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Effect of Hydrazine on DNA Content of the Liver. (31800)

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A single sub-convulsive dose of hydrazine has been shown to produce hepatic lesions when introduced into laboratory animals by various routes of administration. Subcutaneous administration of the compound to dogs resulted in centrolobular fatty degeneration which progressed outwardly(1). Hydrazine exposure produced hypoglycemia and marked reduction of glycogen stores of liver and skeletal muscle(2). The depression of liver glycogen has been correlated biochemically and histologically with accumulation of liver lipid in rats(3). This hydrazine-induced lipid deposition was related to an elevation in plasma free fatty acids(4) and to increased rate of transport from the circulating fatty acid pool into liver(5). Moreover, hydrazine depressed conversion of glycine to liver glycogen(6) and CO_2(7). The incorporation of amino acids into protein of liver slices was enhanced by treatment of the animals with hydrazine(7). The increased incorporation of amino acids into protein was ascribed to an expanded amino acid pool size resulting from hydrazine-induced inhibition of conversion of amino acids to keto acids. For example, liver transaminases are inhibited by hydrazine *in vitro,* presumably *via* formation of pyridoxal hydrazone(8).

Increased protein biosynthesis might be expected to be accompanied by changes in ribonucleic acid (RNA) content of the liver. Hydrazine treatment increased total RNA and protein contents in the liver, but not in kidneys or gonads in rats(9). Since alterations in

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liver protein following hydrazine treatment were opposed to those due to fasting alone, these changes could not be ascribed to protein nutritional factors. Moreover, the change in liver protein correlated directly with increase in liver RNA. These results suggest that hydrazine treatment may stimulate liver protein biosynthesis *in vivo*. The present study demonstrates that hydrazine-induced changes in liver protein and RNA are preceded by alteration in liver deoxyribonucleic acid (DNA) content.

Methods. Four groups, each containing 30 adult male Sprague-Dawley rats, were treated as follows: two groups were injected intraperitoneally with 40 mg/kg of hydrazine (brought to pH of 7.4 with CO_2); of these 2 groups, one was fasted for 4 hours and the other for 24 hours before being sacrificed. The other 2 groups were injected intraperitoneally with isotonic saline, and one was fasted for 4 hours, the other for 24 hours before being sacrificed. At time of sacrifice, animals were anesthetized with sodium pentobarbital (50 mg/kg), and exsanguinated *via* the renal vein. Livers were removed, promptly weighed, and frozen. After 3 days, each liver was thawed, homogenized in distilled water, and concentrations of protein, RNA, and DNA were determined by the procedure of Wannemacher *et al*(10). An additional determination of DNA concentration was performed on an aliquot of the nucleotides liberated by perchloric acid hydrolysis of the DNA using the diphenylamine reaction(11).

Blood was collected in centrifuge tubes and allowed to clot overnight in the refrigerator. Total serum protein concentration was determined by the biuret method of Layne(12). Serum proteins were separated by paper electrophoresis, stained with bromphenol blue and their concentrations estimated using an "Analytrol" densitometer.

Data were analyzed statistically by an analysis of covariance with initial body weight considered as a covariant(13). Means for the various parameters were adjusted to take into account variation of the initial body weight. Standard deviations were calculated from the pooled variance from the 4 groups. Pooled variance represents the best estimate of the true variance of the population for each statistic(13). Prior to experimentation, a $p<0.01$ was selected as the acceptable level of significance.

The effect of hydrazine on the several parameters was obtained by comparing data from the hydrazine treated rats with data from the corresponding fasted controls for each time period. The effect of fasting alone was obtained by comparing data from the 4- and 24-hour control groups.

