Submicroscopic Morphology of Avian Neoplasms. II. Studies on Granuloblastosis (Myeloblastosis).* (24145)

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Much valuable information about size and structure of a number of viruses has been gained by study of ultrathin sections of virus-infected cells. Examination of viruses in ultrathin sections of infected cells constitutes a convenient first step in investigation of ordinary and also of tumor-inducing viruses. This method provides a new approach to the study of viruses, their structure, mode of development, the part they play in the life of the cell, and also for similar investigations of tumors of suspected or unknown viral origin. Information gained in examination of ultrathin sections of tumors of known viral origin will undoubtedly be of help in the study of ultrathin sections of tumors of unknown viral etiology. The present studies were a continuation of investigations on viral agents involved in the origin of different forms of chicken leukosis complex, known to be transmitted by cell-free preparations. These studies constitute a first step in the attempts at characterization of different viral agents of chicken leukosis and assessment of their etiologic relationship in different types of chicken leukoses. A study of ultrathin sections of the affected organs of chickens with erythroblastosis has been reported(1). The present studies were undertaken to obtain information about changes in cells of the affected organs of chickens with granuloblastosis (myeloblastosis).

Materials and methods. The granuloblastosis strain was originated by Hall, Bean and Pollard(2) and studied extensively by Beard and his collaborators(3). Fourteen-day-old chickens of line 15, of the U. S. Regional Poultry Research Laboratory, East Lansing, Mich., were inoculated intravenously with a dose of log-1 ml of plasma from chickens with granuloblastosis. Chickens were killed on 19th day after inoculation and spleen and liver from chickens showing macroscopic and microscopic changes characteristic of granuloblastosis were used for ultrathin section studies. The method of tissue preparation for study in ultrathin sections was the same as for erythroblastosis(1). An RCA EMU 3 A electron microscope was used for study of the sectioned material.

Results. Tumorous† cells of spleen and liver from chickens with granuloblastosis (myeloblastosis) were found in ultrathin sections to have similar changes to those described in cells of the involved spleen and liver of chickens with erythroblastosis(1). In addition to changes in endoplasmic reticulum, mitochondria and the cell membrane, inclusion-like bodies were observed. These appear to be more frequently found in cytoplasm of tumorous cells (in approximately 1 out of 30 cells examined) than similar bodies in cells of erythroblastosis tumors. Characteristic particles with internal structure similar to that of particles in erythroblastosis have been found in inclusion-like bodies, freely scattered in cytoplasm of cells and also in intercellular spaces. The nucleus of the involved cells remained apparently unchanged. The outer diameter of the characteristic particles, showing an internal dense core, varied

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† After this manuscript was completed, a report on ultrathin sections of myeloblasts appeared (D. F. Parsons, Painter, J. C., Beaudreau, G. S., Becker, C., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 839) in which it was indicated that virus particles were only rarely seen in myeloblasts but were numerous in other tissue cells. This is at variance with findings of this study, in which cells with described changes may be regarded as granuloblasts.

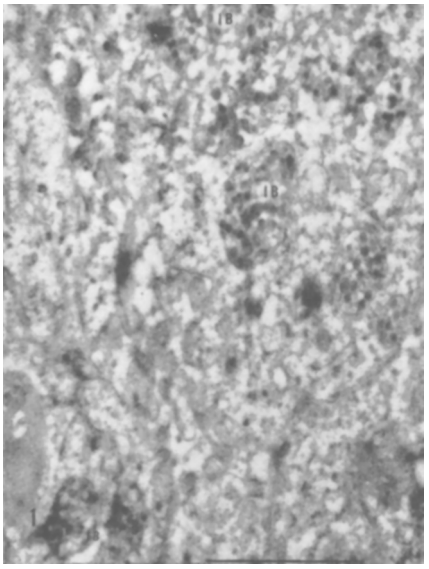

FIG. 1. Electron micrograph of ultrathin section of tumorous cells of spleen from chicken with granuloblastosis. Inclusion-like bodies (IB) with characteristic particles present in cytoplasm. Magnification 20,000 ×.

from 570 Å to 770 Å, with an average of 670 Å. Size of the dense center of the particles varied from 220 Å to 320 Å, averaging 260 Å.

