likely that the reactive groups on the viruses also differ in quality.

Knowledge of the reactive chemical moiety of the RBC which the viruses share contributes little to understanding the infection of host-cells by polyoma virus. Here the RBC is not a useful model. Host-cell receptors of this agent appear not to be mucoproteins, are distinct from those of influenza virus, and presumably operate at 37°C where the RBC receptor complex is unstable. Analogous to polyoma receptors of RBC and host-cells are 2 proteinaceous bovine serum inhibitors. One reversibly (at 4° and 22°) blocks the HA reaction without neutralizing infectivity(13). A second acts at 22° to block both infection and the HA reaction(14). It is anticipated that these substances upon isolation will be valuable for further study of the reactive surface of polyoma virus.

Summary. Evidence is presented for the existence of 2 types of erythrocyte receptors for the PR-8 strain of influenza virus; one of these is available also to polyoma virus. Modifications of the quality of the hemagglutination reaction, resulting from treatment of either virus or red cell, are described. While a similarity in the receptors for influenza virus on the host-cell and the erythrocyte can be demonstrated, these were found to be distinct in the case of polyoma virus.

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Effect of RNA and Yeast Autolysate on Experimental Infection in Irradiated and Unirradiated Mice.* (27308)

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Detre and Finch(1) found a marked reduction in mortality (30 days) from a lethal dose of X-radiation in mice injected intraperitoneally or intravenously with yeast ribonucleic acid (RNA) or yeast autolysate 30 minutes before exposure. The following experiments were undertaken to determine whether this protective effect might have been due, in part at least, to increased resistance to bacterial infection, since the development of endogenous infection plays a significant role in the death of mice exposed to mid-lethal doses of ionizing radiation(2). Mice were accordingly treated with RNA or yeast autolysate 24 hours or 30 minutes before, or on the fourth day after irradiation (475 r) and challenged on the fifth day post-irradiation by intraperitoneal inoculation with *Pseudomonas aeruginosa.*

Materials and methods. Mice. CF-1 females, 10 weeks old, were housed in stainless

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TABLE I. Mortalities among Unirradiated Mice Treated with RNA or Yeast Autolysate (YA) before Challenge with *Ps. Aeruginosa.*

	Treatment 4-5 days before challenge				Treatment 24 hr before challenge			
Mean inoc.*	RNA	YA	Controls	No. mice	RNA	YA	Controls	No. mice
	% mortality				% mortality			
Treatment i.p.								
2.3×10^8	18	20	67	240	20	27	50	120
1.9×10^8	2	12	40	240	2	12	27	120
3.8×10^7	0	5	1	240	0	5	5	120
Treatment i.v.								
1.6×10^8	23	—	53	60	25	—	55	60
1.3×10^8	20	—	50	60	20	—	45	60
2.6×10^7	0	—	0	60	0	—	10	60

* In each experiment, the largest inoculum contained between 1.2×10^8 and 3.3×10^8 *Ps. aeruginosa.* The other 2 inocula in each instance contained, respectively, 5% and 1% of that number. Each inoculum was given to 10 mice.

steel cages and supplied with feed and water *ad libitum.* Water bottles were sterilized daily and cages once a week. All mice in an experiment came from the same shipment.

