species living in quiet pools or aquaria. It is possible that the type of selection used by fanciers in developing the present strains of domesticated *Carassius* has in some way been selective also for a given state of thyroidal activity. Possibly it would be illuminating, therefore, to test the thyroidal activity of wild *Carassius*, or of the closely related carp.

Summary. 1. Normal goldfish, *Carassius auratus* accumulate a peak of 3% of an injected tracer dose of I^{131} intrathyroidally. This small amount of radioiodine remains in the thyroid at the same level for more than a week. Thyroids of thyrotropic hormone-injected goldfish accumulate 9% or more of the tracer I^{131}, but lose at least 40% of it within a week, indicating a greater turnover rate. 2. Radioiodine in normal goldfish thyroids is mostly in the form of monoiodotyrosine and diiodotyrosine, with little, if any, thyroxine formed even after one week. The thyrotropic hormone stimulated goldfish thyroid forms a small proportion of radiothyroxine as early at 24 hours after injected I^{131} and as much as 8% of the I^{131} is in the form of thyroxine by 7 days after injection of I^{131}. 3. Keeping the goldfish in water low in iodine had an effect on I^{131} metabolism similar to thyrotropic hormone treatment.

1. Berg, O., and Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 751.

2. Berg, O., and Gorbman, A., *Canc. Res.*, 1954, v14, 232.

3. Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1940, v45, 772.

4. ———, *Univ. Calif. Publ. Zool.*, 1946, v51, 229.

5. Gorbman, A., Berg, O., and Creaser, C., *Anat. Rec.*, 1953, v117, 535.

6. Gordon, M., In E. J. Farris, *Care and Breeding of Laboratory Animals*, 1950, New York, Wiley and Sons, Inc.

7. Hoar, W., Publications of the Ontario Fisheries Res. Lab., No. 71, 1951, U. Toronto Press.

8. LaRoche, G., *Ann de l'ACFAS*, 1950, v16, 134.

9. Lissitzky, S., Thesis, Paris, A. I. A., 1952.

10. Lynn, W. G., and Wachowski, H. E., *Quart. Rev. Biol.*, 1951, v26, 123.

11. Roche, J., Justisz, M., Lissitzky, S., and Michel, R., *Compt. rend. Soc. Biol.*, 1950, v144, 1321.

Received April 23, 1954. P.S.E.B.M., 1954, v86.

Protective Effect of Hypoxia against Irradiation Injury of the Rat Bone Marrow and Spleen.* (21038)

W. A. RAMBACH, H. L. ALT, AND JOHN A. D. COOPER.†

From Departments of Medicine and Biochemistry, Northwestern University Medical School, Chicago.

The reduction in radiation sensitivity by hypoxia has been demonstrated under a variety of experimental conditions(1). Dowdy, Bennett, and Chastain(2) have shown that the LD_{50} dose of x-irradiation is increased from 800 r to between 1200 and 1400 r when rats are irradiated in a 5% oxygen atmosphere.

In previous studies reported from this laboratory(3-5), the concentration of desoxyribonucleic acid has been used as a measure of the cellularity of the bone marrow and spleen and the rate of incorporation of radiophosphorus into this fraction as an index of mitosis rate. In the present investigation, these methods have been used in a study of the protective effect of hypoxia against irradiation damage to these organs.

Methods. Male rats of the Sprague-Dawley strain, 3 to 4 months old with an average weight of 290 g were used. Hypoxia was produced by submitting the animals to a stimulated altitude of 25,000 feet, previously described(5). Irradiated animals were given 800 r of total body irradiation at the rate of 21 r per minute from a 220 kv Maximar

* This study supported by funds under contracts between the USAF School of Aviation Medicine, Randolph Field, Texas, and between the U. S. Atomic Energy Commission and Northwestern University, and by the Armour Laboratories.

† Markle Scholar in Medical Sciences.

TABLE I. Effect of Hypoxia on DNA Metabolism in Rat Bone Marrow and Spleen following Irradiation.

Group	No.	Phosphorus content, mg/g ASP	Phosphorus content, mg/g DNAP	Specific activity ASP	Specific activity DNAP
		Bone marrow			
Normal	15	.966 ± .088	1.320 ± .295	.481 ± .064	.199 ± .034
Irradiation alone	5	.676 ± .218	.109 ± .088	.366 ± .070	.060 ± .034
" + hypoxia	7	.699 ± .088	*.208* ± .069	.527 ± .062	*.190* ± .057
Irradiation + hypoxia following intermittent hypoxia	6	.525 ± .135	.082 ± .022	.530 ± .141	*.167* ± .049
		Spleen			
Normal	15	.969 ± .107	1.294 ± .244	.468 ± .011	.034 ± .002
Irradiation alone	5	.926 ± .036	.281 ± .037	.635 ± .083	.003 ± .003
" + hypoxia	7	.921 ± .063	*.475* ± .067	.637 ± .061	*.037* ± .014
Irradiation + hypoxia following intermittent hypoxia	6	.840 ± .042	*.460* ± .112	.613 ± .049	*.035* ± .022

Italics indicate significant differences from control irradiated animals (P value >0.98).

operating at 20 M.A. Filtration used was 0.5 mm of copper and 1.0 mm of aluminum. The HVL was 1.15 mm of copper and the target distance was 70 cm. The animals were divided into 4 groups: the first group was untreated and served as a control; the second was irradiated at normal atmospheric pressure; the third group was subjected to hypoxia only while being irradiated; the fourth group was submitted to 10 hours of hypoxia daily for 3 days and 15 hours after completion of the period of intermittent hypoxia were irradiated in a hypoxic atmosphere. Following irradiation all animals were returned to their cages and 96 hours later were injected intraperitoneally with 2 μc of carrier-free $NaH_2P^{32}O_4$‡ per 100 g of body weight. Four hours after administration of the isotope the animals were sacrificed by a blow on the head and tissues removed for chemical, radioisotopic, and morphologic studies by methods previously described(5). The values for acid soluble phosphorus (ASP) and DNAP content are expressed as mg of phosphorus per g wet weight of tissue. The specific activity represents the per cent of the injected dose of radiophosphorus present/mg of phosphorus in the fraction. The significance of differences between the hypoxic animals and control irradiated animals were subjected to "t" analysis by the method of Fisher(6). Differences were considered significant if the probability was 0.98 or greater.

