viruses were considerably more resistant to gamma rays when in frozen media. This latter effect was in contrast to results obtained with poliovirus(9) but similar to vaccinia results obtained by J. F. McCrea Freezing presumably suppresses indirect effects of irradiation either through restricting mobility of radicals formed in the medium, or by reducing transfer of charges from non-essential to essential portions of the virus particle. Regardless of the mechanism, the effect of freezing provides another point of similarity between the viruses under consideration.

Summary. Comparisons of certain physical properties of canine distemper (CDV) and measles (MV) viruses have yielded data compatible with the hypothesis that they belong to the same group. Ultrafiltration of CDV indicated a particle size between 115 and 160 mμ, a value similar to that previously reported for measles. Both viruses were inactivated by ultraviolet light at a rate of 4.5 log units per erg/cm^2 × 10^5. Irradiation with 10^5 roentgens of gamma particles reduced the titer 0.4 log unit at −70° and 1.0 log unit at 0°C for both CDV and MV.

1. Pinkerton, H., Smiley, W. L., Anderson, W. A. D., *Am. J. Path.*, 1945, v21, 1.
2. Adams, J. M., Imagawa, D. T., Yoshimori, M., Huntington, R. W., *Pediatrics*, 1956, v18, 888.
3. Carlstrom, G., *Lancet*, 1957, v273, 344.
4. Adams, J. M., Imagawa, D. T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 240.
5. Warren, J., Nadel, M. K., Slater, E., Millian, S. J., *Am. J. Vet. Res.*, 1960, v21, 111.
6. Cabasso, V. J., Avampata, J. E., Kiser, K. H., Stebbins, M. R., *Bact. Proc.*, 1960, v60, 106.
7. Rockborn, G., *Arch. ges. Virusforsch.*, 1959, v8, 485.
8. Black, F. L. *Virology*, 1958, v5, 391.
9. Benyesh, M., Pollard, E. C., Opton, E. M., Black, F. L., Bellamy, W. D., Melnick, J. L., *ibid.*, 1958, v5, 256.
10. Black, F. L., *ibid.*, 1959, v7, 184.
11. Powell, W. F., *ibid.*, 1959, v9, 1.
12. Musser, S. J., Underwood, G. E., *J. Immunol.*, 1960, v85, 292.

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Disappearance Rate of Normal Bactericidins in Irradiated Mice.* (26697)

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In an earlier publication(1) we reported that normal serum bactericidal activity for a strain of *Escherichia coli* was lost within 9-12 hours after total body exposure of CF-1 mice to a midlethal dose of X-rays and remained absent for about 10 days. The disappearance of bactericidins could not be associated with development of an inhibitor demonstrable *in vitro* or with *in vivo* absorption by enteric microorganisms through the damaged intestinal mucosa.

The rapidity of the loss of bactericidins indicated that their production was promptly interrupted by irradiation and that they must have a very short biological half-life. Recent findings suggested that a block in the synthesis of bactericidins develops after X-ray exposure(2). It is the purpose of this communication to show that normal bactericidins for *E. coli* disappear very quickly after passive transfer in irradiated mice.

Materials and methods. CF-1 mice were exposed to 500 or 700 r total body X-radiation‡ and were used on the following day when no serum bactericidal activity was demonstrable. They were injected intravenously *via* a tail vein with pooled mouse sera of known bactericidin titer and exsangui-

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nated at intervals thereafter. Bactericidal tests were performed with sera from individual mice. Briefly, the test consisted of mixing serially diluted mouse serum, rabbit complement and a suspension of *E. coli*. After incubation at 37°C, the number of microorganisms present in each tube was determined either by plating aliquots on eosin-methylene blue agar and making colony counts or by adding 5 volumes of nutrient broth and continuing incubation until the complement control became turbid. At that time, the presence or absence of turbidity in all other tubes was recorded. These readings closely paralleled the results obtained by plate counts. The reciprocal of the highest dilution of serum showing bactericidal activity was recorded as the titer. Details regarding animals, irradiation factors, bleeding and assay of bactericidins have been reported(1). In one series of experiments, preinjection plasma samples were obtained from each mouse by a slight modification of the orbital bleeding technic described by Riley(3).

