inhibition of DNA synthesis can affect the carcinogenic power through an immunosuppressive effect leading to a decreased immunosurveillance[21-23].

Riassunto. È esaminato l'effetto di sette idrocarburi policiclici, a diverso potere oncogeno, sulla sintesi del DNA nel timo, milza, midollo e fegato rigenerante di ratto. Il DMBA è inibente in tutti i tessuti esaminati, come pure il 1,2,3,4-DBA. Il BP ha un notevole effetto solo sul fegato rigenerante mentre il 1,2,3,4-DBA é più efficace dell'1,2,5,6-DBA in ogni caso. Non é stata stabilita una correlazione tra il potere oncogeno delle sostanze esaminate e la inibizione della sintesi del DNA.

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Cytoplasmic DNA of Hepatoma Tumor Cells Studied by ^{3}H-Actinomycin D Binding

In this study the cytoplasmic DNA content of mouse hepatoma tumor cells was compared with that of normal mouse liver cells. The method chosen was that of ^{3}H-actinomycin D (^{3}H-AD) incubation followed by radioautography. Since ^{3}H-AD binds specifically to DNA[1], it can be used to localize small amounts of DNA with a high degree of specificity in radioautographic preparations at the light microscope level[2-6].

Materials and methods. The tumor used in this study was a mouse hepatoma (Jax Code BW7756), obtained from the Jackson Laboratory, Bar Harbor, Maine and maintained by serial transplant in C57L/J mice. Livers from normal C57L/J mice served as controls All tissues were fixed for 2 h in glutaraldehyde[7], post-fixed for 1 h in osmium tetroxide[8], embedded in Araldite[9], and sectioned at 0.5 μm Control sections from both the hepatoma and the normal liver were incubated in DNase or RNase[10]. All tissues then were incubated in ^{3}H-AD (Schwarz Bioresearch Inc, Orangeburg, N.Y., specific activity 8.4 Ci/m*M*) using methods described in the literature[2-4,6]. Three experimental methods differing in the sequence of fixation and ^{3}H-AD incubation were used. These methods, as well as the methods of light microscope radioautography and Toluidine Blue staining are thoroughly described elsewhere[6].

Silver grains, indicating the sites where ^{3}H-AD had bound to DNA, were counted per unit area (123.4 μm^2) of cytoplasm, using a calibrated grid placed in the ocular. Grain counts were carried out over 20 randomly selected areas of cytoplasm per animal. The mean grain concentrations thus obtained were converted to a standard value: grain counts per 100 μm^2 cytoplasmic area. Standard errors of the means were calculated for all data.

Background counts were made on 'cold' control sections, which were dipped, exposed, developed, fixed and stained in a manner identical to the experimental slides, but which were not subject at any point to incubation in the radioactive compound[11]. Background values were then subtracted from the experimental grain count data.

Results and discussion. The results presented in the Table confirm that in this system ^{3}H-AD binds specifically to DNA. No significant differences were observed between RNase treated sections and non-treated sections, indicating that the presence or absence of RNA has no effect on ^{3}H-AD binding. On the other hand, after DNase extraction, no ^{3}H-AD binding occurred.

The Table shows that ^{3}H-AD binding is more than 2 times greater in the cytoplasm of hepatoma cells (an average of 27 grains per 100 μm^2) than it is in the cytoplasm of normal liver cells (an average of 12 grains per 100 μm^2). This mode of presenting the data compensates for differences in cell size. The Table also shows that the results obtained by all 3 experimental procedures are almost identical. This uniformity suggests that we had obtained maximal binding to DNA in all 3 experimental methods used.

Mitochondrial DNA (m-DNA) is the best documented class of cytoplasmic DNA. Differential H^3-AD binding to the m-DNA of tumor or an increase in amount of m-DNA in the hepatoma could account for the results reported here, especially since the m-DNA of tumors is significantly different from the m-DNA of normal cells.

The m-DNA molecules from normal tissues are circular monomers of double stranded DNA with a remarkably consistent contour length of 5 μm^2 [12,13]. In contrast,

Mean grain concentration per 100 μm^2 cytoplasmic area, representing the amount of ^{3}H-actinomycin D binding to cytoplasmic DNA in normal hepatic cells and hepatoma tumor in 18 mice

	Liver	Hepatoma
Experiment 1	12.41 ± 0.20	27.03 ± 0.18
Experiment 2	12.31 ± 0.19	27.00 ± 0.16
Experiment 3	12.35 ± 0.19	27.00 ± 0.16
Experiment 1 RNase	12.41 ± 0.18	26.97 ± 0.18
Experiment 1 DNase	0.10 ± 0.04	0.11 ± 0.04

Each value represents the average of 120 areas.

