and efferent vessel are available for injection and sampling. A modification applicable to animals for preventing significant recirculation in organs with a large blood flow (such as the kidney) will be described in a subsequent communication.

Summary and conclusions. A method is presented for determining under physiological conditions the net bidirectional exchange of permeable tracer substances across the capillary walls of the human forearm. 1. In the early period, maximum net transcapillary loss for thiocyanate was 49 ± 19% and for deuterium oxide 90 ± 4%. 2. The equilibrium time or period of net loss averaged 111 ± 66 seconds for SCN and 130 ± 86 seconds for D_2O. Equilibrium times for both SCN and D_2O were related directly to blood flow. 3. The half-return time for SCN averaged 3 ± 3.0 times the equilibrium time. In the cases in which simultaneous measurements were made the half-return time of D_2O averaged twice that of SCN.

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Protective Effect of Pre-Irradiation on Lymphocytic Choriomeningitis Infection in Mice. (22425)

WALLACE P. ROWE.* (Introduced by Karl Habel.)

Naval Medical Research Institute,† Bethesda, Md.

X-irradiation has been found to affect the course of experimental viral infections in a variety of ways(1); in some instances the illness is aggravated(2-4), and in others it is ameliorated(5-8). It is the purpose of this communication to describe the striking beneficial effect of pre-irradiation on the course of lymphocytic choriomeningitis (LCM) infection of the adult mouse.

Materials and methods. Five-to 6-week-old white Swiss mice of the NMRI colony were used; the colony was carefully surveyed for the presence of latent LCM infection and no evidence of such was obtained. Guinea pigs were from the NMRI stock. Two strains of LCM were employed. The "Inst." strain is a mouse brain passaged strain which is uniformly lethal for mice after intracerebral inoculation. The "Arm." strain was recovered from house mice by Dr. C. Armstrong,‡ and maintained by 15 to 20 intraperitoneal passages prior to the experiments reported here; following inoculation by this route, it produces pleural and peritoneal effusion, pneu-

* Present address: Department of Health, Education and Welfare, Public Health Service, N.I.H., National Institute of Allergy and Infectious Diseases, Bethesda, Md.

† The opinions expressed are those of the author and do not necessarily represent those of the Bureau of Medicine and Surgery of the United States Navy.

‡ The "Inst." and "Arm." strains were received through the courtesy of Drs. E. Traub and C. Armstrong, respectively.

monitis, and some degree of hepatitis. The immunological identity of these strains was indicated by complete cross immunity in mice and guinea pigs, and by cross complement fixation reactions. Animals were subjected to single-exposure total body irradiation, using the technic of Ellinger, *et al.*(9), with 200 KVP and 25 ma., an HVL of 0.8 mm of copper, and an intensity of 43 r per minute. Mice received 300 r (LD_5), and guinea pigs 150 r. Infectivity titrations were carried out by intracerebral (i.c.) inoculation of 5 mice per 10-fold dilution, 0.04 ml per mouse; criteria of infection were typical cerebral symptoms or death 5 to 14 days after inoculation, or immunity to intracerebral challenge with approximately 10,000 LD_{50} of the Inst. strain, given 14-21 days after the initial inoculation. Fifty per cent end points were calculated by the Reed-Muench(10) method; titers are expressed in terms of 0.04 ml of a 10% organ suspension.

Results. Effect of pre-irradiation on meningitic form of the disease. Two chief experiments have been performed, using the same procedure. Half of a group of mice were irradiated, and 24 or 96 hours later equal numbers of irradiated and non-irradiated mice were injected i.c. with 0.04 ml of a dilution of a freshly prepared Inst. strain infected mouse brain suspension. Animals were examined daily or more often for the appearance of cerebral symptoms, by lifting and spinning by the tail, from the fifth through the twenty-first days after inoculation, and at less frequent intervals thereafter. Experimental details and the outcome of the infection in the irradiated and control groups are presented in Table I.

The infection was uniformly fatal for the non-irradiated mice; in the irradiated groups, however, the onset of the disease was delayed, symptoms were observed in less than half of the animals, and the course of the disease in symptomatic animals was not uniformly fatal. There was no apparent difference in the reaction of the groups inoculated one or four days after irradiation, or between the groups inoculated with greatly different doses of virus. While most of the irradiated mice did not develop cerebral symptoms of infection, they appeared ruffled and moderately emaciated from the second through the sixth week after the inoculation. In another experiment mice were challenged intracerebrally with approximately 10,000 LD_{50} of Inst. strain virus on the fifty-sixth day after irradiation; this group reacted identically with the non-irradiated controls. Thus, the beneficial effect of pre-irradiation had disappeared by the fifty-sixth day.

To compare the growth of the virus in the irradiated and non-irradiated mice, virus titers in the brain were determined at intervals in additional mice inoculated with the 10^{-4} dilution in Exp. 2, Table I. The titers

TABLE I. Effect of Total Body Irradiation (300 r) on Intracerebral Infection with LCM.

