

an increase in concentration of bilirubin¹⁷. It is noteworthy that jaundice which also arises from elevated bilirubin levels occurs consistently in human biliary atresia¹⁸.

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Relationship between A-type and C-type particles in Ehrlich ascites tumor cells

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Summary. Intracisternal and intracytoplasmic A-type particles were discovered in Ehrlich ascites tumor cells. In addition, 'mature' and 'immature' C-type particles were also seen in the intercellular space. It is believed that A particles may represent a precursor or a formative stage of the C particles.

In the Ehrlich ascites (EA) tumor cells, a virus with morphological characters of A-type particles was first described by Yasuzumi and Higashizawa² and Friedländer and Moore³. Since then these particles have been reported in several mouse tumors⁴⁻⁷. The particles are usually intracytoplasmic and/or intracisternally located. Recently Myking and Åbro⁸ were able to demonstrate C-type particles in a transplant of EA tumor cells. The present communication reports the presence of A- and C-type particles in EA tumor cells obtained from peritoneal fluid containing tumor cells and i.m. transplants.

Materials and methods. The Ehrlich ascites tumor cells in suspension were kindly provided by Dr Y.C. Kong of the Department of Biochemistry, The Chinese University of Hong Kong. The tumor is carried in this laboratory by i.m. and i.p. transplantations in WHT/HT (Swiss) mice. Small pieces of solid tumor pellets made from peritoneal fluid containing tumor cells were fixed in 2.5% glutaraldehyde in phosphate buffer and post-fixed in 1% osmium tetroxide. Routine procedures were followed for dehydration and embedding in Epon 812. Sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 300 at 60 kV.

