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Effect of Radiation on Malaria. An Experimental Study in the Chick and Duck.*

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Skinner and Carson¹ reported several cases of malarial infection in man in which roentgen irradiation was applied to the skin over the spleen. This method of therapy in malaria has not been widely accepted, although these investigators were favorably impressed with their results. Reinhard² considered the effect of radiation to be favorable in clinical cases of malaria. Twenty cases of splenomegaly the result of either chronic recurrent or repeated attacks of malaria were treated by roentgen irradiation between 1931 and 1939 by one of us (H. R., Jr.). The splenic tumor was reduced in size in each of the 16 cases that completed the series of treatments. The spleen either decreased in size or returned to normal in all of these cases. The general health of each patient improved following this therapy.

Experiments were performed to determine the effect of radiation on malaria during the time other studies were being made on *P. lophuræ* infection in ducks and chicks.³ The results of these experiments are reported in this paper.

Methods and Materials. The strain of plasmodium used was *P. lophuræ*. These parasites were serially transferred in young chicks for approximately 6 months preceding

the time in which they were used in this experiment to inoculate young chicks. The chicks were 3 to 10 days of age when inoculated. *P. lophuræ* obtained from ducks were used to infect young white Pekin ducklings. The ducks varied in age from 2 to 6 weeks.

Blood for the inoculum was obtained directly from the heart. It was diluted with an equal volume of a 2.0% solution of sodium citrate in physiological saline. Blood from a group of chicks was pooled when a large series of birds were to be inoculated. Injections were made into a vein in the leg. The course of the infection was followed by counting the number of parasitized cells per 500 red blood cells. Blood for these counts was obtained from the toes at varying intervals during the disease. Smears were stained with a combination of Giemsa's and Wright's stains.

Citrated parasitized blood was put into petri dishes approximately 10.0 cm in diameter and varying quantities of roentgen irradiation was given over these dishes. The amount of radiation is given in each experiment. The physical factors were: half-wave, K.V.P. 100, M.A. 5, with 0.5 mm Al. inherent filtration.

In a second group of experiments the effect of roentgen irradiation on malarial parasites was studied *in vivo*. Chicks were infected with *P. lophuræ* by intravenous inoculation. The entire body was irradiated. The amount of irradiation and the number of treatments varied. These data are given in the report of each experiment and are shown in Fig. 2.

The effect of radiation from radium bromide† was studied both *in vitro* and *in vivo*. In the former experiment parasitized

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¹ Skinner, Bruce, and Carson, H. W., *Brit. Med. J.*, 1911, **1**, 431.

² Reinhard, P., *Med. Klin.*, 1918, **14**, 619.

³ Rigdon, R. H., *Am. J. Trop. Med.*, 1944, **24**, 371.

† The Canadian Radium and Uranium Corporation of New York supplied the radium bromide. We wish to acknowledge our appreciation for this assistance.

blood from chicks was diluted with an equal volume of a 2.0% solution of sodium citrate in physiological saline and pooled. Six cubic centimeters of this blood was put into 2 test tubes and centrifuged. Two cubic centimeters of the supernatant was removed from each tube and discarded. Two cubic centimeters of radium bromide in distilled water was added to one of the tubes containing the parasitized blood and an equal volume of distilled water was added to the second tube of parasitized blood. These two tubes were kept at room temperature for $1\frac{1}{2}$ hours. They were shaken at intervals. 0.5 cubic centimeter of these suspensions of parasitized cells were given intravenously to 20 chicks 3 days of age. The above procedure was repeated using parasitized blood cells from ducks. 0.5 cubic centimeters of this irradiated duck blood was given intravenously to 13 ducks 7 weeks of age. The parasitemia was followed with blood smears in these chicks and ducks.

To study the effect of radium bromide on *P. lophuræ* *in vivo* 10 ducks 14 days of age were given 0.5 cc of parasitized blood intravenously. Five of these ducks were given 1.0 cc of radium bromide intravenously 24 hours following the injection of the parasites. A second injection of 1.0 cc of radium bromide was given 30 hours and a third injection of 1.0 cc of radium bromide was given 48 hours following the injection of parasites. Five ducks in this group were carried as the controls. The parasitemia was followed by blood smears in these 10 ducks.

The radium[†] used in these experiments was in the form of a bromide salt dissolved in distilled water. 0.5 of a milligram of the element radium was dissolved in 30.0 cc of distilled water. The amount of this dilution given to the different birds and also mixed with parasitized red cells is given in the different experiments.

