

nificantly more than 5% of ingested fluoride retained by the body, but that retention is a function of age and previous exposure to the element. During the initial metabolism period the animals in this study were 24-28 days of age and were in a rapid stage of skeletal growth. The fluoride data obtained at this time (Table I) indicate that about 75% of the ingested fluoride was incorporated into the skeleton. During the final metabolism period the animals were 120-124 days of age and were in an early stage of maturation which is accompanied by a significantly decreased rate of growth and skeletal exchange. The fluoride data obtained at this time indicate that only 30% of the ingested fluoride was deposited in the skeleton. These data thus suggest not only a decreased rate of fluoride retention as a function of increased age, but in addition, a possible mechanism for the action of molybdenum upon fluoride metabolism.

Summary. The results suggest that administration of molybdenum increased retention of fluoride, but that this effect is more prominent in older animals. Analysis of the soft tissues showed that molybdenum significantly increased concentration of fluoride. However, elevations in fluoride content were not directly proportional to increases in amount of molybdenum administered.

1. Adler, P., *Odont. Revy.*, 1957, v8, 202.
2. Stookey, G. K., Muhler, J. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1959, v101, 379.
3. Weddle, D. A., Muhler, J. C., *J. Nutrition*, 1954, v54, 437.
4. McClure, F. J., in *Dental Caries and Fluorine*, F. R. Moulton, Ed., A.A.A.S., Washington, D. C., 1946, p89.
5. Wagner, M. J., Muhler, J. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1958, v98, 496.

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Activation of *Bartonella muris* Infection in X-Irradiated Rats. (27175)

H. BERGER AND W. H. LINKENHEIMER (Introduced by T. H. Jukes)

Agricultural Division, American Cyanamid Co., Princeton, N. J.

Bartonellosis, caused by *Bartonella muris*, is an endemic disease in many rat colonies. The infected animals are essentially free of symptoms unless splenectomized. It has been reported(1,2) that latent *Bartonella muris* infection in the rat may be activated by exposure to whole body X-irradiation. The presence of this disease may be a complicating factor in radiation experiments when studying diverse protective procedures that may modify the response to the *Bartonella muris* infection. In this investigation, the effect of X-irradiation on latent bartonellosis in the rat was confirmed and the infectivity of whole blood, red blood cells and plasma containing this organism from infected X-irradiated rats was compared.

Methods. Injected donor animals. Female albino rats weighing 80-100 g were obtained from the Royal Hart breeders, Middletown, N. Y. The Royal Hart rats had *Bartonella*

muris infection as diagnosed following splenectomy by anemia, presence of Bartonella bodies in the blood smear and death of the animal. The donor animals were exposed to 2 doses of whole body radiation at 3-day intervals. Each dose was equivalent to 250 rad. Ten to 15 rats were placed in a flat plastic cylindrical container so that movement was limited to a minimum and only in a horizontal plane during the exposure period. X-radiation was delivered by 250 KVP X-ray unit operating at 30 milliamperes with 1.0 mm Cu and 1.0 mm Al filters. Using an absorption value of 0.58 as an average quantity over the plastic container and a transmission factor of 94% for the 1/8" thick plastic top, as determined by dosimetry measurements, average dose rate was 51 rad per minute.

Uninfected receptor animals. Female CFN rats weighing 80-100 g were obtained from

TABLE I. Mortality Data for Splenectomized Bartonella-Free Rats (10/Group) Injected with 2 Dose Levels of Whole Blood from X-Irradiated Bartonella Carrier Rats.

Exp. II										
Origin of blood	Amt, cc	No. deaths on days post inj.								MST*
		1	2	3	4	5	6	7	8	
X-irrad. rats	.2	9	1							1.2
	1.2	9	1							1.1
Non-X-irrad. rats	.2	2	2	2	2		1		1	4.1
	1.0	5	4	1						1.6

* Mean survival time in days.

Carworth Farms, New City, N. Y., and served as receptor animals. These animals were free from Bartonella infection and were housed in quarters not previously used for rodent storage. All CFN rats were splenectomized 2 weeks before intraperitoneal injection of blood or blood components obtained from infected rats with or without X-irradiation.

Identification of Bartonella organisms. Daily blood samples were obtained from tail vessels and smears were made. The dried blood smears were fixed in absolute alcohol and stained with highly alkaline 10% Giemsa stain in tap water for 20 minutes followed by rapid differentiation in tap water.

Results. In Experiment I, one group of splenectomized Carworth *Bartonella muris* free rats was injected intraperitoneally with 1.0 cc of whole blood from 3-day post X-irradiated *Bartonella muris* infected Royal Hart rats. A second group received whole blood from non-irradiated infected Royal Hart rats, and a third group of splenectomized Carworth rats received no treatment. When 1 ml of

blood from X-irradiated rats was injected in 7 splenectomized Bartonella-free rats, all recipient animals died within one day. Peripheral blood smears showed many Bartonella organisms within the red cells before death. A control group of 7 animals injected with blood from non-irradiated infected animals survived without symptoms for a 2-week observation period.