Exp. No.	Virus dilution	Irradiation	No. of mice inoculated	No. dying of cerebral symptoms	No. surviving cerebral symptoms	Late deaths*	Incubation period of cerebral symptoms Median	Range
1	10^{-4}	300 r‡	10	1	4	0	9	8-16
		0	11	11	0		6	6- 7
		300 r§	10	2	2	0	8	7-13
		0	10	10	0		6	5- 7
2	10^{0} †	300 r‡	10	2	0	2	9	9
		0	10	10	0		5	5- 7
	10^{-2}	300 r‡	10	2	2	1	10	8-14
		0	10	10	0		6	5- 7
	10^{-4}	300 r‡	14	4	2	2	12	9-15
		0	14	14	0		6	5- 7
Total		300 r	54	11	10	5	10	7-16
		0	55	55	0		6	5- 7

* Death between 12th and 75th days, not preceded by cerebral symptoms. † Titer—$10^{5.5}$ LD_{50} per 0.04 ml. ‡ Irradiated 24 hr before inoculation. § Irradiated 96 hr before inoculation.

TABLE II. Virus Content of Brains of Irradiated and Control Mice at Intervals after Intracerebral Inoculation.

Days after inoculation	Virus titer* in brains of individual mice	
	Irradiated	Non-irradiated
3	3.6, 3.5, 2.5	3.5, 3.6, 2.8
6	5.4, 5.5, 5.2	5.5, 5.5, 5.4
11	5.0, 5.5, 5.5	
16	4.5,† 4.8	
23	4.2, >5.3	
41	3.5, 4.5	
72	>3.5, 2.2	
157	>3.5, —‡	

* Titer expressed as negative log of ID_{50} dilution of .04 ml of 10% suspension.
† Convulsing at time of sacrifice.
‡ No virus detected.

obtained are recorded in Table II. The virus titers in the brains of the irradiated mice rose with the same rapidity and reached the same levels as in the non-irradiated group; in the former group the titers remained high for 2 to 5 months in some animals, and diminished or disappeared in others. No data are available for the persistence of virus following i.c. infection of non-irradiated animals with this strain of LCM since the disease is uniformly fatal in the doses employed in the present experiments.

Histological examination of the brains of asymptomatic irradiated mice sacrificed at intervals after virus inoculation showed minimal cellular infiltration of the basal meninges with no infiltration of the choroid plexus. Irradiated mice showing some degree of tremors or convulsions demonstrated a mild meningeal and choroid plexus infiltration which was much less severe than that of the non-irradiated controls.

Effect of irradiation on intraperitoneal form of the disease. Two experiments were carried out. Irradiated and non-irradiated mice were infected intraperitoneally with 0.1 ml of a dilution of a freshly prepared suspension of Arm. strain infected mouse spleen, 26 or 48 hours after irradiation. The mice were observed carefully for the appearance of the characteristic labored respiration which had been found to be an indication of pleural effusion(11), and mice dying were autopsied for confirmation of the presence of pleural fluid. On the seventh, ninth, or eleventh days after inoculation, surviving mice were sacrificed and the volume of fluid in the pleural and peritoneal cavities estimated with a one ml pipette equipped with a rubber bulb. One group of mice was set aside for observation without sacrifice. The findings are presented in Table III.

Production of serous exudation by the Arm. strain infection was completely prevented by the irradiation, regardless of the magnitude of the challenge inoculum. Although several of the irradiated mice died during the period of observation, none showed thoracic or peritoneal fluid, whereas all of the non-irradiated mice which died demonstrated large amounts of pleural exudate.

TABLE III. Effect of Pre-Irradiation on Intraperitoneal Infection with Arm. Strain LCM.

Exp.	Virus dilution	Irradiation	No. of mice	Signs of respiratory distress	Deaths	Day of sacrifice	Effusions in sacrificed mice*
3	10^{-3}	300 r†	14	1	0	11	0/14
		0	13	7	1		7/12
4	10^{0}	300 r‡	8	0	1	7	0/ 7
		0	15	13	6		8/ 9
	10^{-3}	300 r‡	12	0	0	9	0/12
		0	16	9	2		7/14
		300 r‡	13	1	3	Not	
		0	17	14	9	sacrificed	
Totals		300 r	47	2	4§		0/33
		0	61	43	18‖		22/35

* Numerator = No. of mice with more than 0.15 ml thoracic fluid. Denominator = No. of mice sacrificed. † Irradiated 26 hr before inoculation. ‡ Irradiated 48 hr before inoculation. § None had thoracic fluid at autopsy. ‖ All had abundant thoracic fluid at autopsy.

In the groups inoculated with the 10^{-3} virus dilution in experiment 4, three additional mice were sacrificed on the sixth day and the virus titer of the pooled liver, spleen, kidneys, and lungs of each mouse determined. The titers (ID_{50}) in the three irradiated mice were 4.7, 4.6, and 4.3, and in the non-irradiated mice, 5.3, 5.0, and 4.9. Thus, the virus titers in the irradiated group appeared to be slightly lower than in the controls.