Irradiation (475 r) was delivered by a 250 kv, 30 ma Maxitron (G.E.) machine using 0.25 mm Cu and 1 mm Al filters at a target distance of 70 cm and a dose rate of approximately 60 r per min. For details of method of exposure see Hammond *et al.*(3). *Injections.* 24 hours or 30 minutes before irradiation or on the fourth day after irradiation, mice were injected intraperitoneally or intravenously with 1.0 ml of a 2% solution of RNA (Schwarz Lab., Inc., Mt. Vernon, N. Y.) or intraperitoneally with 0.5 ml of a 20% solution of yeast autolysate (Albimi Lab., Inc., Brooklyn, N. Y.). RNA was dissolved in N/25 NaOH and adjusted to pH 7.2. It was not autoclaved routinely, but only as noted in specified experiments. Yeast autolysate was dissolved in buffered saline and autoclaved at 15 lb for 15 minutes. It was too toxic to be administered intravenously. Intraperitoneal injection caused moderately severe prostration for 15 or 20 minutes, most marked in irradiated mice. Control mice were injected with buffered saline. *Challenge.* On the fifth day post-irradiation, mice were randomly distributed in order to separate cage mates, and challenged by intraperitoneal injection of graded inocula of a streptomycin resistant strain of *Ps. aeruginosa,* used for a number of years in this laboratory to determine the effect of irradiation on susceptibility to bacterial infection (4). This microorganism was chosen because the infection it produces is rapidly fatal in susceptible animals; *i.e.,* inoculated mice either die within 18-36 hours or survive the observation period of 30 days. Virulence has been maintained by weekly passage in CF-1 mice. An 18-hour culture was washed off an agar plate in 5 ml saline, and any clumps of bacteria were dispersed by shaking in an Erlenmeyer flask containing a few glass beads on an electric rotator for 15 minutes. The suspension was made up to contain 10^9 bacteria/ml by means of a Coleman spectrophotometer and then diluted to provide the desired inocula. Irradiated mice were inoculated with approximately 10^8, 2×10^7, and 10^7 microorganisms in 0.5 ml volume, 10 mice per inoculum. Actual numbers of bacteria inoculated were determined from quadruplicate platings of appropriate dilutions of the suspension used. LD_{50}'s were calculated by probit analysis.

Unirradiated mice were inoculated with somewhat larger numbers of *Ps. aeruginosa* (Table I).

Results. Irradiated mice. In all 4 challenge experiments summarized in Fig. 1, mice treated intraperitoneally with RNA before or after irradiation had higher LD_{50}'s than their controls, indicating increased resistance to the experimental bacterial infection. The increase was greatest in those treated the fourth day post-irradiation—one day before challenge.

Similar results (not included in Fig. 1) were obtained in mice treated intraperitone-

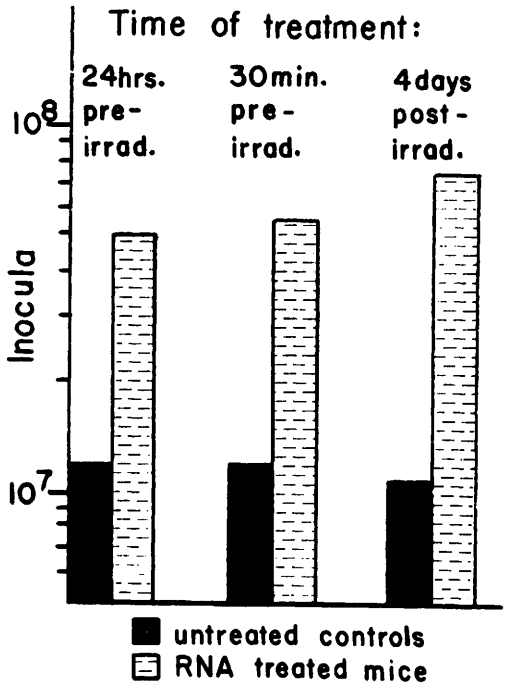

FIG. 1. LD_{50} of *Ps. aeruginosa* in mice inoculated on fifth day post-irradiation (475 r). Treated mice received 20 mg RNA intraperitoneally at times indicated.

ally with yeast autolysate 24 hours or 30 minutes before irradiation, but not in those treated the fourth day post-irradiation, presumably because the latter had not yet recovered from the toxic effect of yeast autolysate on the following day when they were challenged.

Intravenous treatment with RNA 24 hours before or on the fourth day after irradiation increased LD_{50}'s of the challenge inoculation almost, but not quite, as much as intraperitoneal treatment shown in Fig. 1.

Unirradiated mice. Results of a series of experiments summarized in Table I showed that treatment with RNA or yeast autolysate reduced mortality from the experimental infection in unirradiated mice. The reduction was most marked in mice treated intraperitoneally with RNA 4 or 5 days before challenge. Some effect resulted from intravenous treatment with RNA. Yeast autolysate injected intraperitoneally also reduced mortality, particularly when given 4 or 5 days before challenge.

Effect of autoclaving on activity of RNA. In 3 experiments, the relative effectiveness of autoclaved (15 lb for 15 minutes) and unautoclaved RNA was compared in mice treated the fourth day post-irradiation and challenged the following day. In each instance, autoclaved RNA was somewhat more effective than unautoclaved in reducing mortality from the experimental infection.

