able and no consistent relationship has been found between length of darkness and length of recovery period. Temperatures of 3-5° C darken frogs as does light. Light and cold appear to be stimuli which cause release of melanophorotropic hormone (ACTH?) from the pituitary gland. Light-induced changes, opposite to those shown by other frogs, seem explicable on the basis of natural selection during the diurnal period when these frogs are inactive.

1. Parker, G. H., *Animal colour changes and their neurohumors,* Cambridge Univ. Press, 1948.

2. Teague, R. S., Noojin, R. O., and Geiling, E. M. K., *J. Pharmacol. and Exp. Therap.,* 1939, v65, 115.

3. Johnsson, S., and Högberg, B., *ibid.,* 286.

4. Sulman, F. G., *Nature,* 1952, v169, 588.

5. Thing, E., *Acta endo.,* 1952, v10, 295.

6. Sulman, F. G., *ibid.,* 320.

7. Thing, E., *ibid.,* v11, 363.

8. Stebbins, R. B., and Thomas, G. B., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 44.

9. Edgren, R. A., *ibid.,* 1954, v85, 229.

10. Hogben, L., and Slome, B., *Proc. Roy. Soc. London,* 1931, v108, 10.

11. Sulman, F. G., *J. Endo.,* 1952, v8, 275.

12. Thing, E., Birch-Andersen and Ravn, H., *Acta Endo.,* 1953, v14, 113.

13. Köhler, V., *Naturwiss.,* 1952, v39, 554.

14. Selye, H., *Stress,* etc., Acta Inc.: Montreal, 1950.

Received July 14, 1954. P.S.E.B.M., 1954, v87.

Effect of Immunity on Resistance to Infection in Irradiated Mice and Rats.* (21273)

FALCONER SMITH, WILLIE W. SMITH, LEON GONSHERY, AND MARIE M. GRENAN.

From the National Cancer Institute and National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, Bethesda, Md.

Bacteria which are common inhabitants of the intestinal tract are frequently found in blood and various organs of lethally irradiated mice(1-3). The efficacy of antibiotics in reducing mortality(4,5) and the effect of certain of the organisms on survival time(2,6) show that these infections cause or contribute toward death in the irradiated animal. Irradiation reduces the defenses against infection, in part, by decreasing the number of circulating granulocytes and by interrupting the production of antibodies. Granulocyte count has recently been shown to be quantitatively related to survival after midlethal radiation(7). In the present experiments the effect of immunity acquired prior to irradiation on ability to withstand bacterial challenge and on survival following lethal radiation are examined.

Procedures. Male N.I.H. littermate mice and Sprague-Dawley rats from the N.I.H. animal colony, maintained on a diet of Purina Laboratory Chow, were used in these studies. The animals were caged individually from

TABLE I. Effect of Immunization on Survival of Animals Experimentally Infected with *Proteus* after LD_5 Irradiation.

Exp.	Immunizing antigen	No. of animals	% surviving	P†	Days from chal. to death, ± S.E.
	Mice: 6 immunizing inj., challenge 5 days after irradiation				
1	0	47	17		3.3 ± .5
	Prot. Kf 7	50	66	<.001	10.3 ±1.2
2	0	40	2		1.1 ± .1
	Prot. AM	39	31	<.01	5.2 ± .6
3*	0	39	0		1.2 ± .3
	Prot. AM	40	63	<.001	6.5 ± .7
	Rats: 6 to 7 immunizing inj., challenge 4 days after irradiation				
A	0	43	42		2.2 ± .4
	Prot. Kf 7	60	90	<.001	9.8 ±3.4

* Challenge organism was *Proteus* Kf 7 in this experiment.

† Significance of differences in survival of Exp. and Control groups by the method of χ^2.

* Supported in part by the Department of Defense.

TABLE II. Effect of Immunization Schedule on Survival of Mice Challenged with *Proteus* Kf 7 (1:1) after Exposure to 475 r.