Experimental. Effect of Roentgen Irradiation on P. lophuræ in vitro. A series of experimental observations were made in which different amounts of irradiation were given over petri dishes containing parasitized blood. This blood then was injected into chicks. The results of these different experiments are shown in Fig. 1. The radiation

was unfiltered. The smallest amount of irradiation was used in the experiment in which the results are shown in Fig. 1A. In this experiment, the 50, 100, and 500 R were given at the rate of 137 R/min. In experiment B 1000 R and 1500 R of irradiation were given at the rate of 126 R/min. In experiment C, 1000 R and 2000 R were given at the rate of 34.4 R/min. In experiment D, 15,754 roentgen were given over the petri dish at a rate of 787.7 R/min. The results of these experiments show that the degree of parasitemia decreases with an increase in the amount of irradiation applied to the parasitized blood. Apparently there is no reproduction of the parasites when they are treated with 15,754 roentgens preceding the time of inoculation into young chicks (Fig. 1D).

Effect of Roentgen Irradiation on the Parasitemia Produced by P. lophuræ in Chicks. The results obtained in the preceding experiments indicate that roentgen irradiation prevents the normal reproduction of malarial parasites. The effect of roentgen irradiation on *P. lophuræ* was studied *in vivo* by irradiating the entire body of chicks infected with this strain of malaria. The amount and frequency of the radiation varied in the different experiments and was given as follows: Rate 49.5 R/min, 100 K.V.P., 5 M.A., distance 10 inches. A 2 mm al. filter was used in the experiments shown in Fig. 2, A, C, and D. The rate was 137 R/min and no filter was used in the experiment shown in Fig. 2B. The frequency of irradiation and the interval following inoculation is given for each of the four experiments in Fig. 2. The results of the effect of the radiation on the parasitemia are also shown in Fig. 2. The parasitemia is less in the irradiated chicks than in the controls in each of these 4 experiments. The greatest reduction in the number of parasites occurred in the chicks given the larger quantities of irradiation. These larger amounts of irradiation frequently were fatal to uninfected chicks.

Effect of Radium Bromide on P. lophuræ.[‡]

[†] Dr. Jay McLean, Columbus, Ohio, suggested to one of us (R.H.R.) the study of the effect of a radium salt on malaria.

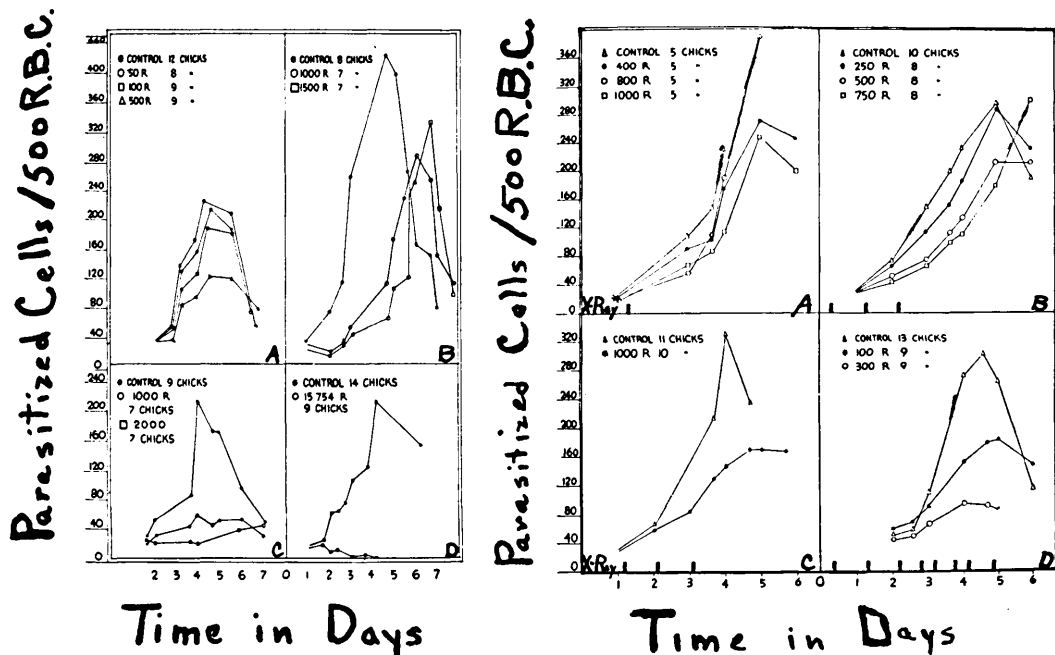


FIG. 1 (Left).

The results of the effect of roentgen rays on the malarial parasites *in vitro*, one shown in each of the 4 graphs. The number of roentgens and the number of chicks used in each experiment are shown above.

FIG. 2 (Right).

The results of the effect of roentgen rays on the malarial parasites *in vivo* are shown in each of the 4 graphs. The number of roentgens and the number of chicks used in each experiment are shown above. The frequency of the treatments and the intervals when given are indicated following the word X-ray.

