feeding of the filtrate is apparently dependent upon individual susceptibility and enterotoxin potency. Reactions have been observed within a range of 45 minutes to 7 hours. After the spasm has subsided, further reactions may be elicited, for at least a 10-day period, by feeding the frog. Therefore, it is possible to feed and then leave the animal until the next morning when a meal is given. A typical spasm occurs within 3 to 4 hours.

Such typical reactions have been obtained with other known enterotoxigenic strains of *Micrococcus pyogenes var. aureus,*† while no such reaction has ever been observed in frogs fed with filtrates prepared from known negative strains of *Micrococcus pyogenes var. aureus* No. 902 and 64, *Escherichia coli, Porteus vulgaris,* and sterile culture medium. The results of these tests are summarized in Table I.

TABLE I.
Effect of Enterotoxigenic and Non-Enterotoxigenic Filtrates Upon the Frog (*Rana pipiens*).

Organism used in filtrate preparation	Total No. of frogs tested	Reaction observed
Micrococcus pyogenes var. aureus		
No. 161	28	Spasms
No. 8	8	,,
No. 432	21	,,
No. 422	5	,,
No. 64	18	None
No. 902	5	,,
Micrococcus pyogenes var. albus	9	,,
Escherichia coli	6	,,
Proteus vulgaris	3	,,
Sterile culture medium	29	,,

A series of frogs was fed known enterotoxigenic filtrates in an effort to show reverse peristalsis of the stomach. At the height of a spasm the animal was decapitated. In each frog studied, the esophageal sphincter was relaxed and the stomach was observed in reverse motion. Controls included unfed frogs, those fed hamburg, and those fed sterile culture medium. One each of these was sacrificed with each test frog. In no control was any reverse motion of the stomach observed.

Summary. Staphylococcal enterotoxins which gave positive results with the kitten test were capable of producing spasms when fed to frogs. It would appear from the results summarized in Table I, that the frog test was specific for no false positive reactions were noted. It is not possible to use a frog more than once when a positive reaction has been obtained and further study is in progress to determine the nature of the injury to the digestive mechanism.

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Epilation in the Non-Irradiated Member of Parabiotically United Rats.* (17402)

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Despite the lack of our ability to explain this phenomenon—the loss of hair in the non-irradiated members of parabiotically united rats—it was thought worthy of record as an observation.

The relatively few known fundamental reactions involved when either particulate or electromagnetic radiation reacts with matter are described in the standard textbooks of Atomic Physics. These explanations are fully

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as applicable to animate as to inanimate objects. Nevertheless much is unknown concerning the chain of events which leads to the eventual dissolution or loss of the integrity of a system.

Specific agents are known which produce in a mammal a syndrome comparable to that seen following radiation. For example, it is possible with folic acid antagonists to produce epilation, lymphopenia, anemia, hemorrhage, diarrhea, anorexia, malaise, wasting and eventual death in much the same sequence as occurs with radiation. This can be done with concentrations of these substances so low in body fluids that they avoid chemical detection.

It is possible that the indiscriminate production of ion pairs and excitation of atoms in animals by means of radiation may be followed by the recombination of ions in such a manner that the probability of the formation of a specific substance causing the sequence of events seen in radiation illness is present. Evidence for toxic recombinations has been gained from the study of the radiation of water *in vitro*.[1,2] Other simple *in vitro* experiments have led to the idea of direct and indirect action.[3] A direct action is one in which ionization produced within the molecule causes the effect while an indirect action is one in which the effect occurs because of ionization produced in surrounding material. Apart from this consideration of whether a toxic substance is produced directly or indirectly, a second, perhaps less fundamental question has concerned investigators: *Is the toxic material during and after formation free to move from one part of the animal's system to another and act there?*

The conviction that the production of a specific harmful substance could not occur without being conveyed throughout the animal's system has led to cross circulation experiments in which the vascular flow of a non-treated animal was connected to that of a radiated animal.[4-8] In general these experiments have been performed with complete disregard for the magnitude of the vascular exchange or with some regard but little evaluation. It is particularly important to realize that negative results of cross circulation radiation experiments are worthless so far as disproving an hypothesis that radiation damage is caused by a circulating agent. Moreover similar physical considerations apply to transfer within a single animal. A physico-mathematical consideration of cross circulation experiments has been presented in which it is shown that the distribution of a substance within a system is a function of at least two turnover rates, (1) that of the blood flow to the area and (2) that of the metabolite.[9]

