callosal) system. It would be, indeed, an interesting problem in mechanism of action to study the interactions of these agents at the enzymatic level.

Summary. 1. It has been demonstrated that chlorpromazine, perphenazine, proclorperazine, promazine, thiopropazate, promethazine and reserpine are effective antagonists of mescaline-induced mortality in CF_1 mice. 2. No significant protection was found with use of sodium phenobarbital, meprobamate, benactyzine, atropine, hydroxyzine, ethoxybutamoxane, SY-21, diphenylhydantoin, trimethadione, lysergic acid diethylamide, serotonin, amphetamine, morphine, pyrilamine, diphenhydramine, iproniazid, pilocarpine, and arecoline. 3. It is suggested that the protective effects of the ataractics against mescaline-induced mortality are centrally mediated.

1. Deegan, J. F., Cook, L., *J. Pharmacol. and Exp. Therap.*, 1958, v122, 17A.
2. Spech, L. B., *idem*, 1957, v119, 78.
3. Litchfield, J. T., Jr., Wilcoxon, F., *idem*, 1949, v96, 99.
4. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., Koetschet, P., *Arch. int. pharmacodyn.*, 1953, v92, 305.
5. Bein, H. H., *Experientia*, 1953, v9, 107.
6. Stone, C. A., Komrad, E. L., Loew, E. R., *Arch. int. pharmacodyn.*, 1952, v92, 7.
7. Slater, J. H., Jones, G. T., *J. Pharmacol. Exp. Therap.*, 1958, v122, 69A.
8. Marrazzi, A. S., *Ann. N. Y. Acad. Sci.*, 1957, v66, 496.
9. Marrazzi, A. S., Hart, E. R., *Science*, 1955, v121, 365.

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Submicroscopic Morphology of Avian Neoplasms I. Studies on Erythroblastosis.* (24144)

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Electron microscope studies of ultrathin sections of cells infected with a number of different viruses have revealed the structure of various submicroscopic cell elements during different stages of infection and have showed the complicated structure of the viruses in cells and tissues. The observation that viruses can be detected in diseased tissues and that they can be differentiated from normal cell constituents has in turn led to electron microscope studies of tumors of known or suspected viral origin(1,2). The neoplastic conditions now classified in the chicken leukosis complex were the first tumors induced by filterable agents(3). Some tumors of the fowl leukosis complex, such as visceral lymphomatosis(4), osteopetrosis(4), erythroblastosis and myeloblastosis (granuloblastosis)(5) have been transmitted by cell-free preparations. Other conditions, such as neuro-lymphomatosis and ocular lymphomatosis, have not as yet been transmitted by cell-free preparations. Extensive studies have been carried out to ascertain the physical, chemical, immunologic and other properties of the viral agents present in cell-free preparations(5). Electron microscope studies on the morphology of particulate material in cell-free preparations, which had been dehydrated and shadowed, were also carried out to characterize agents responsible for erythroblastosis, myeloblastosis and lymphomatosis(5). Recently, the technic of ultrathin sectioning has been applied to ultracentrifugal pellets from plasmas of chickens with myeloblastosis and erythroblastosis(6). These studies revealed the characteristic structure of particles present in the ultracentrifugal

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pellets. Similar particles were also observed in ultrathin sections of spleen and bone marrow of chickens with erythromyeloblastosis (7). The present studies were undertaken to characterize the particles found in tissues of chickens with different forms of chicken leukoses. As a first step in ascertaining the etiologic relationship of the agents to the various chicken neoplasms, studies have been made of ultrathin sections of organs from chickens with erythroblastosis.

Materials and methods. The erythroblastosis strain was started by Engelbreth-Holm (8), and used extensively by Beard(5). Fourteen-day-old chickens of line 15, of U.S. Regional Poultry Research Laboratory, East Lansing, Mich., inoculated intravenously with a dose of log-4.0 ml of plasma from chickens with erythroblastosis, served as donors. Spleen and liver from chickens showing macroscopic and microscopic changes diagnostic of erythroblastosis were used for ultrathin section studies. Most material required, when in 70% alcohol, transportation to laboratory for 48 to 72 hours, after fixation for 1 hour in 1% osmic acid buffered with veronal acetate at pH 7.4. Storage of material in 70% alcohol had no deleterious effect. All steps, from immersion of tissue in a mixture of equal volumes of 100% alcohol and methacrylates (6 parts of n-butyl to 1 part of methyl) through final stage of polymerization at 48°C, following addition of Luperco to the methacrylates, were carried out in vacuum obtained by 15 lb of negative pressure. Sections were obtained with Porter-Blum microtome, and collected in 30% acetone on specimen screens covered with a thin film of collodion and thinly coated with evaporated carbon particles. An RCA EMU 3A electron microscope was used.

Results. Tumorous cells† in spleen and liver from chickens with erythroblastosis showed vacuolization of cytoplasm and alterations in mitochondria, endoplasmic reticulum, and cellular membranes. It should be pointed out that not all tumorous cells showed these changes. Frequently these changes were accompanied by appearance of osmiophilic bodies, often surrounded by single or double membranes. Within these inclusion-like bodies the spheroidal particles showing internal structure have been observed. Approximately one out of 50 cells examined showed the presence of these particles. Following breakdown of membrane surrounding the inclusion-like bodies, the characteristic particles are released into the surrounding cytoplasm. The nucleus of the affected cells remained unchanged. Characteristic particles have also been observed in intercellular spaces.

