Ultrastructure of Viruses of Myeloblastosis and Erythroblastosis Isolated from Plasma of Leukemic Chickens.* (23642)

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Avian myeloblastosis and erythroblastosis are malignant leukemic diseases of the chicken caused by distinct though closely related viruses(1). These agents occur in the plasma of birds with the respective diseases, the virus of myeloblastosis in concentrations as high as 2 x 10^{12} particles per ml(2) and that of erythroblastosis at levels of 10^{10} to 10^{11} per ml(3). In morphology and size the 2 agents, examined by electron microscopy, either in the plasma or in ultracentrifugal concentrates of the plasma, are essentially identical(4). The spheroidal particles representing the respective agents vary widely in size, but average about 120 mμ in diameter(5,6). In both diseases, the particles have been identified by correlations of the results obtained with physical, chemical and biologic methods(7).

Examination of the particles by the usual technics of electron microscopy, involving metal shadowing, yields little evidence of the actual structure of the individual entities(1). Moreover, in the shadowed preparations, it was not always possible to distinguish unequivocally between virus and particles extraneous to the specific elements. In order to obtain information about the ultimate structure of the virus particles and of the relation of viral to non-viral material in concentrates, studies have been made of ultrathin sections of pellets resulting from ultracentrifugation of plasmas containing the agents.

Materials and methods. The myeloblastosis virus was the BAI strain A investigated at Duke University since 1949(1). This disease is a pure strain of myeloblastosis in which no evidence of abnormality of the red cells has been seen in stained smears of the circulating blood. The erythroblastosis virus was strain "R" of Engelbreth-Holm(8) obtained in 1955 from Dr. Astrid Fagraeus. Host response to the virus and the pathologic characteristics of this erythroblastosis have been uniform and wholly different from those seen with myeloblastosis.

Plasmas used as source of myeloblastosis virus were selected for high virus content by adenosinetriphosphatase activity with the micro screening technic(9), cleared of cells by centrifugation and stored under crushed CO_2 ice for about 2 months. Virus was sedimented from 3 plasmas separately by ultracentrifugation at 15,000 x g for 45 minutes. Erythroblastosis virus was obtained in the same way from 4.6 ml of a single plasma from a bird with a high content, approximately 250,000 per ml^3, of erythroblasts in the circulating blood. The actual virus content of the erythroblastosis plasma was unknown since this agent does not dephosphorylate adenosine triphosphate(3). The plasma had been frozen in CO_2 ice for about 6 months. Infectivity measurements have shown that both viruses are quite stable under these conditions. A series of 5 pellets of erythroblastosis virus was obtained from separate plasmas by like methods with a strain of the agent maintained at the Institut de Recherches sur le Cancer, at Villejuif. Here, also, analogous pellets were obtained from the plasmas of 12 apparently healthy chickens.

After centrifugation and removal of the plasma, the pellets were fixed immediately for 1 or 2 hours in 2% osmic acid buffered with veronal acetate at pH 7.3; dehydrated with alcohol; and embedded in methyl methacrylate by the usual procedures. Sections varying in thickness from about 250 to 500 Å were obtained with the Porter-Blum microtome. The electron micrographs were taken

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with the RCA EMU 2† or the Elmiskop I SIEMENS microscope.

Results. An electron micrograph of an ultrathin section obtained from a relatively large pellet derived from myeloblastosis plasma is shown in Fig. 1. The distinctive feature of the picture is the presence of circular or oval images representing cross sections of spheroidal particles. The images were strikingly similar in appearance but of variable diameter, usually about 80 mμ across. Some of the smallest, however, indicated a particle diameter of about 60 mμ, and the largest diameters were in the range of 110 mμ. A finely granular mass comprised the inner material which was of highest density in the central area. This central mass of high electron absorbing properties was about 35 to 40 mμ in diameter and comparable with the nucleoid seen in analogous pictures of other viruses. At a higher magnification (Fig. 2), the ultrastructure of the particles is more clearly visible. No typical structural organization was found in the nucleoid which appeared rather homogeneous. The external limitating membrane seemed to be single in most instances, but in some particles, it was distinctly separated into 2 layers (Fig. 2 and 3). The high degree of homogeneity of most specimens examined should be emphasized. In addition to the specific bodies described here, elements of variable size, corresponding to the debris of disintegrating blood cells, were also found in very small amounts.