In the groups of mice inoculated with the undiluted virus suspension in experiment 4, the livers and lungs of four of the sacrificed mice in each group were examined histologically. All 4 non-irradiated mice showed marked round-cell infiltration of the liver and moderate to severe interstitial pneumonia. On the other hand, the livers from the irradiated mice were normal, and the lungs showed no more than an occasional small area of pneumonic infiltration.

It should be noted that the survivors of the 10^{-3} infection in experiment 4 were challenged intracerebrally 55 days after infection, and both groups were found to be solidly immune. Thus, the modification of the infection by pre-irradiation did not interfere with the development of immunity.

Effect of irradiation on subcutaneous infection of the guinea pig. Five female guinea pigs weighing approximately 400 g were subjected to 150 r total body irradiation. Twenty-four hours later they and three normal guinea pigs of the same sex and weight were infected subcutaneously on the foot pad with 0.25 ml of a 10^{-2} dilution of a fresh 5% suspension of Arm. strain infected mouse spleen. In addition, two normal guinea pigs of approximately 800 g were inoculated with the same material.

All the animals in both the irradiated and non-irradiated groups died of the infection, and there was no significant difference in the times of death of the animals in the 2 groups (7 to 10 days in the irradiated guinea pigs, and 8 to 14 days in the controls).

Discussion. A number of factors are known to affect markedly the clinical reaction of mice to infection with LCM virus. Virus carriers, which may contain high titer virus throughout the body, show no outward response either to the carrier state or to massive virus challenge(12); normal suckling mice, while developing immunizing infection following virus inoculation, similarly demonstrate little or no clinical response to the infection(13). Immunized, non-carrier mice may support high titer proliferation of intracerebrally inoculated virus without showing any cerebral signs(11), and conversely, mice in the "accelerated stage" of immunity(14) may demonstrate cerebral signs at a time when the virus titer in the brain has risen but slightly(11).

The protective effect of irradiation on the meningitic form of LCM infection would appear to be entirely a suppression of reactivity to virus multiplication; despite growth of virus at the same rate as in non-irradiated animals, the irradiated animals generally showed neither clinical nor histological reaction to the virus. These findings are consistent with the hypothesis developed elsewhere (11) that the cerebral signs are a reaction to the presence of a meningeal infiltrate, which in turn is the result of host reactivity to the virus. Interpreted by this hypothesis, the protective effect of irradiation could be viewed as a result either of decreased tissue reactivity(15) or decreased ability of a leukopenic animal to form inflammatory exudates.

The effect of irradiation on the course of the intraperitoneally produced LCM infection was very marked, there being almost complete prevention of serous effusion and inflammatory reaction in the liver and lung. The relative importance of inhibition of viral multiplication, which was slight but probably significant, and of tissue reaction to infection will require further study.

Summary. 1. 300 r total body x-irradiation given to adult mice one to 4 days prior to intracerebral or intraperitoneal infection with LCM virus, produced a marked increase in survival and diminution in objective evidence of the infection. 2. The growth curve of the virus after intracerebral inoculation was identical in the irradiated and non-irradiated groups. After intraperitoneal infec-

tion with a viscerotropic strain there appeared to be a slightly lower virus titer in the pooled organs of the irradiated as compared with the non-irradiated mice.

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Use of Anti-Rh Sera for Demonstrating Agglutination Activating Factor in Rheumatoid Arthritis.* (22426)

MARION V. WALLER AND JOHN H. VAUGHAN. (Introduced by Lynn D. Abbott.)
Departments of Clinical Pathology and Medicine, Medical College of Virginia, Richmond.

There is a substance in the serum of most patients with rheumatoid arthritis that is capable of agglutinating previously unagglutinated, but sensitized red blood cells(1-4). This substance has been called(4) an agglutination activating factor (AAF). Not all red cells nor all anti-red cell antibodies are equally effective in demonstrating this factor. Ouchterlony and Jonsson(5) considered it necessary to use red cells from sheep or from animals closely related to sheep. However, Wager(4) was able to demonstrate AAF with human Group O cells or with the patient's own red cells sensitized with the corresponding rabbit antibody. The use of a human red cell sensitized with human antibody for the demonstration of this factor has not previously been reported.

Methods. 273 undiluted anti-Rh sera were used to sensitize equal volumes of a 2% suspension of washed Rh positive cells. After sensitization for one-half hour at 37° the cells were washed 3 times in saline and to the packed sensitized cells in duplicate tubes were added one volume of a 1:2 dilution of rheumatoid arthritis serum or of a normal control serum. After standing 10 minutes at room temperature, the cells were centrifuged at 1000 r.p.m. for one minute and the reactions read with a hand lens. Ninety-seven of the 273 anti-Rh sera proved to be unsuitable for these studies because of strong agglutination reactions developed by the anti-Rh antibody. Of the remaining 176, 30 provided sensitized cells which were strongly agglutinated by the rheumatoid serum, but not by the normal serum.

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