Some Factors Involved in the Effect of X-Irradiation on the Phosphatase Activity of Hematopoietic Tissues

By Edwin M. Uyeki and Paul R. Salerno

A number of enzymes from hematopoietic tissues have been studied with respect to their altered activities after exposure to x-irradiation. Among the enzymes found to be significantly different in x-irradiated animals are adenosine triphosphatase, 5-nucleotidase, and adenylate kinase. Concomitant with the changes in phosphatases, a decrease in the adenosine nucleotide titer of the spleens of irradiated animals was demonstrated. There appear to be several relationships held in common by the enzymes noted above, namely sensitivity to low doses of x-ray, presence in the hematopoietic tissues and a similar post-irradiation sequential pattern of alteration. Maximum alterations in enzymic activity are observed on about the third day after x-irradiation; after a sub-lethal dose (ca. 400 r) a gradual return to normal levels is observed.

Since these tissues undergo changes in cellular composition, it is possible that changes in biochemical activity reflect alterations in tissue morphology. In view of these considerations, it was of interest to further characterize the nature of these changes. Known factors which alter the composition of hematopoietic tissues were thus investigated, and enzyme studies were conducted on tissues of modified cellular composition.

Methods

Adult male rats weighing from 200 to 300 grams were employed for these studies and were maintained in air-conditioned cages and fed ad libitum. All doses of x-ray were given in single total-body exposures (200 keV, 15 ma., 0.5 mm. Cu and 1.0 mm. Al filters). Radiation was delivered at a dose rate of 130 r per minute. ATPase activity was assayed according to the procedure of DuBois and Potter. To separate the cellular elements from the supportive tissue of the spleen a specially constructed syringe described by Thomas was constructed. Splenic cells were freed by mincing the tissue with a pair of scissors and forcing the brei through variously sized mesh screens (50 mesh, 0.28 mm. opening; 120 mesh, 0.117 mm. opening; 200 mesh, 0.074 opening) by applying pressure on the plunger. Either 0.9 per cent NaCl or 0.40 M sucrose in citrate buffer (pH 6.0) was used as the suspending medium. The cells were counted on a hemocytometer and the ATPase activity of the spleen fraction was expressed in units per 10^7 cells. The isolation procedure was carried out in the cold.

For the isolation of bone marrow cells, the marrow was collected by extruding the marrow from the femur by air pressure. The marrow was collected from the femurs of three to four animals and the cells were further isolated by the use of the wire mesh syringe.

The lymph fluid was collected from the thoracic duct by means of a fine polyethylene catheter leading into a test tube which was kept cold during the collection period.

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lymph was centrifuged for 5 minutes at 200 rpm to separate the supernatant fluid from the lymphocytes.

Peripheral blood leukocytes were isolated by the procedure of Skoog and Beck,* in which a 3 per cent dextran solution in saline was used to accelerate sedimentation of the red blood cells. The supernatant layer was removed and centrifuged; the isolated leukocytes were then analyzed for their ATPase content.

**EXPERIMENTAL PROCEDURES AND RESULTS**

Since the method of determining ATPase activity is convenient and sensitive, this enzyme assay was employed to characterize the nature of the altered phosphatase activity noted above from hematopoietic tissues upon x-ray exposure. Although histochemical methods have revealed the presence of other phosphatases in blood cells, the amounts of 5-nucleotidase, glucose-6-phosphatase and \( \beta \)-glycerophosphatase in peripheral blood leukocytes were not detectable at a significant order of magnitude in cell concentrations and incubation periods employed in our studies. A number of phosphatase enzymes (ATPase, 5-nucleotidase, glucose-6-phosphatase, and \( \beta \)-glycerophosphatase) were not demonstrable in comparable concentrations of erythrocytes.

The prior demonstration of the extreme sensitivity of the lymphoid tissue to ionizing radiations led us to utilize measures other than x-ray for producing dissolution of the lymphoid elements. The relative specificity of x-rays in altering the enzyme content of hematopoietic tissue might thus be further characterized. It has been shown that the hormones of the adrenal cortex (corticotropin as well as the adrenal cortical steroids) cause destruction of the lymphoid tissues. To establish whether or not adrenalectomy results in alteration of the enzyme activity, rats were adrenalectomized by a dorsal incision, and the animals were maintained on a 1 per cent NaCl diet in a constant-temperature room. The animals were sacrificed at 1, 2 and 10 days after adrenalectomy and analyzed for their ATPase content. The results indicated that there was no appreciable change in the ATPase activity of the spleen, thymus and liver (table 1). Thus, although gross organ weights of lymphoid organs are known to increase with adrenalectomy, the ATPase activity was not modified to any appreciable extent.

