from foreign proteins which are unsedimentable in the gravitational fields employed. There is a marked tendency towards aggregation upon storage at low temperatures.

The calculation of the size and mass of the tumor-producing material from the sedimentation constant and density requires a knowledge of the shape of the particles. The absence of birefringence both in flowing solutions and in the gel-like pellets formed during ultracentrifugation, and, more directly, the observation of round, uniform particles in such preparations upon microscopical dark-field examination (Ledingham and Gye, McIntosh, Amies, Claude) indicate a symmetrical shape of the agent, in spite of the relatively pronounced viscosity of its solutions. The application of Stokes's equation for spherical particles yields a mean value for the diameter of the particles of 70 mμ. This, in turn, leads to values for the average weight of the individual particles of 2.3×10^{-16} gm and for the average "molecular weight" of 139×10^6. These figures are in close agreement with the results secured by ultrafiltration[6] and non-optical centrifugation[7] experiments.

KURT G. STERN
F. DURAN-REYNALS
LABORATORY OF PHYSIOLOGICAL CHEMISTRY
AND DEPARTMENT OF BACTERIOLOGY,
YALE UNIVERSITY SCHOOL OF MEDICINE

ON THE NATURE OF THE AGENT CAUSING LEUCOSIS IN FOWLS[1]

THE work of Ellerman, Furth and others has shown that fowl leucosis, in contrast to mammalian leukemia, can be transmitted by cell-free plasma and tissue extracts. The virus strain used in the present investigation was Strain No. 1 of Dr. J. Furth, to whom the authors are greatly indebted for the supply of original donor material. Fifty per cent. of the chickens inoculated intravenously with whole blood and 28 per cent. of those inoculated with whole plasma developed the disease.

Centrifugation of heparinized leucotic plasma in the chilled quantity rotor of an air-driven ultracentrifuge[2] for 30 to 60 minutes at 23,000 r.p.m. (gravitational force at the bottom of the tubes, 37,400 g) yielded a small, colorless flaky sediment in addition to a yellow, translucent jelly-like pellet. Suspension of the sediment in M/15 phosphate buffer (pH 7.3), after removal of aggregated matter in the horizontal centrifuge, gave an opalescent solution showing a pronounced Tyndall effect. Pellet solutions prepared from active plasma have produced leucosis in 3 out of 11 chickens after intravenous injection.

Marrow of the long bones provided a richer source of the macromolecular material. Upon grinding frozen leucotic bone marrow with sand, extraction with 0.005 M. phosphate (pH 7.4) and removing cell debris in the horizontal centrifuge (2,600 r.p.m.), strongly opalescent solutions were obtained. These produced leucosis in 4 out of 10 injected chickens. When such crude marrow extracts were subjected to ultracentrifugation at speeds ranging from 23,000 to 30,000 r.p.m. (max. gravitational force, 37,400 to 63,300 g), substantial pellets were obtained containing all the material responsible for the strong opalescence of the crude extracts. The material was purified by resuspending it in 0.005 M. phosphate (pH 7.4), removal of aggregated matter by low-speed centrifuging (2,600 r.p.m.), resedimentation of the macromolecular material in the ultracentrifuge (30,000 r.p.m.) and repetition of the entire procedure. The average yield of macromolecular material after four ultracentrifugal sedimentations was 11 mg from 1 gm of bone marrow. Only traces of macromolecular material were obtained from normal bone marrow under the same conditions.

Material prepared in this manner from leucotic bone marrow has produced leucosis in 4 out of 19 inoculated chickens. The remaining 15 birds are still under observation. With the exception of one instance, where contamination with pellet material could not be excluded, supernatant solutions from both plasma and bone marrow ultracentrifuge runs were inactive (20 chickens). It should be mentioned that although microscopic examinations of pellet material have definitely excluded the presence of cellular material in a few tests, in other instances cellular debris was observed. It remains to be determined whether this material might be responsible for some of the positive results obtained.

Purified macromolecular bone marrow material contains about 9.5 per cent. nitrogen. Color reactions for thymonucleic acid are positive. Hemin can be demonstrated by the pyridine hemochromogen test. The material possesses cytochrome oxidase and catalase activity (unpublished experiments with Mr. J. L. Melnick). It remains to be determined whether these enzymatic activities are due to the agent itself or to small amounts of associated substances.

In the analytical ultracentrifuge the material sedimented with a single boundary which tended to spread during the course of the runs, indicating a relatively low degree of inherent homogeneity. From microphotometer tracings (Fig. 1) obtained from sedimentation photographs of bone marrow material purified by four ultracentrifugations an average sedi-

[6] W. J. Elford and C. H. Andrewes, *Brit. Jour. Exp. Pathol.*, 16: 61, 1935.

[7] W. J. Elford and C. H. Andrewes, *Ibid.*, 17: 422, 1936; J. McIntosh and F. R. Selbie, *ibid.*, 18: 162, 1937; A. Claude, *J. Exp. Med.*, 66: 59, 1937.

[1] This investigation was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the Fluid Research Fund of the Yale University School of Medicine.