In comparison with preparations of myeloblastosis virus, pellets from the plasma of chickens infected with the *erythroblastosis* virus are much smaller and contain fewer distinctive particles in electron micrographs of shadowed preparations(1,6). It was more difficult, also, to find the representative images in the ultrathin sections of the pellets. However, in the midst of variable quantities of cellular debris such as small mitochondria, ergastoplasmic lamellas with ribonucleo-protein granules, areas were found which contained thousands of particles similar to those seen in the myeloblastosis pellets. Their diameters varied, also, in the range of 75-80 mμ, and they, too, were surrounded by a membrane, which appeared single at low magnification, but often was double in the pictures of high resolution (Fig. 4 and 5). The central core was 35 to 40 mμ in diameter. It can be concluded that neither with respect to size, nor to ultrastructure was there a clear morphologic distinction between the agents of erythroblastosis and myeloblastosis.

The very small pellets from plasmas of the *healthy chickens* used for these experiments did not contain the specific elements.

Discussion. The results of earlier work have served to establish the identity of the spheroidal particles occurring in the plasmas of birds with myeloblastosis and erythroblastosis as the respective specific agents of these avian leukemias. In the instance of the agent of myeloblastosis the evidence is particularly strong because of access to the high concentrations of the virus in plasma and the ready applicability of a variety of technics. Identification of the virus of erythroblastosis has been less secure, but nonetheless convincing. There has been much evidence that the concentrates from myeloblastosis plasma must be of high homogeneity. In contrast, it has been recognized that the concentration of erythroblastosis virus in the plasmas has been of a far lower order than that seen with myeloblastosis, and that, although the erythroblastosis virus was concentrated in the pellets, the proportions of the agent to extraneous substance would be relatively small. The results of the present work corroborate fully the earlier findings and greatly extend the knowledge of the structure of the particles and of the constitution of the concentrates obtained by ultracentrifugation. It is evident that the application of the technic of ultrathin sectioning constitutes an invaluable addition to the technics for establishing the content of virus preparations.

Measurements of the diameters of the images gave essentially identical values for both agents. However, there was a striking difference between the data obtained from the sections and the diameters measured in the