It is well known that the lymphocytic elements in the intact animal are very sensitive to ionizing radiations. The adrenal cortex has been shown to exert a lymphocytolytic effect in tissues of the body. Starvation was utilized as a stress condition to enhance adrenal cortical activity, and thus to produce destructive changes in the lymphoid tissues of the animals. Enzyme studies were conducted at 2, 4 and 6 days after food withdrawal. The results of these studies are shown in table 2.

There is a gradual increase in enzyme activity throughout the starvation period. Six days after food withdrawal there was an increase of 36 per cent in the ATPase activity of spleen tissue of starved rats as compared with controls. The enzyme activity in thymus tissue indicated that there was a twofold increase in activity.

Adrenal cortical steroids such as cortisone have also been shown to produce dissolution of the lymphoid organs. Animals were injected daily (subcuta-
TABLE 1.—Adenosine Triphosphatase Activity of Adrenalectomized Rats*

<table>
<thead>
<tr>
<th>Days After Adrenalectomy</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.9</td>
<td>7.0</td>
<td>11.0</td>
</tr>
<tr>
<td>1</td>
<td>19.3</td>
<td>5.5</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>27.3</td>
<td>7.1</td>
<td>11.8</td>
</tr>
<tr>
<td>10</td>
<td>18.9</td>
<td>7.0</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Data expressed as μg. of phosphorus liberated per mg. of tissue per 15 minute incubation. Each value represents the average of at least 3 animals in which 2 levels of tissue were employed for each animal.

TABLE 2.—Effect of Starvation on the Adenosine Triphosphatase Activity of Rats*

<table>
<thead>
<tr>
<th>Days after Food withdrawal</th>
<th>Spleen</th>
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</tr>
<tr>
<td>2</td>
<td>27.1</td>
<td>7.1</td>
<td>15.7</td>
</tr>
<tr>
<td>4</td>
<td>27.8</td>
<td>9.0</td>
<td>16.6</td>
</tr>
<tr>
<td>6</td>
<td>31.0</td>
<td>14.6</td>
<td>12.4</td>
</tr>
</tbody>
</table>

*Data expressed as μg. of phosphorus liberated per mg. of tissue per 15 minute incubation. Each value represents the average of at least 3 animals in which 2 levels of tissue were employed for each animal.

neously) with a dose of 2 mg. or 5 mg. of cortisone acetate per rat for a period of seven days. In animals injected with a daily dose of 2 mg., only a 10 per cent increase was noted in the spleen, whereas the thymus gland showed an increase greater than twofold. When 5.0 mg. of cortisone acetate was injected daily for a period of seven days, the spleen activity increased by 50 per cent and the thymus tissue activity was three times that of control values.

It has been postulated that the pronounced increase in activity of the spleen is primarily due to depletion of the cellular elements of the spleen. To test this hypothesis a specially constructed syringe was used to separate the cellular elements of the spleen. The results of this study are shown in figure 1, in which the activities of the isolated cells were compared in control and x-rayed animals. Expressed in terms of 10⁷ cells, the table indicates that an increase in excess of fivefold over the control values was noted 3 days post-irradiation. Since these studies were conducted essentially on parenchymal cells, the rise in activity noted in the entire irradiated spleen cannot be attributed solely to the relative increase in the connective tissue elements of the whole spleen on exposure to ionizing radiations.

