Submicroscopic Morphology of Avian Neoplasms. IV. Studies on Erythroblastosis of Strain RPL-12.* (24681)

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Studies have recently been reported on submicroscopic morphology of avian erythroblastosis(1) caused by strain "R" of Engelbreth-Holm(2) and extensively investigated by Beard(3). Erythroblastosis is also caused by strain "RPL-12," which originally caused primarily visceral lymphomatosis, but after serial passages elicited erythroblastosis when given in large doses to susceptible chickens. Both strains in low doses also induce a high incidence of visceral lymphomatosis(4,5). The most notable biological differences between these 2 strains are range of time of death (12-30 days for strain R, 30-90 days for strain RPL-12) and occurrence of osteopetrosis marked with strain RPL-12 and negligible with strain R. Majority of chickens, when inoculated to age of 84 days with cell-free preparations of RPL-12 strain, die of erythroblastosis(4), while older chickens when inoculated with similar preparations, develop more frequently visceral lymphomatosis but only after 100 days, irrespective of age of inoculation. It has been suggested that visceral lymphomatosis virus is a "wild type" virus, since in contrast to erythroblastosis virus it has maintained itself in most poultry populations for several decades(6). The present studies were carried out as a first step in ascertaining relationship of agent or agents causing visceral lymphomatosis and erythroblastosis RPL-12 strain.

Material and methods. Chickens which developed erythroblastosis following intravenous or intraperitoneal inoculation with cell-free preparations of RPL-12 tumor strain, served as donors. Spleen from chickens with macroscopic and microscopic changes diagnostic of erythroblastosis was used for ultrathin section studies in an RCA EMU-3A electron microscope. Method of specimen preparation for electron microscopy was the same as in study of organs from chickens with erythroblastosis caused by "R" strain of Engelbreth-Holm(1), granuloblastosis(7), and visceral lymphomatosis(8).

Results. The general appearance of some tumor cells from spleen of chickens with erythroblastosis RPL-12 strain, as frequently encountered during scanning, may be seen in Fig. 1. Cytoplasm and nucleus of cells appear unchanged. Nuclear and cytoplasmic membranes may be seen clearly delineated and intercellular spaces are well defined. Frequently, however, (Fig. 2), at somewhat higher magnification dense spherical particles have been encountered in clusters or in rows within intercellular spaces and in what appear to be vacuoles in the cytoplasm of some cells. Majority of cells appear unchanged, although some vacuoles may be seen in the cytoplasm of a cell in lower left hand corner. A cluster of particles within what may be a vacuole in the cytoplasm of a cell in Fig. 2, may be seen at higher magnification in Fig. 3. Some particles show a characteristic internal structure, with a central dense core and a limiting membrane surrounding a less dense zone around the central core. Similar spherical or spheroid particles have been observed scattered within the cytoplasm of cells and in inclusion-like bodies, (Fig. 4). Appearance of some tumor cells, frequently observed, may be seen in Fig. 5. Numerous dense osmiophilic bodies of varying size and appearance are a striking feature. Cell membranes have disappeared and cytoplasm of cells contains numerous altered mitochondria and vacuoles, some of considerable dimensions, partly filled with irregularly shaped dense osmiophilic material. In addition, inclusion-like bodies may be seen containing spherical particles. Some particles

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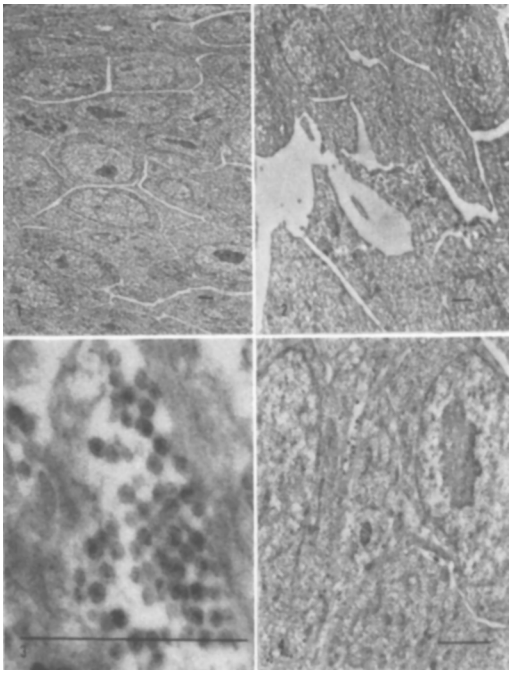

FIG. 1. General view of an ultrathin section of tumorous spleen from chicken with erythroblastosis RPL-12 strain. Magnification 1,700 ×.

FIG. 2. Characteristic particles present among apparently unchanged cells of tumorous spleen. Magnification 2,500 ×.

FIG. 3. Part of Fig. 2 showing characteristic particles. Magnification 28,000 ×.

FIG. 4. Dense inclusion-like body with particles, also present in intercellular spaces. Magnification 6,000 ×.

are also scattered in the cytoplasm and in spaces between broken down cell material. Part of cytoplasm of a tumor cell, with an inclusion-like body containing characteristic particles, some lying free in the cytoplasm, is shown in Fig. 6. Characteristic membranous structures occasionally observed in breakdown cells may also be seen. Comparison of appearance of characteristic particles within an inclusion-like body and those scattered within and outside the cytoplasm of a cell is shown in Fig. 7 and Fig. 8. Particles shown in both Figures reveal the same characteristic structure. Those within the inclusion-like body appear more uniform in size than those outside the body.