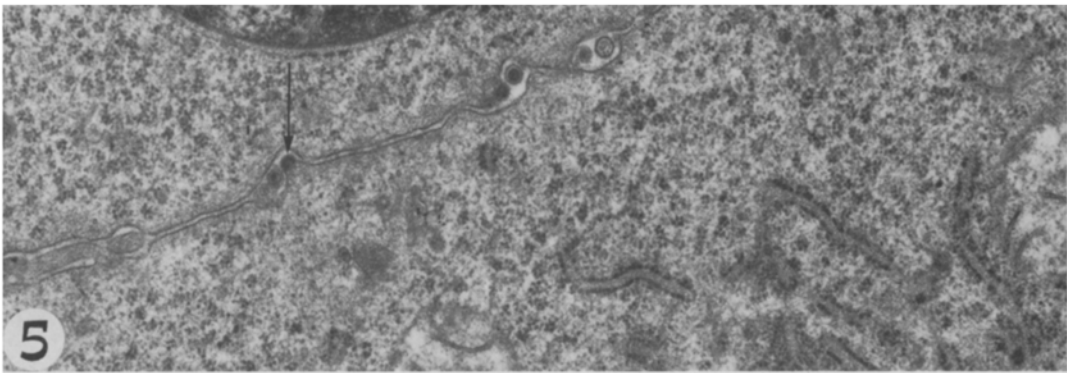
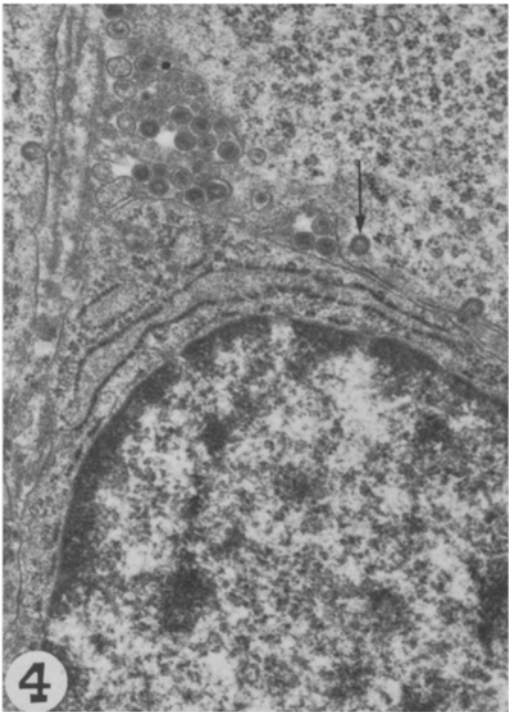
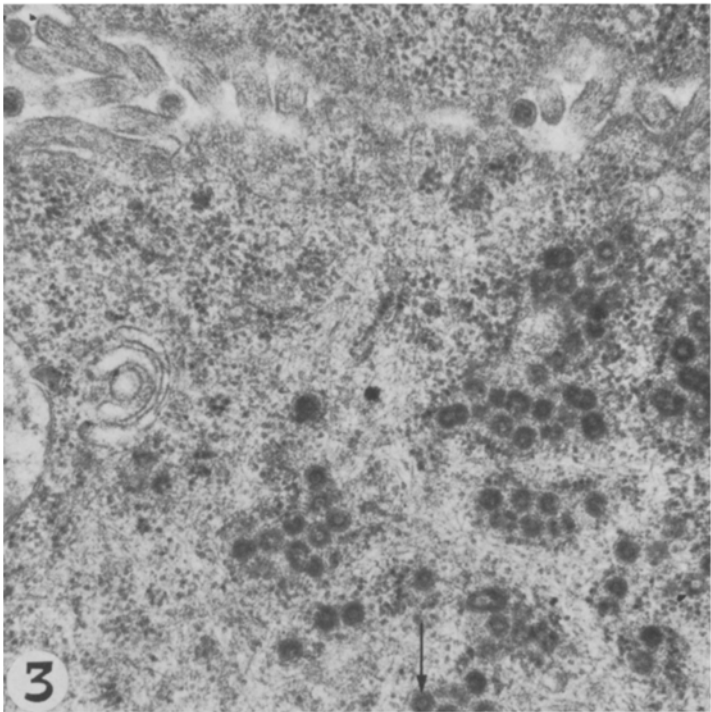
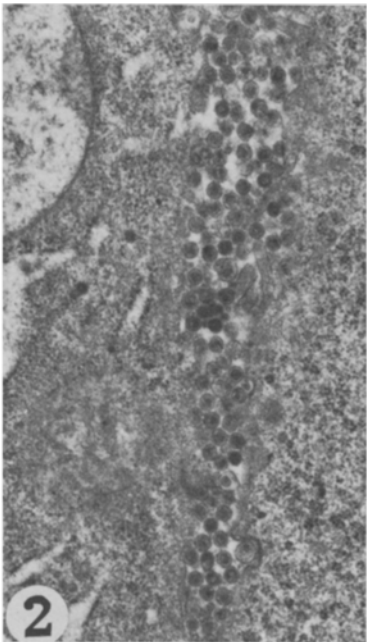
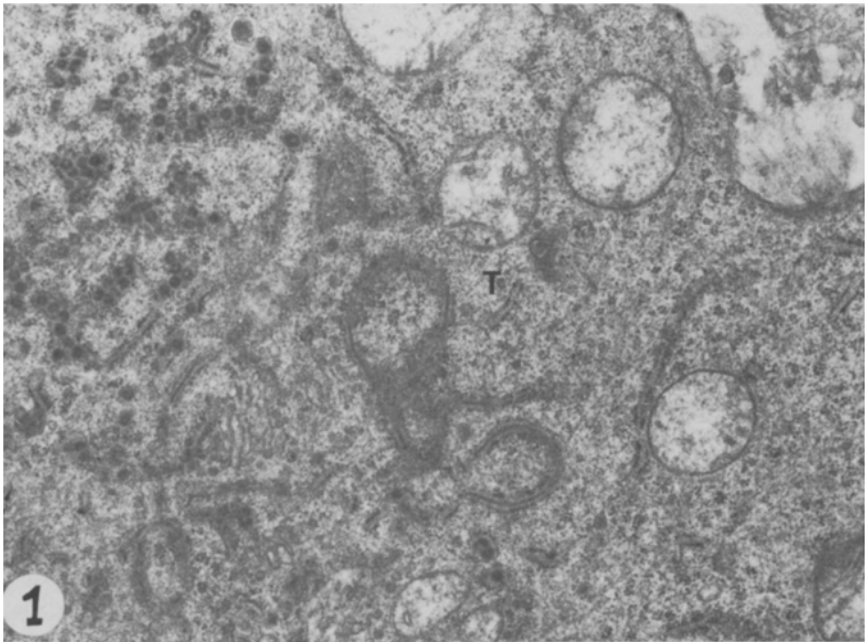
Results. The details of cell organelles of EA tumor cells have been described by many investigators^{2,8-11}. Intracisternal A-type 'virus-like' particles were found in the cytoplasm of most of the tumor cells. On rare occasions, intracytoplasmic A particles were also detected (figure 1). Most of the A particles were in the juxta-nuclear position. The particle consisted of 2 concentric shells and a rather electron-lucent center. The outer and inner shells have diameters of 70 and 40 nm respectively. In addition, 'mature' and 'immature' C-type particles were observed in some tumor cells obtained from solid i.m. transplants and from peri-

