an artifact. Some substance, as yet unidentified, which was produced during cell growth, appears to be the responsible agent. The absence of significant quantities of C^{14} in the AF from both sterile incubated controls and incubated resting cell suspensions seems to preclude improper extraction, interfering substances in the medium used, or autooxidation during incubation and extraction as sources of this effect.

Increased water solubility of cholesterol in biological systems such as, for example, bile and serum(9, 10) has long been known. In addition, it is possible to modify the water solubility of this sterol *in vitro* by use of proteins, phospholipids(11), bile salts(12), or detergents(13).

It seems important, therefore, to use caution in interpretation of results of biological experiments dealing with cholesterol catabolism, in particular, where relatively small changes detectable only by means of tracers are involved. Simple classical extraction procedures, without further fractionation and a more detailed characterization of the products, were shown to be both inadequate and misleading.

Summary. C^{14}-labeled cholesterol was incubated with growing cells of *A. aerogenes*. Following hydrolysis of the incubation medium, a significant portion of the sterol was recovered in the saponifiable fraction. Apparently, the solubility of the sterol was modified by a substance produced during the growth of the *Aerobacter*.

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The Effect of Continuous Antibiotic Feeding on X-Irradiation Mortality in Mice. (27032)

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The occurrence of bacteremia in animals after exposure to lethal or sublethal total body X-irradiation has been reported(1-4). The bacteremia was caused primarily by microorganisms normally present in the lower intestinal tract; however, due to apparent increase in intestinal wall permeability these organisms were dislocated into the peritoneal cavity(1). Since the generalized infection develops during the period of greatest mortality, it may be concluded that infection plays an important role as one of the factors in death following irradiation. Various workers(5-6) have found that clinical doses of chlortetracycline when administered orally in the feed or by injection following irradiation prolonged the lives of rats, dogs and mice. The following experiments were undertaken to determine the effect of low level chlortetracycline feeding administered prior to and after exposure to total body X-irradiation on a lifetime basis. During the early weeks of the first experiment, a *Bartonella muris* infection was detected in the experimental animals. A second experiment was included to determine possible interactions of irradiation, disease, time and chlortetracycline feeding.

Methods. Mice. The mice used in Experiment 295 were young females obtained from Royal-Hart Animal Labs weighing 20-22 g. The mice used in Experiment 300 were young females of the Carworth Farms #1 strain weighing 19-21 g at time of irradiation. Initial group sizes varied with experiment and treatment (Table I).

TABLE I.
Experiment 295

Exp. No.	Group	Strain	No. Animals	Treatment: Irradiation*	Treatment: Chlortetracycline**
	2358	RH	116	−	−
295	2359	RH	111	−	+
	2360	RH	225	+	−
	2361	RH	217	+	+
			Experiment 300		
	2400	CF-1	100	−	−
	2401	CF-1	100	−	+
300	2402	CF-1	191	+	−
	2403	CF-1	191	+	+

* 250 Rad/Wk Total = 750 Rad.
** 20 ppm Chlortetracycline in Diet.

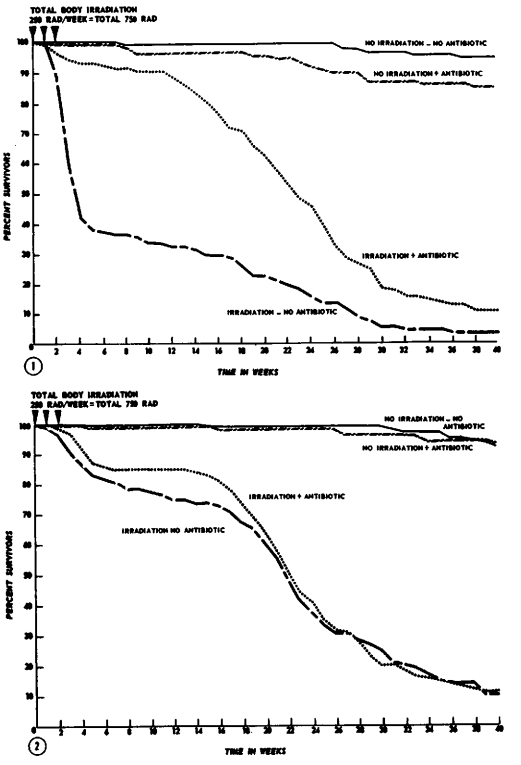
Diet. Ground Wayne rat pellets were fed to all mice upon delivery from the respective supplier for one week. Two weeks prior to X-irradiation diet 288R (Table II) was substituted as the basal diet and continued throughout the experiment. One experimental group and one control group in each experiment received supplements of 20 ppm (parts per million) chlortetracycline in the basal diet for duration of the experiment (Table I).

