with actinomycin D stimulated an increase in alkaline phosphatase in the duodenum to about twice the activity seen during the course of normal development.

The doses of actinomycin D in our experiments, although smaller than those in similar studies (*2*), were toxic to the animals as reflected by their loss in body weight, accumulation of fluid in the peritoneal cavity, and hemorrhagic areas in various tissues. The influence of such toxicity (*11*) on the altered enzymic activity produced by actinomycin D is difficult to evaluate. It is well known that fasting increases the activity of a number of the adaptive enzymes (*12*). The altered enzymic activity produced by the stress of fasting was compared with that found in actinomycin D-treated animals. The weight loss observed when rats were fasted for 2 days was twice that of the actinomycin D-intoxicated animals, but none of the enzymes in the livers of the fasted rats was more than doubled in activity.

The dose of actinomycin D used to inhibit the 5-hour response of tyrosine transaminase and tryptophan pyrrolase to cortisol (*2*) was more than twice the LD_{50} (50 percent lethal dose) of this antibiotic (40 μg/100 g) for the rat (*11*). We have observed that 25 μg of actinomycin D given as a single dose to rats weighing 100 g does not block the rapid induction of these enzymes by cortisol. On the other hand, the cortisone-induced increases in the activity of several glycolytic enzymes were impaired by treatment with as little as 8 μg of actinomycin D per 100 g of body weight given daily for 5 days (*13*). These different observations and the finding that enzyme induction by the antibiotic is achieved with doses in the range of the LD_{50} suggest the importance of comparing the amount of drug required to inhibit RNA synthesis with that necessary to inhibit enzyme induction.

Studies by Schwartz *et al.* (*14*) have indicated that inhibition of RNA synthesis in rat liver to the extent of 90 percent occurs within 30 minutes after the administration of actinomycin D (100 μg/100 g); thereafter this effect is slowly relieved and is no longer apparent after 16 hours. These results suggest that, after a single large dose of this antibiotic, protein synthesis should be markedly inhibited for a period of about 5 hours and not significantly impaired after 12 hours. Thus, different effects of actinomycin D may be obtained, depending on the dose and the intervals at which observations are made. However, the manner by which this antibiotic stimulates the induction of certain adaptive enzymes is not understood. It is conceivable that the negative nitrogen balance and cachexia which occurred after administration of toxic doses of the drug might enlarge the pool of free amino acids in liver and thus selectively promote the induction of hepatic enzymes responsive to increased protein intake or fasting (*12*). The evidence that treatment with actinomycin D induces the synthesis of certain adaptive enzymes in adrenalectomized rats indicates the need for comparative investigation of the mechanism underlying the induction of the same enzymes by either cortisol or actinomycin D.

FRED ROSEN, PREM NATH RAINA
RICHARD J. MILHOLLAND
CHARLES A. NICHOL
Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York

References and Notes

1. I. Slotnick, *Bacteriol. Proc. Am. Soc. Microbiol.* **59**, 130 (1959); A. Korner and A. J. Munro, *Biochem. Biophys. Res. Commun.* **11**, 235 (1963).
2. O. Greengard, M. A. Smith, G. Acs, *J. Biol. Chem.* **238**, 1548 (1963); O. Greengard, G. Weber, R. L. Singhal, *Science* **141**, 160 (1963); H. V. Gelboin and N. R. Blackburn, *Cancer Res.* **24**, 356 (1964).
3. J. W. Uhr, *Science* **142**, 1476 (1963); C. J. Wust, C. L. Gall, G. D. Novelli, *ibid.* **143**, 1041 (1964).
4. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *ibid.* **134**, 556 (1961); J. Hurwitz, J. J. Furth, M. Malamy, M. Alexander, *Proc. Natl. Acad. Sci. U.S.* **48**, 1222 (1962); E. Kahan, F. Kahan, J. Hurwitz, *J. Biol. Chem.* **238**, 2491 (1963); E. Reich, *Science* **143**, 684 (1964).
5. W. E. Knox and A. H. Mehler, *Science* **113**, 237 (1951); E. C. C. Lin and W. E. Knox, *J. Biol. Chem.* **233**, 1186 (1958).
6. F. Rosen, H. R. Harding, R. J. Milholland, C. A. Nichol, *J. Biol. Chem.* **238**, 3725 (1963); F. Rosen and C. A. Nichol, in *Advances in Enzyme Regulation*, G. Weber, Ed. (Pergamon, New York, 1964), vol. 2, pp. 115–135.
7. J. Planelles, N. Ozeretskovsky, O. Djeksenbaev, *Nature* **195**, 713 (1962).
8. H. Nitowsky, S. Geller, R. Casper, *Federation Proc.* **23**, 556 (1964).
9. M. R. Pollock, *Biochim. Biophys. Acta* **76**, 80 (1963).
10. F. Moog, *Science* **144**, 414 (1964).
11. F. S. Philips, H. S. Schwartz, S. S. Sternberg, C. T. C. Tan, *Ann. N. Y. Acad. Sci.* **89**, 348 (1960).
12. F. Rosen and C. A. Nichol, *Vitamins and Hormones* **21**, 135 (1963).
13. G. Weber, R. L. Singhal, N. B. Stamm, *Science* **142**, 390 (1963); G. Weber and R. L. Singhal, *J. Biol. Chem.* **239**, 521 (1964).
14. H. S. Schwartz, J. E. Sodergren, M. Garofalo, *Proc. Am. Assoc. Cancer Res.* **5**, 57 (1964).
15. A. Grollman, *Endocrinology* **29**, 855 (1964).
16. Supported in part by NIH grant AM-04389. We thank L. E. Budnick and Phyllis A. Roswick for their technical assistance.