Exp.	No.	Immunizing injections: Wk pre-irradiation 1st inj.	Immunizing injections: Wk pre-irradiation Last inj.	Conc.*	No. mice	% surviving 4 days†
4	0				44	5
	1	3		2:1	45	64
	6	3	1	2:1	45	71
5	0				40	8
	1	4		1:1	40	45
	6	4	2	1:1	39	67
6	0				30	3
	1	7		1:2	30	63
	6	7	1	1:2	30	80

* Heat killed suspensions of 24 hr cultures in concentration indicated.

† Survival of nonirradiated, nonimmunized challenged mice: *Exp. 4*, 73%; *Exp. 5*, 50%; *Exp. 6*, 33%.

the beginning of immunization and were 15 to 20 weeks old at the time of irradiation. Irradiation was carried out with a 200 Kvp X-ray unit operating at 20 ma with 0.25 mm Cu and 0.51 mm Al added filtration. Mice were irradiated 10 at a time in a partitioned plastic container, the target distance being 50 cm and dose rate in air 55 r per minute. The rats were irradiated in groups of 12 in a partitioned pressed wood box, with target distance 70 cm and dose rate 23 r per minute. The irradiation groups, and in mice the litters, were appropriately distributed among the various treatment groups. Procedures used for bacterial culture have been described elsewhere(8). Briefly, subcultures of the lyophilized organisms were cultured for 24 hours at 37°C in horse meat infusion broth and centrifuged, and the organisms suspended in physiological saline. Vaccines were prepared by heating the suspensions at 60°C for one hour and were given subcutaneously 6 times over a period of 3 weeks, with one week elapsing between the final injection and irradiation (with the exceptions noted in the Tables). The challenge consisted of a single subcutaneous injection of a suspension of living organisms for mice and an intraperitoneal injection for rats. Animals alive 28 days after irradiation were counted as survivors except in Table II where survivors were counted 4 days after challenge. In the challenge experiments irradiation alone (475 r for mice, 500 r for rats) killed only about 5% of the animals.

Results. When mice were treated with a series of immunizing injections prior to LD_5 irradiation, resistance to a challenging infection with the same organism or mixture of organisms was increased as shown in Tables I-III. The differences between vaccinated and nonvaccinated groups were statistically significant in all experiments with *Proteus* alone and with mixed bacterial species. The rats of *Exp. A*, Table I (which represents pooled results of 3 smaller experiments) also responded favorably to the immunizing process. Im-

TABLE III. Effect of Vaccination on Survival of Mice Exposed to 475 r (LD_5) and Experimentally Infected with Single or Mixed Bacterial Species Isolated from Lethally Irradiated Littermates.

Exp.	No. per group	Organism*	Immunizing inj.†	% surviving Irr. control	% surviving Exp.	P
7	24–30	A (*Proteus*)	5	4	40	<.01
		B (*E. coli*)	5	33	60	<.1
		C (*Paracol.*)	5	33	44	<.7
		ABC	5	0	62	<.001
8‡	39–40	D (*Pseudo.*)	6	60	78	<.1
		ABCD	6	20	67	<.001
9§	39–40	ABDE (α-strep.)	6	5	38	<.001

* Organisms used for challenge and their incidence in lethally irradiated littermates: A, *Proteus mirabilis* (20–31%); B, *E. coli* (15–38%); C, *Paracolon coliforme* (0–14%); D, *Pseudomonas aeruginosa* (3–4%); E, α-streptococcus (14–46%).

† Heat killed 24 hr cultures of each organism mixed in equal parts and injected in concentrations ranging from 1:8 to 1:1 over a period of 2 wk with 1 wk elapsing before irradiation.

‡ Of 20 nonirradiated challenged controls in each group all survived.

§ " 30 " " " 22 survived.

TABLE IV. Survival and Occurrence in Post Mortem Heart Blood of Bacterial Species Used for Vaccination in Lethally Irradiated Mice (625 r).

