toxin. Therefore, the lack of effect of this procedure in the experiments presented above suggests that hyperreactivity to endotoxin induced by infection with BCG is a phenomenon which must be considered an entity distinguishable from the Shwartzman reaction. The results presented here and those mentioned in the introduction also indicate that hyperreactivity to endotoxin induced by BCG is not related either to anaphylactic or delayed type of hypersensitivity.

Summary. BCG infection, although previously shown to increase the sensitivity of mice to endotoxin, does not increase the sensitivity of mice to passively induced anaphylaxis to histamine or to serotonin. Heparin, which inhibits the Shwartzman reaction in rabbits, failed to prevent endotoxin hyperreactivity in BCG-inoculated mice.

1. Suter, E., Kirsanow, E. M., *Immunol.,* 1961, v4, 354.
2. Suter, E., to be published.
3. Kind, L. S., *J. Immunol.,* 1953, v70, 411.
4. Kind, L. S., *Bact. Revs.,* 1958, v22, 173.
5. Good, R. A., Thomas, L., *J. Exp. Med.,* 1953, v97, 871.
6. Anacker, R. L., Munoz, J., *J. Immunol.,* 1961, v87, 426.
7. Munoz, J., Anacker, R. L., *ibid.,* 1959, v83, 502.
8. Ribi, E., Haskins, W. T., Landy, M., Milner, K. C., *J. Exp. Med.,* 1961, v114, 647.
9. Stetson, C. A., Jr., *Bact. Revs.,* 1961, v25, 457.

Received July 1, 1963. P.S.E.B.M., 1963; v114.

Bactericidal Activity of Fixed Phagocytes in Irradiated and Unirradiated Mice Treated with RNA.* (28629)

CAROLYN W. HAMMOND
Department of Microbiology, University of Illinois College of Medicine, Chicago

Hammond, Anderle and Miller(1) reported the intravenous or intraperitoneal injection of RNA or yeast autolysate into mice before or after irradiation with 475 r reduced the mortality from experimental infection with *Pseudomonas aeruginosa* initiated by intraperitoneal inoculation on the 5th day post-irradiation. The experiments to be reported here were undertaken to determine whether or not the lower mortality among irradiated mice injected with RNA might not be due, at least in part, to an increased ability of phagocytes in liver and spleen to kill ingested bacteria. This explanation seemed feasible since Gordon, Cooper and Miller(2) had shown that irradiated rabbits were able to clear their blood streams of intravenously injected *Klebsiella pneumoniae* equally as well as unirradiated rabbits. In most of the irradiated rabbits, however, the microorganisms reappeared in the blood stream 8 hours later where they multiplied until the animal died. These investigators concluded irradiation had not damaged the ability of the phagocytes to ingest bacteria but had damaged their ability to destroy ingested microorganisms.

Materials and methods. Mice. CF1 females 9-10 weeks old were housed in stainless steel cages, supplied with food and water *ad libitum,* and kept in an air conditioned, humidity controlled room. *Irradiation.* 550 r were delivered by a Picker X-ray machine using 220 kv, 20 ma and no filtration. The target distance was 94.5 cm and the dose was delivered at approximately 15.4 r per minute. The mice were placed in individual compartments of a lucite box which revolved slowly in the field during exposure. *Experimental procedure.* Thirty minutes before irradiation or on 4th day after irradiation 4 mice were injected intraperitoneally with 1.0 ml of a 2% solution of RNA. RNA was dissolved in N/20 NaOH and adjusted to pH 7.2. At the same time 4 irradiated mice were injected intraperitoneally with buffered saline to serve as controls. Unirradiated mice were

* This investigation was supported by a grant from Graduate College, Univ. of Illinois, Med. Center, Chicago.

TABLE I. Recovery of *Ps. aeruginosa* from Homogenates of Liver and Spleen 24 Hr After Intravenous Inoculation.

Time of RNA inj	No. *Ps. aeruginosa* injected	Mean No. *Ps. aeruginosa* per organ — Liver RNA treated	Liver Control	Spleen RNA treated	Spleen Control	No. mice RNA treated	No. mice Control
30 min pre-irrad	$1.8\text{-}4.1 \times 10^7$	720	5,100	56	175	31	32
4 days post-irrad	$1.4\text{-}4.0 \times 10^7$	12,000	18,000	290	350	30	32
Unirrad 4 days before challenge	$1.0\text{-}4.1 \times 10^8$	20,000	210,000	810	880	32	32

injected intraperitoneally with 1.0 ml of a 2% solution of RNA 4 days before challenge. On the 5th day post-irradiation the 4 mice injected with RNA and 4 controls were inoculated intravenously with approximately 3.2×10^7 *Ps. aeruginosa*† in 0.5 ml saline. Unirradiated mice injected with RNA 4 days before were inoculated intravenously with approximately 3.1×10^8 pseudomonads. One minute after injection of the microorganisms 500 μ polymyxin or 5 mg colymycin (injectable without Dibucaine)‡ were injected intravenously in order to kill any extracellular pseudomonads. Twenty-four hours later the mice were killed by cervical fracture. The chest was opened aseptically and blood was removed from the heart. A drop was cultured on a nutrient agar plate containing 1 μg of streptomycin per ml. The abdomen was opened and the liver and spleen were removed. Livers from 4 mice were put into 5 ml chilled nutrient streptomycin (1 μg per ml) broth in a tissue grinder tube. Spleens were put into 2.5 ml. The tissues were homogenized by means of a mechanically driven teflon pestle. After grinding, appropriate dilutions were made in chilled saline. One-tenth ml was pipetted onto each of 3 nutrient agar plates (1 μg streptomycin per ml) and the inocula spread by means of glass beads. The plates were incubated at 37°C for 24 hours and those showing 30-300 colonies were counted. Experiments in which mice were found to have positive heart's blood cultures were deleted from the data. *Leucocyte counts.* White blood cell counts were made by customary methods the day of challenge on 30 mice injected with RNA 30 minutes before irradiation and on 30 irradiated controls. These mice were used only for leucocyte counts.

