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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globulin. The results were expressed as the percentage of the splenic volume, a method previously shown by Dunnill[9] to be mathematically valid.

Figure 1 shows the typical distribution of slow α_1-globulin containing cells in the red pulp of a normal rat spleen. The white pulp normally contains few if any fluorescent cells. From Figure 2 it is readily apparent that both normal male and female rats show complete absence of fluorescent cells until after 18 weeks of age and it appears to be an age related phenomenon. The rise in the numbers of slow α_1-globulin containing cells between 20 and 40 weeks of age best fits an exponential curve (correlation coefficient males $r = 0.81$, females $r = 0.82$) and after 45 weeks there appears to be a plateau effect. No difference in the frequency of fluorescent cells, however, is observed between males and females. Similar studies have been undertaken to show the frequency and distribution of slow α_2-globulin containing cells. No relationship is apparent between the frequency of slow α_2-globulin containing cells and age nor is there any apparent difference in the frequency of such cells between males and females. Compared with slow α_1-globulin, α_2-globulin containing cells are infrequent in most male and female spleens although, as with slow α_1-globulin,

[9] M. S. DUNNILL, in *Recent Advances in Clinical Pathology*, Series 5 (Ed. S. C. DYKE; Churchill, London 1968), p. 401.

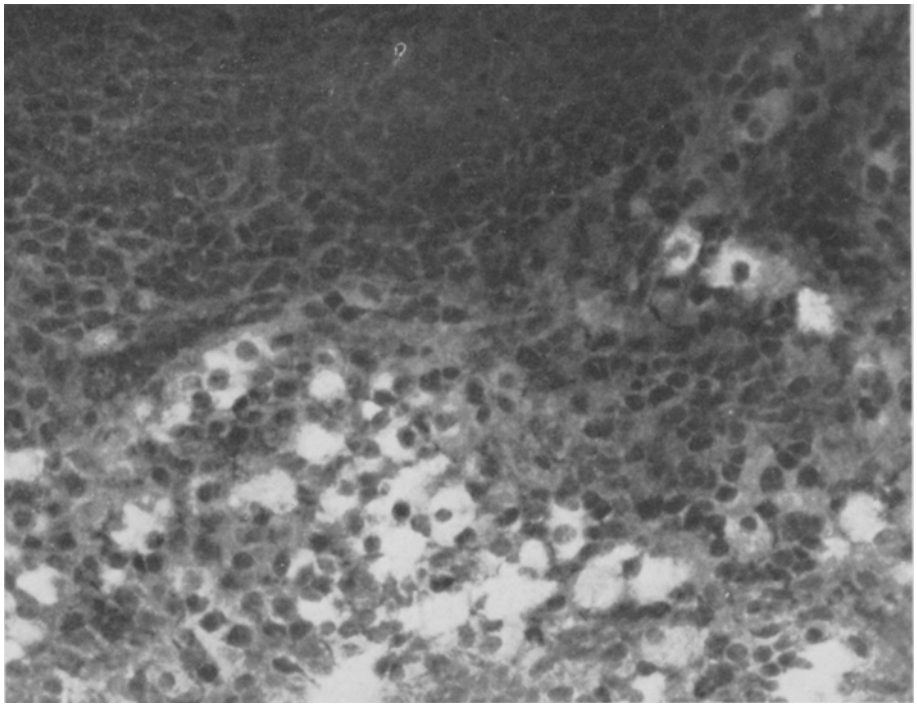

Fig. 1. Normal rat spleen showing fluorescent cells in the red pulp. Note the absence of fluorescent cells in the white pulp. × 380.

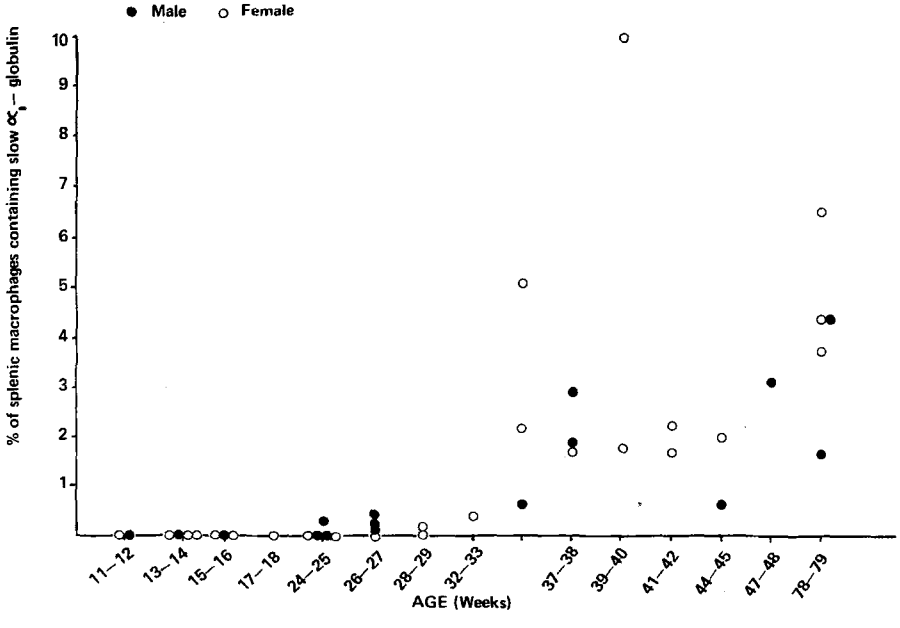

Fig. 2. Diagram to show the frequency of slow α_1-globulin containing splenic cells in normal males and females of varying age.