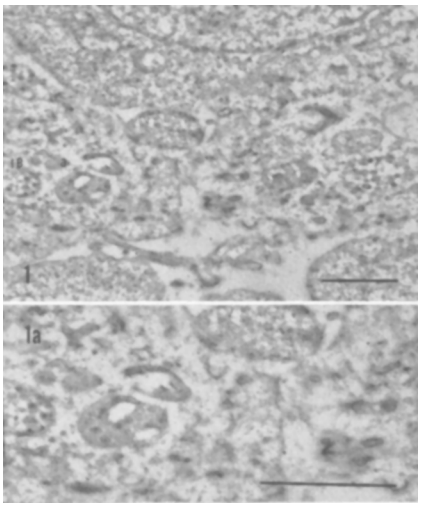

FIG. 1. Electron micrograph of section of tumorous cells of spleen from chicken with erythroblastosis. Inclusion-like bodies (IB) present in cytoplasm of some of the cells. Magnification 10,500 ×.

FIG. 1a. Part of Fig. 1 marked by "a" at greater enlargement. Two inclusion-like bodies with characteristic particles. Magnification 17,500 ×.

The particles have been found to vary in diameter from 570 Å to 760 Å, averaging 670 Å. These particles may be seen in Fig. 1, and at higher magnification in Fig. 1a. Their relationship to other cytoplasmic constituents is shown in Fig. 1. The ultrastructure is more clearly visible in Fig. 2 and Fig.

† Examination by means of the light microscope of spleen tissues from chickens in terminal stages of erythroblastosis reveals that the majority of all cells are primitive cells of erythroid origin. In ultrathin sections of erythroblastosis examined by means of electron microscope, it is difficult to differentiate the cells which show the described changes from those showing similar changes in granuloblastosis, which will be discussed later.

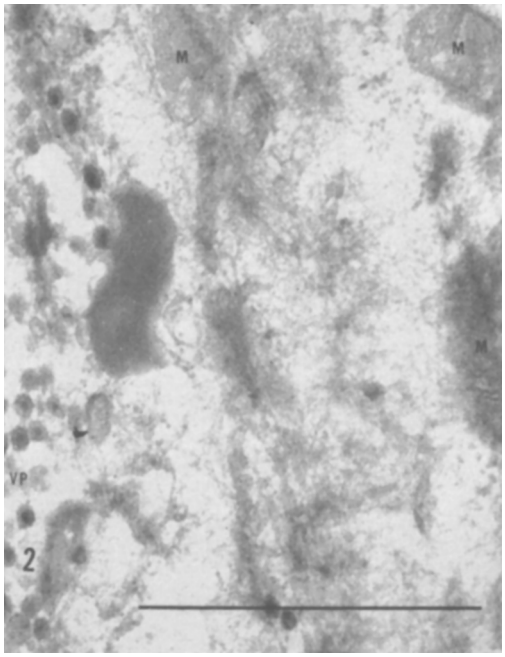

FIG. 2. Characteristic spheroid particles at greater enlargement showing internal structure; mitochondria (M). Magnification 46,667 ×.

3, 3a and 3b. The particles show a dense central mass, surrounded by a lighter zone, which in turn is surrounded by an external limiting membrane. Diameter of the central core was found to vary from 220 Å to 300 Å, averaging 260 Å.

Distribution of the particles both within and outside the cells is shown in Fig. 4, 4a, 4b, and 4c. The external limiting membrane is seen in particles present in inclusion-like bodies within the cytoplasm of cells and in particles found in extra-cellular spaces.

Discussion. The study of ultrathin sections of tumorous spleens and livers from chickens with erythroblastosis revealed a number of changes in the submicroscopic cell elements of some tumor cells. Some changes, like vacuolization of cytoplasm, breakdown of mitochondria, and of endoplasmic reticulum are not considered specific for viral infection. Similar changes have been observed in HeLa cells grown *in vitro*(8) and in cells subjected to starvation(9). However, other changes, like formation of inclusion-like bodies with spheroidal particles resembling virus particles observed in thin sections of other tumors of known viral origin(1) appear to be more specific.