Results and discussion. Table I shows the mean number of pseudomonads recovered per liver or spleen. Twenty-four experiments were carried out: 8 using mice injected with RNA 30 minutes before irradiation, 8 using mice injected on the 4th day post-irradiation and 8 using unirradiated mice injected with RNA 4 days before challenge. In 4 experiments fewer pseudomonads were recovered from both livers and spleens of the controls than from both organs of mice injected with RNA. In 5 experiments fewer pseudomonads were recovered from either livers or spleens of the controls than from either organ of mice injected with RNA. In all other experiments the bacterial counts of livers and spleens of mice injected with RNA were lower than those of the controls.

It was thought the lower mortality observed among mice injected with RNA before or after irradiation and challenged with *Ps. aeruginosa* on the 5th day post-irradiation might be due partly to an increased ability of fixed phagocytes to kill ingested bacteria. The mean number of pseudomonads recovered from the livers and spleens of mice injected with RNA 30 minutes before or 4 days after irradiation was lower than the number recovered from livers and spleens of the controls. The bacterial counts of livers and spleens of unirradiated mice injected

† The strain of *Ps. aeruginosa* was the same the author has used in challenge experiments for many years. It is highly resistant to streptomycin and has been maintained by weekly passage through mice. Dilutions were prepared as previously described(1).

‡ Colymycin was obtained from Warner-Chillcot Laboratories through the courtesy of Dr. Robert M. Gabrielson.

with RNA 4 days before challenge were also lower than the controls. In 9 experiments of 24, however, the bacterial counts of both organs or one organ in the controls were lower than the counts in the RNA injected mice. The difference between the mean bacterial counts was slight and does not warrant the conclusion that RNA had enhanced the bactericidal ability of the fixed phagocytes.

On the day of challenge the mean leucocyte count of mice injected with RNA 30 minutes before irradiation was not significantly different from the mean control count. The former was 700 per mm^3 and the latter 500 per mm^3.

Summary. CF1 female mice 9-10 weeks old were injected intravenously with RNA 30 minutes before or on the 4th day after exposure to a sublethal dose of X-irradiation (550 r). Simultaneously an equal number of irradiated mice were injected intravenously with buffered saline to serve as controls. On the 5th day post-irradiation all mice were injected intravenously with *Ps. aeruginosa* followed one minute later by intravenous polymyxin or colymycin. The next day all mice were killed and heart's blood cultured. Livers and spleens were removed, ground separately in tissue grinders and appropriate dilutions plated. The results of the bacterial counts indicated that the mean number of pseudomonads in livers and spleens of mice injected with RNA were slightly lower than the mean number in livers and spleens of controls but the difference was not significant. Similar results were obtained among unirradiated mice injected with RNA 4 days before challenge with *Ps. aeruginosa.*

The author is indebted to Mr. Richard Haas, Radiology Dept., for x-ray dosimetry and to Miss Mabel Counelis for irradiating the mice. She also wishes to thank Mr. Michael LaPorta for skillful technical assistance.

1. Hammond, C. W., Anderle, S. K., Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 690.
2. Gordon, L. E., Cooper, D. B., Miller, C. P., *ibid.*, 1955, v89, 577.

Received July 8, 1963. P.S.E.B.M., 1963, v114.

Reversibility of Intestinal Absorption of 3-0-Methylglucose.* (28630)

HALVOR N. CHRISTENSEN AND SEYMOUR J. GRAY
Department of Biological Chemistry, University of Michigan, Ann Arbor

The model amino acids, α-aminoisobutyric acid and 1-aminocyclopentanecarboxylic acid, reach much the same distribution between the contents of isolated loops of the small intestine and the blood serum of the rat, whether by absorption or by secretion(1). The distribution ratios reached are far higher than those attained across the walls of isolated intestinal sacs(2). These findings appear to represent the first demonstration and evaluation of "uphill" transport of amino acids from the intestine *in vivo*, and of the reversible action of this transport. We have now made the same demonstration with the unmetabolizable sugar, 3-0-methylglucose(3-6).

Bárány and Sperber showed that absorption of glucose from sulfate- or sorbose-containing solutions in isolated loops of the intestine of the rabbit could bring the concentration of sugar in the loop to approximately one-quarter to one-fifth of the level of the blood sugar(7). These experiments appear to show unequivocally that glucose absorption can occur against a considerable concentration gradient. Campbell and Davson(8) showed in 3 experiments that the 3-0-methylglucose level of the contents of the small intestine of the cat could be lowered by absorption to values in the range of 55 to 78% of simultaneous plasma levels, which were maintained by injection at 5-minute intervals.

* This investigation was supported in part by U.S.P.H.S. grants. Dr. Gray was a guest investigator at Univ. of Michigan during this work.