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].

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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 nm[2]. 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.

1 Acknowledgments. We thank Miss Pirjo Vuori for excellent technical assistance, Engineer Charles Vane-Tempest for providing the necessary microscope equipment and the Finnish Academy for financial support.

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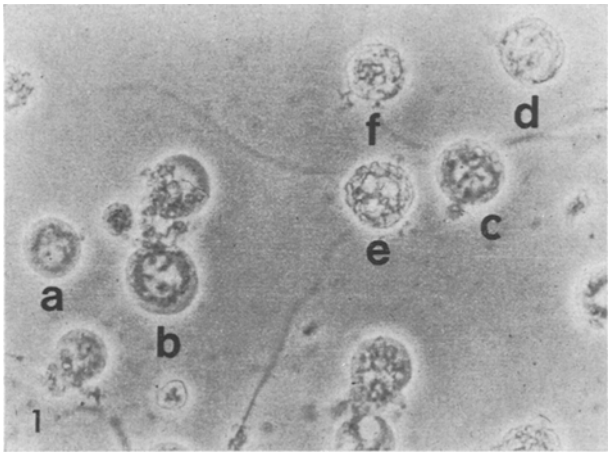

Fig. 1. A phase contrast microscopic picture of early postmeiotic cells from stage I of rat spermatogenesis. Vincristine (0.25 µg) had been injected intratesticularily 24 h earlier. The cells a, b, c and d appear normal whereas the cells e and f show an increased negative phase change indicating cell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

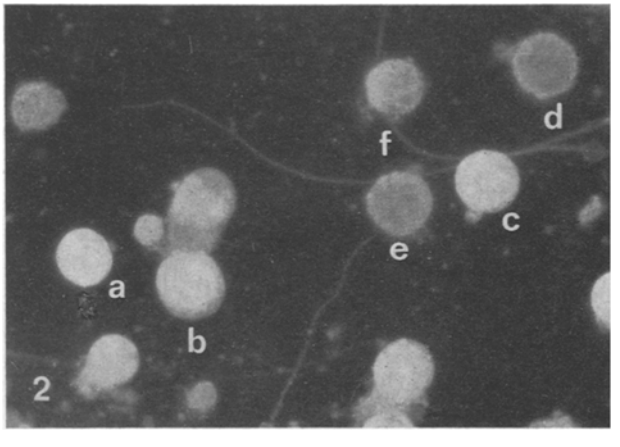

Fig. 2. A picture showing the AO fluorescence of the same early postmeiotic cells as in figure 1. A green nuclear fluorescence typical to normal cells is seen in cells a and b. The cell c has a somewhat increased red fluorescence of the nucleus and the cells e and f are clearly red. Note especially the 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.

In this work, we have studied the early detection of cell damage by supravital acridine orange staining. The cell damage was caused by injecting 0.25 µg of vincristine (Eli Lilly and Co., Indianapolis, Ind., USA) intratesticularily in the rat. This drug causes an arrest of the cells in metaphase during the meiotic divisions, which is followed by a progressive degeneration and death of these cells. The rat was killed by a blow on the head 24 h after the injection and the seminiferous tubules of the testis were dissected free from the interstitial tissue[3]. The isolated tubules were observed under transmitted light in a preparation microscope. The different stages of the seminiferous epithelium containing constant accurately known cell associations[4] absorb light in a different way, permitting the identification of individual stages[5,6]. The stages XIV and I containing the meiotic and early postmeiotic cells were identified and a 1–2 mm long segment was cut and placed on a microscope glass slide together with a few drops of 0.9% NaCl with AO (0.1 mg/ml). A cover-glass was placed on the specimen and the cells were allowed to sqeeze out of the tubulus so that a monolayer was formed. The observations were done with an Olympus Vanox microscope, permitting successive phase contrast and fluorescence observations of the same field.

From previous studies, it is known that the death of the spermatogenic cells can be rapidly detected because of the increased negative phase change[7]. The results in this study show that by using AO fluorescence it is possible to detect cell damage much earlier than by phase contrast optics or by conventional histological methods. Figure 1 shows the phase contrast appearance of early postmeiotic cells from the testis treated for 24 h with 0.25 µg of vincristine. The AO fluorescence of the same cells is seen in figure 2. The nuclei of living cells are characterized by a green fluorescence typical to double helical DNA (cells a and b), whereas with progressive cell damage the red fluorescence of the cell nuclei increases (cells c, d and f). The red fluorescence appears in the damaged cells before any morphological changes or increase in the negative phase contrast can be observed (cell d).

The change in the AO fluorescence during cell death could be explained by the occurrence of a change in the secondary structure of the DNA. During the cell degeneration, the double stranded DNA could be split into pieces of single stranded DNA molecules which would result in a changed AO binding with a red fluorescence. The appearance of the red fluorescence in the cells before morphological or phase contrast changes can be seen indicates that the damage of DNA is a very early phenomenon in the mechanism of this type of cell death.

That the change in the AO fluorescence is a general phenomenon and not specific to cell death caused by vincristine was shown by studies in which cell death was caused by chryptorchism. In the dying cells the same red fluorescence could be observed. It is also known that an increased concentration of AO causes the apparence of a red fluorescence[2]. The red fluorescence in this study was, however, not caused by a concentration effect because cells which were localized closely together showed quite a different fluorescence (figure 2).

This technique for the early detection of cell damage might have important practical applications, while it also gives new information about the order of events in the process of cell death.

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Etude cytofluorométrique et histoautoradiographique de la synthèse d'ADN et de protéines sur des cellules mésothéliales humaines

Cytofluorometric and histoautoradiographic study on DNA and protein synthesis of human mesothelial cells

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Summary. The study of nuclear DNA synthesis of human mesothelial cells showed that cells do not divide by mitosis, even if an important percentage of them have a tetraploid amount of DNA. Concerning the cell cycle of mesothelial cells, the authors conclude that some of them are blocked in postsynthesis.

Le problème de la multiplication des cellules mésothéliales dans la cavité péritonéale n'est pas encore entièrement élucidé[1]. Différents auteurs[2,3] ont observé, sur frottis, des cellules en mitose. Il a aussi été décrit[4] que dans certains cas, tel qu'une ascite d'origine cirrhotique, les cellules mésothéliales présentent une plus forte activité mitotique.

Pour ce travail, nous avons prélevé du liquide ascitique humain chez 6 patients porteurs d'une cirrhose alcoolique. Sur les frottis, tirés du culot après centrifugation du liquide à 1000 tours/min pendant 10 min et fixés avec de l'alcool-acétone (1:1), nous avons effectué les réactions suivantes: BAO (bis-amino phényl-oxadizole) pour les mesures quantitatives d'ADN[5]; sulfaflavine à pH 2,8 pour les protéines totales[6] et à pH 8,2 pour les protéines-histones[6]; isothiocyanate de fluorescéine pour les protéines totales[7]. On a aussi étudié la synthèse d'ADN des cellules prélevées du même culot de centrifugation et maintenues en suspension dans un milieu nutritif (90% 199-Difco, 10% sérum de veau) contenant 0,5 µCi/ml de Thymidine-^{3}H (Th-^{3}H, activité spécifique: 5000 mCi/mmol) pendant 30, 60, 120 min et 12 h à 37 °C. Après l'incubation, nous avons procédé à l'histoautoradiographie des frottis d'après la méthode décrite par Bogoroch[8].