Submicroscopic Morphology of Avian Neoplasms. III. Studies on Visceral Lymphomatosis.* (24680)

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The natural occurrence of visceral lymphomatosis (lymphoid leukosis lymphocytoma), one of the diseases of fowl leukosis, is widespread, the disease being transmitted from parent to offspring *via* the egg and also by contact with infected environment(1). Experimental transmission may be effected by infective material, such as plasma, oral washings, and various extracts (tumors, embryonated eggs, feces) from either naturally infected chickens or those used in serial passage of the virus. In most cases, exposure of susceptible chickens to all the different sources of virus, has resulted in development not only of visceral lymphomatosis but also of erythroblastosis. Relative and absolute occurrence of each disease is subject to marked variation and appears influenced by effective dose of virus and genetic variation of host(2,3). It remains to be determined whether these 2 disease entities are caused by specific viruses or by a single multipotent virus. The present studies were undertaken to characterize any submicroscopic changes and specific structures which may be found in organs of chickens with visceral lymphomatosis acquired after natural and experimental exposure.

Materials and methods. Chickens with visceral lymphomatosis induced experimentally with RPL-12 lymphoid tumor, and others with the disease acquired under natural conditions were used. The RPL-12 tumor strain originated as a field case(4). Cell-free preparations of this tumor fail to induce tumors at site of inoculation but produce a high incidence of lymphomatosis tumors of viscera in chickens(5). Visceral lymphomatosis occurs also in chickens in contact with other birds inoculated with filtrates of strain RPL-12 tumor. They induce, after 4 months, visceral lymphomatosis characterized by extravascular proliferation of lymphoid cells in one or more viscera(2,3). A secondary feature in some chickens is the intravascular involvement with a character of erythroblastic leukosis(2). Donor birds had visceral lymphomatosis induced by intravenous inoculation of chickens at age 1 day to 3 weeks, with cell free filtrates of different passages of strain RPL-12 tumor. Chickens which contracted the disease naturally, or by contact with inoculated birds were also used as donors. Spleen from chickens showing moderate or extensive tumor deposits, histologically diagnosed as visceral lymphomatosis was studied in ultrathin sections by means of RCA EMU-3A electron microscope. The method of tissue preparation for study in ultrathin sections was the same as that used for erythroblastosis(6) and granuloblastosis(7).

Results. The cytoplasm of tumorous cells of spleen from chickens with visceral lymphomatosis showed alterations in mitochondria, endoplasmic reticulum, and cellular membranes, while the nucleus usually remained unchanged. Osmiophilic bodies of varying size and appearance were also frequently encountered. A general view of appearance of sections of the affected spleen from chickens with experimentally induced visceral lymphomatosis is shown in Fig. 1. As can be seen, not all tumor cells show changes. The appearance of several cells at somewhat higher magnification may be seen in Fig. 2. Numerous osmiophilic bodies of varying size and different appearance are present. In addition, structures resembling virus particles may be seen within the osmiophilic bodies and scattered in the cytoplasm (Fig. 2). A cell with only a few inclusion-like bodies is shown in Fig. 3. The cell also contains a number of different size cytoplasmic vacuoles filled with

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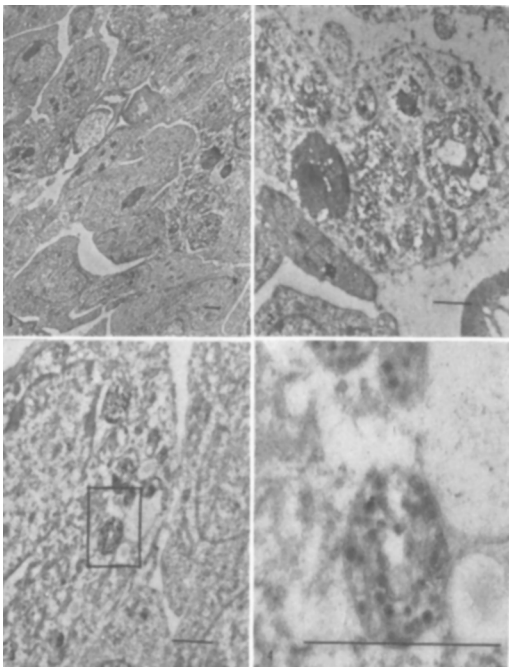

FIG. 1. Electron micrograph of section of tumorous cells of spleen from chicken with visceral lymphomatosis. Dense osmiophilic bodies present in cytoplasm of some cells. Magnification 1,700 ×.

FIG. 2. Cytoplasm of tumorous cells of spleen with inclusion-like bodies of different size and appearance and characteristic particles. Magnification 4,750 ×.

FIG. 3. Inclusion-like bodies with characteristic particles and vacuoles present in the cytoplasm of cell surrounded by apparently unaffected cells. Magnification 4,750 ×.