Early investigators had much difficulty with the parabiotic technique—indeed some of them admitted to more operative failures than successes and with a successful operation there was always the question of the efficacy of the inosculation. Zacherl irradiated parabiotic rats with a lethal and unspecified amount of X-ray.[4] He made no statement concerning the specific nature of the shielding afforded the protected members of the pair. He observed concomitant fall in the temperature and white blood count of the members of the pair; however, the fall of both the white blood count and the temperature was not as great in the protected member. He gave little consideration to the effect of dilution which would occur with mixing. He was convinced of a radiation toxin. Behnes' experiments on parabiotic guinea pigs are less clearly described.[5] Woenckhaus also was convinced that a radiation toxin was produced, but his

1 Risse, O., *Z. Phys. Chem. A*, 1929, **140**, 133.

2 Fricke, H., *J. Chem. Phys.*, 1934, **2**, 556.

3 Lea, D. E., *Action of Radiations on Living Cells*, chap. 2, Macmillan Co., 1947.

4 Zacherl, H., *Beitrag zur Allgemeinwirkung der Röntgenstrahlen Strahlentherapie, Band*, 1926, **23**, 272.

5 Behne, *Deutsche Med. Wochensch.*, 1920, **46**, 223.

6 Woenckhaus, E., *Arch. f. Exp. Path. und Pharmak.*, 1930, **150-152**, 183.

7 Lawrence, J. S., Dowdy, A. H., and Valentine, W. N., Atomic Energy Commission Declassified Document No. MDDC-853, 1947.

8 Barnes, W. A., and Furth, O. B., *Am. J. Roent.*, 1943, **49**, 662.

9 Huff, R. L., Trautman, R., and Van Dyke, D. C., to be published.

experiments are subject to the same criticism as Zacherl's.[6] Lawrence *et al.*[7] reviewed the literature concerning the evidence for a circulating radiation toxin and concluded that the question was not settled. They set out to confirm or deny the presence of such a substance by cross-circulating cats (carotid to carotid anastomoses) at various time periods following radiation of one with 1500 r X-ray for a duration of approximately 10 hours. In spite of the fall in absolute lymphocyte count which occurred during and after the cross circulation in the non-radiated member they concluded that their results did not support the theory of a radiation toxin. Exact interpretation of their experiments requires that the turnover rates of white cells and of the blood flow between the animals be known. Furth and Barnes[8] performed an extensive experiment on parabiotic mice which included much microscopic tissue examination. The non-radiated members of the pairs showed non-specific changes which were similar to but much less than in the radiated animal. They mentioned that the parabiotic combination offered protection from radiation.

In this laboratory an experiment was planned to determine the magnitude of the protection from radiation afforded rats in parabiosis. During the course of this study it was noted that both the radiated and non-radiated members of a pair showed epilation; starting about 4 weeks after radiation and lasting 6 weeks.

Methods. The apparatus used for radiation is shown in Fig. 1. It consisted of a lead chamber in juxtaposition with a lucite chamber between which was a lead divider except in the area corresponding to the connecting tissue of the pairs. The dosage given was determined by inserting a lucite phantom, which has approximately the x-ray absorption equivalent of tissue. Victoreen ionization chambers were placed in holes drilled in the center of the phantom, and a third was placed in an attached lead box to serve as a monitor. Radiation conditions were 200 K.V., 15 ma, 0.5 mm Cu and 1.0 mm Al filters, 37.5 cm tube distance, rate 40 r/min. The time necessary to deliver the desired dose was determined with the ionization chambers in the plastic

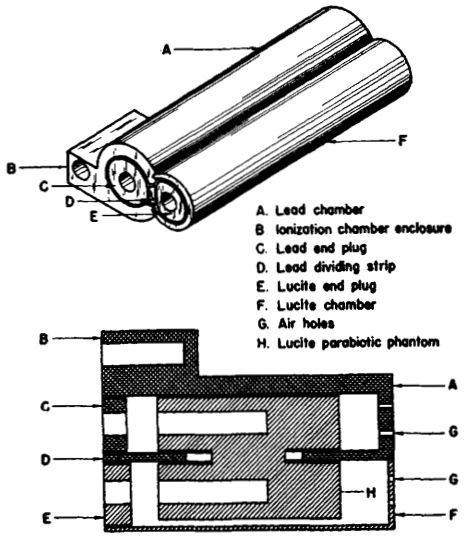

FIG. 1.
Chamber for exposing parabiotic rats to radiation.

phantom. The reading in the lead box was found to be a constant fraction of the dose received inside the phantom, and this reading was used later to check the dose given the animals. The dose delivered inside the phantom protected by the lead chamber was found to be 3% of that given the unprotected member. Readings were made inside the lead chamber by means of the Victoreen Ionization Chambers and also with x-ray film to insure the absence of large amounts of soft radiation from scatter.

