Effects of Hyperoxia and Hypoxia on Mitosis in the Normal and Regenerating Rat Liver (33894)

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In a previous paper (1), we reported that centrifugation at 2.5 and 4.7*g* of partially hepatectomized rats delays the initiation of mitosis in the regenerating rat liver, the delay being proportional to the magnitude of the *g*-load. A possible cause for this delay appeared to be hypoxia either of the whole animal or of the liver *per se*. Cardiovascular and respiratory disturbances are consequences of centrifugation, which could elicit a hypoxic state of the whole animal. Barer *et al.* (2) postulated that acceleration stress interferes in the exchange of gases between the alveolar spaces and alveolar blood vessels. The occurrence of localized ischemia of the liver due to centrifugation was reported earlier (1).

While the effects of hypoxia on mitosis are of great interest, the effects of hyperoxia are also of concern, especially in light of a report by Bullough and Johnson (3) showing that mitotic activity of mouse ear epidermis *in vitro* increases proportionally with increased oxygen tension.

In this report, we demonstrate the effects of hyperoxia and hypoxia on mitotic activity in the regenerating and normal rat liver by exposing partially hepatectomized and unoperated rats to four different environmental conditions: 100% O_2, 760 mm Hg; 100% O_2, 258 mm Hg; 100% O_2, 191 mm Hg; and air, 380 mm Hg. The first condition is hyperoxic, normobaric; the second, hyperoxic, hypobaric; the third, normoxic, hypobaric, and the fourth, hypoxic and hypobaric.

Methods. Two low-pressure chambers with a capacity of 13 ft^3 each were used in this study. The chambers were illuminated with fluorescent light automatically set for on–off cycling at 6:00 a.m. and 6:00 p.m., respectively. The temperature and relative humidity in the chambers were maintained at 21–24° and 40–70%, respectively. On occasion, the relative humidity approached 100% in the pure oxygen experiments; however, control animals were exposed to the same conditions. The barometric pressure was automatically controlled within ±5 mm Hg of the desired pressure by a Hg manometer-solenoid valve switching device similar to that described by Dowsett *et al.* (4).

Male Sprague-Dawley rats, weighing 140–180 g, were partially hepatectomized under ether anesthesia by the procedure of Ralli and Dumm (5). The animals were sacrificed between 8:30 a.m. and 10:30 a.m. to control the effects of diurnal variation in mitosis. Histological preparation of the liver and the method of mitotic analysis have been described in an earlier paper (1).

The partially hepatectomized animals were operated on in groups of 6–8, housed in individual cages, and then placed in the chamber within 1 hr after surgery. Partially hepatectomized and unoperated rats were exposed for 18, 22, 26, and 34 hr to the four environmental conditions described above. Control groups for both the operated and unoperated animals were placed in one chamber for identical time periods but kept at ambient conditions (air, 760 mm Hg). All animals were sacrificed within 1 hr after removal from the chambers. In this report, all animals exposed to any of the four environmental conditions will be referred to as experimental animals, while all animals kept at ambient conditions will be referred to as control animals.

All experimental rats were given food and water *ad libitum*. Since the rats exposed to the pure oxygen environments maintained weights comparable to those of the *ad libitum* fed controls, no pair-fed controls were used for these groups. However, pair-fed rats were used as controls for the rats exposed to the hypoxic environment (air, 380 mm Hg) since

TABLE I. Mitotic Indices[a] of Regenerating Livers from Partially Hepatectomized Rats Exposed to 100% O_2 at 760, 258, and 191 mm Hg.

