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Hydrocarbon-deoxyribonucleoside products present in the DNA of mouse embryo cell cultures that had been treated with [^3H]-7-methylbenz(a)anthracene were isolated from enzyme digests of the DNA by Sephadex LH20 column chromatography. Similar digests were prepared from DNA reacted in solution with either 7-bromomethylbenz(a)-anthracene or the K-region epoxide of 7-methylbenz(a)anthracene and from DNA from cells treated in culture with either 7-hydroxymethylbenz(a)anthracene, 5-hydroxy-7-methylbenz(a)anthracene, *cis*-5,6-dihydro-5,6 dihydroxy-7-methylbenz(a)anthracene, or the K-region epoxide of 7-methylbenz(a)anthracene. These digests contained no products identical with those obtained from hydrocarbon treated cells.

These results suggest that none of the above compounds is directly involved in the binding of this hydrocarbon to DNA.

THE TARGET CELL IN 7,12-DIMETHYLBENZ(A)ANTHRACENE CARCINOGENESIS IN THE MOUSE SUBMANDIBULAR GLAND *IN VIVO* AND *IN VITRO*. C. B. WIGLEY. Department of Cellular Pathology, Imperial Cancer Research Fund, Lincoln's Inn fields, London.

Direct applications of a carcinogen (DMBA) on mouse submandibular gland epithelium *in vivo* and *in vitro* were compared. The granular tubule cell is the primary target cell in both systems. *In vivo* it shows degranulation, squamous metaplasia and increased DNA synthesis after 3-4 weeks. This is followed by the development of squamous carcinoma at 14-20 weeks. *In vitro*, untreated cultures show epithelial outgrowths which arise largely from granular tubule cells. These undergo a regeneration process in the explant before attachment to the substrate occurs.

Cultures in this regeneration phase were treated with DMBA at 0.1 $\mu\text{g}/\text{ml}$ of medium. Approximately 10 weeks later foci of cells appear in some outgrowths which grow rapidly and have morphological features consistent with neoplastically transformed epithelium. Experiments are in progress to test the tumorigenicity of these cells.

STUDIES OF THE MECHANISM OF TUMOUR INITIATION. M. P. RAYMAN and A. DIPPLE. Chester Beatty Research Institute, London.

7 - Bromomethyl - 12 - methylbenz(a) - anthracene is a more effective carcinogen than 7-bromomethylbenz(a)anthracene (Dipple and Slade, *Eur. J. Cancer*, 1971, 7, 473). Comparison of the products of reaction of each bromo compound with DNA *in vitro* and *in vivo* indicates that similar products are formed in each case through reaction on the amino groups of the DNA bases; that 7-bromomethyl - 12 - methylbenz(a)anthracene reacts less extensively with DNA than does 7-bromomethylbenz(a)anthracene; that no correlation exists between the amounts of any hydrocarbon-DNA product formed and carcinogenic potency; but that 7-bromomethyl-12-methylbenz(a)anthracene does exhibit a greater preference for attack on adenine residues in DNA than does 7-bromomethylbenz(a)anthracene. These findings do not support the view that DNA is the critical receptor for chemical carcinogens, unless the attack of these carcinogens on DNA exhibits a differential specificity for chromosomal sites which are specifically relevant to tumour initiation.

MITOTIC INHIBITION IN MAMMALIAN CELL CULTURES EXPOSED TO HYPERTONIC MEDIA. D. N. WHEATLEY. Department of Pathology, University Medical Buildings, Aberdeen.

An almost immediate cessation of mitotic division can be brought about by increasing the tonicity of the medium in cell cultures (Hughes, *Quart. J. microsc. Sci.*, 1952, 98, 207; Stubblefield and Mueller, *Cancer Res.*, 1960, 20, 1646). The effects of raising tonicity with Na^+ , K^+ , Li^+ , choline, urea and other substances will be discussed, particularly the ways in which they affect the flow of cells from G_2 into mitosis as well as their action in mitosis itself. After an initial period of perturbation following treatment, cells adapt to the hypertonic medium (that is, within certain limits depending upon the substance used) and may begin to accumulate in metaphase. Several hours later some cells recover their ability to move out of metaphase into interphase.

