Although an occasional rabbit died of intercurrent infection, there was no "clinical" evidence that any of the 3 types of tissue culture virus was pathogenic for the rabbit, thus confirming the results of earlier workers(1). The variability in antibody responses of individual rabbits emphasizes the need of using adequate numbers in each test—possibly 5 animals per specimen. The response of the rabbit to the trivalent irradiated vaccine suggests the possible use of a rabbit test for assay of the immunogenic potency of different batches of vaccine for the selection of more antigenic strains of virus for vaccine production, and for determining the antigenicity of vaccines inactivated by various chemical or physical methods. Finally, storage tests of different types of poliomyelitis vaccine may be carried out.

Summary. Type specific neutralizing antibodies were produced in rabbits inoculated with the 3 types of poliomyelitis virus grown in tissue culture. Rabbits were successfully immunized by repeated intravenous or intramuscular inoculations of active or ultraviolet irradiated virus suspensions. The possibilities of a rabbit test for the assay of poliomyelitis vaccine are discussed.

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Endogenous Infection in Mice Irradiated with Fast Neutrons or Gamma Rays.* (21304)

HOWARD H. VOGEL, JR., JOHN W. CLARK, CAROLYN W. HAMMOND, DOROTHY B. COOPER, AND C. PHILLIP MILLER.

From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill. and the Department of Medicine, University of Chicago.

The development of generalized infection has been shown to play a significant role in the death of mice exposed to moderate doses (450-600 r) of total body x-irradiation in a single exposure(1,2). Such infections, which were always caused by bacteria of enteric origin, were demonstrated by blood and spleen cultures of mice killed at daily intervals after x-irradiation, and in a more recent study, by serial blood cultures(3). Bacteremia occurred most frequently during the second week following x-irradiation. In mice exposed to an equivalent dose of fast neutrons, death has been found to occur much earlier—between the 4th and 8th day(4), *i.e.*, before bacteremia becomes an important factor as a cause of death following x-irradiation. Field studies utilizing nuclear test devices in 1951 and 1952 had demonstrated this difference in mortality time between neutron- and γ-irradiated mice(5). This shorter survival time of neutron-irradiated mice raised the question whether generalized infection has time to develop and contribute to the lethal effect as it does in x-irradiated mice. The following study was undertaken to determine the incidence of bacteremia in mice irradiated with fast neutrons and, for comparison, mice irradiated with gamma rays from Cobalt 60.

Methods and materials. Female CF No. 1 mice, 5-6 weeks old, were shipped† to the laboratory by air express and placed in isolation for 3 weeks. They were maintained on a diet of Rockland mouse pellets and water

* Part of this investigation was carried out under contract between the U. S. Atomic Energy Commission and the University of Chicago.

† From Carworth Farms, New City, N. Y.

