APF of 20% is not a large increase and, considering variations, would not be significant from a few pairs of calves. The results become impressive with larger numbers, however. Rusoff and Haq(3) used Merck's APF (a vitamin B_{12} supplement) and added it only to the grain mixture, which is usually consumed in rather small amounts by calves during the first 4 to 6 weeks. It is not surprising that no response was observed from vitamin B_{12} under these conditions. The effect observed in our experiments appears to be largely antibiotic since crystalline aureomycin gave a response equal to the APF supplement alone, both on the milk and milk substitute diets. The response observed in these studies appears to agree with the results reported by Dyer *et al.*(5) using pigs.

Summary. Adding an APF supplement (Lederle) and crystalline aureomycin to the diet of young dairy calves significantly increased the rates of growth and reduced the incidence and severity of diarrhea. The data suggest that the responses observed may be largely the result of an antibiotic effect of aureomycin.

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Received October 9, 1950. P.S.E.B.M., 1950, v75.

Particulate Component of Plasma from Fowls with Avian Erythro-Myeloblastic Leucosis.* (18255)

DOROTHY BEARD, EDWARD A. ECKERT,† T. Z. CSÁKY, D. G. SHARP AND J. W. BEARD.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

Recently, studies were undertaken in this laboratory directed toward the purification and the determination of the chemical and physical properties of the virus of avian erythro-myeloblastic leucosis. With the progress in the work there has been encountered a particulate component in the blood plasma of birds affected with this disease. The component cannot be identified unequivocally with the virus without quantitative biological correlation based on infectivity measurement. Though investigations are underway for the development of suitable procedures for such correlation, the results will not be available for some time. Meanwhile, the possible significance of the component would appear to warrant a preliminary account of the results thus far observed.

Experimental. The condition studied was the erythro-myeloblastic form of avian leucosis identified as the BAI Strain A(1) obtained from a diseased bird provided by Dr. E. P. Johnson.‡ The disease, characterized by the appearance of exceedingly large numbers of primitive cells in the blood stream, has been maintained for the past year by continual passage either by whole blood or filtered plasma injected intravenously in 0.1 or 0.05 ml volumes in 3-day-old New Hampshire Red chicks. The procedures employed in the isolation and partial purification of the component may be illustrated by those of a typical experiment. Blood was drawn into heparin (10 ml heparin, Liquaemin, "Roche-Organon," 1 ml = 10 mg, per 100 ml blood) from 15 chicks by heart puncture 39 days after intravenous injection of 0.05 ml of filtered plasma each. The individual samples yielded a pool of 255 ml whole blood

* This work was supported by a research grant to Duke University from the American Cancer Society, on recommendation of the Committee on Growth and by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

† Predoctorate Fellow of the U. S. Public Health Service, Natl. Cancer Inst.

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‡ The authors wish to express their deepest appreciation for the interest and encouragement of Dr. Johnson in the undertaking of this work.

with a count of $10^{8.9}$ primitive cells per ml and a volume of 124 ml of plasma after removal of cells by low-speed centrifugation. The plasma was passed immediately through 02 Selas (Selas Corporation of America, Philadelphia, Pa.) filter candles under 4 pounds pressure, yielding a volume of 106 ml. The plasma was distributed in 15 ml quantities in celluloid tubes and spun in a chilled head at 20,000 X G in the vacumn type air-driven ultracentrifuge for 60 minutes. The resulting supernate was poured off, and the pellets were resuspended by gentle pipetting in 5.5 ml Simms'(2) solution, pH 7.3. After centrifugation of the suspension in the horizontal head of an International Centrifuge No. 2 at 2300 X G for 15 minutes, the supernate was taken off with a pipette, diluted to 15 ml and spun again in the ultracentrifuge, this time at 15,000 X G for 30 minutes. The supernate was poured off, and the pellets were taken up in 1.0 ml of 0.07 M NH_4Cl-NH_4OH buffer solution, pH 7.3. The suspension was spun in the horizontal head as before, the supernate was taken off and, after dilution to 15 ml with the same diluent, ultacentrifuged for the third time. The sequence of high and low-speed centrifugation was carried through the third and fourth cycle as in the second cycle. The final pellet was taken up in 0.5 ml of the NH_4Cl-NH_4OH buffer solution. In control experiments, identical procedures were applied to plasma from birds failing to develop the disease on injection with the same infectious material and to plasma from birds obtained from a different source receiving no injection. Nitrogen was determined by direct nesslerization(3) and desoxyribonucleic acid (DNA) by the method of Stumpf(4).