Connective tissue fragments in these preparations may constitute some source of enzymic activity. Further, the identification of the spleen cells isolated by this technic was difficult, owing to injury to some of the cells. By utilizing peripheral blood leukocytes for the enzyme source, one can eliminate connective tissue as a possible contaminant of the isolated cells which could give rise to the increased activity. Peripheral blood leukocytes isolated as outlined above from normal and irradiated rats were assayed for the adenosine triphosphatase activity, and the data were expressed in terms of units of activity per 10⁷ cells. Concomitant cell type analyses were also conducted in
it undergoes profound changes in cellular content on exposure to x-rays. Rats were used for these studies since their bone marrow composition is such that little or no lymphoid tissue is present. The differences in bone marrow cell types are essentially degrees of maturity of the erythroid and myeloid cells. The marrow was therefore analyzed on the basis of units of activity per unit weight, and the following observations were made (figure 3). Two and five days following 780 r of x-ray, the ATPase activity was actually found to decrease by 47 and 27 per cent of the control values, respectively. If, however, the cells of the bone marrow were isolated and the units of activity were expressed in terms of cellular population, the ATPase content of the bone marrow showed a very marked rise in activity. The results of this study are shown in figure 3. Twelve hours after doses of 390 and 780 r there were maximum increases in excess of five times those of control values. In the case of

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**Fig. 3.—Effect of total-body x-irradiation on the ATPase activity of rat bone marrow.** Each point represents the average of two experiments.
780 r, the activity continued to rise, whereas the 390 r values indicated that five days following 390 r of x-irradiation the ATPase activity returned to control values. In the rat bone marrow, this marked rise in enzyme activity cannot be caused by lymphocytic dissolution since lymphocytes comprise but a small portion in normal marrow.

**Discussion**

The observations of DuBois and Petersen\(^1\) have indicated that in spleen and thymus tissues of irradiated animals, the enzymes—adenosine triphosphatase and 5-nucleotidase—have an increased ability to hydrolyze these phosphate esters—adenosine triphosphate and 5-adenylic acid. We have continued these studies to include blood leukocytes and bone marrow tissues. Our studies on heterogeneous cell mixtures have indicated that several factors are involved in the altered enzyme activity of hematopoietic tissues after total-body x-irradiation.

Factors which modify the lymphoid distribution of the tissues under investigation will modify the adenosine triphosphatase activity of these tissues. Thus, with the use of procedures such as starvation or cortisone injection which promote lymphocytic destruction, a higher adenosine triphosphatase activity in the spleen and thymus was observed in the control animals. Moreover, the higher activity observed in the thymus tissue as compared with the spleen corroborates the idea that the thymus, having greater amounts of lymphoid elements than does the spleen, will display a greater rise in activity.

The increased enzyme activity of the spleen in x-irradiated animals cannot be attributed solely to the relative increase in the connective tissue fraction. The cellular fraction of the spleen after total-body x-ray revealed an adenosine triphosphatase activity in excess of five-fold greater than comparable controls. In the case of bone marrow ATPase activity, for which the values were expressed in terms of whole organ weight, a decrease was noted in the values of x-rayed specimens, as compared to the control values. However, when the cellular elements were isolated, the bone marrow cells indicated a fivefold increase, based on cellular population.

The increase in adenosine triphosphatase activity of hematopoietic tissues after total-body x-irradiation appears to be associated with the type of cells present in the assay medium. With respect to the peripheral blood leukocytes of the rat, the cell type is confined largely to lymphocytes and granulocytes. The increase in adenosine triphosphatase activity of peripheral blood leukocytes after total-body x-ray was seen to parallel the increase in granulocytes present in the assay medium. Moreover, in dogs the preponderant type of leukocyte in the blood is the granulocyte, and the ratio of granulocytes to lymphocytes is not appreciably altered by x-rays. The adenosine triphosphatase of leukocytes in dogs after exposure to total-body x-rays is likewise unaltered, which indicates that the primary change in enzyme activity after irradiation is brought about through changes in the cell types of the assay medium and not in the enzymes per se. Studies of the lymphocytes from the thoracic duct of the rat before and after x-ray indicated that there was no significant change in ac-
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activity. Thus, it appears that in cases where the cell composition of the assay medium is essentially unaltered one finds little effect on the adenosine triphosphatase activity.

