

## Effect of irradiation on electrophoretic properties of enzymes in haemopoietic cells of opossum

N. Prasad, R. Prasad, S. C. Bushong and L. B. North

Department of Radiology, Baylor College of Medicine, Texas Medical Center, and Veterans Administration Hospital, Houston (Texas 77030, USA), 26 July 1976

**Summary.** 5-month-old male opossums were exposed to 5000 rads whole body  $^{60}\text{Co}$  radiation and sacrificed at 16, 40 and 90 h following irradiation. Starch gel electrophoretic studies of dehydrogenases of lactate, malate, 6-phosphogluconate and glucose-6-phosphate and  $\alpha$ -esterases and acid phosphatase were conducted on the homogenates of spleen and bone marrow cells. Expressions of LDH and G6PD were affected by irradiation in bone marrow and spleen cells.

Opossums (*Didelphis virginiana*) are unique animals for experimental biology because their litters are directly accessible prenatally to experimental manipulation without the interference of maternal tissues<sup>1-3</sup>. Their anatomical and physiological features at birth are similar to a 12-day-rat or an 8-week-human<sup>1,4</sup>. Research involving opossum has been widely used in the fields of immunol-

ogy<sup>5,6</sup>, neurology<sup>7</sup>, physiology<sup>8</sup>, and embryology<sup>9</sup>. Studies dealing with enzyme systems and radiation have not been conducted on this animal. The purpose of this paper is to present data on the effect of ionizing radiation on electrophoretic properties of enzymes in bone marrow and spleen cells of opossum.

**Materials and methods.** 7 male opossums, *Didelphis virginiana*, ranging in weight from 0.75 to 1.0 kg and approximately 5 months old, were used in this experiment. All were born of the same mother and 5 were irradiated while 2 served as controls. The animals were exposed to a single dose of 5000 rads whole body  $^{60}\text{Co}$  radiation at a dose-rate of 125 rads/min.

Irradiated animals were sacrificed by cervical dislocation at 16, 40 and 90 h (2, 1 and 2 animals respectively). Control animals were also sacrificed. Spleen and bone marrow cells were removed and were homogenized in deionized distilled water. Crude extracts of the tissues were obtained by centrifuging the homogenates at  $10,000 \times g$ . All crude extracts were subjected to vertical starch gel electrophoresis. The gel slices were stained for  $\alpha$ -esterase, acid phosphatase and dehydrogenases of lactate, malate, 6-phosphogluconate and glucose-6-phosphate, according to the method of Shaw and Prasad<sup>10</sup>. The electrophoretic studies were repeated three times for all the samples.

**Results.** Bone marrow and spleen tissues of all control animals showed five distinct bands of lactate dehydrogenase (figure 1). LDH-1 was absent in spleen homogenates of all irradiated animals. In bone marrow of irradiated animals both the LDH-1 and LDH-2 were absent. In the control animals glucose-6-phosphate dehydrogenase was expressed as double bands in spleens and triple bands in bone marrow cells (figure 2). In bone marrow homogenates of all irradiated animals, only one of the isozymic forms (medium band) was expressed (figure 2). In spleens of irradiated animals both isozymes were expressed, but in these animals usually the fastest moving band was more prominent, whereas in the spleen of the control animal, both bands were distinctive.

The 6-phosphogluconate dehydrogenase was expressed as a single band in all the tissues studied of irradiated and control animals. No significant changes were seen in the expression of this enzyme, following irradiation.

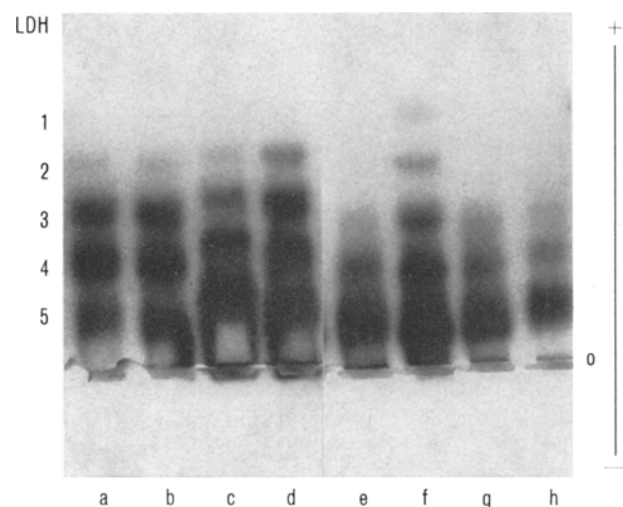


Fig. 1. Starch gel electrophoretic patterns of lactate dehydrogenase in opossum spleen and bone marrow homogenates following whole body irradiation (5000 rads). The samples are: a, b, c spleen homogenates at 16, 40 and 90 h respectively following irradiation; d control spleen homogenate; e, g, h bone marrow homogenates at 16, 40 and 90 h respectively following irradiation; f control bone marrow homogenate (o, origin).

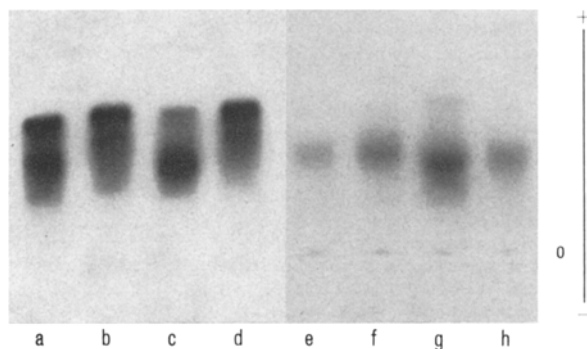


Fig. 2. Starch gel electrophoretic pattern of glucose-6-phosphate dehydrogenase in opossum spleen and bone marrow homogenates following whole body irradiation (5000 rads). Samples are: a control spleen homogenate; b, c, d spleen homogenates at 90, 40 and 16 h respectively following irradiation; e, f, h bone marrow homogenates at 90, 40 and 16 h respectively following irradiation; g control bone marrow homogenate (o, origin).