Antigen*	No. of mice	% surviving	% positive†
Single species			
Control	45	4	15
Vaccinated	110	3	10
Mixed species			
Control	136	34	41
Vaccinated	139	28	34

* *Proteus, E. coli, Paracolon,* as single species (Exp. 7); mixed species as in Exp. 7, 8 and 9 of Table III.

† Post mortem heart blood positive for bacterial species used in immunizing injections.

munization with *Proteus mirabilis* of mouse origin (AM) was effective against challenge with *Proteus vulgaris* of human origin (Kf 7, from the Kauffman collection) as shown in *Exp. 3,* Table I. In addition to increasing the number of challenged animals that survived 28 days, the immunization consistently prolonged the survival time of those that died.

The results of one and 6 immunizing injections, given according to the schedules and concentrations indicated, are compared in Table II. The groups given 6 injections had a slightly higher survival than those given a single injection. No quantitative relationship was observed between serum agglutinin titer and the effect of immunization on resistance to bacterial challenge.

The beneficial effect of vaccination with the bacterial species commonly found in the blood of lethally irradiated mice (Table III) suggested that such vaccination might also increase survival following lethal irradiation. Groups parallel to those of the experiments shown in Table III (with the exception of D, *Pseudo., Exp. 8*) were given the same course of immunization but were exposed to 625 r and were not challenged. The pooled results from these groups are shown in Table IV. In no case was any degree of protection evident, nor was there a significant reduction in the number of mice showing blood cultures positive for the bacterial species used in the vaccines.

Since a beneficial effect of antibodies on survival in these experiments must be implemented by the action of phagocytic cells, granulocyte counts after lethal (600 r) and sublethal (475 r) irradiation were compared (Fig. 1). During the period of maximum depletion median counts were about 170 per cu mm in the sublethally irradiated mice and 30 per cu mm in the mice exposed to 600 r (57% mortality).

Discussion. It has long been known that specific antibodies persist following whole-body X-irradiation(9), and the present experiments show that antibodies formed prior to sublethal irradiation increase resistance to bacterial challenge. Hatch has recently reported similar results(10). The fact that the vaccinations neither increased survival nor brought about significant shifts in the specific infections of mice exposed to 625 r suggests either that the effective antibody content was lower after 625 r than after 475 r or that a content which affords protection in sublethally irradiated animals is totally inadequate in animals exposed to the higher radiation dose. At such doses the number of circulating granulo-

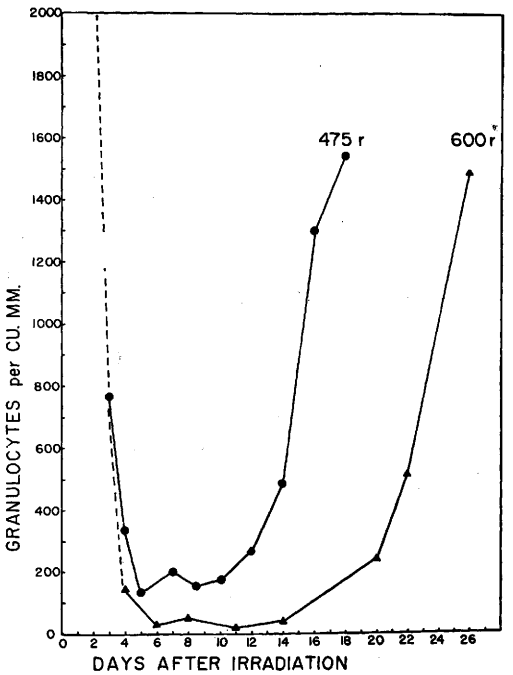

FIG. 1. Granulocyte counts of N.I.H. mice following 475 r or 600 r. The 600 r data are from a single experiment, each point representing the median of 8 to 12 counts. The 475 r data are compiled from 8 experiments (635 counts), each point representing the weighted average of the group medians.

cytes is severely depleted over a relatively long period of time and this with possible impairment of phagocytic function(11,12) may account for the difference in effectiveness of antibodies under the 2 conditions studied.