Immune sera for sheep cells were obtained from CF-1 mice given 1 ml of a 1% suspension of washed sheep erythrocytes intraperitoneally 12-13 days earlier. For hemagglutination tests, equal volumes (0.1 ml) of serial 2-fold serum dilutions and 1% washed sheep cells were mixed and allowed to stand at room temperature for 30 minutes. The tubes were then centrifuged for 5 minutes at 1000 rpm and gently shaken. The reciprocal of the highest dilution of serum showing agglutination distinctly visible to the naked eye was recorded as the titer.

Experimental. Disappearance of normal bactericidins following a single intravenous injection. One day postirradiation, mice were injected intravenously with 0.25 ml of mouse serum having a bactericidin titer of 128. Control mice were given 0.25 ml of negative serum (titer<2). Table I lists the titers of the recipients' sera at various intervals after injection. Bactericidins were not demonstrable in sera of uninjected mice or of those given negative serum. Bactericidins were present in the circulating blood for a short time after injection of positive serum and were lost so rapidly as to be undetectable 20 minutes later.

Disappearance of normal bactericidins following a second intravenous injection. To rule out the possibility that the rapid disappearance of bactericidins might be due to equilibration between intravascular and extravascular fluids, a second intravenous injection of bactericidal mouse serum was given 30 minutes after the first. The results of 5 separate experiments are summarized in Table II. After one injection, passively transferred bactericidins always disappeared within 30 minutes. After 2 injections, passively transferred bactericidins had disappeared by 30 minutes in half of the animals, and by 60 minutes in all those tested. It is interesting to note that the mean titers of sera from

TABLE I. Disappearance of Passively Transferred Bactericidins after a Single Intravenous Injection.*

Serum inj. .25 ml	Bleeding time, min. after inj.	No. mice tested	No. of mice with titer of: <2	2	4	8	16	32	64	Geometric mean titer
Positive (titer 128)	1	20		2		2	9	4	3	17.1
	10	6				2	3	1		14.2
	15	6	3			1	2			
	20	6	6							
	30	10	9		1					
	45	3	3							
	60	8	8							
	120	3	3							
Negative (titer <2)	1	11	11							
	10	6	6							
	30	3	3							
	60	3	3							
None		18	18							

* Mice were exposed to 500 r 24 hr before inj.

TABLE II. Disappearance of Passively Transferred Bactericidins after 2 Intravenous Injections 30 Minutes Apart.*

No. of injections†	Bleeding time, min. after inj.	No. mice tested	No. of mice with titer of: <4	4	8	16	32	64	Geometric mean titer
1	1	17			3	12	2		15.3
	30	9	9						
2	1	12		1	3	4	3	1	16.0
	30	13	7	1	2	2	1		
	60	12	11	1					

* Mice were exposed to 700 r 24 hr before inj. All mice had preinjection titers of <4.
† 0.25 ml mouse serum (titer 128).

mice sacrificed immediately after injection were essentially the same for both groups. In this series of experiments, a bleeding was obtained from each mouse before the first injection of serum. All initial plasma samples were devoid of bactericidal activity.

Disappearance of immune hemagglutinins after passive transfer. Dixon *et al.*(4) reported that homologous gamma globulins (antibodies) had a half-life of 1.9 days in mice. This value differs considerably from that we obtained for normal bactericidins. Consequently, disappearance rates of normal bactericidins and immune hemagglutinins were compared under identical experimental conditions. A mouse antiserum for sheep cells which was also bactericidal was given intravenously to irradiated mice. This serum had a hemagglutinin titer of 1024 and a bactericidin titer of 128. The recipients were sacrificed at intervals after injection and their sera were tested for the presence of both *E. coli* bactericidins and sheep cell agglutinins. The results are presented in Table III. In this experiment, as in those described above, a loss of passively transferred bactericidins corresponding to about 3 serum dilutions occurred within 30 minutes. In contrast, 5 days were required for a comparable decline in sheep cell agglutinins.