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Malate dehydrogenase seems unaffected by irradiation as the isozyme patterns from irradiated animals were similar to the control samples. Similarly, expression of  $\alpha$ -esterase and acid phosphatase was unaltered by irradiation.

**Discussion.** This study indicates that 5000 rads inhibits the expression of some forms of LDH and G6PD in bone marrow and spleen cells. In both types of cells, only the B-subunit of LDH is affected by radiation. Radiation induced inhibition of only the LDH-1 band in spleen cells and of both LDH-1 and LDH-2 in bone marrow cells suggests that the B-subunit of LDH from bone marrow cells is more sensitive to radiation than that from spleen cells.

The presence of 2 isozymic forms of G6PD in spleen but 3 such forms in bone marrow cells perhaps indicate tissue specificity of this enzyme. The expression of 2 isozymic forms is inhibited by radiation in bone marrow cells while only one of the isozymic forms is affected in the spleen cells. This again indicates that G6PD from bone marrow cells is more sensitive to radiation than that from the spleen cells.

In our previous studies the LDH<sub>50/30</sub> of opossum was reported to be 511 rads<sup>11</sup>, however, this dose of radiation does not affect the electrophoretic property of enzymes. Our enzyme study in opossum testes indicates that electrophoretic pattern of LDH is affected at 16 and 40 h following 5000 rads whole body irradiation<sup>12</sup>. After 90 h following the radiation, the electrophoretic pattern was the same as in the control, indicating recovery or repair. In spleen and bone marrow cells, this affect of radiation was not observed.

It is concluded that among the 6 enzymes studied, only LDH and G6PD are affected by radiation. Also LDH and G6PD from bone marrow cells are more sensitive to radiation than that from spleen cells.

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## Nitrate fertilizers as environmental pollutants: Positive correlation between nitrates (NaNO<sub>3</sub> and KNO<sub>3</sub>) used per unit area and stomach cancer mortality rates

R. ZALDÍVAR<sup>1</sup>

*Epidemiological Research Unit, Regional Hospital, National Health Service, Antofagasta (Chile), 29 June 1976*

**Summary.** Since a significant positive correlation between nitrate fertilizer exposure and stomach cancer mortality has been found, a study of nitrate fertilizer pollution in arable land in terms of amount of nitrates used per unit area (kg/ha) and stomach cancer death rates was carried out. The regression of death rates for stomach cancer, standardized for age, on the nitrates used per unit area, by province ( $N = 25$ ), exhibited a significant association between the 2 variables ( $p < 0.004$ ). When industrialized provinces ( $N = 3$ ) were not considered, a highly significant association was found ( $F = 16.47$ ; d.f. = 1, 20;  $p < 0.0006$ ). The correlation coefficient was + 0.672.

In previous communications<sup>2-4</sup>, a positive relationship has been established between exposure to nitrate fertilizers (NaNO<sub>3</sub> and KNO<sub>3</sub>) and age-adjusted death rates per 100,000 population for stomach cancer, by province, in Chile. Since nitrates and nitrites are precursors of nitrosamines, a hypothesis of a nitrosamine biosynthesis in the stomach of Chileans was advanced<sup>5</sup>.

In order to learn more on this environmental health problem, the nitrate fertilizer pollution in the various provinces ( $N = 25$ ) was studied. Nitrates (NaNO<sub>3</sub> and KNO<sub>3</sub>) per unit area, expressed as kilograms per hectare, were computed for each province. The present communication provides information on nitrate fertilizer pollution in arable land and stomach cancer mortality.

**Material and methods.** Data on the area of arable land, by province, were taken from the 4th National Agricultural Census for the year 1964-5. The amounts of nitrate fertilizers (NaNO<sub>3</sub> and KNO<sub>3</sub>) used for the 1960-4 period, by province, were kindly provided by The Chemical and Mining Society of Chile, at Santiago. Data on the age-adjusted death rates per 100,000 population, both sexes combined, for stomach cancer (mean rates for the years 1960, 1962 and 1964), by province, were taken from a previous communication<sup>5</sup>. This gastric cancer mortality study was timed to coincide with a census year, in order to provide a satisfactory population base for computing rates. The square roots of mean annual values for nitrates used per unit area (kg/ha) in a 5-year-period (1960-4) for each one of the 25 provinces were obtained. Then, the

death rates for stomach cancer were regressed on the square roots of nitrates used per unit area (kg/ha).

**Results and discussion.** The regression of stomach cancer mortality rates on the nitrates used per unit area exhibited a significant association between the 2 variables ( $F = 10.05$ ; d.f. = 1, 23;  $p < 0.004$ ). The equation of the regression line is:  $Y = 35.554 + 1.781 X$ , where  $Y$  = age-adjusted mortality rates for gastric cancer and  $X$  = nitrates used per unit area.

In order to eliminate the interference of occupational or industrial factors in the aetiology of gastric cancer, such as iron dust<sup>6</sup>, coal dust<sup>7,8</sup>, and suspended particulate air pollution<sup>9</sup>, industrialized provinces (Concepción, Santiago, Valparaíso) were not included in the analysis.

<sup>1</sup> Requests for reprints should be addressed to: R. Zaldivar, 2170 N.W. 11th Street, Suite 53, Miami (Florida 33125, USA).

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