Summary. Immunization with *Proteus* prior to LD_5 irradiation increased resistance of mice and rats to challenge with the living organism. Immunization with mixtures of the bacteria which occur most frequently in post-mortem blood increased resistance to challenge in mice. Immunization with these mixtures did not increase survival in mice exposed to LD_{65-95} radiation.

1. Miller, C. P., Hammond, C. W., and Tompkins, M., *J. Lab. Clin. Med.*, 1951, v38, 331.

2. Gonshery, L., Marston, R. Q., and Smith, W. W., *Am. J. Physiol.*, 1953, v172, 359.

3. Congdon, C. C., Uphoff, D., and Lorenz, E., *J. Nat. Cancer Inst.*, 1952, v13, 73.

4. Miller, C. P., Hammond, C. W., Tompkins, M., and Shorter, G., *J. Lab. Clin. Med.*, 1952, v39, 462.

5. Smith, W. W., Smith, F., Ruth, H. J., Canter, Y., and Grenan, M. M., *Am. J. Physiol.*, 1953, v172, 351.

6. Hammond, C. W., Tompkins, M., and Miller, C. P., *J. Exp. Med.*, 1954, v99, 405.

7. Smith, W. W., Gonshery, L., Alderman, I. M., and Cornfield, J., *Am. J. Physiol.*, 1954, v178, Sept.

8. Marston, R. Q., Gonshery, L., Alderman, I. M., and Smith, W. W., *ibid.*, 1953, v172, 365.

9. Hektoen, L., *J. Infect. Dis.*, 1915, v17, 415.

10. Hatch, M. H., *Bact. Proc.*, 54th General Meeting, Soc. Am. Bact., 1954, p. 61.

11. Schechmeister, I. L., Fishman, M., and Yunker, R., *Fed. Proc.*, 1954, v13, 511.

12. Donaldson, D. M., Marcus, S., and Gyi, K. K., *ibid.*, 1954, v13, 490.

Received July 16, 1954. P.S.E.B.M., 1954, v87.

Effect of Xenoplastic Adrenal Transplants upon Limb Regeneration in Normal and Hypophysectomized Newts (*Triturus viridescens*). (21274)

OSCAR E. SCHOTTÉ AND DONALD A. B. LINDBERG.

From the Department of Biology, Amherst College, Amherst, Mass.

The following experiments arose from investigations concerned with the role of the pituitary as a requisite for regeneration in newts (1,2,3,4,5). When, in addition, it was recently shown that the administration of ACTH and also of several cortical extracts to hypophysectomized newts, otherwise incapable of regeneration, reinstated in these animals the capacity for regeneration(6), it became probable that the adrenal glands might be an important or even essential hormonal factor in limb regeneration. Unfortunately, the participation of the adrenals in regeneration is not demonstrable by adrenalectomy because of the diffuseness of these glands in urodeles. As one of the methods to explore the premise that purely adrenal products may replace the missing pituitary in limb regeneration, this research has investigated the effects of xenoplastically transplanted frog adrenals into previously hypophysectomized newts.

Materials and methods. All the experiments were performed upon normal adult or upon hypophysectomized newts (*Triturus viridescens*) and the adrenal transplants were obtained from adult *Rana pipiens* and *R. palustris* kidneys by dissection immediately before transplantation. For control purposes muscle transplants have been used. The frog adrenal or muscle transplants were introduced under a skin pocket of the heavily vascularized lower jaw. Using this technique several series of transplantations were made. In a first group the hypophysectomized newts received a single adrenal transplant; in another group one adrenal was implanted at the time of amputation and additional frog adrenals were implanted at the fifth and also at the tenth day following amputation. In a final group the transplantation, limited to one adrenal only, was delayed until the sixteenth day after amputation at which time the second limb of the newt was freshly amputated. All limbs were studied histologically and the heads of