One practical application of the accumulation of metaphases in hypertonic medium

has been the successful collection of large numbers of mitotic cells over a 3-4 hour period using a constant gentle washing procedure for large monolayers. The arrested cells show almost perfect metaphase synchrony and pass out of mitosis synchronously with similar kinetics to isotonicity collected mitoses.

AGGLUTINATION OF NORMAL AND MALIGNANT CELLS BY CONCAVALIN A IN RELATION TO CELL SURFACE STRUCTURE. N. E. PAYNE, P. WHUR and R. T. ROBSON. Cell Biology Unit, Marie Curie Memorial Foundation, Research Department, Oxted, Surrey.

Our Ehrlich ascites tumour cells do not aggregate *in vivo*. However, when washed and resuspended in tissue culture medium aggregation occurred at 37°C and the cells are agglutinated by concanavalin A. When pre-incubated in trypsin inhibitor (soybean) the cells show markedly less tendency to aggregate but in the presence of concanavalin A agglutination is greatly enhanced. This suggests that trypsin inhibitor interacts with a cell surface component, possibly a protease.

In related studies, concanavalin A agglutination was investigated using trypsinized BHK 21 cells. After exposure to trypsin these cells become agglutinable, but lose this property, apparently as the cell coat is resynthesized. The time course of this event is compared with polyoma-transformed BHK 21 cells, which remain agglutinable after coat resynthesis.

EXAMINATION OF EXFOLIATED CERVICAL CELLS BY THE SCANNING ELECTRON MICROSCOPE (SEM). J. F. MURPHY, J. M. ALLEN, J. A. JORDAN and A. E. WILLIAMS. Departments of Obstetrics and Gynaecology and Microbiology, University of Birmingham.

This study examines the ability of the SEM to differentiate exfoliated cells from the cervix uteri into benign and malignant types.

Three basic methods were used to obtain cell samples: (1) the cells were scraped from the cervix, examined by phase contrast microscopy and then processed for examination in the SEM; (2) cells were washed from the cervix with tissue culture medium and similarly treated and examined; (3) a membrane filter was used to remove cells

from a colposcopically directed area on the cervix.

The results using techniques 1 and 2 often showed good cell preservation but were not sufficiently reproducible to be of clinical value. The membrane filter technique provided much more satisfactory results though there was some loss of surface detail at higher magnification.

THE TEMPORAL AND SPATIAL DISTRIBUTION OF OESOPHAGEAL CANCER AMONG MINeworkERS IN SOUTHERN AFRICA. J. S. HARINGTON. National Cancer Association of South Africa, Johannesburg and N. D. McGLASHAN, University of Tasmania, Hobart.

The initial analysis of cancer among mineworkers recruited from several rural areas of southern Africa (Robertson *et al.*, *Br. J. Cancer*, 1971, **25**, 395) has been tested for significance of time trends and spatial distribution. 48.8% of all cases of oesophageal cancer diagnosed in this medically well recorded population of 2.9 million men came from home addresses in the Xhosa-speaking Transkei, and a further 18.5% from neighbouring areas of the Eastern Cape Province, mainly the Ciskei, also a Xhosa area. Only 4.3% of oesophageal cases came from Mozambique. In contrast, 68.6% of cases of liver cancer occurred in Mozambique miners and only 10% in miners from the Transkei.

Regressions of annual rates per 100,000 employees for the Transkei and Eastern Cape for the 8 years, 1964-71, have been found to be similar and 7 times as high as those for all other areas of recruitment. Within the Transkei the districts with the highest rates, and significantly higher case numbers, lie in the extreme south-west. The spatial and temporal patterns, taken together, emphasize the high incidence both east and west of the Kei River. They suggest that the populations there are under uniform conditions of environmental risk and provide basis for aetiological enquiry amongst these general Xhosa populations.

CHILDHOOD CANCER: AN EPIDEMIOLOGICAL STUDY. J. POWELL. Birmingham Regional Cancer Registry, Queen Elizabeth Medical Centre, Birmingham.