There was no variation in the degree of parasitemia in the chicks and ducks injected with parasitized blood to which radium bromide was added when compared with the controls. Furthermore, the ducks with malarial infections, and given radium bromide intravenously did not show any variation in the degree of parasitemia from the control birds.

Discussion. The variation in the development of parasitemia in chicks inoculated with irradiated blood when compared with the parasitemia in control chicks indicate that the roentgen ray does injure *P. lophuræ*. Only a few parasites appear to reproduce in the blood stream of the chick when the blood is treated with 2000 roentgens preceding inoculation. Apparently all the parasites are destroyed when 15,754 roentgens are used for treating the parasitized blood. The rapid decrease in the total number of parasites in the blood stream of chicks as shown in Fig. 1D, would indicate that the parasites were dead when injected. The number of chicks used in a

single experiment is small; this method for determining the degree of parasitemia is not very accurate, however, when all the data in Fig. 1 are considered they show the injurious effect of this type of irradiation on *P. lophuræ* in the chick.

The results of the *in vivo* study of roentgen rays on *P. lophuræ* in the chick as shown in Fig. 2 also indicate that this irradiation injures *P. lophuræ*. Many of the chicks died in these experiments when large amounts of irradiation were given either in multiple or single doses. The large quantity of irradiation necessary to obtain evidence of injury to *P. lophuræ* and its lethal effect on normal chicks would indicate that this form of therapy in malaria is impracticable. It would be interesting, however, to know if there is any variation in the susceptibility of different strains of plasmodia to irradiation. Furthermore, it would be interesting to know if the trophozoites varies from the sporozoites in their susceptibility to irradiation.

The quantity of radium bromide used in these experiments may have been insufficient to affect *P. lophuræ*. The amount used, however, was sufficient to produce severe degeneration in the tissues of the chick and duck.⁴ The variation in the type of irradiation from radium and the roentgen rays also must be recalled in considering the differences observed in their effect on *P. lophuræ*.

The clinical observation that irradiation reduces the size of the spleen and improves the general health of the patient with malaria is of interest, however, the number of cases treated is small and the method of evaluating the results is unsatisfactory. If different stages of the plasmodium or different plasm-

dia vary in their susceptibility to irradiation, then irradiation of the spleen in chronic malaria might be of some value.

Summary. *P. lophuræ* are injured by roentgen irradiation *in vivo* and *in vitro* as indicated by the development of the parasitemia in the chick. The amount of irradiation necessary to produce a significant effect *in vivo* is near the lethal dose of radiation for the chick.

Radium bromide as used in these experiments has no effect on *P. lophuræ*. Severe degenerative changes occur in the birds given radium bromide.

Since this paper was completed Bennison and Coatney have published their results of the effect of radiation on *P. gallinaceum*. (*Public Health Rep.*, 1945, **60**, 127.)

⁴ Rigdon, R. H., and Fletcher, D. C., to be published.

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Effect of Glycine on Peripheral Blood Flow.

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Peripheral blood flow is an important mechanism for the regulation of heat loss from the body. Under conditions of increased heat production as in fever¹ or hyperthyroidism² the blood flow to the periphery is greatly augmented. A linear correlation has been found to exist between the metabolic rate and peripheral blood flow over a wide range of metabolic levels in subjects with hyperthyroidism² and myxedema.³

In the present investigation, utilization has been made of specific dynamic action to increase heat production in the body. The increase in metabolic rate following a protein meal is due almost entirely to a few amino acids, notably, glycine, alanine, phenylalanine, and tyrosine; the increased heat production

occurring chiefly in the liver.⁴ A study was carried out to determine to what degree the dissipation of this excess heat might be attended by changes in the blood flow to the extremities.

Twenty grams of glycine* dissolved in 200 cc of water were administered to 15 subjects in the basal state. Observation was made of oxygen consumption, skin temperature of toes, fingers, and forehead, and oscillometric pulsation in the calf and forearm. Readings were made in the basal state and over a period of 4 hours following administration of glycine. Skin temperature was measured with the Leeds-Northrup potentiometer. Observations on the skin temperature were carried out under conditions of approximately constant environmental temperature ($22^{\circ}\text{C} \pm 1.3^{\circ}$), and with the extremities exposed for a period

¹ Bierman, W., *J. A. M. A.*, 1936, **106**, 1158.

² Stewart, H. J., and Evans, W. F., *Am. Heart J.*, 1940, **20**, 715.

³ Stewart, H. J., and Evans, W. F., *Arch. Int. Med.*, 1942, **69**, 808.

⁴ Wilhelmj, C. M., *Physiol. Rev.*, 1935, **15**, 202.

* A preparation of glycine supplied by Sharp and Dohme was employed.