ad libitum, and weighed 20-25 g when irradiated. Eight mice were housed together in a plastic box (11 x 7 x 5½ inches) containing wood shavings(6). *Methods of irradiation.* The mice were irradiated at the Argonne National Laboratory in the gamma-neutron radiation chamber described by Vogel *et al.* (7). During exposure the animals were kept at approximately 75°F and 50% relative humidity. Fast neutrons were produced through fission, by allowing neutrons from the thermal column of the Argonne CP-3′ reactor to impinge on uranium. Appropriate shielding devices of boron carbide prevented slow neutrons from reaching the animal exposure position. The gamma contamination of the fast neutrons was approximately 7%(8). Pure gamma radiation was obtained from 18 sources of Cobalt 60 located at the opposite end of the chamber from the uranium. Mice were irradiated in a lucite chamber, 16 in. x 16 in. x 2 in., containing 66 exposure cells, each of which could hold one animal. The intensity at each animal position was determined and isodose groups were established for both neutron and gamma radiations(9). *Dosimetry.* During irradiation, Victoreen condenser r-chambers were inserted into standard positions in the dosimetry columns of the lucite animal cage(7). With the aid of Dr. H. H. Rossi of Columbia University, a Victoreen chamber was calibrated with a tissue equivalent chamber of 10.1% hydrogen composition so that doses in the fast neutron field could be expressed in terms of roentgens-equivalent-physical (rep) (8). This calibration has recently been confirmed by Mills and Blosser of Oak Ridge National Laboratory using a Hurst proportional counter for neutron dosimetry(10). One of the Victoreen 25 r thimble chambers with 4 mm plastic cap has also been calibrated, for gamma radiation from Co^{60} in terms of International Roentgens, by the National Bureau of Standards. *Dose range.* 188 mice were irradiated with fast neutrons in 4 experiments. The dose rate was 2.5-3.0 rep/min. and the time of exposure varied from 75 to 90 minutes. Previous work had established the LD_{50}—30 days for this strain to be 210 rep of fast neutrons(11). The dose range used (216-265 rep) was thus in the upper lethal range, between approximately the LD_{60} and the LD_{98}—30 days. 111 mice were exposed to Co^{60} γ rays in 2 experiments. The duration of the first exposure was 75 minutes (dose rate: 12-12.5 r/min.). The second irradiation lasted approximately 45 minutes, since the dose rate was increased to 20.4-21.7 r/min. The LD_{50} (single 90-minute exposure) for this strain to Co^{60} γ rays, is approximately 920 r (11). Thus the exposures used in these experiments (900-942 r) were in the mid-lethal range ($LD_{40\text{-}60}$—30 days), and were comparable to, but lower than, the fast neutron exposures. *Animal transportation.* Immediately after irradiation the mice were transported 25 miles to the University of Chicago in an air conditioned truck(12). Thereafter they were housed in a mouse room‡ maintained at 75°F and 52% relative humidity. Their plastic cages were chemically sterilized and changed twice a week. Sterilized water bottles were refilled daily and replaced weekly; food was provided in mangers. *Autopsy and culture methods.* On the days indicated below, mice were killed with ether, the abdomen opened aseptically, the spleen removed, cut in several places, and dropped into a tube of brain-heart infusion broth. The chest cavity was opened, the heart seared with a hot spatula and as much blood as possible drawn into a capillary pipette. One drop was streaked on each of 3 agar plates—blood, eosin-methylene-blue and Salmonella-Shigella agars. The remainder of the blood was cultured in brain-heart infusion broth. A record was kept of the number of colonies that developed on the agar plates. As soon as growth appeared in the broths, subcultures onto differential media were made. If no growth was visible by the 3rd day, they were subcultured onto blood agar before they were discarded. All microorganisms recovered from these cultures were identified by their morphology, gram reaction and characteristic appearance on appropriate media. These included the ones already mentioned as well as triple sugar-iron agar, urea broth and the usual carbohydrate broths. Anaerobic cultures were not

‡ The authors wish to acknowledge the various facilities that were made available to them in the Argonne Cancer Research Hospital.

made because anaerobes were recovered from only 0.3% of blood and/or spleen cultures of x-irradiated mice(1). *Neutron-irradiated mice.* From the 3rd day post-irradiation through the 11th, 5 mice a day (each from a different cage) were killed for culture of heart's blood and spleen. This procedure was followed for each of the 4 neutron experiments until 129 of the 188 exposed mice had been cultured, a total of approximately 20 per day during the period of greatest mortality—3rd-8th days post-irradiation (Table III). Forty-six of the mice that died were also autopsied for inspection of the viscera and for heart's blood culture. *Gamma-irradiated mice.* From the 6th through the 15th days post-irradiation, 5 to 10 mice (from different cages) were killed each day for culture of heart's blood and spleen. *Control cultures on normal mice.* Blood and spleen cultures were also made on 36 unirradiated control mice which were transported to and from the reactor together with the irradiated animals. These control mice were sacrificed and cultured at daily intervals using the methods described. All of their cultures remained sterile.