[2] J. W. Beams, F. W. Linke and P. Sommer, *Rev. Sci. Instr.*, 9: 248, 1938.

mentation constant $s_{20} = 582 \times 10^{-13}$ cm dynes^{-1} sec^{-1} has been calculated. On the assumption that the particles possess spherical shape, which is supported by the absence of flow double refraction and by the low relative viscosity of their dilute solutions (1.09), the average diameter of the individual particles, as derived from the sedimentation constant with the aid of Stokes's equation, is 72 mμ. Using the value of 1.22 for the density of the material (kindly determined for us by Mr. P. H. Barbour, Jr., by the falling drop method of Barbour and Hamilton) an average particle weight of 2.6×10^{-16} gm and a "molecular weight" of 146×10^6 is obtained.

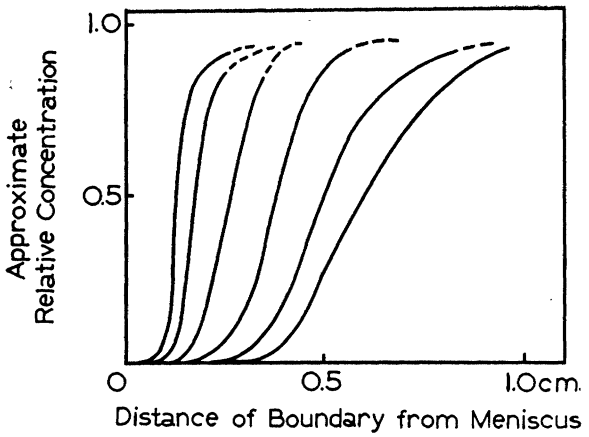

FIG. 1. Tracings made from the microphotometer curves of the sedimenting boundary of macromolecular material isolated from bone marrow of leucotic chickens. Material purified by four alternate high-speed (30,000 r.p.m.) and low-speed (2,600 r.p.m.) centrifuge runs; concentration, 0.5 per cent., in 0.005 M. phosphate (pH 7.4); mean gravitational force during analytical run, 3,500 g (7,200 r.p.m.); interval between exposures, 6 min.; $\lambda = 2{,}480 - 3{,}600$ Å; photographic magnification, 1.5; magnification ratio during recording, 1: 6.

With respect to its chemical and physical properties the material described above resembles the causative agent of the Rous chicken tumor I[3] and also the macromolecular material isolated by Claude[4] from normal chick embryos. In contrast to the virus proteins isolated by Stanley and others from plants, these materials do not represent pure nucleoproteins but much more complex chemical structures of the type encountered in the analysis of protoplasm.

KURT G. STERN
ARTHUR KIRSCHBAUM
LABORATORY OF PHYSIOLOGICAL CHEMISTRY
AND DEPARTMENT OF ANATOMY,
YALE UNIVERSITY SCHOOL OF MEDICINE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE SIMPLEST TRANSPARENT ULTRACENTRIFUGE

WE have developed a form of transparent ultracentrifuge which permits of photographic recording of the contents of a glass or quartz tube while spinning at 110,000 times gravity. It is so simple that it dispenses entirely with accessories, not even a camera being required. The complete equipment can be made in fifty to sixty hours of a mechanician's time. Nevertheless, it gives useful results of fair accuracy.

The device is a development of ideas previously used by Elford, McIntosh and Selbie, and Ford, but applied to obtaining a record without stopping the rotor. This consists, Figs. 1 and 2, of a solid piece of duralumin, 7 cm in diameter, and directly air-driven. In the upper surface two channels are milled on the same diameter; one carries a dummy to balance the cell. In the other is placed first a piece of photographic film and upon it a block of hard rubber with a horizontal hole to hold a tube of 1.5 mm internal and 7 mm external diameter, containing the solution to be measured. The rubber block is sawn nearly in two, giving a radial vertical slit 0.6 mm wide. The tube is sealed at the outer end and closed at the other end with a piece of gummed paper with a piece of cork pressing against it to assist insulation. The length of column of liquid we have used is 8 mm, but may readily be longer. It therefore has ample resolving power for the smallest proteins. The rotor has a cover screwed on with a central screw. In the cover is a window, set in with Duco or similar

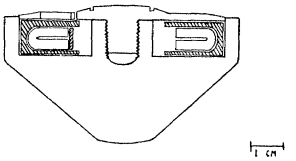

FIG. 1. Cross section through assembled rotor.

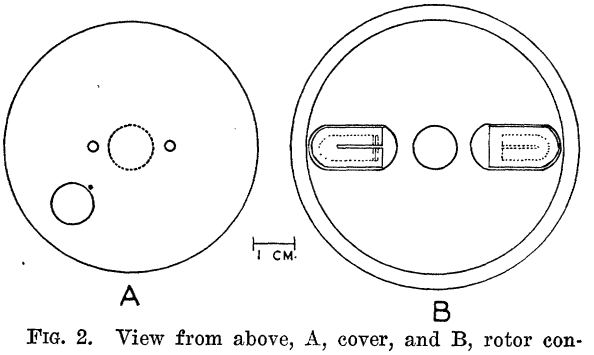

FIG. 2. View from above, A, cover, and B, rotor containing cell and dummy.

[3] K. G. Stern and F. Duran-Reynals, SCIENCE, 89: 2322, 609, 1939.

[4] A. Claude, *Proc. Soc. Exp. Biol. and Med.*, 39: 398, 1938.