Particles of similar internal structure but of somewhat larger diameter have been observed in plasma of chickens with erythroblastosis(6). The question arises about the relationship of the particles observed in ultrathin sections of tumorous spleens of chickens with erythroblastosis and those found in plasmas of chickens with erythroblastosis. The difference in size between particles in ultrathin sections of spleen and liver of chickens with erythroblastosis (670 Å) and those in ultrathin sections of ultracentrifugal pellets of plasma of chickens with erythroblastosis (800 Å) may be due to differences in preparation of the material and in calibration of the microscope. Spheroidal particles of similar ultrastructure and average diameter varying 750 Å to 800 Å were observed in ultrathin sections of spleen and bone marrow of

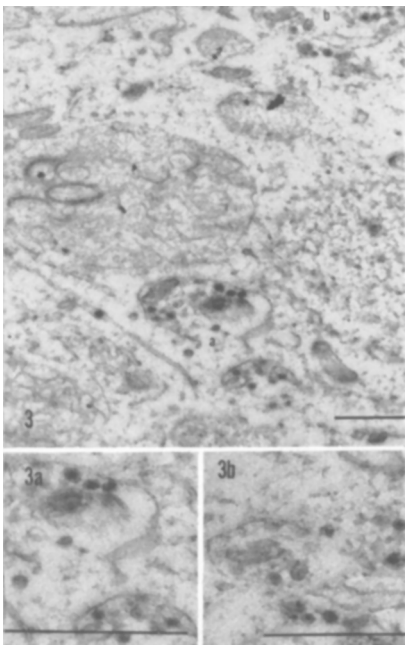

FIG. 3. Ultrathin section of several tumor cells in spleen of chicken with erythroblastosis. Characteristic "whorl-like" formation of membranous structures in cytoplasm of 2 cells. Inclusion-like bodies (a, b) with characteristic particles. Magnification 30,000 ×.

FIG. 3a and 3b. Parts of Fig. 3 enlarged. Characteristic structure of particles and membranes, in part broken-down, surrounding inclusion-like bodies. Magnifications 25,000 ×.

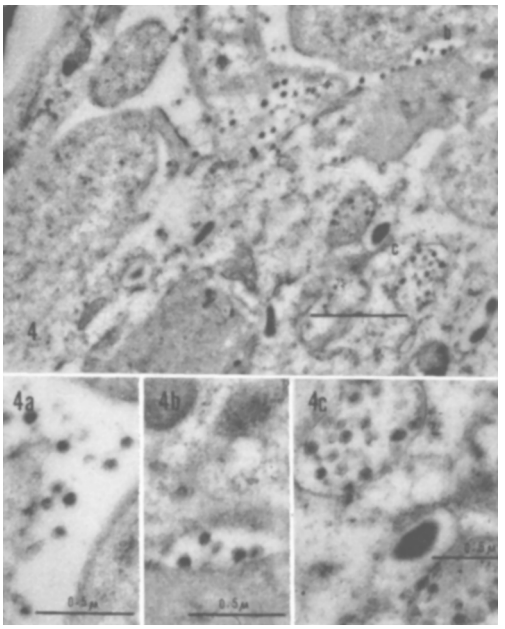

FIG. 4. Characteristic particles present both within and outside cytoplasm of cells of a tumorous spleen from a chicken with erythroblastosis. Magnification 25,000 ×.

FIG. 4a and 4b. Same particles in intercellular spaces. Magnification 25,000 ×.

FIG. 4c. Particles within inclusion-like bodies. Magnification 25,000 ×.

chickens with erythromyeloblastosis(7). Similar particles were also found in spleen and bone marrow of 3 out of 24 normal chickens (7). The size of these particles (750 Å-800 Å) resembles more closely the size of those (570 Å-760 Å) observed in the present study of thin sections of cells in erythroblastosis. It is possible that particles observed by Bernhard *et al.*(6) and by Benedetti *et al.*(7) and those in our studies represent the same agent. Inclusion-like bodies were also described in erythromyeloblastosis and their origin attributed to mitochondria(7). Similar inclusion-like bodies have been observed in the present study. Their origin will be discussed later.

The study of ultrathin sections of spleen and liver of control, young chickens of the same line, failed to reveal similar changes in the cells of these organs.

Summary. Ultrathin sections of tumorous spleen and liver from chickens of the so-called line 15 with erythroblastosis have been examined in the electron microscope. In cells of these organs spherical or spheroid structures were observed, composed of a central dense core of about 260 A, surrounded by a less dense material and limited by an outer membrane, varying in diameter from 570 Å to 760 Å. The structure of these particles resembles that of particles described in other tumors of chickens and in other tumors of known viral origin. No particles of similar character were observed in cells of organs from control healthy chickens.

1. Dmochowski, L., *Cancer,* v1, 216, Butterworth & Co., (Publishers) Ltd., London, 1957.

2. Swerdlow, M., Dalton, A. M., Birks, L. S., *Anal. Chem.,* 1956, v28, 597.

3. Ellermann, V., Bang, O., *Zentr. Bakteriol. Parasitenk.,* 1908, v46, 595.

4. Burmester, B. R., *Ann. N. Y. Acad. Sci.,* 1952, v54, 992.

5. Beard, J. W., *ibid.,* 1957, v68, 473.

6. Bernhard, W., Bonar, R. A., Beard, D., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 48.

7. Benedetti, L. E., Bernhard, W., Oberling, Ch., *C. R. Acad. Sci.,* 1956, v242, 2801.

8. Mcrgan, C., Rose, H. M., Moore, D. H., *Ann. N. Y. Acad. Sci.,* 1957, v68, 302.

9. Gansler, H., Rouiller, C., *Schweiz. Z. Allgem. Path. u. Bakt.,* 1956, v19, 217.

Received May 14, 1958. P.S.E.B.M., 1958, v98.