Post-operative time (hr)	Exposure time (hr)	Group I			Group II	
		Control: ambient; *ad libitum* fed	Experimental: 100% O_2		Control: ambient; *ad libitum* fed	Experimental: 100% O_2 at 191 mm Hg
			760 mm Hg	258 mm Hg		
20	18	0.24 ± 0.11[b] (8)[c]	0.06 ± 0.02 (8)	0.28 ± 0.11 (8)	0.09 ± 0.03 (8)	0.05 ± 0.02 (7)
24	22	5.88 ± 1.00 (8)	6.11 ± 1.10 (8)	5.74 ± 1.16 (8)	5.47 ± 0.92 (8)	5.18 ± 0.99 (7)
28	26	6.60 ± 0.65 (8)	6.87 ± 0.70 (8)	7.04 ± 0.84 (8)	7.06 ± 0.81 (8)	7.89 ± 1.39 (8)
36	34	3.71 ± 0.74 (8)	4.07 ± 0.32 (8)	5.60 ± 0.73 (7)	5.04 ± 0.73 (8)	3.12 ± 0.72 (6)

[a] Mitoses/100 nuclei.
[b] Mean ± SE.
[c] Number of animals.

food intake of these animals is reduced by 50% or more.

Results. The mitotic indices of regenerating livers from partially hepatectomized rats exposed to 100% O_2 at 760, 258, and 191 mm Hg and their corresponding *ad libitum* fed control groups are shown in Table I. Two different control groups were used in this study because the 100% O_2 experiments at 760 and 258 mm Hg (group I) were conducted at a different time than the 100% O_2 experiment at 191 mm Hg (group II).

When analyzed by Student's t test for differences between experimental and control values, the data show that 100% O_2 at normobaric and hypobaric pressures has no effect on mitosis in the regenerating rat liver during any of the time periods studied. One can also conclude that hypobaric pressure *per se* has no effect since the alveolar partial pressure of oxygen in a 100% O_2, 191 mm Hg environment is 104 mm Hg, which is equivalent to that in air at 760 mm Hg. Therefore, the hypobaric pressure is the only changed environmental condition in the 100% O_2 experiment at 191 mm Hg.

The mitotic indices of livers from unoperated rats exposed to 100% O_2 at 760, 258, and 191 mm Hg and their corresponding *ad libitum* fed control groups are shown in Table II. The study was divided into groups I and II for the same reasons stated above. Each unoperated control group consisted of data from 3 rats or less for each time period studied, the data being combined to form one group of 8–9 rats. Therefore, each unoperated control group does not represent any particular time interval studied.

The unoperated experimental groups showed a general reduction in mitotic activity after the 18-hr time period. The mitotic activity of the unoperated groups exposed to 100% O_2 at 258 and 191 mm Hg for 26 hr and the mitotic activity of the unoperated group exposed to 100% O_2 at 760 mm Hg for 34 hr were significantly different ($p < 0.05$) from the mitotic activity of their corresponding unoperated control groups as judged by the t test. However, this reduction in mitotic activity is not thought to be caused by the pure oxygen condition, but rather by other factors (see "Discussion"). Therefore, there appears to be no change in mitotic activity in the normal liver because of the pure oxygen environment.

Table III shows the mitotic indices of regenerating livers from partially hepatectomized rats that were exposed to air at a barometric pressure of either 380 or 760 mm Hg. The control group (760 mm Hg) was pair-fed to the experimental group (380 mm Hg). The data show a definite delay in the initiation of mitosis in the experimental group, the initiation not occurring until after the 28-hr

TABLE II. Mitotic Indices[a] of Livers from Unoperated Rats Exposed to 100% O_2 at 760, 258, and 191 mm Hg.

Exposure time (hr)	Group I			Group II	
	Control:[d] ambient; *ad libitum* fed	Experimental: 100% O_2		Control:[d] ambient; *ad libitum* fed	Experimental: 100% O_2 at 191 mm Hg
		760 mm Hg	258 mm Hg		
18		0.18 ± 0.06 (8)	0.16 ± 0.04 (8)		0.29 ± 0.06 (8)
22	0.11 ± 0.03[b] (9)[c]	0.08 ± 0.03 (8)	0.05 ± 0.02 (8)	0.33 ± 0.12 (8)	0.27 ± 0.12 (8)
26		0.07 ± 0.04 (8)	0.03 ± 0.01[e] (8)		0.04 ± 0.02[e] (8)
34		0.02 ± 0.01[e] (8)	0.05 ± 0.02 (8)		0.14 ± 0.05 (8)

[a] Mitoses/100 nuclei.
[b] Mean ± SE.
[c] Number of animals.
[d] Mean for each control group represents three rats or less from each exposure group.
[e] Significantly different from the control, $p < 0.05$.

postoperative time period, while mitosis has begun between the 20- and 24-hr postoperative time periods in the control group. The difference in mitotic activity was significant ($p < 0.01$) as the 28-hr postoperative time period.