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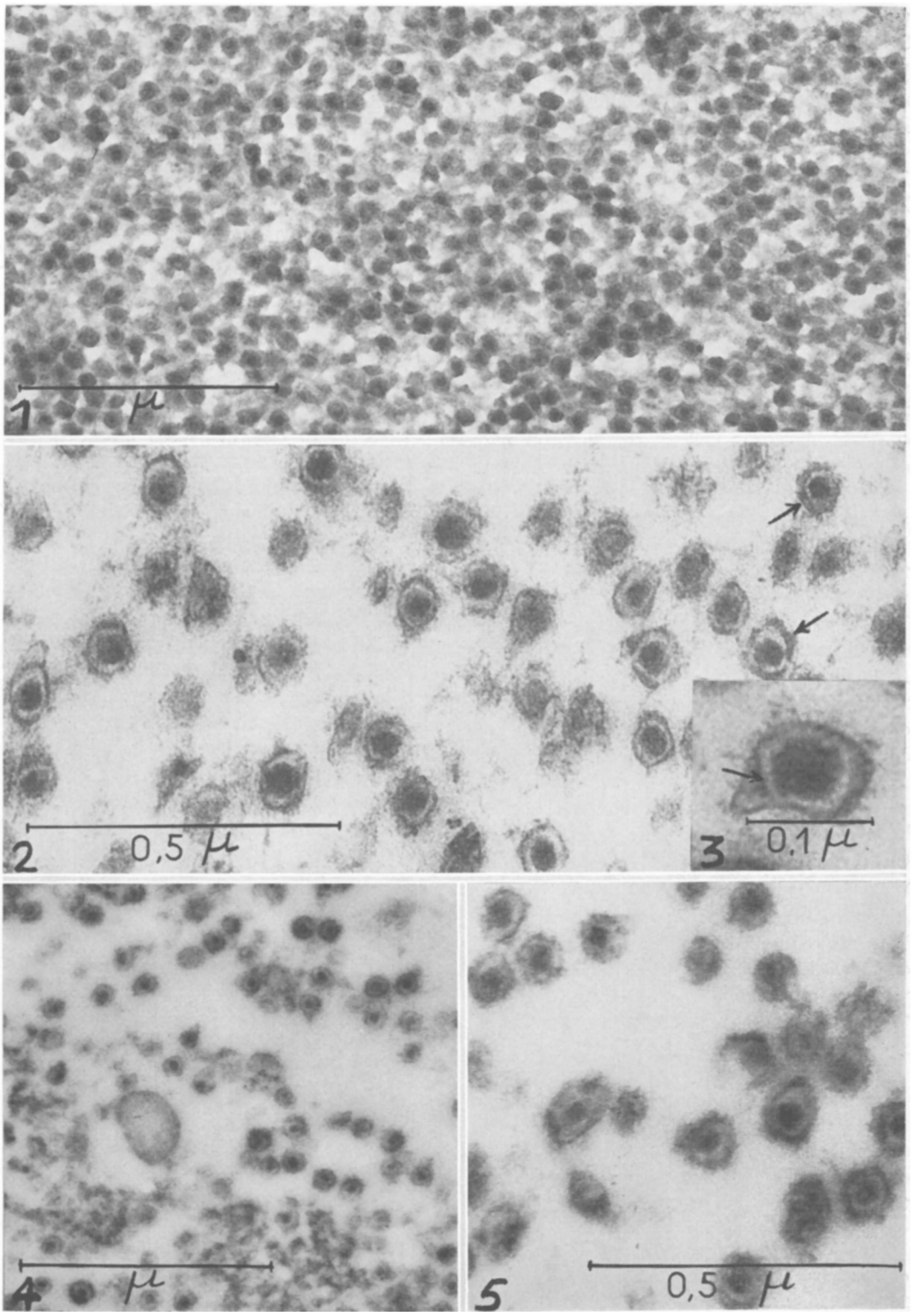
1 μ
2 0,5 μ
3 0,1 μ
4 μ
5 0,5 μ

micrographs of previous studies, namely, about 120 mμ(1,6), in which the estimates were made on dehydrated virus shadowed with metal. Sizes determined in this way may have been relatively large because of flattening of the particles and the thickness of the metal. It is unlikely that the diameter indicated in the present studies is indicative of the sizes of the agents *in vivo* which, for the myeloblastosis virus, was 144 mμ measured by sedimentation analysis(5). The dehydration and embedding in preparation for thin sectioning certainly produce shrinkage of variable degree, which is very difficult to evaluate in each single case. It is nevertheless striking that the virus particles as shown in thin sections of spleen and bone marrow from chickens with erythroblastosis(10), from the Rous sarcoma(11,12), and from the Murray-Begg endothelioma(13) are of approximately the same size as that of particles outside the cells. In addition, the inner structure of the viruses described in this paper was very similar, perhaps identical, to that of the particles found in all these tumors. Slight structural differences may exist between the various agents. For instance, it is not known why, in the sections of pellets of the leukemia viruses, the two layers of the double membrane were so closely adherent and appeared separated only occasionally, whereas in the particles visible in tissue sections of erythroblastosis and other chicken tumor viruses the two membranes were clearly distinct entities(14). It seems possible that these ultrastructural differences may have been related to storage in the frozen state or to the ultracentrifugal packing and did not represent the real differences *in vivo*. There is increasing evidence that all chicken tumor viruses belong to the same family as far as their fine structure is concerned(11,14). It is highly probable that the differences in size(11,13) or ultrastructure are due to differences in the technics applied by the various investigators. This is emphasized by recognition of immunologic interrelationships between the agents of the leukosis complex and those of the chicken tumors(1).

