concomitant resistance to chloramphenicol and to aureomycin. No cross resistance was indicated, with the possible exception of a slight cross resistance (4x) of the 2-(2-hydroxy ethyl) substituted semicarbazone (NF-67)-resistant strain to chloramphenicol.

These studies, added to those of Green and Mudd(10), indicate that those bacteria studied, with developed resistance to chloramphenicol, aureomycin, penicillin, streptomycin, and sulfonamides, retain unaltered their susceptibility to the nitrofurans.

Summary. (1) Bacteria can develop only a limited resistance to the nitrofurans *in vitro*. On the basis of a bacterial cross resistance study of a series of eighteen nitrofurans, it was found that the compounds investigated could be divided into two classes. The second class differs in chemical structure by having a carbon atom between the carbonyl group and the terminal group of the nitrofuran side-chain. Bacteria becoming resistant to the first class are reciprocally cross resistant to all members of this class but remain susceptible to nitrofurans of the second class. (2) Bacteria in which resistance was developed to chloramphenicol or to aureomycin remain susceptible to the nitrofurans. The converse is also true.

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Particulate Component of the Plasma of Fowls with Avian Lymphomatosis.* (19322)

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The avian leukosis complex consists in two chief pathological types of neoplastic disease (1). One of these is an "intravascular" process characterized by the occurrence in the blood stream of large numbers of primitive cells of erythroblastic or myeloblastic characters. The other type, lymphomatosis, is manifested in various forms dependent on localized or widespread overgrowths of primitive cells of the lymphoid type which may reach the circulation in only small numbers or not at all. Evidence of the viral etiology of both types of the complex has been demonstrated repeatedly, and a principal problem in the study of the complex is the possible relationships of the respective causative agents.

One approach to this problem is the purification of the viruses associated with the diverse forms of the complex and the direct comparison of their physical, chemical, and immunological properties. Progress in this direction has been made in recent work(2,3) in the purification and study of the virus of erythromyeloblastic leukosis which has been found to be present in large amounts in the plasma of affected chicks. It has been shown (4), also, that the infectious agent of some

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forms of lymphomatosis is likewise present in the plasma of chickens with this form of the complex and can be sedimented by centrifugation(5). In the present work, studies have been made on such plasmas by ultracentrifugal and electron microscopic procedures, and the results have revealed particles of characters differing from and in numbers far greater than those of any other particles of comparable size in the plasma of normal chickens. The findings in this work are described briefly here.

Materials and methods. The plasmas investigated were obtained from chickens affected with the RPL 12 strain(6,7) initially classified(4) as visceral lymphomatosis and from other chickens judged to be normal. The condition has been readily transmissible(7) by means of cell-free filtrates of both tumor extracts and plasma. In order to obtain the plasma as nearly as possible at the height of the disease in the donor, repeated examinations were made of blood smears. The criterion employed to judge the time of bleeding, in addition to clinical evidence of the disease, was the appearance of a few primitive cells of the lymphoid type in the blood stream. Blood was drawn into heparin as previously described(2), and the cells were removed by centrifugation. All of the plasmas had been frozen from 9 to 19 days before the studies were made. Immediately before use, the frozen plasma was thawed and spun in the cold room at 2000 X g for 15 minutes. The investigation consisted in the search of 3 series of individual plasmas for particulate material which might represent the virus. For this, the technic of sedimenting the particles onto an agar surface was employed, as in similar studies on erythromyeloblastic leukosis(3). Counts of the particles were made from micrographs of particles spun down from plasma diluted 1-10 in all instances. To obtain purified concentrates, plasma was spun at 20,000 X g for 60 minutes, and the sediment was resuspended in Ringer's solution. The process was repeated a second time by spinning at 20,000 X g for 30 minutes, after which the final concentrate, taken up in Ringer's solution, was spun in the horizontal centrifuge at 2,000 X g for 10 minutes, leaving the particles suspended in the supernatant fluid. Electron micrographs were obtained (3) by drying this particle suspension on an agar surface, fixing the particles with osmic acid and coating the particles with chromium.

TABLE I.