When a comparison is made of the partially hepatectomized control animals in Table III, which were pair-fed, with those in Table I, which were *ad libitum* fed, the effect of reduced food intake can readily be seen; the mitotic response of the pair-fed controls appears delayed when compared to that of the *ad libitum* fed controls. The values of the partially hepatectomized control groups in Table I are higher at the 20-, 24-, and 28-hr postoperative time periods than the values of the corresponding group in Table III. At the 36-hr postoperative time period, the values of the former groups are lower because the mitotic activity is declining, while in the latter group, the mitotic activity probably has just reached its peak and is beginning to decline.

Table IV shows the mitotic indices of livers from unoperated rats exposed to air at 380 mm Hg and of pair-fed unoperated controls exposed to air at 760 mm Hg. The mitotic indices of the experimental group and the pair-fed control group are significantly different at the 18 ($p < 0.05$), 22 ($p < 0.05$), and 34 ($p < 0.01$) hr time periods. The hypoxic condition appears to have caused a

TABLE III. Mitotic Indices[a] of Regenerating Livers from Partially Hepatectomized Rats Exposed to Air at 380 mm Hg.

Postoperative time (hr)	Exposure time (hr)	Control: ambient; pair-fed	Experimental: air at 380 mm Hg
20	18	0.05 ± 0.02[b] (6)[c]	0.01 ± 0.004 (6)
24	22	1.86 ± 0.92 (8)	0.05 ± 0.02 (7)
28	26	5.23 ± 1.35 (6)	0.02 ± 0.004[d] (6)
36	34	5.74 ± 0.65 (6)	6.30 ± 0.79 (6)

[a] Mitoses/100 nuclei.
[b] Mean ± SE.
[c] Number of animals.
[d] Significantly different from the control, $p < 0.01$.

TABLE IV. Mitotic Indices[a] of Livers from Unoperated Rats Exposed to Air at 380 mm Hg.

Exposure time (hr)	Control: ambient; pair-fed	Experimental: air at 380 mm Hg
18	0.12 ± 0.04[b] (8)[c]	0.01 ± 0.004[d] (8)
22	0.02 ± 0.01 (7)	0.004 ± 0.003[d] (8)
26	0.04 ± 0.03 (8)	0.004 ± 0.002 (8)
34	0.04 ± 0.01 (6)	0.004 ± 0.002[e] (8)

[a] Mitoses/100 nuclei.
[b] Mean ± SE.
[c] Number of animals.
[d] Significantly different from the control, $p<0.05$.
[e] Significantly different from the control, $p<0.01$.

reduction in mitotic activity during the time periods studied.

Discussion. The data demonstrated that hypoxia delays the initiation of mitosis in the regenerating rat liver and causes a reduction in mitotic activity in the normal liver. No change in mitotic activity occurred in rats exposed to a hyperoxic condition. The purpose of conducting the 100% O_2, 191 mm Hg experiment was to determine whether a hypobaric pressure at a normal alveolar partial pressure of oxygen would have an effect on mitosis. Since the results showed no change in mitotic activity due to the hypobaric pressure, it could be eliminated as a cause for the changes observed in the mitotic activity of the rats exposed to the hypoxic environment.

One noteworthy trend in the unoperated groups is the general reduction of mitotic activity after the 18-hr time interval. Since this reduction occurs in experimental as well as in control animals, the reduction must be attributed to a condition that is common to all of the unoperated rats. The housing of the animals in individual cages and the new environment of the chambers may possibly have precipitated the reduction in mitotic activity since environmental changes are known to influence the regenerative process (6).

