5-Azacytidine is incorporated in vivo into RNA of mammalian cells[11], in distinction to 5-iodo-2′-deoxyuridine[7] which is taken up into DNA. It suppresses the growth of L1210 and of AKR leukemia[12,8] and inhibits the synthesis of antibodies[13]. Accordingly, the possibility must be considered that AKR leukemia could have been evoked on account of the immunosuppressive effects of 5-azacytidine by the impairment of the synthesis of corresponding antibodies. However, the immunosuppressive effect of 5-azacytidine is inferior to that of cyclophosphamide[13] which has failed to activate the virus synthesis in vitro[14]. Similar negative results in tissue culture were obtained also with cytosine arabinoside, 5-fluoro-2′-deoxyuridine, 6-azathymine, 6-azauridine, 6-mercaptopurine and 6-azaguanine[14]. It is clear that leukemia virus is present in tissues of AKR mice from the time of birth[15]. It is difficult to conclude at present whether the chemical treatment described is analogous to the induction of virus synthesis in vitro. However, our preliminary studies of virus titers in respective mouse tissues indicate that virus is being induced by 5-azacytidine also in vivo.

Zusammenfassung. Nachweis der leukämogenen Wirkung von 5-Azacytidin und 5-Jod-2′-Desoxuridin beim Stamm der AKR-Mäuse. Es wird angenommen, dass es sich hierbei um eine Virusinduktion handelt.

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A Study of the Cytology and Cell Culture of the Canine Transmissible Venereal Sarcoma

Canine transmissible venereal sarcoma (CTVS) is a neoplasm occurring naturally on the genitals of both male and female dogs. It is the oldest transplantable tumor known and is readily transplanted by sexual contact and by parenteral injection of viable tumor cells. The etiology, histogenesis, and the mechanism of universal 'take' and spontaneous regression of the tumor are poorly understood[1–3]. Although there is no serious objection to the belief that the CTVS is truly neoplastic, it has been considered by some competent pathologists to be infectious granuloma[4]. Ultrastructural examination of the tumor cells by previous workers has not revealed distinctive features and the reports of the presence of virus-like particles have been controversial[5]. We now report that the CTVS cells behave like tumor cells in culture and that a distinctive ultrastructural feature is indeed present.

Materials and methods. Canine transmissible venereal sarcoma. The original tumor (kindly provided by Dr. G. Brown of Farragut Animal Hospital, Knoxville, Tennessee) from the vagina of a poodle of mixed ancestry was removed surgically and portions were processed immediately for cell culture, chromosome analysis, histopathology, and electron microscopic studies. Other portions of the tumor were kept in Medium 199 overnight before being transplanted into dogs. Single cell suspensions and tumor pieces were prepared by mincing the tumor tissues with scissors without the use of trypsin. Non-preconditioned, apparently healthy, dogy of different ages were used for in vivo tumor passage. Either single cell suspensions or tumor pieces of (1–2 mm^3) suspended in serum-free Medium 199 were injected s.c. into the interscapular region. Chromosome, histopathology, and transplantation studies of the original and transplanted tumors confirmed that the tumor was indeed canine transmissible venereal sarcoma[1–7].

Electron microscopic studies. The original tumor was fixed in 3.5% glutaraldehyde and processed as previously described[8].

Cell culture. The tumor cells were cultured as monolayers in Medium 199 and Minimum Essential Medium (MEM) supplemented with 20% fetal calf serum or as suspensions in RPMI 1640 supplemented with 20% fetal calf serum. Final cell concentration of 1×10^6 and 2×10^6 viable cells per ml, as determined by trypan blue dye exclusion test, were used for initiation of the cultures. Trypsinization was done at the time of subculturing monolayer cell cultures.

Results and disscussion. Electron microscopic studies showed that large round or polygonal cells were most predominant in the original donor tumor. The nuclei were large, vesicular and with prominent nucleoli. Mitochondria, smooth endoplasmic reticulum and ribosome granules were sparse but the Golgi apparatus was relatively well developed and there were many small cytosomes in the cytoplasm. Mitotic figures were frequently found. In addition, cells resembling plasma cells, lymphocytes, and fibroblasts were mingled within the main tumor mass. Tumor cells with increased rough endoplasmic reticulum occasionally showed whorl like formation (Figure 1). To our knowledge, this structure had not been recorded by previous workers for this tumor. Similar, but not identical, concentric formations of rough endoplasmic reticulum have recently been observed in human osteosarcoma cells in culture[9], in the oocyte and folicular cells of the lizard *Lacerta sicula* during winter rest[10], and in the border cells of the adrenal glands of the Mongolian gerbil[11].