Results. The neutron irradiated mice, despite their earlier deaths, showed conclusive evidence of the development of generalized infection. The mortality figures, represented by the line graphs in Fig. 1, were taken from data used to construct survival curves for CF No. 1 female mice, exposed to single 90-minute radiations of fast neutrons (948 mice) or to Co^{60} gamma rays (1154 mice)(11). Each daily mortality figure is the per cent of the total number which died in the 30-day post-irradiation period (447 deaths following fast neutrons; 579 after gamma rays). The peak of the daily mortality curve for fast neutrons occurred 4-6 days after irradiation, and for gamma rays 12-14 days post-irradiation. No significant difference in the mortality pattern was observed between the mice which died in these experiments and those presented in Fig. 1. The results of the heart's blood and spleen cultures are shown in the bar graphs as per cent of the mice killed and cultured each day which had positive cultures of heart's blood and spleen (stippled bars) or positive cultures of spleen only (striated bars). The per cent

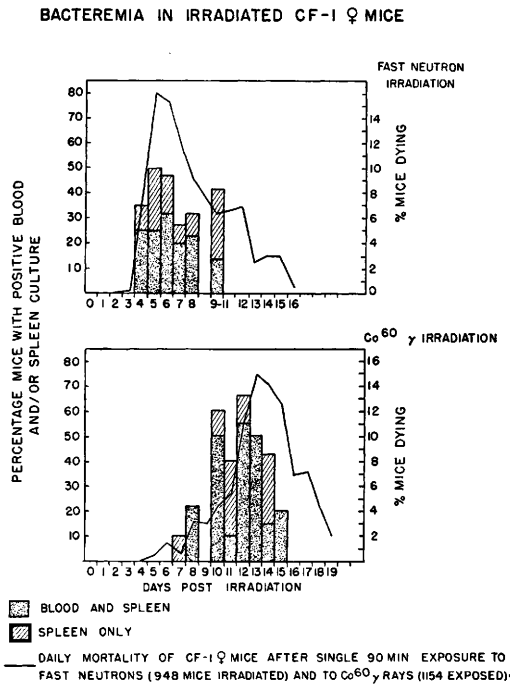

FIG. 1. Bacteremia and mortality in CF #1 mice irradiated with fast neutrons and gamma rays.

of mice with positive blood cultures would have been increased if the figures had included cultures of the mice which died. The results of these post mortem cultures have been omitted from the data illustrated in Fig. 1, but are shown in Table III.

Neutron-irradiated mice. Beginning on the 4th day, heart's blood and/or spleen cultures were positive in ⅓ to ½ of the sacrificed mice. The onset of bacteremia, therefore, coincided with the steep rise in the mortality curve, and both maxima occurred on the 5th or 6th day. It is worth noting that on the 3rd day, when mortality was insignificant, cultures on all 20 of the mice were negative. Of the neutron-irradiated mice killed for bacteriological examination, 26 had positive cultures of blood and

TABLE I. Numbers of Bacteria Recovered from Blood Cultures.

Colonies/drop of blood	Neutron mice, %	Gamma mice, %
Innumerable	—	70
>50	—	15
25-50	4	10
1-25	46	—
Only broth positive	50	4

TABLE II. Classification of Microorganisms Recovered from Heart's Blood and Spleen.

	Neutron-irradiated mice, %	Co^{60} γ-irradiated mice, %
E. coli	55	18
Proteus	20	4
α-Streptococcus	11	71
Paracolobactrum	7	7
Alcaligenes	4	—
Gram neg. bacilli unclassified	3	—

spleen, 17 of spleen only. The numbers of colonies which developed from a capillary drop of blood are given in Table I, which shows that the severity of the bacteremia was less in the neutron- than in the gamma-irradiated mice. The microorganisms recovered from these cultures are listed in Table II according to their per cent distribution among the several genera or species. All of the microorganisms were members of the normal intestinal flora of these mice. The cultures obtained from the neutron-irradiated mice were, with few exceptions, pure cultures. One blood culture and 3 spleen cultures contained 2 kinds of microorganisms. As shown in Table III, the fraction of positive blood cultures obtained from the mice which died was nearly twice that obtained from those which were sacrificed for culture. The results on cultures of dead mice have been excluded from the data presented in the graph in order to eliminate the consideration of post mortem contamination.

Gross pathological findings. All mice were carefully autopsied, but no histopathological studies were made. The following changes were noted: Evidence of diarrhea; distention of small and large bowel with fluid after the 4th day post-irradiation; progressive decrease in size of the spleen to the 9th day; obvious anemia after the 6th day; rare petechial hemorrhages beneath the visceral peritoneum after the 6th day.