Calculation of size of 700 particles present within inclusion-like bodies and outside the cells, showed average size of smallest to be approximately 650 Å and that of largest particles approximately 830 Å. The overall average size of all particles has been estimated at 730 Å. The size of their dense core ranged from 280 Å to 380 Å, average 320 Å.

Discussion. Electron microscope studies of spleen affected by visceral lymphomatosis(8) and erythroblastosis RPL-12 strain, revealed an essential similarity in changes of tumor cells in these 2 conditions, and also a similarity in size and frequency of appearance of characteristic virus-like particles present in affected organs. Average size of particles, based on estimation of 700 particles in each of the 2 diseases was similar, 720 Å and 730 Å respectively. The morphological similarity of particles, assuming they are the causative agent, could conceivably indicate that the same agent

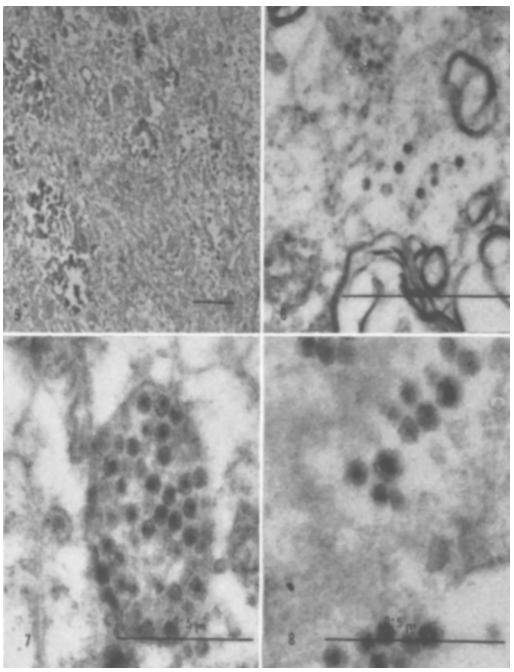

FIG. 5. General view of an ultrathin section of tumorous spleen from chicken with erythroblastosis RPL-12 strain, showing breakdown cells with dense osmiophilic bodies and particles. Magnification 4,800 ×.

FIG. 6. Part of a cytoplasm of breakdown cells with characteristic particles within an inclusion-like body and in the cytoplasm. Whorl-like formations of membranous structures. Magnification 21,000 ×.

FIG. 7. An inclusion-like body with characteristic particles. Magnification 37,000 ×.

FIG. 8. Characteristic particles lying free in and outside the cytoplasm of an affected cell. Magnification 45,000 ×.

is involved. Frequent induction of visceral lymphomatosis and erythroblastosis RPL-12 strain by the same cell-free preparations from naturally occurring cases of visceral lymphomatosis may indicate variation or mutation of the same agent(4). Further studies, however, are required to settle the problem.

Changes observed in organs of chickens with erythroblastosis "R" strain(1) and granuloblastosis(7) are essentially similar to those seen in visceral lymphomatosis(8) and erythroblastosis RPL-12 strain. However, the average size of the characteristic particles, based on a count of approximately 700 particles, is 640 Å in erythroblastosis "R" strain and 620 Å in granuloblastosis. A similarity in size and structure of particles present in granuloblastosis(7) and erythroblastosis "R" strain(1), however, does not necessarily indicate their identity, in view of observed chemical and serological differences between the agents of these 2 diseases(9).

The average size of particles in visceral lymphomatosis and erythroblastosis RPL-12 strain, based on the average of 700 measurements, would appear to be larger than the average size of particles in erythroblastosis "R" strain and granuloblastosis, but further statistical analysis will have to be made before unequivocal conclusions can be drawn about the observed difference in size of the particles.

It has been suggested, although evidence is far from conclusive, that all forms of avian leukosis and related neoplasms are caused by a single multipotent virus(10,11,12). Evidence accumulated during recent years indicates, however, that viruses of avian leukemias, erythroblastosis, and myeloblastosis (3), virus of lymphomatosis(13), and viruses responsible for numerous avian sarcomas, although related agents, are in fact separate and distinct biological entities(3). The simultaneous application of as many technics as possible to the study of these viruses, such as biological, biophysical, biochemical as well as others and also of electron microscopy of ultrathin sections of tissues and organs infected by these viruses, may possibly settle the problem of the relationship of these agents.

Summary. Tumorous spleen from chickens with erythroblastosis RPL-12 strain were examined in ultrathin sections. The cytoplasm of cells of the affected spleens showed changes similar to those observed in visceral lymphomatosis. Characteristic particles were observed in cytoplasm of cells similar in structure and size to that of particles in visceral lymphomatosis. The diameter of the particles was found to vary from 650 Å to 830 Å, with an average of 730 Å, and that of the internal dense core varied from 280 Å to 380 Å, with an average of 320 Å. The average size of the characteristic particles in erythroblastosis RPL-12 strain and visceral lymphomatosis is similar and appears to be larger than that of similar particles in erythroblastosis "R" strain and granuloblastosis (myeloblastosis).

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