FIG. 4. Inclusion-like body from Fig. 3 with particles showing characteristic internal structure. Magnification 24,000 ×.

homogeneous material, and is surrounded by cells showing no apparent alterations. An inclusion-like body, similar to one shown in Fig. 3, may be seen at higher magnification in Fig. 4. It is in part surrounded by what appears to be a membrane, and contains a number of characteristic particles, showing a dense central core with an outer lighter zone, surrounded by external limiting membrane. Appearance of a disintegrating cell or cells is shown in Fig. 5. An inclusion-like body, marked by letter "a", may be seen at higher magnification in Fig. 6a. Characteristic particles, resembling virus particles, are surrounded by what appears to be a limiting membrane. Part of Fig. 5, marked by letter "b", may be seen at higher magnification in Fig. 6b, which shows characteristic membranous structures, frequently encountered in cytoplasm of affected cells. The changes described and characteristic particles were found in approximately one out of 50 tumor cells examined. The characteristic appearance of particles within inclusion-like bodies and in intercellular spaces may be seen in Fig. 7 and Fig. 8. The particles, whether within inclusion-like bodies or outside the cells, are of similar appearance. An estimation of the size of 700 particles, measured both within cells and in intercellular spaces, showed average size of smallest particles to be approximately 640 Å and that of largest particles approximately 820 Å. Overall average size of particles was 720 Å and average size of internal dense core approximately 300 Å.

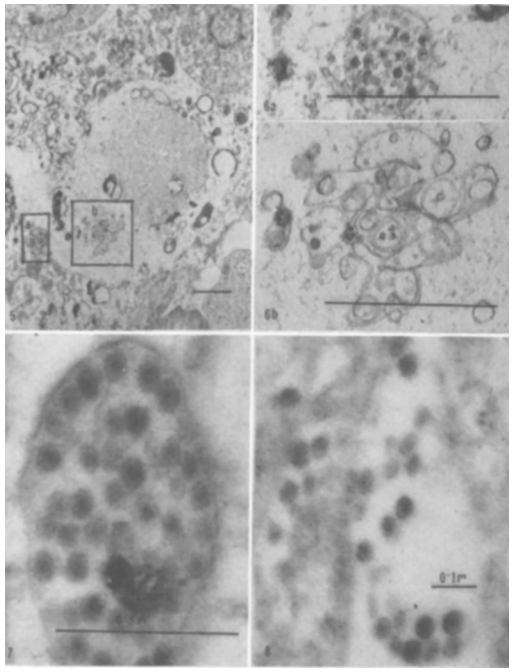

FIG. 5. Appearance of breakdown cell or cells, surrounded by comparatively unchanged cells. Magnification 4,250 ×.

FIG. 6a and 6b. Parts of Fig. 5 enlarged. Characteristic structure of particles within inclusion-like body. Membranous structures probably originating from endoplasmic reticulum. Magnification 21,250 ×.

FIG. 7. Appearance of characteristic particles within an inclusion-like body surrounded by a membrane. Magnification 56,250 ×.

FIG. 8. Appearance of similar particles in an intercellular space. Magnification 45,000 ×.

Discussion. Study of ultrathin sections of tumorous spleens from chickens with naturally acquired and experimentally induced visceral lymphomatosis showed a number of similar characteristic changes in cytoplasm of some tumor cells. Inclusion-like bodies, some with spherical or spheroidal structures resembling virus particles were frequently observed. The particles were also found in cytoplasmic vacuoles, occasionally in cytoplasm and frequently in intercellular spaces. Other changes in cytoplasm of tumor cells, such as vacuolization, breakdown of mitochondria, swelling and breakdown of endoplasmic reticulum, and of cellular membranes, although not strictly specific for viral infections, have usually been observed in association with the presence of inclusion-like bodies and characteristic viral structures. Close examination of the inclusion-like bodies revealed the surrounding membrane to be composed in places of 2 membranes and presence of what could be interpreted as parts of broken-down cristae. It appears that some inclusion-like bodies may be of mitochondrial origin. Similar bodies were described in erythroblastosis and their origin attributed to mitochondria(8). Characteristic "whorl-like" formations of membranous structures have frequently been observed in some tumor cells of the affected spleen. Their origin appears to be from endoplasmic reticulum and they may be an expression of cellular defense mechanism. Somewhat similar membranes have been observed in other virus-cell systems, such as Ehrlich ascites tumor cells infected with Newcastle Disease Virus(9) and in monkey kidney cells infected with mumps virus(10).

Similar changes and characteristic particles have been observed in erythroblastosis(6) and in granuloblastosis(7). The particles observed in these 2 diseases, although similar in structure were consistently somewhat smaller in size than those encountered in visceral lymphomatosis.

Spleen of young control chickens failed to reveal similar particles, although they have been observed by others in approximately 10% of control birds(8).

Summary. Ultrathin sections of tumorous spleen from chickens with visceral lymphomatosis acquired after natural and experimental exposure have been examined in the electron microscope. Spherical and spheroid particles were observed within inclusion-like bodies present in cytoplasm of cells, in cytoplasmic vacuoles, and in intercellular spaces. The particles have an internal dense zone of about 300 Å, surrounded by a less dense zone and limited by an outer membrane of a size varying from 640 Å to 820 Å, and average diameter of 720 Å. These particles resemble in appearance but not in size particles observed in erythroblastosis and granuloblastosis of chickens.

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