TABLE III. Positive Cultures on Sacrificed Mice and on Mice Which Died following Neutron Irradiation.

Post-irradiation day	No. positive cultures/No. mice cultured Sacrificed mice	Dead mice
4	7/20	4/13
5	10/20	11/12
6	9/19	6/10
7	4/15	6/8
8	7/21	2/3
Totals	37/95 39%	29/46 63%

Gamma-irradiated mice. The highest incidence of positive cultures and of deaths did not occur until the latter part of the 2nd week following the gamma irradiation (Fig. 1). Since these results, as well as the severity of the bacteremia (Table I), duplicated so closely the earlier findings on x-irradiated mice (1,2), it seemed unnecessary to secure additional data. The principal difference between the results on the gamma-irradiated mice and those already published on x-irradiated mice was the recovery of alpha streptococci from a large proportion of blood cultures in the present series (Table II). Most of these streptococcal bacteremias occurred in one of the 2 groups of gamma-irradiated animals. We have previously observed differences, though usually less striking than this, in the percentage distribution of the several microorganisms recovered in blood cultures from different groups of mice. That was one of the reasons for repeating the earlier experiments on so many groups of animals.

Discussion. It is apparent from these experiments that mice irradiated with fast neutrons, despite their shorter survival time, developed generalized infection, demonstrated by the presence of bacteria in blood and/or spleen, as did mice exposed to gamma rays from Co^{60} or to X-rays(1,2). The data illustrated in Fig. 1 give the impression that the onset of bacteremia in the neutron-irradiated mice coincided in time with the steep rise in the mortality curve but did not precede it, as in the case of the gamma-irradiated mice. The reality of this apparent difference was questioned for the following reasons: (a) Although both radiations were administered in the upper lethal range, the neutron doses used (mean dose: 235 rep (LD_{85}—30 days)) were higher than were the gamma ray doses (mean dose: 935 r (LD_{52}—30 days)); (b) the vertical scale in Fig. 1, showing per cent positive cultures, was drawn arbitrarily at 5 times the scale representing per cent mortality of the mice; (c) the mortality data were represented as per cent decedent mice dying each day rather than as daily probability of death.

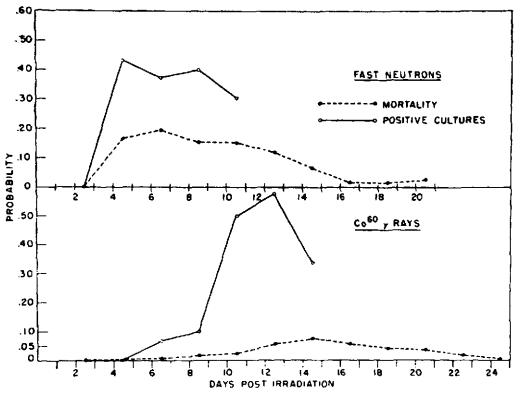

FIG. 2. Comparison of daily probabilities of death and of positive cultures of blood and/or spleen.

Therefore, a comparison was made (Fig. 2) between the daily probabilities of death and of positive cultures in the mice, following each ionizing radiation. By this means 2 rates could be directly compared. The daily probabilities, represented in this figure, have been normalized over 2-day periods. The mortality data have been carefully selected for the actual dose ranges of each radiation used. Since the dose was higher, the probability of death was higher in the neutron-irradiated than in the γ-irradiated mice. The peak of positive cultures preceded the peak of mortality in both cases. In other words, following either radiation, the maximum incidence of positive cultures occurred slightly before the maximum mortality. More frequent sampling between the 3rd and 5th days after neutron exposure, or serial blood cultures on individual mice, might have detected the onset of generalized infection before any deaths had occurred.

Bacteremias in the neutron-irradiated mice were less severe, as measured by colony counts, than bacteremias in the gamma-irradiated mice, or the x-irradiated mice previously reported. This difference is an interesting one and deserves further investigation. It suggests that among their injurious effects which lead to death, neutrons cause relatively less impairment of the mouse's resistance to infection than do gamma or X-rays.