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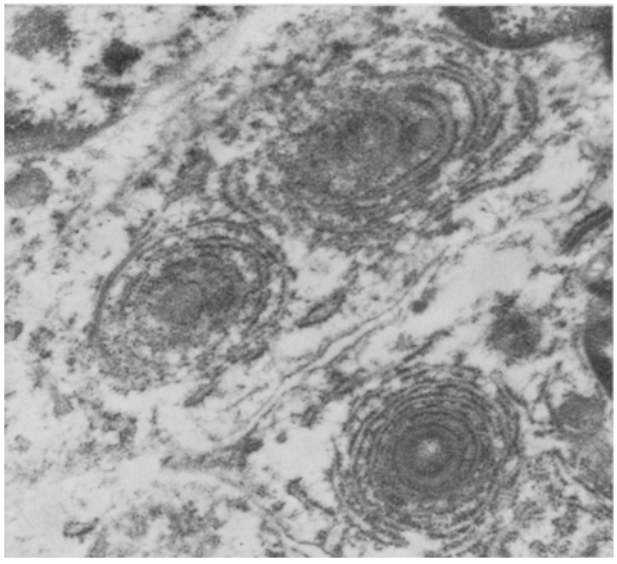

Fig. 1. Whorls of rough endoplasmic reticulum seen in canine transmissible venereal sarcoma cells. × 20,500.

The mechanism of their formation is unknown. The 'ribosomal bodies' in the hibernating lizard undergo progressive transformation into rough endoplasmic reticulum and free ribosomes during spring and the 'membranous whorls' in the border cells of the Mongolian gerbil adrenal gland transform to smooth endoplasmic reticulum in response to the stress of sham injections with accompanying increase in cortisol production. Teleologically it is tempting to speculate that the 'whorls of rough endoplasmic reticulum' we have observed in CTVS are functionally useful for the resting old tumor cell to adapt quickly to the new host and to initiate rapid growth.

Although not shown here, preliminary studies of the original tumor revealed the presence of virus-like subcellular particles. The tumor is an 'old' tumor and a study has been extented to transplanted tumors at different stages of growth and regression in order to see whether a virusevoked regression mechanism such as viral oncolysis or antigenic heterogenization is involved.

At the start of the monolayer cultures, all the cells were round in shape, although some fusiform and spindle-shaped cells began to appear after several days. Occasionally they were surrounded by or intermingled with, healthy round cells. Generally, spindle-shaped cells became dominant in 2 to 3 weeks. However, occasional cultures with predominantly fusiform cells were found. Apparently, many of the spindle-shaped cells were tumor cells because they developed into foci of randomly oriented, crisscrossing, round to irregular-shaped cells piling up on one another after 2 to 3 weeks (Figures 2–4). Although cell culture studies of CTVS have been made previously[12,13] similar foci of piled-up cells without contact inhibition have not been reported for this tumor. Definitive proof that these cells are indeed tumor cells has to be made by in vivo tests. The present finding, however, argues strongly against the suggestion made by some workers that the venereal sarcoma might only be a granulomatous growth[4]. Furthermore, the presence of these foci raises the possibility of cloning or isolating the tumor cells or for obtaining more tumorigenic cells. Suspension cultures of the tumor usually die out after several weeks[14].

Résumé. Un cas avancé de tumeur canine spontanée et transmissible par voie vénérienne a été examinée au microscope électronique. Ce sarcome est caractérisé par la présence dans les cellules tumérales de rouleaux de rédiculum endoplasmique et de nombreuses inclusions cytoplasmiques. Les cultures de la tumeur originale et ses transplants forment des zones de cellules superposées sans indications d'une inhibition de contact après 2 à 3 semaines.

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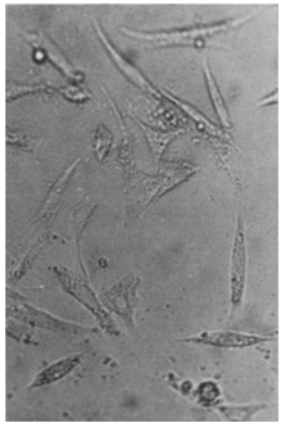

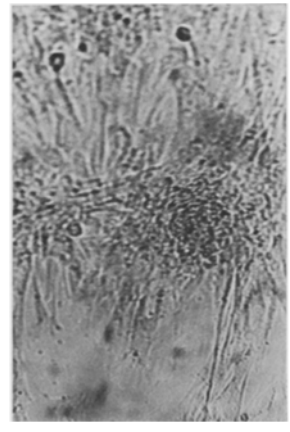

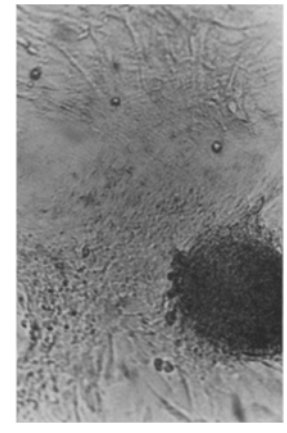

Figs. 2, 3, and 4. Typical sequential stages of the growth of canine transmissible veneral sarcoma in cultures which lead to formation of foci of piled-up cells in 2–3 weeks. ×100.