Discussion. No explanation can be offered for the increased resistance to experimental infection with *Ps. aeruginosa* in irradiated and unirradiated mice. The effect of treatment with RNA and yeast autolysate may have been due to non-specific increase in host resistance. In this connection, Smith, Smith and Alderman(5) found that intraperitoneal or subcutaneous injection of finely ground glass or other inorganic particles, within a few hours after irradiation reduced the mortalities of mice challenged with *Proteus vulgaris* or *Pseudomonas aeruginosa.* The protective activity was attributed to the resulting inflammatory reaction. In the experiments reported here, however, RNA and yeast autolysate or their breakdown products may possibly have played a more specific role. Taliaferro and Jaroslow(6) found that injection of DNA partially degraded *in vitro* restored to a considerable degree the ability of rabbits to produce hemolysins.

Whatever interpretation of our results will ultimately seem appropriate, they nevertheless suggest a possible explanation of the findings of Detre and Finch(1).

Summary. Intraperitoneal or intravenous injection of RNA or yeast autolysate before or after irradiation with 475 r reduced the mortality of mice from experimental infection with *Pseudomonas aeruginosa* initiated by intraperitoneal inoculation on the fifth day post-irradiation. Mortality was also reduced in unirradiated mice treated with RNA or yeast autolysate before inoculation with *Ps. aeruginosa.*

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Production of Dense Red Band Around Growth of *Staphylococcus aureus* on Blood Agar Plates. (27309)

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When certain strains of *Staphylococcus aureus* are grown on blood agar plates, a dense red band develops around the growth. The band appears around the hemolytic zones produced by strains that are hemolytic for human erythrocytes and directly around the bacterial growth of strains that are not hemolytic. Investigations have been carried out to determine the conditions under which this band develops and grows, and to define some of the factors necessary for its appearance.

Materials and methods. Most of the work was carried out with 2 coagulase-positive laboratory strains of *S. aureus* previously isolated from human beings—Strain Guy which hemolyzed and Strain Oxford which did not hemolyze human erythrocytes. The culture medium consisted of BBL trypticase soy agar to which 10% human blood (ACD anticoagulant) was added. Approximately 16 ml of this blood agar was poured into plastic petri dishes 9 cm in diameter. For inoculation, one loop of undiluted 18-hour meat infusion broth culture was spread on the center of the plate. After inoculation, the plates were incubated at 37°C for 4 to 6 days, and then kept at room temperature.

Experimental procedures and results. S. aureus (*Guy Strain*). On blood agar after 3 days' incubation at 37°C definite hemolysis appeared around the growth of *S. aureus*. In addition, a dense red band 0.2 to 0.4 cm wide, with indistinct outer margin, developed around this hemolytic zone. On further incubation, 5 days in all, the hemolytic zone around the growth measured 0.2 to 0.3 cm. (There was always an opaque ring in the approximate middle of this hemolytic zone. Since this observation is not germane to this study, it will not be discussed here.) At this time, a conspicuous well demarcated red band 0.8 to 1.0 cm in width encircled the hemolytic zone. The medium immediately around the red band was a paler red than the rest of the medium, which was bright red and partially hemolyzed (Fig. 1). After the plate had been kept at room temperature for several days, the red band gradually decreased in width, apparently due to extension of hemolysis encroaching on the inside of the band. When sealed by tape and kept at room temperature, these plates retained conspicuous though narrowing dense red bands, 0.2 to 0.3 cm wide, for at least 4 weeks.

Cutting of agar during growth of S. aureus (*Guy Strain*). Blood plates were inoculated centrally and then the agar was cut with a thin scalpel immediately after inoculation, and after 1, 2 or 3 days of incubation at 37°C. The cuts were made 0.5 cm from the growth or hemolytic area and at right angles to the growth and measured 1.5 cm in length.

When the agar was cut immediately after inoculation, the hemolytic zone reached to the cut but no hemolysis with red band developed beyond the cut even after long incubation at 37°C. However, a typical dense band developed around the growth away from the cut. Moreover, no clearing of the agar