of activity which more than compensates for the inhibitory effect of the streptomycin present. We are not aware of any observations suggesting that radiation stimulates this enzymatic reaction. It seems more likely that streptomycin itself is inactivated in some way in the irradiated egg, but not directly by the x-rays, since the inactivation occurs even when streptomycin is injected several days after irradiation of the egg. The inactivation might result from a simple combination with degradation products, as has been observed in the complexes known to occur between streptomycin and nucleic acids and nucleoproteins (3, 4, 6, 14, 15, 25).

Another possibility is that (while there may be combining or complexing action between streptomycin and other compounds) there may be an anti-

metabolite action of streptomycin that effectively inhibits the growth of rickettsiae in unirradiated cells at the dosage levels given. The reversal of the inhibitory effect in irradiated cells may result from the presence of higher levels of the compound or compounds with which streptomycin is in competition so that the original level of the antibiotic is no longer effective. Supporting this are two facts. First, there appears to be a quantitative balancing out of amounts of streptomycin and x-rays. Second, it has been shown that in embryos, x-irradiation is followed by definite increases in plasma levels of such compounds as taurine, $\beta$-alanine, $\gamma$-amino-butyric acid, methylhistidine, cystine (as cysteic acid), hydroxyproline, ethanolamine, and phosphoethanolamine (19). Perhaps streptomycin acts in competition with one or more of these substances.

## SUMMARY AND CONCLUSIONS

The effect of x-rays on the rickettsiostatic activity of streptomycin, penicillin and aureomycin in the embryonate egg was investigated.

Only a slight reversal of the rickettsiostatic action of 5 mg. of streptomycin occurred in embryonate eggs given 100 r of x-rays at 4 days of incubation, whereas complete reversal occurred in those given 1000 r. Groups of eggs irradiated with intermediate doses of x-rays showed a graded response.

The rickettsiostatic activity of 5 mg. (1660 Oxford units/mg.) of penicillin or of 5 mg. of aureomycin was not reversed by doses of x-rays ranging from 100 to 1000 r.

500 r of x-rays reversed the rickettsiostatic activity of 2 mg., 4 mg., 8 mg., or 16 mg. of streptomycin per egg in proportion to the amount of streptomycin injected. The rickettsiostatic activity of 2 mg. of streptomycin was completely reversed and the activity of 16 mg. only partially reversed.

Reversal of the rickettsiostatic activity of streptomycin by x-rays was observed when radiation was given up to 6 days after the injection of the antibiotic into 7-day-old embryos.

Reversal of the rickettsiostatic activity of 4 mg. of streptomycin could be detected when the antibiotic was injected 3 days after 500 r of x-radiation.

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

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