Discussion. Taliaferro and Talmage(5) have pointed out that the half-life of antibodies should be calculated from disappearance rates after passive transfer, not from rates of antibody decline after immunization. Values based on the latter are generally too high as they reflect rates of synthesis as well as of decay. The same considerations apply if the half-life of naturally occurring antibodies is calculated from disappearance rates after whole body irradiation(6).

To determine the rate of metabolic decay of normal bactericidins for *E. coli,* passive transfer experiments were carried out in irradiated mice whose sera were not bactericidal. Presumably, bactericidins were not syn-

TABLE III. Disappearance of Passively Transferred Bactericidins and Sheep Cell Agglutinins.*

Bleeding time after inj.†	No. mice tested	Bactericidins No. mice with titer of: <4	4	8	16	32	Hemagglutinins No. mice with titer of: 8	16	32	64	128	256
1 min.	5		1	1	3						3	2
30 "	6	6									6	
6 hr	6	6									6	
1 day	6	6								6		
2 days	4	4							1	3		
3 "	4	4						1	1	2		
4 "	4								4			
5 "	3							1	2			
6 "	4						1	3				
7 "	4							4				

* Mice were exposed to 500 r 24 hr before inj. Uninjected mice had bactericidin and hemagglutinin titers of <4.
† 0.20 ml mouse serum (bactericidin titer 128; hemagglutinin titer 1024).

thesized during the period of observation, therefore their half-life could be estimated from the time of their disappearance after intravenous administration.

The data presented indicate that the half-disappearance time of homologous bactericidins after passive transfer in irradiated mice was a few minutes. Equilibration between intravascular and extravascular fluids accounted for the distribution of bactericidins immediately after intravenous injection, but not for their complete absence 20 minutes later. Estimating the plasma volume of a 25 g mouse to be 1.5-2 ml, 0.25 ml of intravenously injected serum would be diluted 6-8-fold. Thus, a serum of titer 128 should produce titers of approximately 16 in the recipients. Mice sacrificed 1 minute after injection of such a serum had mean bactericidin titers of 17.1 and 15.3 (Tables I and II).

If the loss of bactericidins during the first 30 minutes after intravenous administration had been solely the result of equilibration, it should have been possible to prolong their survival considerably by reinjecting a bactericidal serum at the end of this interval. However, after the second injection, bactericidins disappeared almost as rapidly as after the first.

Data obtained with the sheep cell immune serum provide additional evidence that the rapid disappearance of bactericidins is not due to equilibration. The initial dilution factors were the same for bactericidins and hemagglutinins. Thereafter, the half-disappearance times were a few minutes for the former and approximately 2 days for the latter.

Our findings with hemagglutinins are in accord with the results of Dixon, *et al.*(4) who reported that the half-life of homologous antibodies in mice was 1.9 days, and with those of Smith and co-workers(7) who studied passively transferred antisheep hemolysins.

Our data are consistent with the premise that the rapid disappearance of passively administered bactericidins in irradiated mice is due to metabolic decay. They do not, however, provide information on whether such decay is more rapid in irradiated than in normal mice. Hollingsworth(8) found that irradiation did not alter the rate of disappearance of passively transferred antibodies, but it is not known whether this holds true also for other serum proteins.