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several investigators[14–17], using great variety of tumors, found that the m-DNA in tumor cells show considerable variability, and may be present in multiple copies, as dimers or oligomers. Dimeric and oligomeric forms of m-DNA in cells thus appear to be generally associated with malignancy[16,17]. Each mitochondrion of the tumor cell may thus contain an elevated level of m-DNA as compared to mitochondria from normal cells.

However, 2 other clasess of cytoplasmic DNA have also been reported to occur specifically in tumors or rapidly proliferating cells[18,19]. The first is a cytoplasmic membrane associated DNA from the post-microsomal fraction of Novikoff hepatoma and of rapidly dividing normal tissue. This fraction was reported to stimulate DNA synthesis; it has been identified as DNA on the basis of its DNase sensitivity[18]. The other class reported is communication DNA (c-DNA), which is believed to originate in the nucleus, and to transport information from nucleus to cytoplasm in tumor and fetal cells in the form of unique nuclear sequences[19].

Although in this study, using ^{3}H-AD binding, we cannot distinguish between the various classes of cytoplasmic DNA, we have furnished additional proof that cytoplasmic DNA is elevated in hepatoma cells as compared to their normal counterparts.

Résumé. L'ADN cytoplasmique d'hépatome fixe deux fois plus de ^{3}H-actinomycin D que le foie normal. Cette différence peut être expliquée soit par l'augmentation de l'ADN cytoplasmique du tumeur ou par l'augmentation de fixation de ^{3}H-actinomycin D à l'ADN du tumeur.

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Association Between Rat Serum α-Macroglobulins and Splenic Macrophages

Using an immunofluorescent technique MCCORMICK et al.[1] have recently shown, both in man and the mouse, that α_2-macroglobulin is present on the cell surfaces of a percentage of lymphocytes. From their studies they conclude that the lymphocytes stained in this way are most likely to be a subpopulation of B-lymphocytes.

We should like to draw attention to an association between two α-macroglobulins, slow α_1 and slow α_2-macroglobulin[2–4] and another cell population, splenic macrophages. During a search for α-macroglobulin associated lymphoid cells in normal thymus, spleen, lymph nodes and bone marrow, obtained from Hooded Lister or Sprague-Dawley rats, we observed a well defined population of cells whose cytoplasm stained positively for slow α_1- and α_2-globulin in the splenic red pulp. These cells were identified using both indirect immunofluorescence[5] and immunoperoxidase techniques[6]. They were not present in thymus, lymph nodes or bone marrow.

Antiserum to slow α_1-globulin was prepared in rabbits as described previously[7] and antiserum to slow α_2-globulin by immunising rabbits with a macroglobulin containing fraction (first fraction ex. G.200 Sephadex column). Inflammatory rat serum (rats injected with complete Freund's adjuvant into one hind footpad 5 days prior to bleeding) was used as a source of slow α_2-globulin (a known acute phase reactant). The resultant antiserum was absorbed with freeze dried normal male rat serum. Both antisera were shown to be monospecific by gel diffusion and immunoelectrophoresis. The fluorescein-labelled goat antirabbit IgG antiserum was obtained from Behringwerke AG (Marburg, Germany). For conjugation with horseradish peroxidase the IgG fraction of a goat antirabbit IgG (heavy chain) was obtained from Cappel Laboratories (Downningtown, USA), the actual conjugation being as described by NAKANE and PIERCE[6].

In the splenic localization studies 4 μm thick frozen sections, washed in phosphate buffered saline pH 7.1, were used. The indirect immunofluorescence procedure then carried out was as described by BECK[5]. The indirect immunoperoxidase technique was that of NAKANE and PIERCE[6].

To demonstrate the presence of both slow α-globulins within the cell cytoplasm a photo-oxidation technique was used. This consisted of prior staining of a spleen section for slow α_1-globulin using the indirect fluorescence technique. A carefully defined area containing several fluorescent cells was identified and photographed. Then, having removed the coverslip this area was exposed for some 12 h to a UV-light source (epi-illumination from a mercury lamp C 5200 W – 4 Leitz Orthoplan). The section was finally stained for slow α_2-globulin as previously and the defined area was rephotographed to allow comparison.

For electron microscopic studies whole spleens were teased apart in phosphate buffered saline pH 7.1. The white cells were then separated from red corpuscles on a Ficoll-Triosil gradient[8]. The indirect immunoperoxidase staining was carried out as before except that prior to staining for the reaction product the cells were fixed in 1% glutaraldehyde for 30 min at room temperature. The cells were finally treated with 2% osmium tetroxide and embedded in epon resin.

All fluorescent preparations were examined in blue light (BG 12) using a Zeiss Standard Universal microscope. Using point counting histometry we assessed the number of cells showing fluorescence either for slow α_1 or α_2-

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