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Effects of Extreme Environmental Oxygen Tensions on Periosteal Proliferation of Mouse Femora.* (31801)

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The periosteum has a vast osteogenetic potential which is manifested in the healing of bones. Changes in rates of proliferation of the periosteum during growth, aging, and repair of bone have been described by the use of tritiated thymidine coupled with autoradiography(17-21). Further studies have shown that the periosteal response to trauma is not directly related to the type of exudate which accumulates following injury(21). However, it was felt that with the interruption of circulation and stagnation of blood, the resultant "local anoxia" could lead to the presence of a tissue factor capable of stimulating periosteal cell proliferation. The present study is an extension of this thesis and is designed to evaluate the effects of oxygen on the periosteum. More specifically, young mice were exposed to minimum and maximum oxygen tensions tolerable at 1 atmosphere, and the effects of this treatment on the mitotic activity of the periosteum were determined autoradiographically following tritriated thymidine (H^3TDR) administration.

Methods and materials. Since in 5- to 6-weeks-old mice the maximum periosteal response post-fracture occurred at about 30 hours, this time was chosen to evaluate the effects of oxygen on the periosteum. Pilot studies showed that at 1 atmosphere pressure, the minimum and maximum oxygen levels tolerated were 5% and 100% oxygen. Thirty Swiss albino mice, age 5- to 6-weeks-old, were placed in a respiratory chamber in groups of 5 and exposed to 5% O_2, 100% O_2, and air. The chamber was adjusted to an inflow and outflow of 1.5 l/min. Soda lime was added to the chamber to absorb the expired CO_2. All the mice had their left femora fractured at the midshaft by digital pressure under ether anesthesia, and their right femora were left intact. One hour before sacrifice the mice were given a subcutaneous injection of 0.5 μc of H^3TDR (Sp. Act. = 1.9 C/mM) per g of body weight. At the end of 30 hours the mice were killed and both femora and proximal tibiae were removed for fixation and decalcification. The tissues were prepared for autoradiography as previously described(17-21). Twenty more mice were exposed to similar environmental conditions after fracturing the left femora and injecting 0.25 ml of physiological saline just above the periosteum of the right femora. To determine the earlier effects of 100% O_2, the left femora of 10 additional animals were fractured; of these, 5 were exposed for 8 hours and the remaining 5 for 12 hours in the respiratory chamber. The percentage labeling of the osteogenic layer of the periosteum was determined from autoradiographs prepared with NTB_3 Kodak liquid emulsion(21). The ratio of the number of labeled cells to the total number of cells counted in a given cell population in an animal sacrificed 1 hour after administration of H^3TDR constitutes the labeling index, which is in turn multiplied by 100 to obtain the percentage of labeled cells.

Results. The autoradiographs of mice exposed to air revealed a cellular labeling of

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TABLE I. Periosteal Cell Labeling with Tritiated Thymidine in 5-6-Week-Old Mouse Femora with Intact and Fractured Limbs.

		Air	100% Oxygen	5% Oxygen
Intact femora	No. of animals	10	10	10
	No. of cells counted	5666	6648	7499
	Labeling index	.015	.019	.007
	% Labeling	1.5	1.9	.7
Fractured femora	No. of animals	10	10	10
	No. of cells counted	6006	7481	4000
	Labeling index	.250	.318	0
	% Labeling	25.0	31.8	0

TABLE II. Periosteal Cell Labeling with Tritiated Thymidine in 5-6-Week-Old Mouse Femora with Saline-Injected and Fractured Limbs.

		Air	100% Oxygen	5% Oxygen
Saline-injected femora	No. of animals	10	5	5
	No. of cells counted	384	1088	150
	Labeling index	.221	.195	0
	% Labeling	22.1	19.5	0
Fractured femora	No. of animals	10	5	5
	No. of cells counted	4099	4974	2000
	Labeling index	.314	.356	0
	% Labeling	31.4	35.6	0

TABLE III. Periosteal Cell Labeling with Tritiated Thymidine in 5-6-Week-Old Mouse Femora Exposed to 100% Oxygen at Different Time Intervals.