In the bone marrow of the rat, the normal composition is such that the lymphocytes present represent a small percentage (up to 10 per cent of the total cells as compared with the large percentage of lymphocytes present in the peripheral blood (approximately 80 to 85 per cent). Thus, the fivefold increase in enzyme activity resulting from whole-body radiation cannot be due to the destruction and removal of the lymphocytes from the assay medium. A considerable portion of the composition of rat bone marrow consists of the immature forms of the erythroid and myeloid series. Thus, in the primary hematopoietic tissue of the rat, the increased adenosine triphosphatase activity parallels the percentage increase of granulocytes, macrophage and stem cells present in the assay medium.

In such a heterogeneous cell mixture it is not possible to clearly estimate the activity of the different cell types. However, studies of such cell mixtures do reveal that x-radiation of the whole animal results in an increased enzyme activity of isolated cells of the peripheral blood, bone marrow and spleen tissue.

SUMMARY

Factors which modify lymphoid distribution of tissues were found to modify the adenosine triphosphatase activity of these tissues. Starvation or cortisone injection, which produces destructive changes in lymphoid tissues, was found to increase the enzyme activity of spleen and thymus tissues. The greater increment of enzyme activity of the thymus as compared to that of the spleen was correlated with its normally higher content of lymphoid tissue.

The increase in adenosine triphosphatase activity of hematopoietic tissues appears to be associated with the type of cells present in the assay medium. With respect to peripheral blood leukocytes of the rat, the cell type is confined largely to lymphocytes and granulocytes. The increase in adenosine triphosphatase activity of the leukocytes after total-body x-ray was seen to parallel the increase in granulocytes present in the assay medium. The ratio of granulocytes to lymphocytes is not appreciably altered in dog peripheral blood after exposure to total-body x-ray; the adenosine triphosphatase activity similarly was not significantly altered. After total-body x-ray (390 r and 780 r), cells isolated from the rat bone marrow displayed a fivefold increase in adenosine triphosphatase activity. This increase was seen to correspond with an increase in the ratio of segmented leukocytes and reticuloendothelial cells and a decrease in the immature forms of the erythroid and myeloid cells.

The heterogeneous cell mixtures used for our assay procedures permit the observation that total-body x-irradiation results in an increased enzyme activity of the isolated cells of the peripheral blood, bone marrow and spleen tissue of the rat. The increased enzyme activity was associated with the increased ratio of cells with high enzyme activity present in the assay medium.
SUMMARIO IN INTERLINGUA

Esseva constatate que factorcs que modifica le distribution lymphoide del histos effectua un modification del activitate de adenosino-triphosphatase in ille histos. Esseva trovate que affamation o injectiones de cortisona, que produce alterationes destructive in histos lymphoide, augmenta le activitate enzymatic in le histos del splen e del thymo. Le plus marcate augmento del activitate enzymatic in le thymo in comparation con illo in le splen se monstrava correlati de sum normalmente plus alte contento de histos lymphoide.

Le augmento del activitate de adenosino-triphosphatase in le histos hematoopoietic pare esser associate con le type de cellula que es representate in le medio de esssayage. Quanto al leucocytos de sanguine peripheric de rattos, le typo de cellula es restringite in grande mesura a lymphocytos e granulocytos. Esseva notate que le augmento del activitate de adenosino-triphosphatase de leucocytos post roentgeno-irradiation del corpore total esseva parallel al augmento del granulocytos presente in le medio de esssayage. Le proportion inter granulocytos e lymphocytos in le sanguine peripheric de canes non es alterate appreziabilemente post roentgeno-irradiation del corpore total. Similemente, il esseva trovate que le activitate de adenosino-triphosphatase non esseva alterate significativemente. Post roentgeno-irradiation del corpore total (de 390 e 780 r), cellulas isolate ab le medulla ossee de rattos manifestava un quintuple augmento del activitate de adenosino-triphosphatase. Esseva notate que iste augmento corrispondeva a un augmento in le proportion inter leucocytos segmentate e cellulas reticuloendothelial e a un diminution del formas immatur de cellulas erythroide e myeloide.

Le heterogenee mixturas cellular quc esseva usate in nostre labores de esssayage permette le observation que le roentgeno-irradiation del corpore total resulta in un augmentate activitate enzymatic del cellulas isolate de sanguine peripheric, de medulla ossee, e de histo splenic de rattos. Le augmentate activitate enzymatic esseva associate con un augmentate proportion de cellulas a alte activitate enzymatic presente in le medio de esssayage.

REFERENCES

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