Results and discussion. Hydrazine treatment produced increases in total liver protein and RNA after 24 hours. These increases were similar in magnitude to those reported previously(9). Changes in liver protein and RNA correlated with an increased liver weight in the 24-hour animals (Fig. 1A, B, and C). Elevation in the quantity of liver protein and RNA 24 hours after hydrazine treatment was accompanied by a significant increase in protein/DNA and RNA/DNA (Table I). Similar changes in liver protein and RNA were not observed at 4 hours following hydrazine treatment (Table I and Fig. 1B, and C). In fact, protein/DNA was significantly lowered by hydrazine treatment at 4 hours in comparison to the control value (Table I).

Fasting alone produced a significant reduction in total protein (Fig. 1B) as well as protein/DNA (Table I). This result confirms previous findings in fasting animals(14). Therefore, the observed effects of hydrazine treatment on liver protein cannot be ascribed to lack of nutritional factors associated with fasting. This conclusion is supported by the fact that liver protein/RNA was constant for all 4 groups (Table I), indicating that protein found in the liver was quantitatively related to one component of protein synthesizing capacity of the organ. This ratio has been shown to be a constant *in vivo* when rats have been fed protein regimes of widely varying nutritional value(15).

Even though liver protein content was not affected at 4 hours, hydrazine treatment produced a significant increase in total quantity of liver DNA at this early time period (Fig. 1D). Increased liver DNA produced by hydrazine treatment persisted for at least 24

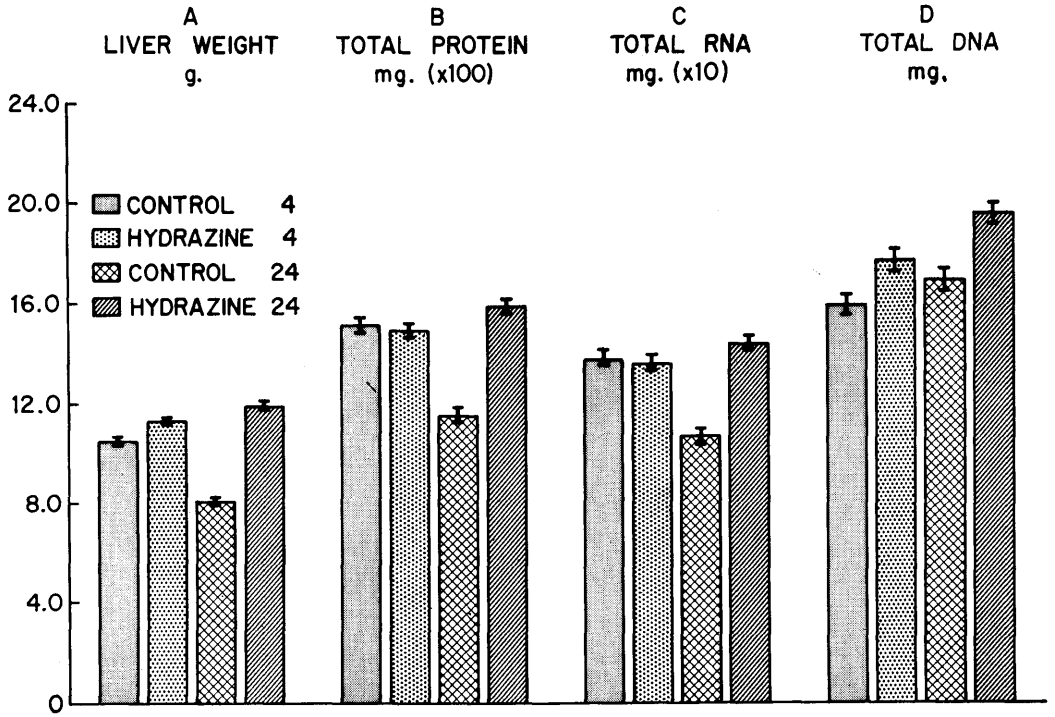

FIG. 1. Effect of hydrazine treatment on liver weight, total liver protein, total liver RNA and total liver DNA at 4 and 24 hours following treatment. The bar represents mean values (adjusted for initial body weight covariance). Standard error of mean is indicated at top of each bar(13).

hours, but this quantity was not affected by fasting alone. Although the increase in liver DNA following hydrazine treatment was not large, any significant alteration in quantity of functional DNA could produce profound alterations in the cellular protein biosynthetic mechanism.