		8 hours	12 hours	30 hours
Intact femora	No. of animals	5	5	10
	No. of cells counted	4414	2955	6648
	Labeling index	.033	.041	.019
	% Labeling	3.3	4.1	1.9
Fractured femora	No. of animals	5	5	10
	No. of cells counted	1658	1823	7481
	Labeling index	.017	.147	.318
	% Labeling	1.7	14.7	31.8

1.5% for the intact femora and 25.0% for the fractured femora. Autoradiographs of the saline-injected and corresponding fractured femora subjected to air had a labeling of 22.1% and 31.4% respectively. Animals exposed to 5% O_2 showed a suppression of labeling in the intact limb (0.7%) and the absence of labeling in fractured limbs (Tables I and II). At 100% O_2, 1.9% labeling was observed in the intact limbs and 31.8% for the opposite fractured femora, 19.5% for the saline-injected femora and 35.6% for their opposite fractured limbs. The quality of labeling over the cell nucleus in animals exposed to 5% O_2 was poor, moderate in those exposed to air, and good in mice exposed to 100% O_2 (Fig. 1-3). It is interesting to note that in the intact femora of mice subjected to 100% O_2 for varying time periods the labeling was higher at 8 hours (3.3%) and 12 hours (4.1%) than at 30 hours (1.9%) (Table III). As previously reported(21), no evidence of an increased periosteal response was observed at 8 hours post-fracture in the corresponding fractured femora.

Discussion. When one decreases the inspired oxygen concentration the change produced is a decrease in the arterial oxygen concentration or hypoxia. Eventually the pO_2 of the tissue environment is lowered, and a point is reached at which the cellular oxygen consumption starts to fall(9). This point is known as the "critical oxygen level" and represents the point at which the pO_2 available to the intracellular site of oxygen reduction (mitochondrion) is no longer adequate(5,6,9,13,15). A 5% O_2 level is probably below the critical oxygen level for most tissues(4-6,15) and