An electron micrograph of an ultrathin section of several tumorous cells in spleen of a chicken with granuloblastosis is shown in Fig. 1. The characteristic feature of the picture is the considerable number of inclusion-like bodies frequently encountered in cytoplasm of the cells. At times, this cytoplasm was found almost entirely filled with these bodies containing the characteristic particles. Occasional particles lying free in the cytoplasm or within what may be extended endoplasmic reticulum may be seen in Fig. 1. Size of the inclusion-like bodies and number of particles within these bodies vary considerably. Most of the bodies are surrounded by a membrane, occasionally by a double membrane. Similar bodies at higher magnification are shown in Fig. 2. Some of the bodies show the characteristic particles in indistinct outlines. Some of the disorganized endoplasmic reticulum can also be seen. Inclusion-like bodies of somewhat different appearance are shown in Fig. 3 and 3a. These bodies, still surrounded by a distinct membrane, appear to be vacuoles in the cytoplasm containing large numbers of the characteristic particles. Some mitochondria showing changes are also seen. Altered mitochondria, and size which the inclusion-like bodies may reach are shown in Fig. 4. Particles at higher magnification present within the inclusion-like body are shown in Fig. 4a, an enlargement of Fig. 4. The ultrastructure of some of the particles may be clearly seen; also the dense material forming the central core of the particles may be seen either alone or partly surrounded by a limiting membrane. Some membranes without the dense center are also present. The same particles as they appear in intercellular spaces are shown in Fig. 5. There appears to be no difference in the ultra-structure between these particles and those within the inclusion-like bodies. However, a greater variation in size was encountered in particles lying outside the cells than in those present within the inclusion-like bodies.

Neither with respect to size nor to internal structure was there a distinction between particles found in ultrathin sections of the affected organs from chickens with erythroblastosis and granuloblastosis.

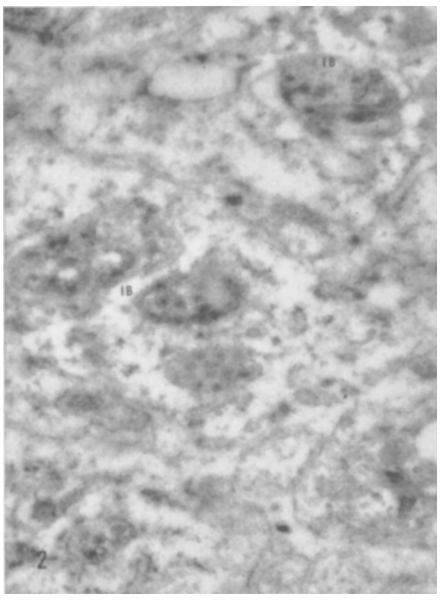

FIG. 2. Inclusion-like bodies (IB) in cytoplasm of tumorous cell with particles showing central core and peripheral membranes. Magnification 30,000×.

Discussion. Measurements of the size of characteristic spherical or spheroid particles observed in erythroblastosis and granuloblastosis gave almost identical values for both types of particles. Similar characteristic particles were observed in ultracentrifugal pellets from plasma of chickens with erythroblastosis and myeloblastosis (granuloblastosis) by Bernhard *et al.*(4) and in erythromyeloblastosis(6). It is possible that particles observed in our study and those found in myeloblastosis(4) represent the same agent. Measurements of the particle size in ultrathin sections of tumorous cells in erythroblastosis (1) and granuloblastosis differ somewhat from those reported by Bernhard *et al.*(4). Possible reasons for the difference were given previously(1).

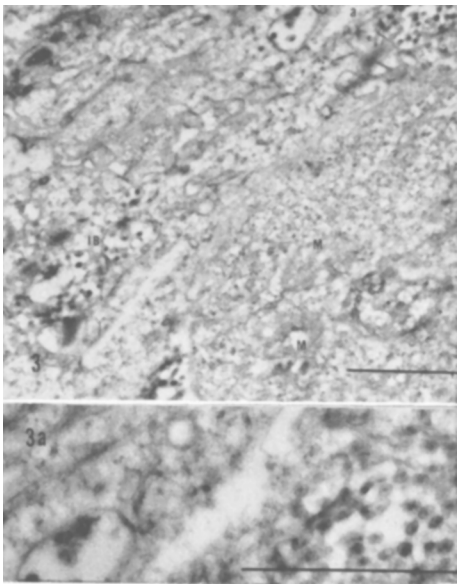

FIG. 3. Inclusion-like bodies (IB) containing characteristic particles; mitochondria (M). Magnification 15,000 ×.

FIG. 3a. Part of Fig. 3 marked "a" at greater enlargement. Ultra-structure of particles is seen and membrane surrounding the vacuole. Magnification 35,000 ×.

