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NUCLEIC ACID METABOLISM OF THE BONE MARROW AND SPLEEN IN THE RAT

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NUCLEIC ACID METABOLISM OF THE BONE MARROW AND SPLEEN IN THE RAT

The Effect of X-ray and the Effect of Hypoxia

W.A. RAMBACH, M.D.
J.A.D. COOPER, M.D., Ph.D.
H.L. ALT, M.D., Ph.D.

Departments of Medicine and Biochemistry,
Northwestern University Medical School,
Chicago, Illinois

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The distribution and metabolism of the nucleic acids in various tissues and cells have been the basis of a number of investigations (1–4). Desoxyribose nucleic acid (DNA) is found in the cell nucleus where it forms a major component of the chromosomes. Although its rate of synthesis appears to be small in mature cells which do not undergo mitosis, its renewal rate is high in tissues with rapid mitotic rates. Ribose nucleic acid (RNA) occurs predominantl in the cytoplasm and is found in large amounts in the microsomes. It has been related to the synthesis of protein, whether for growth, maturation, or secretion.

A relation between the concentration of DNA and cellularity has been established for normal and pathologic human marrow by Davidson and coworkers (5, 6, 7) and Metais and Mandel (8), and for rat and rabbit bone marrow, spleen, and thymus by Lutwak-Mann and her associates (9–12).

The rate of incorporation of radiophosphorus and radiocarbon into the nucleic acids has been used as an index of their rate of synthesis. Andreasen and Ottesen (13) reported briefly on the rapid turnover rate of nucleic acids, particularly DNA, in the rat bone marrow. Martin et al. (14) measured the turnover rate in rat bone marrow following x-irradiation and bleeding. Lowrance and Carter (15) studied the effect of nitrogen mustard on nucleic acid turnover in the rabbit spleen and bone marrow and demonstrated a diminished incorporation of P^{32} into the DNA fraction of these organs. Abrams (16) followed the rate of incorporation of C^{14} labeled glycine into the nucleic acid purines of rabbit bone marrow and was able to demonstrate a depression of DNA and RNA synthesis after x-irradiation. In a recent study reported from this laboratory, a decrease in the rate of incorporation of radiophosphorus into the DNA fraction of rat bone marrow and spleen was observed following the administration of a surgical dose of sodium pentobarbital (17).

In the present study, combined morphologic, chemical, and tracer techniques have been used in a study of the effects of x-irradiation and hypoxia on the bone marrow and spleen of the rat.

METHODS

Sprague-Dawley strain male rats, approximately 3 to 4 months old, were employed. Their weights varied from 228 grams to 345 grams with an average of 284 grams. The rats received a diet of Rockland Dog Blocks and were fasted for 12 hours before injection of radiophosphorus.* The control animals were injected intraperitoneally with 2 microcuries of carrier-free radiophosphorus as sodium phosphate per 100 grams of body weight. The experimental rats received a similar dose of radiophosphorus, but were injected 24 or 96 hours postirradiation or 15 hours posthypoxia. All animals were sacrificed 4 hours after the administration of P^{32}. In general, one control animal was studied with each group of 2 or 3 experimental animals.

The irradiated animals received a dose of 800 r total body x-irradiation delivered at the rate of 22 r/min. from a 220-kv. machine operating at 20 ma. The filtration was 0.5 mm. of copper and 1.0 mm. of aluminum. The HVL (half value layer) was 1.15 mm. of copper. The target distance was 70 cm. The irradiated animals lost, on the average, 7 percent of their body weight in 24 hours and 25 percent in 96 hours.

Animals subjected to hypoxia were placed in a low-pressure chamber at a simulated altitude of 25,000 feet for 10 hours a day for 3 consecutive days. At this altitude, the partial pressure of oxygen is 58.89 mm. of Hg and is equivalent to an oxygen concentration of 7.74 percent at 760 mm. of Hg. (The latter figures are based on dry air and neglect

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immersion field was found, and the number of cells counted in control animals was arbitrarily designated as 100 percent cellularity. The cellularity of the experimental animals was expressed as a percentage of the control count. From this data, the differential cell counts were adjusted to a standard volume by the equation:

$$\text{Number of cells per standard volume} = A \times \frac{B}{100}$$

where A is the percentage of the cell type in the differential count and B is the total cellularity expressed as the percentage of the control. Adjustment of the differential count offers an index of the absolute change in the number of various cells for a unit bone marrow volume.