As discussed above, one purpose of the present study was to correlate the changes found in mitotic activity due to centrifugation (1) with changes found due to hypoxia (air, 380 mm Hg). Both centrifugation and hypoxia caused a delay in the initiation of mitosis after partial hepatectomy. This finding is probably not coincidental, but due rather to a common cause, namely, a lack of sufficient oxygen tension in the liver to support the level of oxidative metabolism necessary for the hyperactive regenerative state of the liver.

Glaister (7) showed that dogs subjected to a $3g$ positive acceleration display a drop in arterial oxygen tension and Trojan and Jilek (8) observed that radial acceleration of rats produces hypoxia of all organs including the central nervous system. Mazia (9) pointed out that, in general, studies on mitosis indicate that respiration is lowered during the visibly active stages of mitosis, but during the period just preceding mitosis, when the cell is preparing for division, oxidative metabolism is increased. Therefore, any condition that might reduce the oxidative activity of the cell might also have a deleterious effect on mitosis.

Summary. Unoperated and partially hepatectomized rats were exposed to 100% O_2 at 760, 258, and 191 mm Hg for various time periods up to 34 hr. No change in mitotic activity was noted in these animals when compared to appropriate controls. Similar groups of rats were exposed to a hypoxic condition (air, 380 mm Hg) for the same time periods as above. The partially hepatectomized group showed a delay in the initiation of mitosis, while the unoperated group showed a reduction in mitotic activity due to the hypoxic environment.

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Received Oct. 24, 1968. P.S.E.B.M., 1969, Vol. 131.

Alanine: Identification as Compound Interfering with Chromatographic Assays of Formiminoglutamic Acid in Urine* (33895)

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The estimation of urinary formiminoglutamic acid (FIGLU) has become widely used as an index of folic acid deficiency in man. FIGLU is a catabolic product of histidine metabolism which requires a folic acid coenzyme for its normal degradation to glutamic acid. When folic acid coenzymes are in short supply, FIGLU accumulates. Its level in urine, following a standardized histidine oral loading procedure, generally parallels the degree of folic acid deficiency (1).

Among the methods that have been employed for the detection and assay of urinary FIGLU are chromatograms on paper (2) and more recently on thin-layer cellulose (3). Paper chromatograms have not been popular because they lack sufficient sensitivity to assay FIGLU in the range of concentrations necessary for an accurate diagnosis of folate deficiency. The thin-layer technique is of considerable interest, however, because it is sufficiently sensitive for this purpose. However, in both paper and thin-layer chromatography, a ninhydrin reactive material migrates with the same R_f value as FIGLU, and is in sufficient cocentration so that its color interferes seriously with the estimation of the FIGLU present in the urine.

Since FIGLU does not have a free alpha-amino group which can react with ninhydrin, chromatograms for FIGLU are exposed to alkali prior to ninhydrin staining. Alkali removes the formimino moiety converting FIGLU to glutamate, which is ninhydrin reactive (Fig. 1, Channel 2a, 2b). To estimate FIGLU, the color intensity at the FIGLU position is compared with a known FIGLU marker. In *chromatograms of urine*, however, there is invariably considerable ninhydrin staining in the FIGLU position prior to alkali treatment (Fig. 1, Channel 4a). As a result, the color intensity of the FIGLU position *before* alkali treatment, must be subtracted from that *after* alkali treatment and compared with appropriate markers in order to estimate FIGLU concentration. This introduces significant error, even if the material is eluted and the color intensities, before and after alkali, are read in a spectrophotometer. In this report, results of studies to isolate and identify this interfering urinary compound are presented and its significance for the chromatographic detection of urinary FIGLU in clinical folic acid deficiency states is discussed.

Experimental Methods. To identify the unknown ninhydrin-positive compound(s) in urine interfering with FIGLU estimation the following plan was employed:

1. The urinary constituents were initially separated by one-dimensional paper chromatography and the material at the FIGLU locus was eluted.

2. This material was then subjected to two-dimensional chromatography and the compounds present were identified chromatographically by comparison of their position with those of known reference standards.

* Supported in part by Grants, AM-01778, National Institute of Arthritis and Metabolic Diseases, USPHS, and U-1275, New York City Health Research Council.