Donor	Interval since inoculation, days	Pathology	Particles/ml plasma ($\times 10^7$)
M 130 Q_2	42*	L†	32
1415 D	105	L and O	29
,,	107	L and O	47
119 R_2	66	L	4
1305 A	110	L and O	160
727 B_3	98	L	5
119 I_2	77	L	1

* All chicks were inoculated at 1 day of age.
† The lymphoid involvement in all cases was confined to vascular networks (see text). L = Lymphomatosis; O = Osteopetrosis.

Results. In all of one series of plasmas from affected birds, all in advanced stages of the disease as determined at necropsy, there were observed significant and, in some instances, as shown in Table I, very large numbers of particles of similar characters with respect to size and shape. The characters of the particles are illustrated in Fig. 1(A) and (B) which reveal the presence of nearly spherical or spheroidal entities of 70 to 160 mμ, with an average of about 117 mμ diameter. The similarity, both in size and appearance, of these particles to the virus of the erythromyeloblastic type of leukosis obtained and portrayed by the same technic (Fig. 6 of (3)) is clearly evident.

The character of the material obtained by the ultracentrifugal procedures with 5 ml of a third plasma, M 1305 A, are shown in Fig. 2. It is evident that a high degree of purification was not effected since many masses apparently not agglomerates of the particles were present. It is still clear, nevertheless, that this small volume of plasma contained very large amounts of the particulate component identical in appearance with the particles shown in Fig. 1 (A) and (B). Again the particles seen in this concentrate are essentially indistinguishable from those (Fig. 4 of (3)) in the concentrates of plasmas from birds with erythromyeloblastic leukosis.

Examination of a second series of 6 plasmas from birds which had been inoculated with

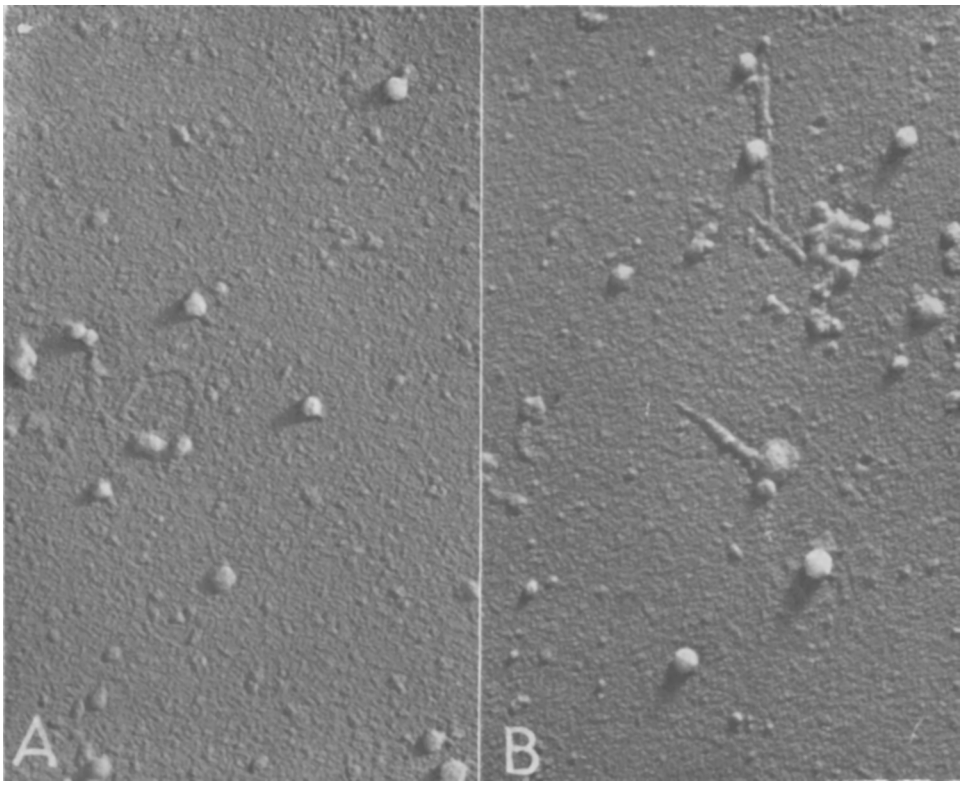

FIG. 1. (A) Electron micrograph of particles centrifuged directly from plasma M 1415 D and (B) the micrograph of particles obtained in the same way from plasma M 130 Q_2, from which the particles were counted as shown in Table I. Magnification 24000 ×.