toneal fluid containing tumor cells (figure 2). The C-particle has an envelope of 90 nm in diameter. Intracisternal and intracytoplasmic A-type particles were sometimes located near the cell membrane and were released as 'doughnut-shaped' enveloped nucleoids called 'immature' C-type particles (figure 5). Many 'mature' C-type particles were located only in the intercellular space (figures 2-5).

Discussion. Previous investigations have revealed the presence of intracisternal and intracytoplasmic A particles in EA tumor cells⁹⁻¹¹. Myking and Åbro⁸ also reported the presence of C-type particles in EA tumor cells. However, the relationship of the A- and C-type particles in this tumor line was not discussed. The present study has revealed a possible gradual 'shift' of the intracisternal and intracytoplasmic A-particles from the juxta-nuclear position to the cell periphery and eventually to produce the 'immature' C-type particles (figure 5). 'Mature' C-type virion is later formed by condensation of the nucleoid.

Guili et al.¹² suggested a relationship between cytoplasmic A particles and the C-type Rous sarcoma virus in chicken cells after revealing that the A particles contain components immunologically related to the protein of C-type virus. However, Dalton believes that no true intracellular A-type particle is ever involved in C particles formation¹³. Our results seem to indicate a gradual transformation of A particles to C particles. This view is also shared by Bibby and Smith¹⁴ in the study of neoplastic transformation of epidermal cell of BalB/c Mice. Intracisternal A particles have never been shown to possess biological activity, whereas C-type particles have been demonstrated as causative agents in avian, murine and feline leukemia and sarcomas¹⁵. The presence of C-type particles in Ehrlich ascites tumor cells may also imply that it is likely to be one of the causative oncogenic agents. Perhaps it is also reason-

Fig. 1. An EA tumor cell (T) showing intracisternal A particles. $\times 12,300$. Fig. 2. 'Mature' and 'immature' C particles in the intercellular space. $\times 19,400$. Fig. 3. Higher power view of an EA tumor cell showing many A particles at the cell periphery. Intracytoplasmic A particles are also seen (arrow). $\times 37,600$. Fig. 4. Higher power view of EA tumor cells showing an intracytoplasmic A particle (arrow) adjacent to the cell membrane. $\times 23,900$. Fig. 5. C-type particles budding from cell membrane (arrow). $\times 23,900$.



able to suggest that A particles may represent a precursor or a formative stage of the C particles.

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The EM immunocytochemical demonstration of lysozyme in macrophage giant cells in sarcoidosis

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Summary. The giant cells (multinucleate macrophages) of human sarcoidosis have been shown by the unlabelled antibody immunoperoxidase technique at electron microscope level to contain lysozyme within cytoplasmic granules.

It is now widely held that macrophages are secretory cells producing a wide variety of substances - hydrolytic enzymes, elastase, collagenase and lysozyme among others³⁻⁸. Evidence has recently been submitted that in an animal model granuloma the aggregate of macrophages may act as an endocrine gland secreting lysozyme into the blood and lymph, and causing an elevation of serum lysozyme⁹. In human sarcoidosis¹⁰ a similar situation probably exists.