Three groups of animals were prepared for the experiment. Group I consisted of 6 parabiotic pairs which were subjected to radiation, Group II was 6 parabiotic pairs not radiated and serving as controls to parabiosis and Group III was composed of 12 single animals radiated in the same manner as those of Group I. All animals were of approximately the same weight so as to insure comparable depth dose. The rats were females of the Slonaker strain which had been propagated by brother and sister inbreeding for 50 generations. This degree of inbreeding should result in an almost homozygous genetic pattern which would tend to eliminate the antigen specificity of their nuclear proteins. Pairs prepared from these animals remain in perfect health for the normal life

expectancy. In none of them is observed the wasting and anemia so often described for parabiotic rats. The vascular exchange rate is .6% of the blood volume per minute.[10] Litter mates were surgically united (coelio-anastomosis) at 20-30 days of age.

After the pairs of Groups I and II had been joined for 2 months the members of Groups I and III were radiated alternately on the same day. Precisely: one pair was placed in the chamber, one animal receiving 27 roentgens and the other 900 roentgens; then 2 single animals were placed in the container one receiving 27 roentgens and the other 900 roentgens, etc. Following radiation 2 animals from each of the 3 groups were maintained in the same cage. Treatment with DDT was carried out weekly.

Results. For the most part the protected members of the radiated pairs and the single animals receiving only 27 roentgens showed no gross signs of radiation injury. However, about 4 weeks following radiation both members of the six pairs in Group I started to lose their hair. Such a pair is shown in Fig. 2. The epilation was observed simultaneously but was first much more marked in the protected members. Rough estimation of the degree of epilation (1 to 4+) was made and the tabulation of these estimations for the 6 pairs, Fig. 3, gives the course of the epilation in the 2 animals. The protected animals were affected to a greater degree but for a shorter time than the unprotected members. Neither the single controls, receiving 27 roentgens, nor the non-radiated parabionts of Group II showed any sign of epilation. The single controls receiving 900 roentgens died within one week after radiation. The protection afforded the radiated parabiont will be discussed in a later communication.

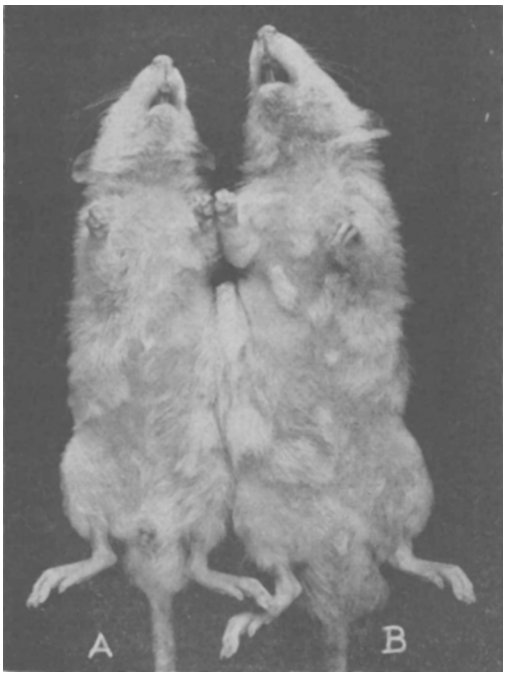

FIG 2.
Parabiotic rats showing epilation of both (A) irradiated and (B) non-irradiated members.

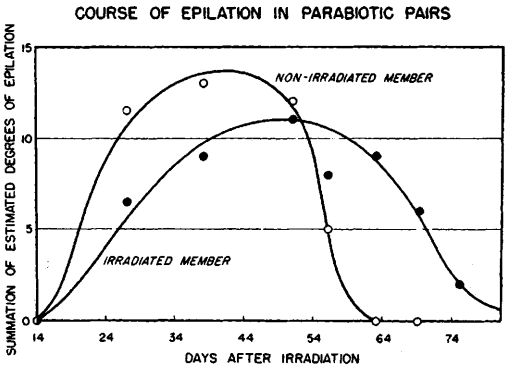

FIG. 3.

Discussion and Conclusion. Parabiotically united rats influence their partners by the mixing of their body fluids through their common circulation. Therefore, an effect such as epilation must result from the transfer of material from one member to the other. Whether the epilation represents the passage of physiologically active material from the radiated to the non-radiated or is a manifestation of loss of material from the non-irradiated to the irradiated is not demonstrated.

[10] Van Dyke, D. C., Huff, R. L., Evans, H. M., *Stanford Med. Bull.*, 1948, **6**, 271.

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