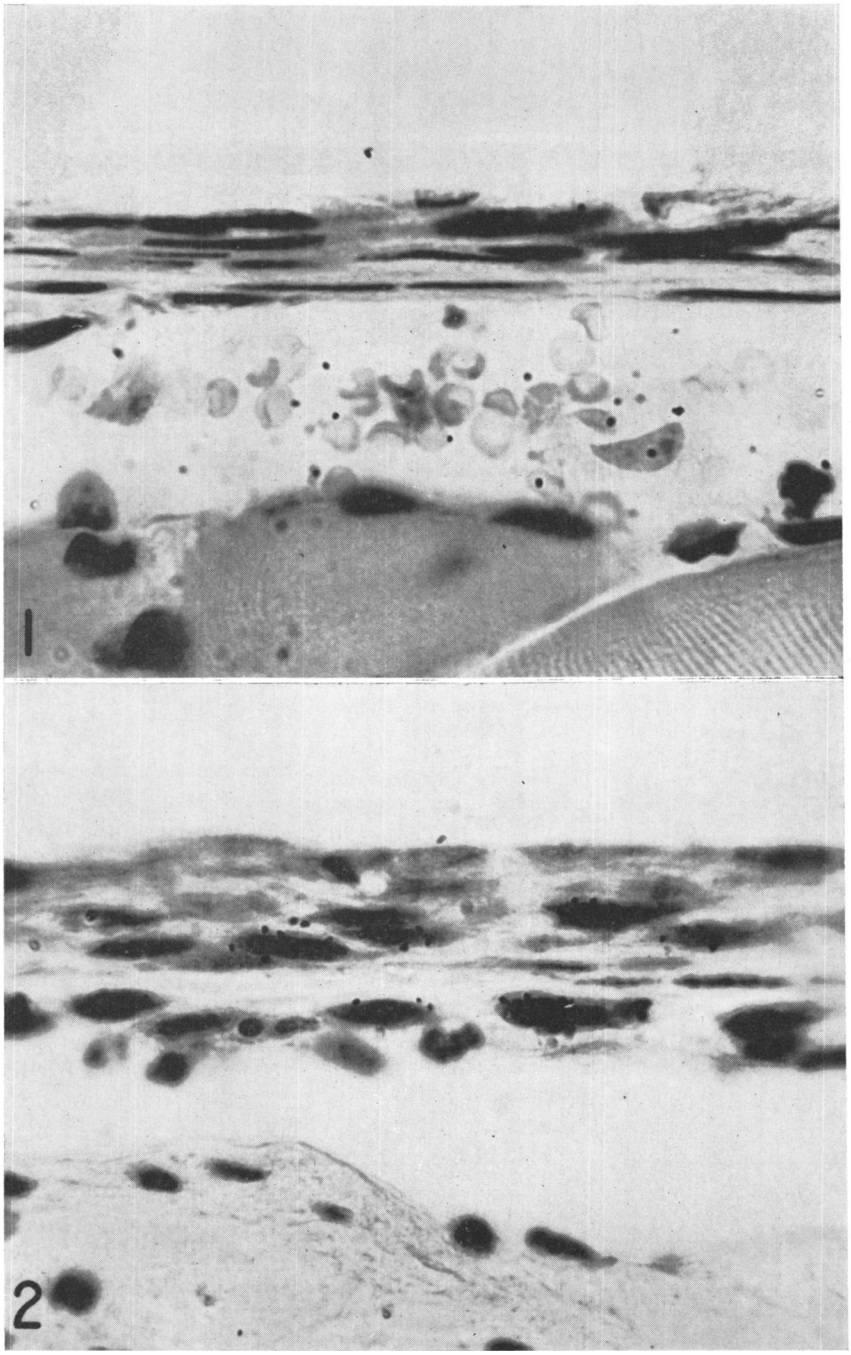

FIG. 1. An H^3TDR autoradiograph of mouse femoral periosteum is shown previously exposed to 5% O_2 at 1 atmosphere for 30 hr. No labeled periosteal cells are observed. Hematoxylin and eosin stained. × 1000 oil immersion.

FIG. 2. An H^3TDR autoradiograph of mouse femoral periosteum is shown previously exposed to air at 1 atmosphere for 30 hr. A moderate number of reduced silver grains are noted over periosteal cells. Hematoxylin and eosin stained. × 1000 oil immersion.

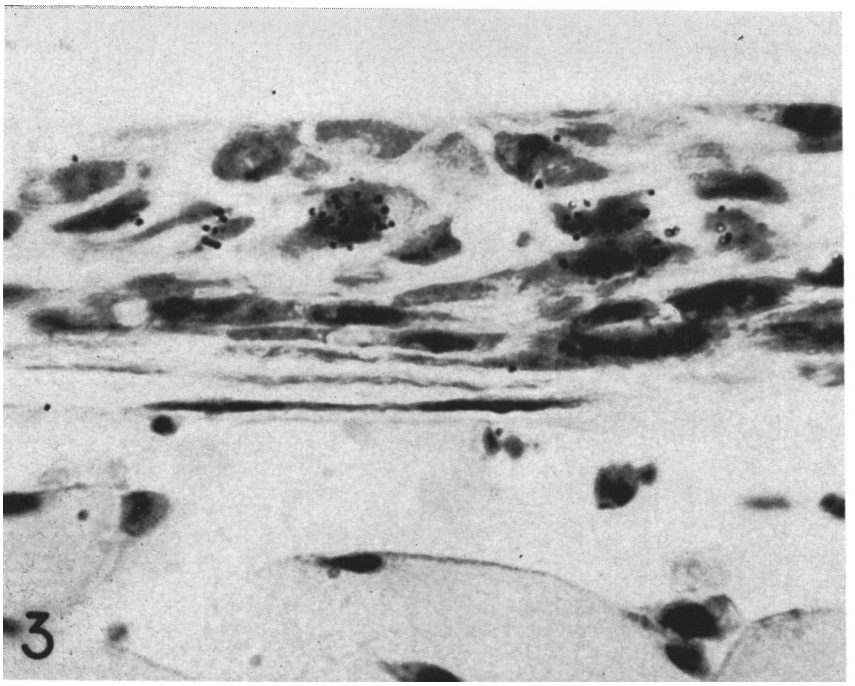

FIG. 3. An H^3TDR autoradiograph of mouse femoral periosteum is shown previously exposed to 100% O_2 at 1 atmosphere for 30 hr. Highly labeled periosteal cells are observed. Hematoxylin and eosin stained. × 1000 oil immersion.