Results. Ninety-six hours post-irradiation the DNAP concentration of the bone marrow and spleen was markedly depressed but was significantly higher in the animals irradiated in a hypoxic atmosphere than in the controls (Table I). The cellularity as determined by direct counting on imprints followed closely the changes in the DNAP concentration. The rate of incorporation of the P^{32} into the DNA (specific activity) of the bone marrow and spleen was near normal in the "hypoxic" animals but was markedly depressed in the irradiated controls.

Preliminary exposure of animals to intermittent hypoxia before irradiation at reduced oxygen tension does not result in any additional protection to the bone marrow and spleen over that observed in animals subjected to hypoxia only during irradiation (Table I). The differential counts of cells in the bone marrow and spleen in normal, control irradiated and protected animals is shown in Table II.

Discussion. The protective effect of hypoxia has been attributed to the reduction in the number of free radicals produced from the interaction between the radiation and water in the biological system(7). In theory hypoxia would reduce the biological effect of a given dose of x-irradiation to that which would be produced by a lower x-ray dose without hypoxia. The pattern of DNAP con-

‡ Obtained on allocation from the U. S. Atomic Energy Commission.

TABLE II. Effect of Hypoxia on Differential Counts in Rat Bone Marrow and Spleen following X-Irradiation.
Major cell types in %.

Group	Bone marrow				Spleen			
	E	G	L	R.E.	E	G	L	R.E.
Normal	46	32	16	.3	5	8	82	4
Control x-ray	1	20	2	59	0	4	5	91
Acute hypoxia	1	56	2	15	1	6	30	61
Chronic "	7	43	2	34	0	5	8	86

E = Erythrocytic series; G = Granulocytic series; L = Lymphocytic series; R.E. = Reticuloendothelial cells.

centration, specific activity and distribution of cell types in the bone marrow and spleen in the animals receiving 800 r at low pressures corresponds to that which would be expected in unprotected animals receiving 600 r(8). Thus hypoxia appears to have reduced the biological effectiveness by 200 r as measured by its action on the bone marrow.

Previous investigators have found a high radiosensitivity of cells of the erythrocytic and radioresistance of cells of the reticuloendothelial series(9). The findings are substantiated in the present study and there was no significant difference between the control irradiated animals and the protected animals. This indicates that hypoxia does not alter the relative radiosensitivity of the various cell types present in the bone marrow and spleen. Although there is little difference in the degree of hypocellularity that develops in the control irradiated and protected animals, the return of mitosis in the latter group probably represents one manifestation of the protective action of hypoxia.

We have observed previously that 30 hours of intermittent hypoxia produced a moderate increase in cell mitosis in the bone marrow and a marked increase in mitosis in the spleen (5). The failure of hypoxia induced stimulation of the bone marrow and spleen to increase protection following irradiation was probably due to the relative sensitivity of the young dividing cells of the erythrocytic series.

The results obtained in animals exposed to hypoxia only during irradiation suggests that the protection observed by Jacobson *et al.* (10) in animals pretreated with phenylhydrazine may have been due to tissue hypoxia resulting from the anemia present in these animals rather than to the hyperplastic bone marrow as they suggested.

Summary. 1. Rats irradiated in a hypoxic atmosphere show a slightly higher cellularity and much greater rate of DNA synthesis in the bone marrow and spleen after 96 hours than rats irradiated at ground level. 2. Thirty hours of intermittent hypoxia preceding irradiation at low oxygen tensions does not give additional protection. 3. A striking parallelism exists in the cellular reactions in the bone marrow and spleen following irradiation, both at normal atmospheric pressures and at low oxygen pressure. 4. Irradiation of animals at low oxygen pressures appears to reduce the biological effectiveness of 800 r on the bone marrow to 600 r.

1. Selle, W. A., Mason, G. D., Neuman, R. H., Atomic Energy Commission, Univ. of Calif. at Los Angeles, UCLA 264, 1953.

2. Dowdy, A. H., Bennett, L. R., and Chastain, S. M., *Radiology,* 1950, v55, 879.

3. Martin, J. S., Alt, H. L., and Cooper, J. A. D., *J. Lab. Clin. Med.,* 1950, v36, 961.

4. Rambach, W. A., Moomaw, D. R., Alt, H. L., and Cooper, J. A. D., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 59.

5. Rambach, W. A., Cooper, J. A. D., and Alt, H. L., U.S.A.F. School of Aviation Med., 1953, Project No. 213501-001, Report No. 1.

6. Snedecor, G. W., *Statistical Methods,* Collegiate Press, Ames, Iowa, 1946.

7. Patt, H. M., *Ann. Rev. Nuclear Science,* 1952, v1, 495.

8. Robbins, G. P., Alt. H. L., Cooper, J. A. D., unpublished data.

9. Rosenthal, R. L., Pickering, B. I., Goldschmidt, L., *Blood,* 1951, v6, 600.

10. Jacobson, L. O., Marks, E. K., Gaston, E. O., Simmons, E. L., *Science,* 1948, v107, 248.

Received April 30, 1954. P.S.E.B.M., 1954, v86.