Experiment II of the series was a slight modification of the first in that 2 dose levels were administered. Splenectomized *Bartonella muris*-free rats were injected intraperitoneally with 0.2 ml and 1.0 ml of whole blood from Bartonella infected X-irradiated rats. A similar group was injected with 0.2 ml and 1.0 ml of whole blood from non-irradiated Bartonella infected rats. The data (Table I) suggest that X-irradiation activated the Bartonella organism to give a more lethal infection. Rats remaining alive for approximately 3-4 days post-infection exhibited hematuria, reticulocytosis, leucocytosis and Bartonella organisms in the blood smears.

The third experiment was designed to compare the virulence of the infection in blood components. Splenectomized *Bartonella muris*-free rats were injected intraperitoneally with 0.2 ml of whole blood, plasma or red blood cells suspended in 0.9% NaCl from infected irradiated rats or from infected non-irradiated animals. Non-splenectomized infection-free rats were injected with similar fractions. The results given in Tables II and III suggest that whole blood and its fractions from infected irradiated animals were equally virulent when injected into uninfected splenectomized rats. The data demonstrate the

TABLE II. Mortality Data for Splenectomized Bartonella-Free Rats (8/Group) Injected with Whole Blood or Fractions from X-Irradiated Bartonella Carrier Rats.

Exp. III										
Material inj. (.2 cc)	Previous treatment	No. deaths on days post inj.								MST*
		1	2	3	4	5	6	7	8	
Whole blood	X-irrad. rats	8								1.0
Red cells	" "	8								1.0
Plasma	" "	8								1.0
Whole blood	None	6			2					2.0
Red cells	" "	1	1		1	1	4	1		4.9
Plasma	" "						1	4	2	6.3+

* Mean survival time in days.

TABLE III. Mortality Data for Non-splenectomized *Bartonella*-Free Rats (8/Group) Injected with Whole Blood or Fractions from X-Irradiated *Bartonella* Carrier Rats.

Exp. III		No. deaths on days post inj.							
Material inj. (.2 cc)	Previous treatment	1	2	3	4	5	6	7	8 9
Whole blood	X-irrad.	1				1			
Red cells	"							1	
Plasma	"	2				1			
Whole blood	None	No deaths in any group							
Red cells	"								
Plasma	"								

protective effect of an intact spleen. The results of the previous experiments, that X-irradiation activated the *Bartonella* organism to give a more lethal infection, were confirmed.

Discussion. The existence of *Bartonella muris* infections in rodents is well established (3). It has also been established (1,2) that X-irradiation activates this infection. Treatment with antibiotics and hematological responses have been reported (4) but actual transmission of the disease by inoculation is presented here. The results indicate the protective role of the spleen in controlling the disease, since activated plasma was not rapidly lethal in non-splenectomized animals as was observed in the splenectomized group. The results also suggest that the *Bartonella* infective response may be obtained with equal

efficiency whether plasma or washed-cells from irradiated infected animals are used as the transmission medium. Since the spleen appears to be essential in controlling the effects of this infection, it is possible to suggest a dual role for this organ as a radiosensitive tissue: first, as a hematopoietic organ; secondly, as an organ exerting a controlling effect on latent *Bartonella* infection.

Summary. The experimental results confirm previous reports that X-irradiation activates latent bartonellosis in rats to produce a fulminating infection. It was also found that whole blood, red blood cells or plasma from infected irradiated rats were equally lethal when injected into *Bartonella muris*-free rats. The lethality was much less when the recipient rats had not been splenectomized.

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1. Scott, J. K., Stannard, J. N., *J. Infect. Dis.*, 1954, v95, 302.
2. Rekers, P. E., *ibid.*, 1951, v88, 224.
3. Weinman, D., *Trans. Am. Phil. Soc.*, 1944, v33, 243.
4. Stanton, F. S., Laskowski, L., Pinkerton, H., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 705.

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Effect of Caffeine on Spinal Reflexes.* (27176)

G. SANT'AMBROGIO,[†] D. T. FRAZIER AND L. L. BOYARSKY
(Introduced by L. D. Carlson)

Department of Physiology, University of Kentucky Medical Center, Lexington

There have been few studies of the effect of caffeine on the mammalian spinal cord. Wood (1) found an increase in the patellar reflex in man following caffeine ingestion, which lasted for several hours. Gualtierotti *et al.* (2) observed an increase in the patellar

response and a decrease in latency in man. Danilov (3) reported a decrease of tendon jerks in spinal and intact dogs and an increase in flexor responses. The present study is an attempt to examine the effect of caffeine on monosynaptic and polysynaptic reflexes of decapitate cats using electrophysiological methods. The amplitude of the responses, the input-output relation of the polysynap-

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[†] Present address: Istituto di Fisiol. Umana, Univ. of Milano, Italy.