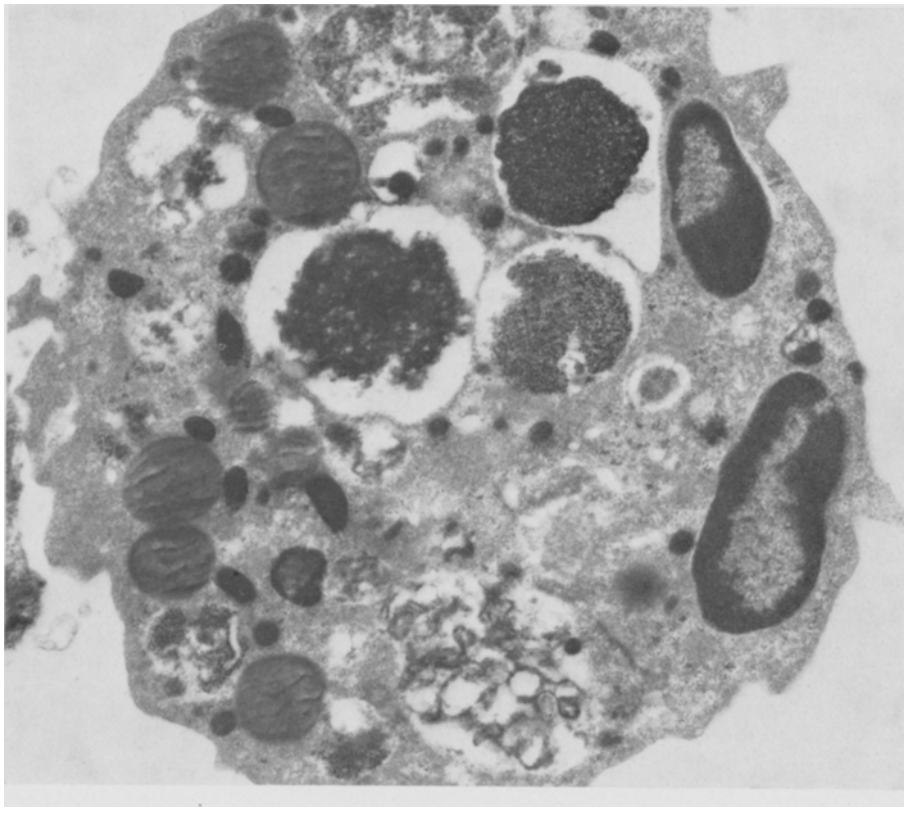

Fig. 3. Indirect sandwich technique to demonstrate the presence of rat slow α_1-globulin within splenic cells using peroxidase-coupled sheep antirabbit IgG. Note the presence of several vacuoles containing the peroxidase-label. ×46,000.

such cells are seen in the red pulp. However, using the photo-oxidation technique a few fluorescent cells have been shown to contain both slow α_1- and α_2-globulin within their cytoplasm.

Since at higher magnification the fluorescent cells contained fluorescent 'granules', it was considered that these cells might prove to be macrophages. Confirmation of this is shown in Figure 3. By means of electron microscopy and the indirect immunoperoxidase technique the peroxidase-label is noted to be within vacuoles, presumably phagocytic vacuoles. All peroxidase-labelled cells identified were similar in appearance to that illustrated.

The functional significance of these findings is uncertain. The marked decrease in the numbers of slow α_2-globulin containing cells compared with slow α_1-globulin containing cells is perhaps attributable to the serum levels. While they may have similar functional roles[10], slow α_2-globulin, unlike slow α_1-globulin, is present in normal male and female rat serum in only trace quantities. That the splenic macrophages phagocytose rather than produce the slow α-globulins is further suggested by preliminary studies using ^{14}C labelled isoleucine and lysine in which we failed to show any evidence of production of these globulins during culture of splenic macrophages. This finding together with our electron microscopic study suggests that these α-macroglobulins are phagocytosed although why this should occur is at present unknown. It is of interest, however, that α_1-antitrypsin has been shown to be present within pulmonary alveolar macrophages[11]. In this context it is perhaps relevant that, like α_1-antitrypsin, slow α_1- and α_2-globulin are functionally similar in that they are antiproteases (antiplasmins)[10].

Finally, in view of the known inhibitory effect of mouse spleen macrophages on the response of lymphocytes to various stimuli[12,13] and the association between mouse and human α_2-macroglobulin and B-lymphocytes, it would seem reasonable to suggest an immunological function for this subpopulation of splenic macrophages. Recent work by Nelson[14] has also shown a powerful inhibitory factor in the mouse serum which is macrophage derived. Could this subpopulation of macrophages identified by us in the rat spleen be responsible for this inhibitory effect and might the macroglobulin found in their cytoplasm play a role in this inhibition? This and their possible role in the decline of the immune response with age are currently being investigated.

Résumé. La présence des globulines lentes α_1 et α_2 dans la rate des rats normaux est révélée par les techniques immunofluorescentes et immuno-péroxydasiques. En microscopie électronique, les globulines α-lentes semblent se trouver dans les phagosomes. Dans le cas de la globuline α_1-lente, le nombre des phagocytes varie avec l'âge du rat. Ils sont presque absents jusqu'à la 18ième semaine. Par contre le nombre de phagocytes contenant la globuline α_2-lente reste constant.

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