

Malate dehydrogenase seems unaffected by irradiation as the isozyme patterns from irradiated animals were similar to the control samples. Similarly, expression of α -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_{50/30} of opossum was reported to be 511 rads¹¹, 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 irradiation¹². 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 (NaNO₃ and KNO₃) used per unit area and stomach cancer mortality rates

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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 communications²⁻⁴, a positive relationship has been established between exposure to nitrate fertilizers (NaNO₃ and KNO₃) 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 advanced⁵.

In order to learn more on this environmental health problem, the nitrate fertilizer pollution in the various provinces ($N = 25$) was studied. Nitrates (NaNO₃ and KNO₃) 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 (NaNO₃ and KNO₃) 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 communication⁵. 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 dust⁶, coal dust^{7,8}, and suspended particulate air pollution⁹, industrialized provinces (Concepción, Santiago, Valparaíso) were not included in the analysis.

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The regression of mortality rates for gastric cancer on the nitrates used per unit area showed a highly significant association between these variables ($F = 16.47$; d.f. = 1, 20; $p < 0.0006$). The linear regression equation is: $Y = 33.826 + 2.161 X$, where Y = age-adjusted mortality rates for stomach cancer and X = nitrates used per unit area. The correlation coefficient was $+ 0.672$.

It is interesting to note that Curicó Province, a part of the Central Valley, which showed a mean annual value of 82.2 kg/ha, had one of the highest age-adjusted mortality rates per 100,000 population for stomach cancer in the world (84.1 for males, 40.8 for females) in the years 1960, 1962 and 1964. Also, Talca Province, located in the Central Valley, which showed a mean annual value of 64.3 kg/ha, had extremely high age-adjusted death rates per 100,000 population for stomach cancer (72.3 for males, 35.9 for females) in 1960, 1962 and 1964.

The gastric cancer mortality rate amongst males from Curicó Province (84.1/100,000) is surpassed only by the male rate from Miyagi Prefecture in Japan (95.3/100,000)¹⁰. The gastric cancer mortality rate amongst females from Curicó Province (40.8/100,000) is the highest in the world.

Curicó and Talca Provinces, together with the other provinces of the Central Valley, have a Mediterranean agriculture. Curicó and Talca have, as main crops, wheat, beans, maize, rice, a wide variety of fruits (grapes, oranges, lemons, apples, peaches, apricots, plums, figs, pears, cherries, etc.), and green vegetables such as lettuce, cabbage, onions and celery. The author believes that the estimation of total nitrate levels of the diet of people living in high- and low-risk areas for gastric cancer may provide data of key interest.

Because of the serious biological effects of nitrogen fertilizers on the environment, elimination of fertilizer nitrogen use for 10 years has been called for in order to restore the balance of nature^{11, 12}.

¹⁰ R. DOLL, C. S. MUIR and J. WATERHOUSE (eds), in *U.I.C.C. Cancer Incidence in Five Continents*, vol. 2 (Springer Verlag, Berlin 1970).

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¹² B. COMMONER, in *Global Effects of Environmental Pollution* (Ed. S. F. Singer; Springer Verlag, New York 1970), p. 70.

Early detection of cell damage by supravital acridine orange staining

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Summary. Cell damage can be detected in living cells by acridine orange fluorescence earlier than with phase contrast microscopy or with conventional histological methods. The change in the acridine orange fluorescence from green to red indicates that the secondary structure of the DNA is altered very early during the cell death.

Acridine orange (AO) is a commonly used fluorescent dye. It has been shown that AO binds to the nucleic acids in a way which is dependent upon their secondary structure. It binds to double stranded helical DNA in a monomer molecular form causing a fluorescence emission maximum at 522 nm. With decreasing structural organization of the nucleic acid chains, the AO binds in an associated molecular form with a higher emission maximum. For instance, in random coiled poly (U) the emission maximum is about

665 nm². Usually these properties of AO can be seen in the fluorescence microscope as a green nuclear fluorescence of DNA and as a red cytoplasmic fluorescence of RNA.

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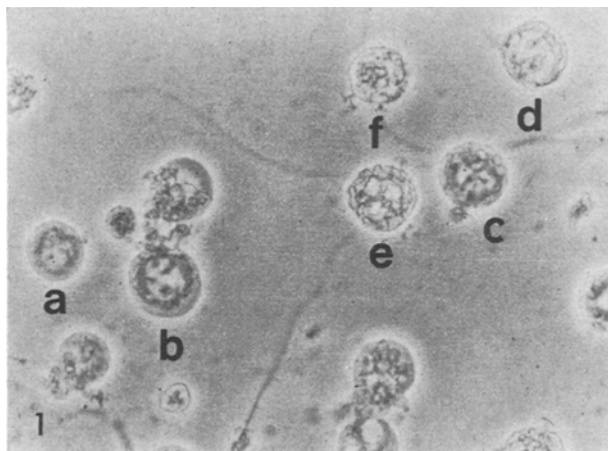


Fig. 1. A phase contrast microscopic picture of early postmeiotic cells from stage I of rat spermatogenesis. Vincristine (0.25 μ g) had been injected intratesticularly 24 h earlier. The cells a, b, c and d appear normal whereas the cells e and f show an increased negative phase change indicating cell damage.

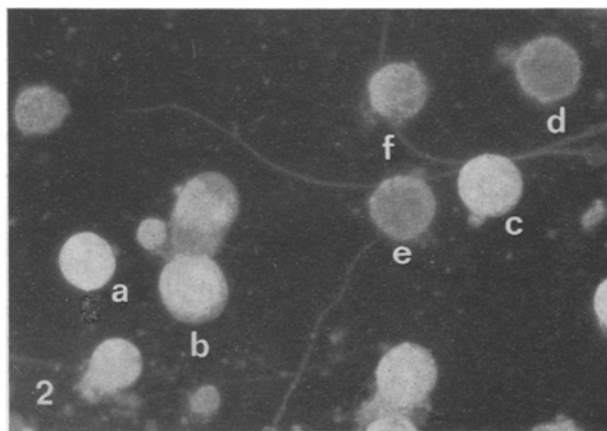


Fig. 2. A picture showing the AO fluorescence of the same early postmeiotic cells as in figure 1. A green nuclear fluorescence typical to normal cells is seen in cells a and b. The cell c has a somewhat increased red fluorescence of the nucleus and the cells e and f are clearly red. Note especially the cell d, which has a normal structure in the phase contrast picture but stains red with AO fluorescence as a sign of early cell damage.