Results. The plasma of birds with this disease differs visibly from normal chick plasma, appearing to be of much greater viscosity and seeming to contain relatively large amounts of mucin-like material. Filtration was accomplished without great difficulty, however, 50 ml of plasma passing through each 2-inch candle in about 45 minutes.

The initial high-speed centrifugation yielded large, well-packed pellets, about 8 mm in diameter, with red and brown pigment in the center surrounded by clear, gel-like material at the periphery. With pipetting, most of the material was easily resuspended to give much opalescence, a small amount remaining as visible flakes. Nearly all of the pigment and much colorless material were removed by the low-speed centrifugation. Subsequent cycles resulted in progressive loss of material and increasing transparence of the pellets. The final suspension, approximately 175 X concentrated with respect to the starting volume (taking account of loss due to sampling), was highly opalescent, and no pigmented material was evident.

An electron micrograph of the material of the fourth cycle placed on the screen in the conventional way, washed with distilled water and shadowed with chromium, is shown in Fig. 1. There are seen the images of large numbers of particles of unusual morphology. The majority of particles vary in size and are spheroidal in shape with smooth or slightly roughened surfaces. Many forms are seen, however, which exhibit a definite tail structure varying somewhat both in length and thickness. The form of these particles seems to lie between that of the virus of Newcastle disease(5) and the forms of some of the tailed bacteriophages(6). There are seen also numerous short, thread-like particles which have the appearance of the tail structure possibly torn from the head pieces. The particles without tails exhibit the size and appearance of the heads of the tailed forms. An approximate size range of these heads and particles was 60 to 100 mμ, and the lengths of the tails were from 100 to 200 mμ. While the preparation was by no means homogeneous with respect to the char-

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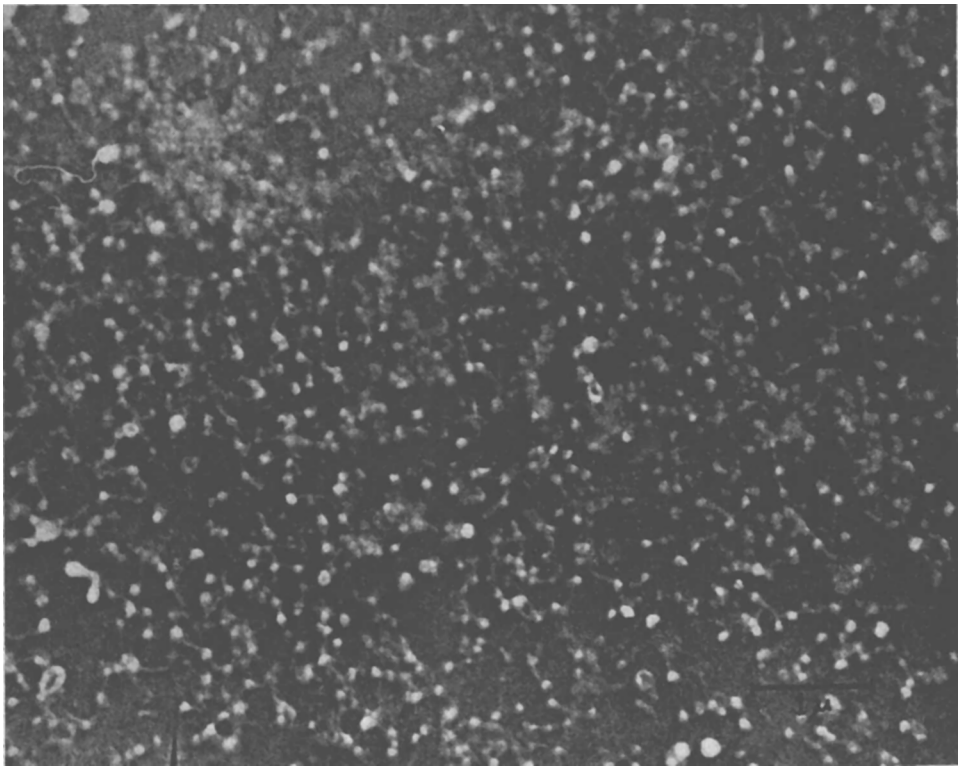

FIG. 1.
Electronmicrograph of a concentrate from the plasma of fowls with avian erythro-myeloblastic leucosis. Magnification 15,000×.

acteristic particles, the latter constituted the major part of the material.