The similarity in size and structure of particles observed in these 2 forms of the chicken leukoses need not necessarily indicate that they are the same agent. Chemical differences have been shown to exist between agents of erythroblastosis and myeloblastosis (granulo-

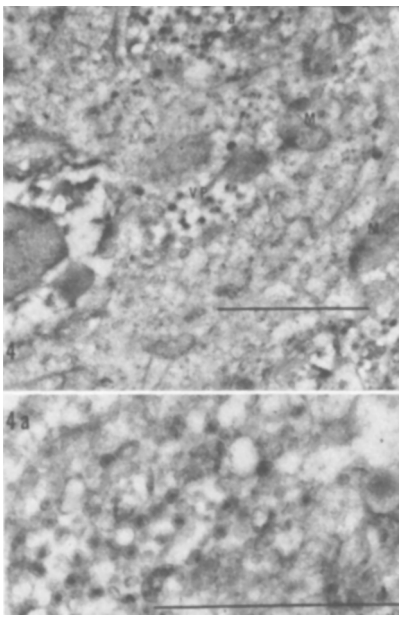

FIG. 4. Appearance of cytoplasm of tumorous cells from spleen of chicken with granuloblastosis. Mitochondria (M) showing changes. Characteristic particles (VP) scattered throughout cytoplasm and also present in inclusion-like body "a." Magnification 20,000 ×.

FIG. 4a. Part of Fig. 4, showing ultrastructure of characteristic particles. Magnification 35,000 ×.

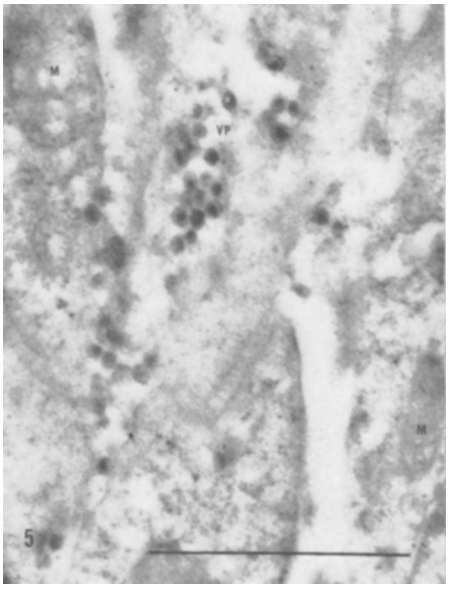

FIG. 5. Electron micrograph showing particles with similar ultra-structure present outside tumorous cell; mitochondria (M). Magnification 35,000 ×.

blastosis) by Beard and his collaborators(3). Whether the close immunologic relationship between the different agents of the leukosis complex(3) is indicatory of a close relationship between these agents and those of chicken tumors can only be settled finally when sufficiently pure preparations of the respective agents become available.

It does not appear probable that all agents involved in the origin of several chicken leukoses are identical, at least, in size. Electron microscope studies of ultrathin sections of spleen and liver of chickens with visceral lymphomatosis have been reported(5). The particles observed in the cells of the affected spleen were found to vary from 740 Å to 940 Å in diameter, with an average diameter of 840 Å. The size of their dense core ranged from 280 Å to 350 Å, with an average of 320 Å. These particles appear, therefore, to be considerably larger than those observed in erythroblastosis(1) and in granuloblastosis (myeloblastosis). It should be pointed out that the method used for preparing the material of visceral lymphomatosis for electron microscope studies was the same as that used for ultrathin section studies of erythroblastosis and granuloblastosis.

Summary. Tumorous spleens and livers from chickens with granuloblastosis were examined in ultrathin sections. In the cytoplasm of the affected cells changes were observed similar to those seen in erythroblastosis, another member of the fowl leukosis complex. Particles were observed in the cytoplasm of cells of similar size and structure to that of particles in erythroblastosis. Average total diameter of the particles was found to be 670 Å and that of the central dense core was 260 Å. No particles of similar size or structure were observed in ultrathin sections of organs from young healthy chickens of similar breed.

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Molt of Capon Feathering with Prolactin.* (24146)

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Our previously reported findings have shown that Progesterone (Pg) will initiate proliferation of the feather papilla with consequent molt in both sexes of fowl(1). Although the treatment interrupted ovulation for a period in the laying hen, the response of the male nonetheless made it seem possible that this activation of the papilla could be a direct effect of progestin. We attempted, unsuccessfully, to demonstrate a local effect of Pg upon the feather papilla(2); hence we made another approach to the question. Laying hens were treated with Pg and with other compounds of anticipated progestational activity while concurrently run experiments tested the effects of follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (LTH) when administered singly and in conjunction with a Pg dose of established potency in molt(3). The various treatments (cortisone acetate excepted) all caused molt and also interrupted lay. There were, however, 2 important developments. With

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