Since the total quantities of DNA were altered by hydrazine treatment at both time periods, one cannot assume that DNA concentration was a direct function of the number of cells as has been reported for normal liver(16). This alteration in liver DNA content may represent an increased number of cells or it may be the result of a metabolic aberration such that some cells were stopped in a phase in which DNA was replicated, but the cell had not divided. In either event, results of these experiments indicate that hydrazine treatment produced a liver which contained a significantly increased ratio of protein to DNA after 24 hours.

Since Freese *et al*(17) reported that ultraviolet absorption of acid-liberated pyrimidine nucleotides was altered when isolated from T_4 phages after they were exposed to hydrazine, DNA concentrations were estimated by two methods; *viz*, the diphenylamine color

TABLE I. Comparison of Liver Protein and RNA Ratios for Control and Hydrazine-Treated Rats.

Treatment	Time (hr)	Protein/DNA*	RNA/DNA*	Protein/RNA
Control	4	100.37	8.950	11.48
Hydrazine	4	86.94	7.895	10.98
Control	24	70.88	6.512	10.83
Hydrazine	24	83.34	7.442	11.29
Standard deviation†		16.88	1.078	1.995

* Diphenylamine method.
† Calculated from the pooled variance. See Ref. 13.

TABLE II. Comparison of Liver DNA Concentration of Control and Hydrazine-Treated Rats by Two Assay Procedures.

Treatment	Time (hr)	DNA* (mg/g liver)	DNA† (mg/g liver)
Control	4	1.320	1.515
Hydrazine	4	1.399	1.569
Control	24	1.872	2.066
Hydrazine	24	1.402	1.623
Stand. dev.‡		.237	.220

* Ultraviolet absorption method.
† Diphenylamine method.
‡ Calculated from the pooled variance. See Ref. 13.

Summary. Hydrazine treatment resulted in increased levels of liver protein and RNA in rats at 24 hours following a single sub-convulsive dose of the compound. These changes were not observed as early as 4 hours after hydrazine treatment. However, increased levels of liver DNA were observed at 4 hours as well as at 24 hours in comparison to control values. These results might suggest that hydrazine treatment produced an initial aberration in liver DNA metabolism which resulted in subsequent alterations in the RNA and protein content of that organ.

TABLE III. Serum Protein Concentrations* of Control and Hydrazine-Treated Rats.

Treatment	Time (hr)	Total protein	Alb.	α_1	α_2	β	γ	A/G
Control	4	5.493	2.53	.99	.56	.81	.61	.86
Hydrazine	4	5.734	2.59	1.00	.58	.86	.70	.84
Control	24	5.602	2.52	1.03	.58	.85	.62	.82
Hydrazine	24	5.819	2.53	.96	.70	.93	.69	.78
Standard deviation†		.419	.10	.13	.12	.13	.17	.14

* Concentrations are given in g/100 ml serum.
† Calculated from the pooled variance. See Ref. 13.

reaction(11), which is specific for the deoxyribose portion of the molecule, and the method(10) based upon ultraviolet absorption of nucleotide bases. In our experiment the results from these two methods correlated very well with one another. The diphenylamine results were consistently higher, but the statistical significance of the several comparisons between experimental groups was unaffected by the assay method which was employed (Table II).

Results of these experiments are consistent with the hypothesis that hydrazine produced an initial alteration in DNA metabolism which resulted in an increased RNA content and consequently an increased protein content of liver. One might speculate that the increased protein may not include all liver proteins but only a selective group. Of the several serum proteins which appear to be produced in liver(18), only the *alpha* 2 globulin concentration was increased by hydrazine treatment after 24 hours (Table III). Concentrations measured are circulating levels; therefore, any inference concerning selective alteration of protein biosynthesis by hydrazine would need to consider the possibility of a selective release of these proteins from liver into serum.

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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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