There have been few previous studies on the role of infection as a factor in the mortality of mice after fast neutron irradiation. Lawrence and Tennant(13) exposed Swiss mice to 200 kv X-rays and to neutrons generated by a cyclotron (beryllium bombarded by 5 mev deuterons). All animals dying after irradiation were carefully examined post mortem, and bacteriological studies were included. The organisms isolated were found to be normal inhabitants of the gastrointestinal tract. They concluded that infection was not a consistent finding in mice dying within a few days after large doses of X-ray or neutrons, but that bacteremia was commonly found if mice were exposed to smaller doses of radiation which permitted them to live longer. They described numerous ulcers in the radiosensitive intestinal mucosa and focal necroses in the livers of animals dying 9-14 days after irradiation, all of which had positive blood cultures.

Silverman, Bond, Chin, and Greenman(14) have recently reported on the role of infection in the death of mice (CF No. 1 males) exposed to high doses of fast neutrons from the 60-inch cyclotron at Berkeley. The estimated dose was 9.5-10.6 x 10^{10} n/cm^2 measured by sulfur threshold detectors. It should be noted that this neutron dose probably exceeded 500 rep and might well be termed supra-lethal since, under our experimental conditions, 250 rep is the LD_{99} (20 days). It should be appreciated, however, that spectral differences existed between the 2 radiation sources employed. The biological significance of these differences, in terms of their effect on the ultimate biological response to measured rep doses, is not fully understood. These workers also found that bacteremia and mortality were correlated in time, and they recovered enteric bacteria in approximately the same proportion as reported here(15). Their results, however, differed from those herein reported in 2 respects: a higher incidence of mixed cultures, *i.e.*, containing 2 or more kinds of microorganisms, and an earlier onset of bacteremia and death (mean survival time 3-4 days). These differences can probably be attributed to the greater dose of neutrons used. None of the mice in their experiments survived more than 4 or 5 days.

It seems to be established, therefore, that generalized infection including invasion of the blood stream, occurs in mice following a single acute irradiation with fast neutrons. The cause-effect relationship, *i.e.*, the relative im-

portance of infection in the death of neutron-irradiated mice, will be discussed in a later communication which will report the results of antibiotic therapy.

Summary. 1. Single, 90-minute irradiation with fast neutrons within the lethal range (175-250 rep) resulted in early deaths among CF No. 1 female mice with a significant peak in the mortality curve 4-8 days after exposure. Among 129 neutron-irradiated mice (216-265 rep: LD_{60-98}—30 days), sacrificed for bacteriological examination, positive cultures of heart's blood and/or spleen were first obtained on the 4th day, and coincided with the onset of mortality. Between the 4th and 8th days after exposure, approximately 40% of the sacrificed mice showed positive cultures. 2. After comparable irradiation with gamma rays (Co^{60}), from 750-1300 r, deaths occurred later, with a peak approximately 12-14 days post-exposure. Gamma-irradiated mice (900-940 r) were found to have positive blood and/or spleen cultures during the latter part of the second week. There seems to be a close correlation between bacteremia and time of death following these 2 ionizing radiations. 3. All the microorganisms recovered in cultures were found to be members of the normal enteric flora of the mouse. 4. The bacteremia, as measured by colony counts on heart's blood, was less severe in the neutron-irradiated than in the γ-irradiated mice.

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Measurement of Glomerular Filtration Rate in the Dog.*† (21305)

RUTH S. HARE AND KENDRICK HARE.

From the Department of Pharmacology, Medical College of Alabama, Birmingham.

Although it is well established that the clearances of inulin and exogenous creatinine in the dog are essentially identical, there is no direct evidence that either of these substances is excreted solely by glomerular filtration in the mammalian kidney. There is, however, direct histochemical evidence that the ferrocyanide ion is filtered at the glomerulus and is neither reabsorbed nor secreted by the tubular epithelium. This has been demonstrated in the rabbit(1,2), in the dog(3) and in the puppy(4). Van Slyke *et al.*(5) compared the renal clearances and extraction percentages of ferrocyanide, exogenous creatinine and inulin in the dog and concluded that they were excreted by the same mechanism. The wide spread of their data, however, and their use of the single intravenous injection technique, leave their conclusion open to criticism. Berliner *et al.*(6) reported that 109 clearance

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