Summary. Ultrathin sections of the pellets formed by ultracentrifugation of plasmas from birds with myeloblastosis and erythroblastosis have been examined by electron microscopy. In the material from both diseases there were observed structures of similar appearance represented by images of round or oval shape consisting centrally of a dense core of about 40 mμ diameter. These were surrounded by a less dense material and were limited by an outer membrane-like structure with a total particle diameter of about 80 mμ. In many instances the limiting membrane appeared to be double. The ultrastructure of these agents of the avian leukemic diseases was very similar, if not identical, to that of the viruses of other chicken neoplasms. No particles of these characteristics were obtained from the plasmas of apparently healthy chickens.

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FIG. 1. Electron micrograph of a relatively thick section of a pellet of myeloblastosis virus. Virus particles of similar size and structure are practically the only constituent of this sample. Magnification 40,800 ×.

FIG. 2. Ultrathin section of another pellet of myeloblastosis virus at a higher magnification. The ultrastructure of the particles with their central core (''nucleoid'') and the peripheral membranes, is clearly visible. Arrows indicate regions of particles where the membrane appears duplicated. Magnification 100,000 ×.

FIG. 3. A single myeloblastosis virus particle at greater enlargement. Magnification 200,000 ×.

FIG. 4. Pellet from plasma of a chicken with erythroblastosis. Images characteristic of virus particles are clearly visible. In addition there are present disintegrating cell structures. Magnification 40,800 ×.

FIG. 5. Micrograph of the pellet from plasma of another chicken with erythroblastosis seen at higher magnification showing the ''nucleoids'' and outer membranes. These particles cannot be distinguished morphologically from the virus of myeloblastosis. Magnification 100,000 ×.

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Influence of *in vivo* Administration of Adrenocorticoids on Metabolism of Thymus, Liver and Tumor Tissues.* (23643)

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Prominent among approaches employed in investigations of the metabolic functions of adrenocortical hormones have been measurement of various metabolic products following administration of the hormones to live animals, and measurement of activity of specific enzymes or enzyme groups after hormone administration *in vivo* or *in vitro*(1,2). In the present studies, the hormones were administered *in vivo,* usually after adrenalectomy, and tissues were removed 4-6 hours later for *in vitro* metabolic studies. Tissues of adrenalectomized animals were employed for obtaining low-hormone control data. Because of the lability of lymphoid tissue to the action of adrenal corticoids, thymus tissue was given particular attention in anticipation of high metabolic sensitivity to cortisone preceding morphological changes(3).

Methods. Cortisone acetate (cortisone) or cortisone in combination with hydrocortisone was administered to 125-175 g male rats of the Holtzman strain or young adult male mice from the Stokely-Peterson colony 4 to 6 hours before the animals were killed and the tissues removed. The hormones, 10 mg per rat and 1 mg per mouse, were injected half by the intraperitoneal and half by the subcutaneous routes(4). When adrenalectomized animals were employed the operations were performed 3 to 5 days previous to hormone administration to allow for recovery from the operation with negligible increase in accessory corticoid-secreting tissue. After adrenalectomy the animals received 1% NaCl and 5% glucose in the drinking water for 24 hours and 1% saline thereafter. Purina laboratory chow was fed *ad libitum.* No adrenalectomies were performed in tumor-bearing animals. Cell suspensions of thymus, liver, or Murphy-Sturm lymphosarcomas from rats, prepared by the procedure of Kit and Barron(3), and Ehrlich ascites cells, harvested from mice, were washed and pipetted into Warburg vessels containing the radioactive substrate in a side arm. The flasks were covered with serum caps through which a 95% O_2-5% CO_2 mixture was introduced by hypodermic needle. All preparative manipulations were performed in the cold, and incubations were carried out in Krebs-Ringer bicarbonate buffer(5) for 60 minutes at 37.5° with shaking. At the end of the incubation 0.2 ml of 10% KOH was added to the center well and 0.3 ml of 70% perchloric acid (PCA) to the incubation medium by hypodermic syringe and long needle. The side arm was rinsed with the acidified mixture, and the flasks were shaken for an additional hour to insure the complete libera-

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