Granuloma macrophages have been shown immunocytochemically to contain lysozyme¹¹, and serum lysozyme levels are elevated in sarcoidosis and other granulomatous lesions¹²⁻¹⁴.

Ultrastructural examination of sarcoid granulomas has shown the component macrophages to contain several types of inclusion of which electron dense and electron lucent types are of present interest. The electron lucent inclu-

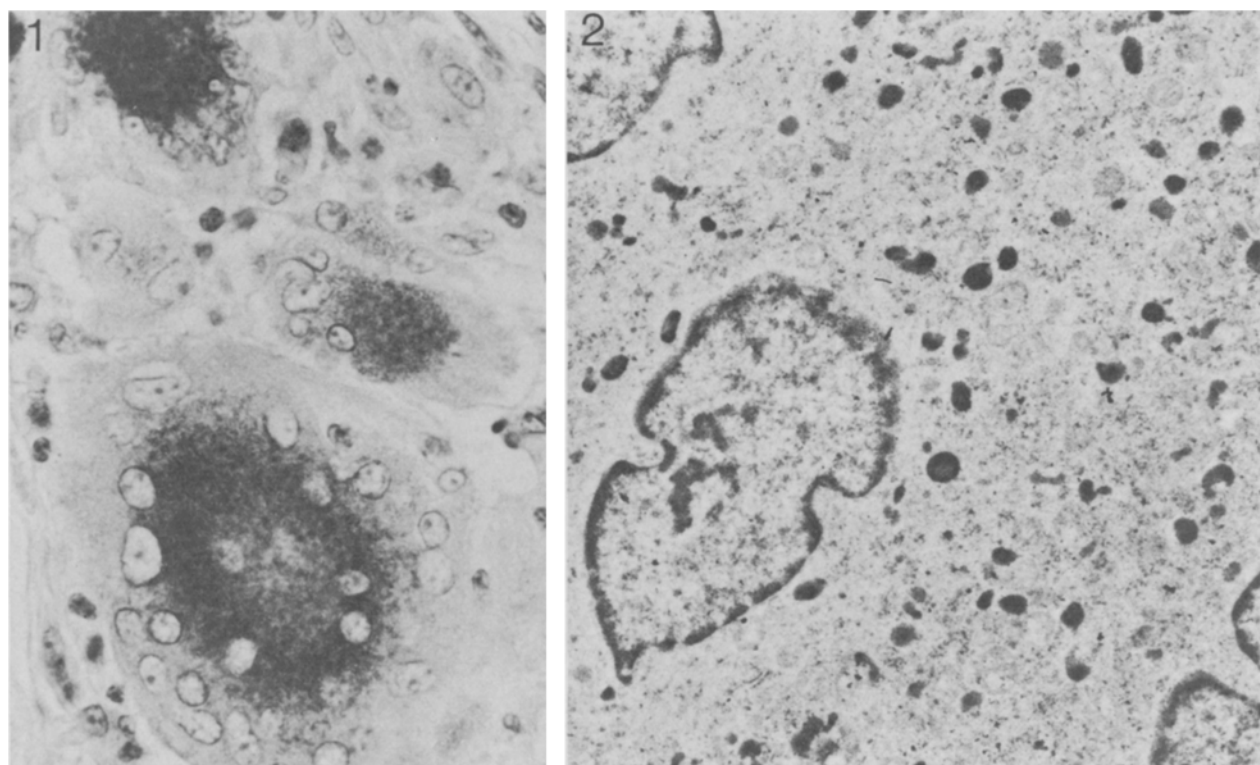


Fig. 1. Light photomicrograph of giant cells in sarcoid lesion stained by immunoperoxidase using antilysozyme antiserum. There is a granular deposit in the centre of the giant cells. 6 μ m paraffin section. $\times 624$. Fig. 2. Electron micrograph of giant cell in sarcoid lesion stained by immunoperoxidase using antilysozyme antiserum. There is strong positive staining in many but not all lysosomes. Mitochondria do not stain. $\times 11,500$.