low enough to have a marked effect on the normal oxidative pathways(1,8). In the present experiment, 5% O_2 most likely represents a "critical oxygen" level in which the oxygen tension is perhaps too low to maintain the metabolic pathways necessary for the incorporation of H^3TDR into the cell and the synthesis of DNA. The effects of this low oxygen tension are more apparent at the fracture site where anoxia is present than at the intact side (Tables I and II). These results, however, do not rule out the thesis that local anoxia may provide a "tissue factor" or environment capable of stimulating cell proliferation, since 30 hours of exposure to extreme hypoxic conditions is unphysiological, and because there are known conditions of hypoxia above "critical oxygen" levels which co-exist with increased cell proliferation, *e.g.*, foetal growth, secondary polycythemia, and pulmonary osteoarthropathy.

Ordinarily, continuous exposure to high oxygen tensions is deleterious to tissues and can lead to convulsions, loss of spermatogenesis, metabolic acidosis, hypertrophy of the adrenal glands and a pneumonitis(3,10). These findings are believed to be caused by an oxidation of thiol (SH) groups(10,14). Gerschman(10) proposed that oxygen toxicity is similar to x-irradiation in that oxidized free radicals are formed at the expense of the SH groups. Work with radiation has shown that hypoxic conditions afford some degree of physiochemical radioprotection because of an abundance of thiol (SH) substances which provide for hydrogen donation to detrimental free radicals formed during irradiation(2,12, 16). In our present study, the deleterious effects of 100% O_2 are not seen at the fracture site, perhaps because of the existing anoxia and the presence of thiol groups. Another possibility is poor diffusion of oxygen at the fracture site. Goldhaber(11) showed that a stagnant bone culture medium was necessary for maintenance of cell viability in an environment of 100% O_2, but cell death occurred if oxygen diffusion was encouraged by the use of a roller-drum device. Drew(7) exposed HeLa cells to 95% O_2 and showed that up to 12 hours there is an increase in incorporation of H^3TDR. This is followed by a decrease in its incorporation, a fall in

labeling indices, and a reduction in DNA synthesis. It was suggested that pershaps the burst of incorporation of H^3TDR within the first 12 hours was due to an initial depletion of endogenous DNA precursors. Similar results were observed in the intact femora exposed to 100% O_2, since at 8 and 12 hours the labeling was higher than that observed at 30 hours and also in the control group subjected to air.

Summary. It is concluded from the present H^3TDR autoradiographic study of mouse femora that 5% oxygen leads to suppression of proliferation in an intact periosteum and cessation of proliferation when the periosteum is injured. The deleterious effects of 100% oxygen on the proliferation of cells are not realized at the fracture site, perhaps because of the protection afforded by the existing anoxia. In the intact limb there is evidence to show an early deleterious effect of 100% oxygen.

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Suppression of Toxoplasmin Skin Reactivity in Sensitized Guinea Pigs by Multiple Inoculations. (31802)

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An earlier publication described a laboratory procedure for determining the potency of toxoplasmins used as intradermal test antigens for diagnosis of toxoplasmosis(1). Subsequent experiments have demonstrated an inhibitory effect on skin reactions when 2 or more toxoplasmin preparations or dilutions of the same preparation are tested simultaneously in the same animal. The results presented here show that strongly reactive antigens may suppress lesser reactive antigens and that mutual suppression may occur between preparations of approximately equal potency.

Materials and methods. Sensitization of guinea pigs. Toxoplasmin prepared from