10 July 1964

Transplantation of Rat Bone Marrow in Irradiated Mice: Effect of Exposure Rate

Abstract. *Mice were irradiated at several different exposure rates so that they received a total of 900 roentgens over the whole body. Within 2 hours they were injected with rat bone marrow. The success of the transplantation depended upon the rate at which the animals had been irradiated: the higher the rate, the greater the success of the grafts. Failure of grafts in mice subjected to low exposure-rates was associated with antibody formation. The data indicate existence of an immune mechanism which is dependent on the radiation exposure-rate.*

In a study of the survival of irradiated rats injected with allogeneic (homologous) bone marrow, Courtenay (*1*) found a relation between survival and the exposure rate. Her study suggested, by virtue of hematopoietic failure and death, that the lower rates of 0.28 r/min and 1.4 r/min (Cs^{137} γ-rays) were less effective in depressing the host's immune mechanism than the higher exposure rate of 29 r/min [250 kv (peak) x-rays]. In the study described here, heterologous (xenogeneic) transfer of rat bone marrow into irradiated mice was used to demonstrate the dependency of an immune mechanism on the exposure rate in the rejection of a foreign graft.

Mice of the $C3BF_1$ [$(C3H \times C57Bl)F_1$] strain were exposed to γ-radiation from two different sources. The first was a total-body irradiator designed for the treatment of patients; it consisted of eight 500-curie Cs^{137} sources (*2*). The positions of the sources ensured a uniform total-body exposure of mice in a compartmented lucite cage continuously rotating at 0.2 rev/min. The exposure rate, as measured in air with a Victoreen roentgen chamber, was 3.75 r/min. For greater exposure rates, a Co^{60} unit with a 260-curie source was used. By altering the distance between the lucite cage and the source, groups of mice could be irradiated at exposure rates of 19.8 r/min, 39.7 r/min, or 53.4 r/min. These values represent the mean of the center and end positions in a single

Table 1. Tests for the presence of rat erythrocytes, and agglutinins for rat erythrocytes, in the blood of irradiated mice injected with rat bone marrow. All mice received a total of 900 r.

Days after injection of bone marrow	Exposure rate (r/min)			
	3.75	19.8	39.7	53.4
	*Tests for rat erythrocytes**			
10	0/34†	1/24	10/33	18/32
20	0/20	1/20	3/13	8/17
30	0/16	0/19	1/13	5/12
	Tests for rat erythrocyte agglutinins			
20	9/20 (1–4)‡	1/19 (1)	0/10	0/9
30	10/16 (2–6)	3/19 (2–4)	2/12 (3,4)	1/7 (5)
50	11/15 (2–9)	4/18 (1–5)	2/10 (2,3)	2/6 (4,6)

* Exposure-rate differences significant by chi-square test. † Fractions represent number of animals showing positive test over number tested. ‡ Values in parentheses represent agglutinin titer ($\log_2$) or range in titer on positive serum samples.

Table 2. Tests for granulocytes characteristic of rats in bone marrow and spleen of irradiated mice injected with rat bone marrow; determinations by the cytoplasmic alkaline phosphatase stain. All mice received a total of 900 r.

