THE INDUCTION AND REPAIR OF DIFFERENT CLASSES OF SINGLE-STRAND BREAKS IN CULTURED MAMMALIAN CELLS TREATED WITH ETHYLATING AGENTS. T.Lakhanisky[1], E.Dogliotti[2] G.P.van der Schans[3] and P.H.M.Lohman[3]. [1]Institut d'hygiène et d'epidémiologie, Brussels, Belgium; [2]Instituto Superiore di Sanita, Roma, Italy; [3]Medical Biological Laboratory TNO, Rijswijk, The Netherlands.

Cultured Chinese hamster ovary cells were treated with ethylating agents. DNA lesions giving rise to single-strand breaks (ssb) or alkali-labile sites were measured by alkaline elution at pH 12.0 and pH 12.6 and by centrifugation in alkaline sucrose gradients after 1 h and 21 h lysis in alkali. 2 agents with different tendencies to ethylate preferentially either at N or O atoms were compared, namely N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) and diethylsulphate (DES). The compounds differed greatly in their potency to induce lesions but the ratios of ssb, measured with the different methods, did not differ significantly after a treatment time of 30 min. Nevertheless, after a short treatment time, it could be shown by alkaline elution at pH 12.0 that only ENNG gave rise to rapid repairable ssb. Most of them rejoined within 5 min after treatment.

These results suggest that rapid repairable lesions relate to lesions at O atoms after treatment of mammalian cells with ethylating agents.

FLOW CYTOMETRIC DISCRIMINATION OF MITOTIC CELLS
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A new method for flow cytometric discrimination of mitotic cells from G1, S and G2 cells is presented. After fixation with formaldehyde and staining with mithramycin the fluoresence of mitotic cells or nuclei is enhanced to an intensity 20-30% higher than that of G2 cells. The method has been applied to mitogen stimulated human lymphocytes and Yoshida ascites tumour cells. In experiments with vinblastine induced mitotic arrest we found a significant correlation between the mitotic index counted by microscopy on smears and the fraction represented by the histogram peak of enhanced fluorescence. FACS sorting demonstrated that mitoses were confined exclusively to this peak of enhanced fluorescence. It can be concluded that a simple flow cytometric analysis on a sample of formaldehyde and mithramycin treated nuclei allows the fractions of G1, S, G2 and M cells to be specifically estimated.

IN VITRO METABOLISM OF ARYLHYDRAZINE INGREDIENTS OF THE MUSHROOM OF COMMERCE AGARICUS BISPORUS. Terence Lawson and Bela Toth.
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The aim of this investigation was to reveal the biochemical conversion of β-N-[γ-L(+)-glutamyl]-4-hydroxymethylphenylhydrazine [synonym agaritine (A)], N'-acetyl-4-(hydroxymethyl)phenylhydrazine (HMPH) and 4-methylphenylhydrazine (MPH) ingredients of the cultivated mushroom Agaricus bisporus. The cytochrome P-450-mediated mixed function oxidase fraction was isolated from the livers of Swiss mice and the preparation was used for the study. The experiments have indicated that 0.5 mM is the approximate optimum substrate concentration. Incubations were conducted for 5 minutes at 37°C in air and were terminated by rapid filtration. The reaction mixture was reacted with β-naphthol in ethanol and the complex thus formed was measured spectrophotometrically. The data obtained thus far were analyzed by Lineweaver-Burke plots and gave Km values of 1.3×10^{-5}M for HMPH, 2.6×10^{-5}M for A, and 0.9×10^{-5}M for MPH. It is conceivable that these reaction products in vivo could interact with cellular macromolecules and may induce malignant transformation. Earlier studies by us have shown the carcinogenicity of HMPH and MPH, while A gave negative results in Swiss mice. It may be significant that the two carcinogens were metabolized more efficiently than the non-carcinogen.