This procedure has been adapted by us for a study of splenic morphology. The cut surfaces of the spleen immediately adjacent to the section removed for chemical studies were each lightly touched 6 to 8 times to a clean, dry slide. The two portions of the spleen were then sectioned transversely and a similar number of imprints were prepared from the freshly cut surfaces. The imprints were stained with Wright's stain and differential counts were done. In order to obtain a fair sample of the morphologic divisions of splenic structure, eyepieces with restricted fields were used to make a "strip" type count. That is, the differential count of 200 cells was made along a straight line or strip beginning in the follicle and passing through the perifollicular envelope (24) into the red pulp. The distinction between these three zones is readily apparent in the imprint of the normal spleen. However, following irradiation, it was sometimes difficult to sharply delineate these areas because of the destruction of splenic architecture. In such instances the differential counts were made through areas of representative total cellularity.

In order to estimate splenic cellularity, areas of uniform distribution were selected and the average number of cells per oil immersion field were counted on several imprints. The average count of the control animals was designated as 100 percent cellularity and the count of the experimental animals was expressed as a percentage of the control count. Since hypoxia and irradiation produced marked changes in spleen size, the counts were corrected further for spleen volume by the following formula:

$$\text{Corrected cellularity} = \frac{A}{B/C}$$

where: A = Cellularity as percent of control.
B = Control spleen weight as percent original body weight.
C = Experimental spleen weight as percent original body weight.

We have assumed that changes in spleen volume are proportional to changes in spleen weight. The spleen differential count was adjusted by use of the value obtained in this formula rather than by the percentage cellularity as was done for the bone marrow. Adjustment of the cellularity and differential count for changes in spleen size allows an estimate to be made of the total change in cellularity and cell types which occurs during a change in size of the spleen.

Peripheral blood studies were made on tail blood before the animals were sacrificed. Platelet counts were estimated by the indirect method on films stained with Wright's stain.

RESULTS AND DISCUSSION

The immediate precursor of nucleic acid phosphorus is not known, but it probably occurs in the acid soluble phosphorus (ASP) fraction (4). Since the specific activity (SA) of the precursor influences the SA of the nucleic acid fractions, Lowrance and Carter (15) have presented the results of their nucleic acid turnover studies in terms of SA_{RNAP}/SA_{ASP} and SA_{DNAP}/SA_{ASP} ratios. In the present study, the specific activities of the ASP are presented to show that changes in this fraction are not responsible for the alterations observed in the SA_{RNAP} and SA_{DNAP} following irradiation and hypoxia.

Effect of X-Irradiation

Twenty-four hours following 800 r of x-ray, there is a decrease in the RNAP and DNAP content of bone marrow which becomes more marked in 96 hours (table I). The fall in DNAP is paralleled by the decrease in cellularity. Although there is also a decrease in RNAP following irradiation, the magnitude of the change is about half that of the DNAP. The decrease in RNAP is probably due both to the loss of cells and to a change in the relative proportion of cell types (table II). Certain cells, particularly the erythrocytic series, decrease rapidly, and at 24 hours the marrow consists largely of mature granulocytes, lymphocytes, and plasma cells. At 96 hours, reticulum cells, plasma cells, and young granulocytes are predominant. The fact that these cells have either a larger cytoplasmic to nuclear ratio, or have higher RNA content per se is probably responsible for the increase in the RNAP/DNAP ratio.

The marked drop in the SA_{DNAP} 24 hours after irradiation is considered to be due to both inhibition of mitosis and to loss of dividing cells, mainly of

TABLE II

Spleen differential counts; normal values and the effect of x-ray and hypoxia

	Control		24 hours postirradiation			96 hours postirradiation			30 hours hypoxia		
Number of animals	15		6			5			6		
Cells/oil immersion field (mean and range)	233 (208–235)		78 (61–115)			37 (26–55)			242 (228–254)		
Percent of control	100		33			16			104		
Percent of control corrected for spleen volume	100		17			3			184		
Differential count	Average (percent)	Range	Average (percent)	Range	Adj. Av.*	Average (percent)	Range	Adj. Av.*	Average (percent)	Range	Adj. Av.*
Blasts	1.0	1	1.4	1–2	0.2	1.3	1–2	0.0	0.3	0–1	0.6
Neutrophils (young)	3.3	3–4	0.0	0	0.0	0.3	0–1	0.0	10.0	9–11	18.4
Neutrophils (old)	3.3	2–5	26.3	18–34	4.5	2.3	1–4	0.1	10.0	7–13	18.4
Eosinophils	0.7	0–1	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Lymphocytes	82.4	80–84	15.0	8–23	2.5	5.4	4–7	0.2	50.0	45–56	92.0
Monocytes	0.3	0–1	0.3	0–1	0.1	0.0	0	0.0	0.0	0	0.0
Plasma cells	0.0	0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Megakaryocytes	0.0	0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Mast cells	0.0	0	0.3	0–1	0.1	0.0	0	0.0	0.0	0	0.0
Basophils	0.0	0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Mitoses	0.0	0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Reticulum cells	4.0	3–5	55.7	48–63	9.4	90.7	88–94	2.7	5.0	4–6	9.2
Prorubricytes	0.0	0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0
Rubricytes	5.0	3–7	1.0	1	0.2	0.0	0	0.0	24.7	22–28	45.4
M./F. ratio	19.0:1		99.0:1						3.0:1		