TABLE II.
Diet No. 288R Formulation

Material	%
Yellow Corn Meal	61.0
Soybean Meal (44% Protein)	20.0
Corn Gluten Meal	5.0
Fish Meal (Menhaden)	5.0
Alfalfa Meal	2.0
Distillers Solubles	2.5
Pulverized Calcite Limestone	2.0
Steamed Bone Meal	1.5
Sodium Chloride (Iodized)	.5
Vitamin Mix	.5
Vitamin Mix	Mg/Kg
Choline (25%)	206
Nopco (A + D dry)	206
Delamix + 2% Zinc	206
Crystalline B_{12}	2.3 (μg)
Riboflavin	.9
Niacinamide	.2
Ca Pantothenate	8.1

Method of Irradiation. All irradiated mice were exposed to 3 doses of 250 rad per week for 3 weeks for a total dose of 750 rad. Twenty-five to 30 mice 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. The X-radiation was delivered from a 250 KVP X-ray unit at 30 ma. with 1.0 mm Cu + 1.0 mm Al filtration. Using an absorption value of 0.58 as an average quantity over the plastic cage and a transmission of 94% for the ⅛" thick Acrylic Resin top, as determined by dosimetry measurements, the average dose rate was 51 rad per minute*.

Bartonella Detection. Blood samples were obtained from the tail and smears made. Staining of the organism was accomplished with



FIGS. 1, 2. Effect of irradiation and chlortetracycline feeding on survival.

* The authors wish to express thanks to Drs. R. F. Stamm and L. A. Siegal, American Cyanamid Co., Stamford, Conn. for dosimetry and irradiation service.

TABLE III.
Analysis of Variance

Source of Variance	Degrees Freedom	Variance
Overall Main Effects		
Radiation (R)	1	1606.88***
Chlortetracycline (C)	1	0.11
Disease (D)	1	10.58
Interaction		
(R) x (C)	1	12.50
Error	3	3.81
Least Required Variance	p = .05 = 38.60*	
	p = .01 = 130.00**	
	p = .001 = 638.18***	
Period x Treatment		
Main Effect		
Period (P)	3	257.10
(linear effect P′)	1	397.78**
(quadratic effect P″)	1	12.45
(cubic effect P‴)	1	361.08**
Interactions		
Period x Radiation		
(P′ x R)	1	339.54**
(P‴ x R)	1	210.13**
Period x Chlortetracycline		
(P′ x C)	1	31.90
(P″ x C)	1	174.85*
Period x Disease	3	47.73
Period x Chlortetracycline x Disease		
(P′ x C x D)	1	87.68
Period x Radiation x Chlortetracycline		
(P′ x R x C)	1	171.31*
(P″ x R x C)	1	177.85*
Error (Residue)	11	29.37
Least Required Variance	p = .05 = 142.14*	
	p = .01 = 283.42**	
	p = .001 = 578.30***	

10% alkaline Giemsa. Visual observation of the organism was used as positive identification of infection.

Results. The 40-week data are presented in Fig. I for Experiment 295 using mice with known *Bartonella muris* infection. One week following the final irradiation dose, a 60% mortality was observed. Following the initial high mortality, a decrease in rate was observed; however, mortality rate was in excess of that for the non-irradiated controls. Irradiated animals continuously fed 20 ppm chlortetracycline did not demonstrate the initial high mortality, although the overall mortality was in considerable excess of the controls.