The material sedimented in the analytical ultracentrifuge with a single, though greatly diffuse boundary, at a rate of 630 S, and there was evidence of unsedimentable material in the diagram. Although the preparation contained sufficient material to be opalescent, the absorption of ultraviolet light in the ultracentrifuge seemed relatively low. The yield of nitrogen in the pellet of the fourth cycle taken up in Simms' solution in a similar experiment was 210 μg from 120 ml of plasma. Desoxyribonucleic acid was too small in amount for measurement, but the amount was less than 125 μg. The ratio N/DNA was not less than 1.

No material of this sort was seen in the plasma of birds refractory to infection or in that obtained from birds of comparable age from a source outside the laboratory. Small, clear pellets were observed with the first centrifugal cycle, but all visible evidence of a component was lost in the subsequent cycles. Electron micrographs showed only an occasional spheroid particle in the size range of the specific component.

Discussion. The results demonstrate the presence in the plasma of birds with this form of avian leucosis of an abnormal component in amounts great enough to be carried readily through 4 ultracentrifugal cycles. The large number of particles seen in the electron micrographs and their unique morphology permits recognition, unequivocally, of a specific component not present in the plasma of normal or undiseased birds.

There is good reason for supposing that the component is of viral nature, though it may not be a virus of avian leucosis. The resemblance of the particles to some of the bacteriophages(6) and to a lesser extent to

Newcastle disease virus(5) is obvious. Like these viruses, it appears that the particles are probably tailed structures and that the rounded forms represent heads from which the tails were torn during purification. The particles differ greatly from the Newcastle disease virus, however, in size and sedimentation properties(5) and somewhat in appearance. No evidence of disease other than leucosis was seen in the birds. It is not impossible that the component represents some form of bacteriophage; if so, the phage must have been present in the plasma. Cultures from whole blood yielding such plasma have been essentially sterile, and the volume of concentrates and the technique of handling the concentrates would cause doubt of multiplication of bacteriophage in significant amounts during the centrifugal processes. The lack of homogeneity of particle size and shape in electron micrographs and the absence of a sharp boundary in the sedimentation diagram constitute great differences between the characters of this component and analogous properties of the bacteriophages already studied(7-8). Moreover, the high N/DNA ratio indicates a low content of DNA in contrast with the high content of this material in bacteriophages(9,10,11).

In view of its character, source and the conditions under which it was obtained, the component is of much interest whether or not further study indicates it to be the etiological agent of erythro-myeloblastic avian leucosis. As a possible nonviral material, it is unique among the large particles thus far isolated. Should it prove to be a bacteriophage, its association with avian leucosis and its presence in such amounts in plasma would likewise be unique. If the component is a virus not related to avian leucosis, it represents a form hitherto not isolated. Further studies are being made in an attempt to learn the relation of the component to avian leucosis.

Summary. A particulate material has been isolated by ultracentrifugal procedures from the filtered plasma of chickens diseased with avian erythro-myeloblastic leucosis. The particles were either spheroid in shape and of size varying from 60 to 100 mμ diameter or tailed structures with spheroidal heads and tails of 100 to 200 mμ lengths. Preliminary studies on small amounts of material yielded sedimentation diagrams showing definite, though diffuse, boundaries and an approximate sedimentation rate of 630 S. The ratio of nitrogen to desoxyribonucleic acid of the concentrates indicated a low content of the latter. Material of this sort was not found in the plasma of either normal chicks or chicks refractory to infection with the blood or plasma from birds with avian leucosis. The significance of the component in relation to the etiological agent of avian leucosis is discussed.

Received October 23, 1950. P.S.E.B.M., 1950, v75,

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