Days after injection of bone marrow	Exposure rate (r/min)			
	3.75	19.8	39.7	53.4
	*Bone marrow**			
6 to 10	10/13†	23/29	22/25	3/3
11 to 20	1/13	6/8	29/35	15/15
Percentage	42	78	85	100
	Spleen			
6 to 10	1/13	14/29	15/25	2/3
11 to 20	0/13	2/8	9/35	10/15
Percentage	4	43	40	67

* Exposure-rate differences significant in 11- to 20-day period by chi-square test. † Fractions represent number of animals showing positive test for rat-type granulocytes over number tested.

compartment of the cage, the variation being approximately 5 percent of the mean. All mice received a total of 900 r over the whole body; this dose, when given with a conventional 250-kv (peak) x-ray machine at 160 r/min, is 100 percent lethal for $C3BF_1$ mice. Within 2 hours after irradiation, groups of mice from each exposure rate received intravenously 100×10^6 nucleated rat (Carworth Farm Nelson) bone marrow cells (*3*). On the 10th, 20th, and 30th day after they received the bone marrow cells, some of the mice in each group were tested for the presence of rat erythrocytes by means of a mouse antiserum that would agglutinate such cells. Reciprocal tests were made with a rat antiserum that agglutinated mouse erythrocytes (*3*). Animals having no erythrocytes characteristic of the donor on the 20th day were bled for serum then and on the 30th and 50th days. The serum was titrated for agglutinating antibody to rat erythrocytes. Bone marrow and splenic tissue from mice dying within 20 days of receiving bone marrow cells were tested for the presence of granulocytes characteristic of rats by the cytoplasmic alkaline phosphatase stain (*4*).

Table 1 shows that the success of the foreign marrow transplantation depended on the radiation exposure rate to which the recipient mice had been subjected. Erythrocytes characteristic of the donor were found in none of the mice receiving 900 r at 3.75 r/min. Of recipients irradiated at 19.8 r/min, only one mouse out of 24 tested on day 10 was positive for rat erythrocytes, and this animal was negative when tested on day 30. Of mice irradiated at 39.7 and 53.4 r/min, increasing numbers showed some signs of a transplant. Among all four groups, only six mice were true radiation chimeras, and five of these had been subjected to the highest rate of exposure.

Failure of the transplantation indicated an immunologic rejection phenomenon, as shown by the presence of agglutinating antibodies in the serum of some of the treated mice (Table 1). This was most evident in the group subjected to 3.75 r/min, where nine of 20 animals tested on day 20 showed some agglutinating antibodies to rat erythrocytes. As the exposure rate increased, the number of animals showing agglutinins decreased.

Tests for the persistence of the donor graft in the bone marrow and splenic tissues of mice dying within the first 20 days after treatment also indicated that the success of transplantation depended on the rate of exposure: the lower the exposure rate, the lower the frequency of animals showing the persistence of foreign cells (Table 2).

The combined data for several experiments at each rate of exposure showed that the percentage mortality after 30 days increased with increasing exposure rate: 3.75 r/min, 61 percent; 19.8 r/min, 72 percent; 39.7 r/min, 86 percent; 53.4 r/min, 93 percent. Only in the mice subjected to the highest rate of exposure did injections of rat bone marrow show any beneficial effect in terms of survival for 30 days, the percentage mortality being reduced to 67 percent. At the other exposure rates, injection of rat marrow did not alter the 30-day mortality. In contrast, when mice were injected with syngeneic (isologous) marrow after receiving 900 r at 3.75 r/min and 39.7 r/min, the mortality in both groups was less than 10 percent at 30 days.

These results are of great importance for transplantation studies. Many irradiators used for studies of transplantation of hematopoietic tissues in larger experimental animals operate at low exposure rates with Co^{60} or Cs^{137} sources. The conventional 250-kv (peak) x-ray machine, with its higher exposure rate, is used almost exclusively for smaller species such as the rat and mouse. Direct comparison between the soft 250-kv (peak) x-rays and the more penetrating γ-rays of Co^{60} (1.17- and 1.33-Mev) and Cs^{137} (660 kev) in terms of their biologic efficacy is not warranted, but the effect of the exposure rate as observed in this study was obtained even within the spectrum of one type of radiation, Co^{60}. It is not yet known, however, whether the exposure-rate-dependent immune phenomenon shown here in mice also operates in larger animals such as dogs and primates, including man. Studies are also needed to determine the association of mortality with immunologic suppression, in view of the increased mortality obtained with increased exposure rates.

NAZARETH GENGOZIAN

Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee 37831

References and Notes

1. V. D. Courtenay, *Brit. J. Radiol.* **36**, 440 (1963).
2. A. C. Morris, Jr., *Intern. J. Appl. Radiation Isotopes* **11**, 108 (1961).
3. N. Gengozian, *J. Immunol.* **83**, 173 (1959).
4. G. Gomori, *J. Lab. Clin. Med.* **37**, 526 (1951).
5. Under contract with the AEC.

4 August 1964