Fig. 2 presents the total 40-week data for Experiment 300 with mice apparently free of *Bartonella muris* infection. The effects of irradiation are markedly different in the non-antibiotic group during the first 10 weeks compared with Experiment 295 since no initial acute mortality was observed. Under the conditions of this experiment there appeared to be little effect of the chlortetracycline on the increased mortality due to irradiation. Because of the obvious interactions involved, the data were statistically evaluated by the analysis of variance†. The statistical results are presented in Table III and Fig. 3. The effect of irradiation and period or time interval on survival indicated that mortality of the irradiated group is significantly greater during the entire period at the 0.001 level; however, it is interesting to note that the difference in mortality between irradiated and control animals is significant only during the first 30 weeks. The rate of mortality does not significantly differ during the final 10 weeks of the experiment. The overall and period effects of chlortetracycline without irradiation were without statistical significance. The effect of chlortetracycline on irradiation significantly modified the death rate ($p = 0.05$) during the first 20 weeks of the

† The authors wish to express gratitude to Dr. R. H. White-Stevens for assistance with the statistical procedures.

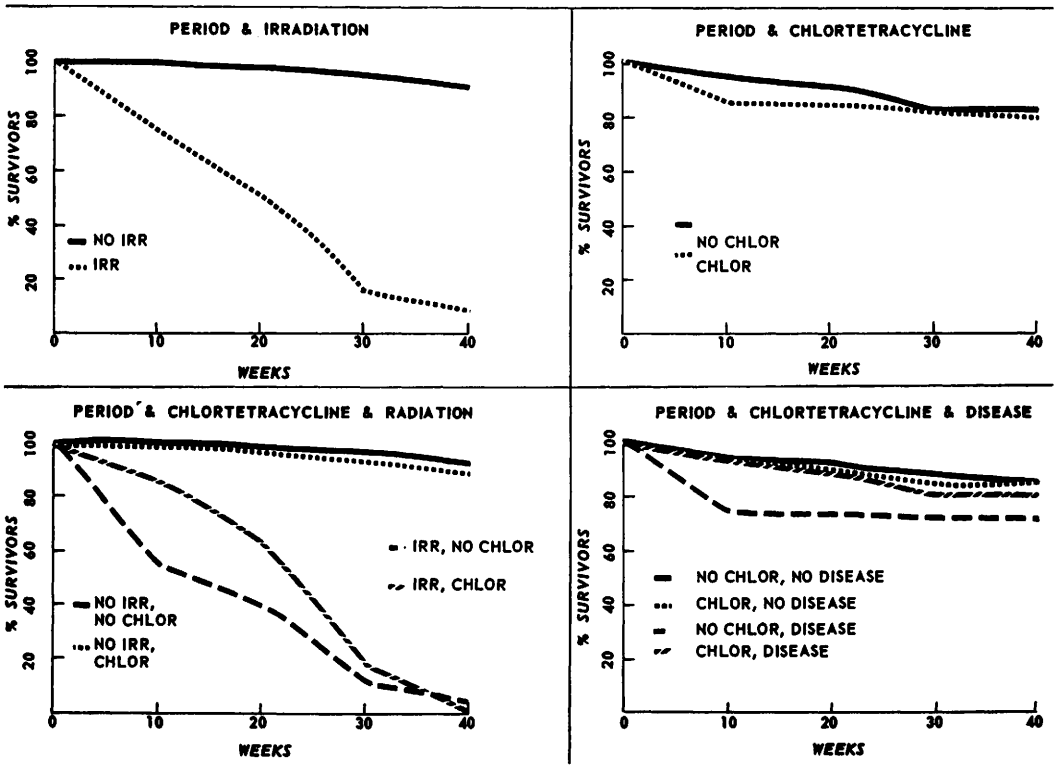


FIG. 3. Analysis of variance.

experiment; however, this effect was not significant during the latter one-half of the experimental period. The effect of the chlortetracycline was the result of a modification of disease during the first 10 weeks presumably due mainly to the effective control of *Bartonella muris* infection.