*Average adjusted for standard volume by correction for changes in spleen cellularity and volume.

the erythrocytic series. The fall in SA_{DNAP} precedes the morphologic change, for we have seen a sharp decline of the SA_{DNAP} 4 hours after x-irradiation when there is only slight change in the cellularity and differential count (25). Persistence of a normal SA_{RNAP} value at 24 hours can be explained by continuing maturation of the older cell elements. These changes are compatible with the known effect of x-irradiation in inhibiting cell division without altering maturation.

At 96 hours the SA_{DNAP} is significantly elevated over the 24-hour value and represents an increase in the average mitotic rate. Since the reticulum cells of the bone marrow, known to be radio-resistant (23, 26), are the only cells to show an absolute increase, tenfold at 96 hours, it may be postulated that they are the predominant elements engaged in mitosis. At 96 hours the SA_{RNAP} is above the normal level, indicating a continuation of growth and maturation of the cells present in the marrow.

Nucleic acid metabolism and its correlation with cellularity following x-irradiation is more difficult to evaluate in the spleen than it is in the bone marrow because of alterations in spleen size (table V). Twenty-four hours after irradiation the splenic volume is reduced to 50 percent of normal and at 96 hours it is only 20 percent of the control value. Twenty-four hours and 96 hours post-irradiation there is a progressive decline in the DNAP concentration in the spleen, and this closely parallels the decrease in cellularity (table III). In terms of the total spleen, the decrease in DNAP exceeds that of the bone marrow and mirrors a greater total loss of cellularity in the spleen.

As in the bone marrow, the fall observed in the RNAP represents a decrease in cellularity and an alteration in the relative proportion of cell types. At 24 hours lymphocytes and rubricytes have decreased sharply, and the relative number of old granulocytes and reticulum cells has shown a marked increase. By 96 hours the spleen is largely populated by reticulum cells. The change in cell types is reflected by an increased RNAP/DNAP ratio. This increase is essentially the same as that found in the bone marrow and indicates the similar morphologic alterations produced by x-irradiation in both organs.

The changes in the SA_{DNAP} and SA_{RNAP} in the spleen following irradiation resemble those of the bone marrow with the exception of the SA_{DNAP} at 96 hours which does not show an increase. Its failure to rise in 96 hours is consistent with the morphology, which shows an absolute decrease in all cell types.

The changes in the peripheral blood following irradiation differ from those reported by Rosenthal et al. (23) only in the failure to note an absolute rise in granulocytes 24 hours after irradiation (table IV). The increase in the red cell count and hemoglobin seen 96 hours postirradiation may be attributed to hemoconcentration associated with diarrhea and weight loss.

Effect of Anoxia

Fifteen hours following exposure to 30 hours of intermittent hypoxia, the DNAP content and cellularity of the bone marrow appear to be somewhat increased. The RNAP content rises about 35 percent and there is a small increase in the RNAP/DNAP ratio. These changes are compatible with the relative increase in the erythrocytic series, predominantly in the prorubricytes and the basophilic rubricytes. The SA_{DNAP} doubles following hypoxia and there is a smaller increase in the SA_{RNAP}. These changes represent an increased rate of mitosis and maturation and reflect the functional state of the marrow more clearly than morphologic studies. More active division in the erythrocytic series probably accounts for the greater proportion of the increased turnover rate.

An evaluation of the changes in nucleic acid metabolism in the spleen following hypoxia must take into account the increase of 84 percent in spleen volume. The DNAP content and cellularity per unit volume show little change, but the total DNAP content and cellularity are greatly increased. As in the bone marrow, the RNAP content per gram increases significantly. The SA_{DNAP} of the spleen increases eight times and the SA_{RNAP} doubles. This is correlated with a marked increase in the percentage of rubricytes and to a lesser extent of the the granulocytes. The absolute increase in these cells may be partly responsible for the increase in spleen size.

TABLE V

Normal spleen weights and the effect of x-ray and hypoxia

Group	No. of animals	Average weight (grams)	Average percent of body weight
Control	15	0.84	0.26
24 hours x-ray	6	0.33	0.13
96 hours x-ray	5	0.15	0.05
30 hours hypoxia	6	1.11	0.46