We reported earlier(1) that normal serum bactericidins for *E. coli* were lost 9-12 hours after total body exposure of CF-1 mice to X-rays. The data presented here show that such bactericidins, when injected into irradiated mice, were lost in less than 1 hour. In other words, the disappearance of passively transferred bactericidins in previously irradiated mice was much more rapid than was the disappearance of normally circulating bactericidins from mice following their exposure to total body irradiation. Similar observations on hemolysins in rabbits were made by Taliaferro and Talmage. They found the mean half-disappearance time of rabbit antisheep hemolysins (large molecules), as determined by passive transfer, to be 2.8 days(5), but that of natural hemolysins in irradiated rabbits to be 7 days(6). If a comparison may be made between hemolysins in rabbits and bactericidins in mice, the loss of normal serum bactericidal activity 9-12 hours after total body exposure of CF-1 mice to 600 r(1) may be explained as follows: production of bactericidins is impaired shortly after irradiation, resulting in an overall rate of decline more gradual than in absence of bactericidin synthesis. A few hours post-irradiation, bactericidin production ceases altogether for approximately 10 days.

Summary. X-irradiated CF-1 mice lacking normal serum bactericidal activity for *Escherichia coli* were injected intravenously with highly bactericidal mouse serum. Passively transferred bactericidins were lost so rapidly from the circulating blood as to be undetectable 20-30 minutes later. Following 2 intravenous injections 30 minutes apart, bactericidins disappeared almost as quickly as after a single injection. The disappearance rates of normal bactericidins and immune hemagglutinins were compared under identical experimental conditions by injecting a mouse antiserum to sheep cells which was also bactericidal. The half-disappear-

ance time of sheep cell agglutinins in mice was approximately 2 days, that of normal bactericidins, only a few minutes.

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1. Kornfeld, L., Hammond, C. W., Miller, C. P., *J. Immunol.*, 1960, v84, 77.

2. Kornfeld, L., Miller, C. P., *ibid.*, 1961, v86, 215.

3. Riley, V., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 751.

4. Dixon, F. J., Talmage, D. W., Maurer, P. H., Deichmiller, M., *J. Exp. Med.*, 1952, v96, 313.

5. Taliaferro, W. H., Talmage, D. W., *J. Infect. Dis.*, 1956, v99, 21.

6. Talmage, D. W., Freter, G. G., Taliaferro, W. H., *ibid.*, 1956, v99, 241.

7. Smith, F., Grenan, M. M., Owens, J., *J. Nat. Cancer Inst.*, 1960, v25, 803.

8. Hollingsworth, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 477.

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Lipid Metabolism in Cultured Cells. II. Cholesterol Uptake from Serum of Normal and Atherosclerotic Human Adults.*† (26698)

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It has been shown that mammalian cells in culture take up considerable amounts of cholesterol from the serum used in the growth medium(1). It was found that the cholesterol content of cells was dependent both upon the type of cell cultured, and upon the type of serum used. Cholesterol uptake by cells was only indirectly related to cholesterol level in the medium, and appeared to be related more directly to the ratio of cholesterol to other unidentified components of the serum(2). The primary process in development of atherosclerotic lesions appears to be related to cellular deposition of cholesterol in the intima of the arterial walls(3). It was of interest therefore to determine if any differences could be found in deposition of cholesterol in cells cultured *in vitro* upon serum taken from normal subjects as compared with serum from atherosclerotic individuals.

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Materials and methods. Blood samples taken from normal and atherosclerotic subjects following overnight fasting were allowed to clot for 24 hours at 4° before centrifuging and collection of serum. Details of age, sex and clinical diagnosis of experimental subjects are given in Table I. Sterile conditions were maintained during collection, and serum samples were stored frozen at –40° until use. The strain of cells used in the studies reported here, the MB III strain of mouse lymphoblasts, was grown in roller tube cultures by methods described previously(1). Serum samples were deactivated by heating at 55° for 30 minutes before use. This heating procedure destroyed a toxic factor associated with most fresh serum samples. It did not affect the cholesterol content of cells grown on non-toxic serum.

Stock cultures of MB III cells in logarithmic growth phase, were harvested and washed and resuspended in Gey's balanced saline solution. Cell populations were determined by counting in a hemocytometer chamber, and aliquots of the suspension containing approximately 1 million cells were dispensed in duplicate into roller tubes containing the experimental serum samples. Gey's balanced salt solution was then added