Discussion. The relationship of irradiation damage and bacteremia have been reported (1-3,7). Most of this literature deals with survivors of atomic bomb blasts or controlled irradiation exposure of relatively massive single doses with observations taken during the acute 30 day period following exposure. Other literature concerning the effects of antibiotics in conjunction with irradiation damage is primarily concerned with high doses of antibiotic and dislocation of intestinal bacteria. The original purpose of this experiment was to attempt to demonstrate a possible antibiotic effect on low-grade infections possibly due to irradiation damage of the immune mechanism. Unexpectedly, the acute mortality observed in Experiment 295, should not have occurred with this dosage procedure. *Bartonella muris* infection was suspected and established. The protective effect of antibiotic was due primarily to the susceptibility of this organism to chlortetracycline. The second experiment is also of interest because without the presence of the *Bartonella muris* infection, chlortetracycline did provide a protective or modifying effect on mortality during the early stages of the experiment, presumably due to antibacterial activity; however, overall effect of continuous chlortetracycline feeding was not statistically significant.

Summary. 1. X-Irradiation administered by the reported procedure significantly increased mortality over controls during a 40 week period. 2. Chlortetracycline administered in the feed at 20 parts per million significantly decreased irradiation mortality during the first 20 weeks of the experiment. 3. The marked chlortetracycline effect in Experiment 295 was due to modification of disease primarily due

to *Bartonella muris* infection. 4. The lesser effect on mortality observed in Experiment 300 was probably due to antibacterial activity of the chlortetracycline on minor incident infections antagonized by the X-irradiation.

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Anticonvulsant Effect of Nialamide and Diphenylhydantoin.*‡ (27033)

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Evidence was accumulated during the past decade indicating that 5-hydroxytryptamine (5-HT), a normal constituent of the central nervous system, is of importance in the functioning of the system. Among its multiple functions, several investigators have suggested a possible interrelation between the levels of brain 5-HT and the excitability of the central nervous system. Prockop *et al.*(1) reported that the monoamine oxidase inhibitors have a pronounced anticonvulsant effect and that this effect is closely associated with an elevation of brain 5-HT and norepinephine (NE). Reserpine which releases brain amines was reported to facilitate convulsions(2). Bonnycastle and co-workers(3,4) and Anderson *et al.*(5) have shown that a number of anticonvulsant compounds including diphenylhydantoin, mesantoin, trimethadione and paramethadione caused a significant elevation of brain 5-HT levels in rats and suggested a relationship between an increase in 5-HT in the brain and the anticonvulsant action of these drugs. Prockop *et al.* (1), on the other hand, observed that diphenylhydantoin does not increase amines in the brain of rats.

The purpose of this paper is to report the influence of pharmacological agents such as nialamide or reserpine which increase(6,7,8) or decrease, respectively, the levels of brain amines on the anticonvulsant action of diphenylhydantoin. A study of the interaction of diphenylhydantoin with these agents was of particular interest because nialamide has been reported to have anticonvulsive action(7,9) and reserpine to facilitate convulsions. Simultaneous measurement of brain amine levels under the various experimental conditions was also made.

Experimental. A total of 595 Swiss-Webster male white mice weighing 17 to 25 g and 125 Wistar male white rats weighing 100-120 g were used in these studies.

Two methods were used in testing the anticonvulsant action: 1. Protection against maximal electroshock seizures (MES) as described by Swinyard, Brown and Goodman(10) in which alternating current of 50 mA intensity in mice and 150 mA in rats with 0.2 second duration was delivered through corneal electrodes from a Hans Technical Associates Stimulator. The dose of anticonvulsant drug necessary to protect 50% of animals (ED_{50}) from the hindleg tonic extensor component of the maximal

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