lymphomatous material revealed parallel results in but a single case. In four instances, no particles of this sort were seen. An occasional particle was observed in one case. In only one plasma of this series was there a significant number of particles observed, but here the number was high in the range of Table I. The bird providing this plasma was also the only one of this series that had unquestionably gross pathology at necropsy; all others were diagnosed by microscopic examination and presumably were cases very early in the lymphomatous process. Thus, it would appear that the differences obtained between the first and second series of plasmas were related, in part, if not altogether, to the stage of the disease.

Plasmas from 6 birds regarded as normal were examined in the present work, and many others have been studied in the investigation of the virus of erythromyeloblastic leukosis. In only one instance of the present series were particles observed in significant numbers; in this case, however, the characters of the particles differed somewhat from those of the entities present in the plasmas of Table I. Significant particles have not yet been observed with normal young birds of the sort used in the study of erythromyeloblastic leukosis.

Discussion. There has been demonstrated a particulate material in the plasma of birds inoculated with RPL 12 lymphoid tumor strain. While a variation of approximately 100-fold in the number of particles was found in the group of animals with advanced disease, the occurrence in some instances of large amounts of the material, as in donors M 1305 A (Fig. 2), M 130 Q_2 and the 2 specimens from M 1415 D (Table I), is strongly suggestive of a specific relationship of the particles with the lymphomatous process. These findings are closely similar to those with erythromyeloblastic leukosis in which there is now little doubt that the plasma particles rep-

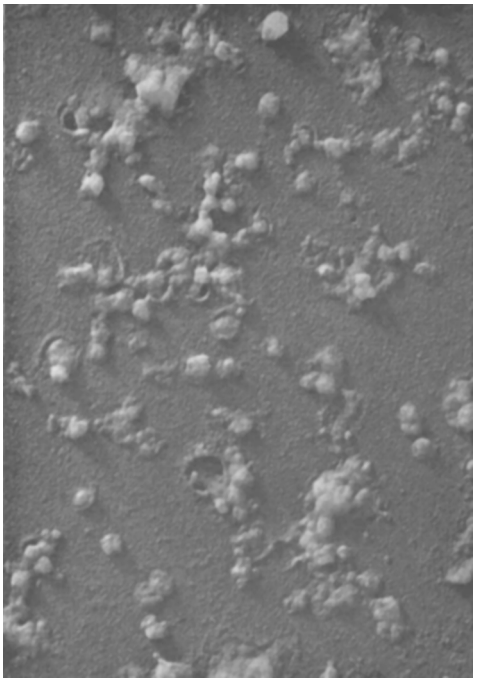

FIG. 2. Electron micrograph of the particles of the concentrate obtained from plasma M 1305 A (see Table I). Magnification 24000 ×.

resent the specific viral agent of that disease. There seems no reason for evading, at least tentatively, the interpretation of the particles in the present work as the virus of one form of lymphomatosis. The diversity of the findings with the individual plasmas is not difficult to explain; variation in the numbers of virus particles is considerable in erythromyeloblastic leukosis in which the stage of the disease is readily diagnosed and in which the cells probably yielding the virus circulate in the blood stream. In contrast, the diagnosis of lymphomatosis in live birds and determination of the optimum time for bleeding is very difficult.

It should be emphasized that no criterion is available by means of which a single particle can be regarded in the electron micrographs as specifically significant. Further, it cannot be said that the particle counts of Table I are of high accuracy. Counts of the particles were made from micrographs of which those of Fig. 1 (A) and (B) represent greatly magnified small areas. Inspection shows that the selection of the particles counted is a matter of judgment based on the resemblance of the one to the many. The counts recorded in Table I indicate, essentially, the minimum number of characteristic particles present.

In the period of about 7 years preceding the present work, during part of which time the tumor material was kept in storage at —76°C, the disease studied here has been passed 12 times in White Leghorn chickens of 1 to 3 days of age by means of plasma, serum or extracts of lymphomatous liver tissue from which the cell material was removed by filtration or centrifugation at 4,000 X g for 40 minutes in the cold. During this time, the disease process has varied somewhat from its original character. Until recently, the primary pathological change was the formation of lymphoid tumors in the viscera, especially the liver, which were typical of those occurring naturally in visceral lymphomatosis. The tumor cells were located extravascularly and accumulations resulted in tumor areas varying in size from those containing only a few dozen cells to others of several cm in diameter. In the last two passages of this tumor strain, most of the deaths occurred in a much shorter time than in previous passages; there was only a moderate enlargement of visceral organs and the same malignant lymphoid cells were confined almost entirely to the vascular networks. Thus, the changes in the liver resemble that found in leukemia though a true leukemic condition in the peripheral blood was seldom seen. It cannot be said at present whether this change is due to an alteration of the virus, an expression of a latent virus or merely an alteration in the reaction of the host. Nevertheless, this form of leukosis is wholly different in its manifestations from the erythromyeloblastic disease studied in this laboratory, and in no instance was there evidence of erythromyeloblastic leukosis in the donors of the plasmas studied here.

If the particles seen in these studies on lymphomatosis are actually the virus responsible for the disease, it would seem that a beginning has been made in the elucidation of the problem of the relations between the virus strains or types causing the various forms of the avian leukosis complex. It is evident that these particles are of the same characters with respect to size, shape and general appearance

as those of the virus of erythromyeloblastic leukosis.

Summary. A particulate material has been obtained from the plasma of chickens affected with the RPL 12 lymphoid tumor strain. Electron micrographs of the particles sedimented directly from plasma and of those in ultracentrifugal concentrates reveal spherical or spheroidal particles of sizes varying from 70 to 160 mμ in diameter and an average diameter of about 117 mμ. Particle counts on the individual plasmas of birds with advanced disease gave values of 1 x 10^7 to 160 x 10^7 particles per ml in contrast with the normal plasmas in which only occasional or no particles of this size range were observed. The findings with this form of the leukosis complex are remarkably similar to the results of analogous studies on erythromyeloblastic leukosis. The particles seen in the present work are interpreted, tentatively, as representing the virus of one form of avian lymphomatosis.

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Serum Precipitable and Butanol Extractable Iodine of Bovine Sera. (19323)

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Dairy and beef cattle differ widely in their ability to secrete milk. Reece and Turner(1) have reported that the thyrotrophic content of the anterior lobe of the pituitary gland of cattle is greater than that in beef cattle. Throughout lactation objective measures of thyroid activity have not been satisfactory. Ruminants, especially lactating ones, are not good subjects for the determination of basal metabolic rates because rarely are they in a post-absorptive state. B.M.R., therefore, cannot be used as a measure of thyroid activity in these animals. Reports in the literature indicate a good relationship between thyroid activity and the serum precipitable iodine (SPI) of men and women(2). In this investigation an attempt was made to study thyroid activity of beef and dairy cattle by comparing sera of 20 dairy cows with sera of 20 beef cows for the content of iodine precipitated with protein. After these 40 sera had been analyzed for SPI the butanol extractable iodines (BEI)(3) of 20 sera were compared with the SPIs of the same sera. The BEI is thought to contain the iodine of "thyroxine-like" compounds(3,4). Comparison of SPI and BEI of men and women had shown that ingestion of inorganic iodine might elevate the iodine precipitated with protein above the "thyroxine-like" iodine compounds by as much as 10 μg %. The BEI correlated more closely than the SPI with the clinical state of patients(3).

Methods. Sera from venous blood were maintained at about 40°F for 1 to 4 days before analysis. SPIs of the first 20 samples were measured by a permanganate acid ashing technic(5,6). Subsequent SPIs were determined by the micro modification of this technic, which requires only 1 ml of serum for each of the duplicate aliquots(2). The BEI(3) values as well as the SPI measurements are the average of duplicate analyses.

Results. The BEIs of nonpregnant, lactat-

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