tion of heart and serus coat of duodenum, sub-periosteal hemorrhage of flat bones, hemorrhage into the lungs and an acute catarrhal or hemorrhagic duodenitis. Various combinations of these lesions will be encountered. If the bird is not sacrificed the lesions will tend to disappear by the seventh day after inoculation. A microscopic examination of the liver of birds sacrificed at the end of 96 hours reveals foci of cloudy swelling. These swollen liver cells also reveal contracted pyknotic nuclei. The kidney changes are more pronounced but are also limited to foci. The epithelial cells lining the tubules show stages of degeneration varying from swelling with contracted pyknotic nuclei to actual destruction of the cells. The glomeruli are markedly swollen and contain few erythrocytes. There is round cell infiltration between the tubules in these foci.

A suspension of the feces and intestinal contents of experimentally infected birds is infective to other birds of the same age when given by way of the mouth. A bacterial free filtrate of the feces is infective when injected intraperitoneally. Bacterial free fecal filtrates are also capable of establishing the virus in incubating chick embryos. The virus is readily filterable through either or both the Seitz E-K 3 and the Chamberland-Pasteur L-3 filter.

Since this filterable virus alone does not produce death when injected into birds of a susceptible age we can not, at this time, say with any certainty that it is the sole etiological agent of the so-called "blue comb" disease.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE ULTRACENTRIFUGE WITH PLASTIC ROTOR¹

THE ultracentrifuge constructions presently in use are based either on the principle of the oil-turbine velocity centrifuge, equipped with mechanical bearings and a vertical steel rotor, or on the principle of the bearing-less "spinning top," which represents an air turbine floating on a cushion of compressed air. The analytical centrifuges of McBain and the concentration and optical centrifuges of Beams, Wyckoff and Pickels and Bauer are derived from the latter type. The most frequently employed rotor material in these constructions is duralumin.

During a recent visit to the Svedberg centrifuge laboratory of Professor J. W. Williams,² at the University of Wisconsin, it occurred to the writer that the use of materials of very low density for the construction of ultracentrifuge rotors might result in a considerable simplification of centrifuge design and obviate the necessity of employing expensive steel and aluminum alloys which are now difficult to procure on account of the National War effort. The first trials were made in Madison with 0.5 inches thick discs of polystyrene and of polyacrylic, transparent resins of 1.5 and 2 inches diameter, respectively. With the mechanical assistance of Messrs. E. Hanson and L. Henke these discs were transformed into simple airturbines. Employing a 2-inch Lucite disc, tank nitrogen as propellant and a primitive optical set-up with a spectacle lens as objective, the sedimentation of aggregated earthworm hemoglobin within the cylindrical

fluid cell was photographed with the kind help of Mr. Ch. Vilbrandt. Since the Lucite turbine showed no signs of irreversible deformation, even when spun for 10 minutes at approximately 40,000 r.p.m., the writer felt encouraged to continue these experiments with plastic rotors after his return to New Haven. A 2-inch Lucite turbine was accelerated to 57,000 r.p.m. with the aid of 80 pounds air-pressure per square inch, as measured with the Kahler-Hunt photoelectric speed-measuring circuit. After the mechanical features of the centrifuge had been improved in various respects, the construction of a 6-inch plastic turbine was undertaken with the mechanical aid of Mr. H. Nelson. Throughout the later phase of this work much benefit was derived from the expert advice of Professor F. W. Keator, of the Department of Mechanical Engineering, and several improvements, based on his suggestions, were incorporated in the design.

A schematic drawing of the centrifuge at its present stage of development is reproduced in Fig. 1.

The top speed, thus far attained with this model, has been 17,400 r.p.m. at 48 lbs/sq. inch air-pressure and an estimated free air flow of 40 to 60 cubic feet per minute, yielding a force of 20,200 times gravity at the center of the analytical fluid cell which is situated at a distance of 6 cm from the center of rotation. This speed is sufficient to cover practically the entire size range of plant and animal viruses as given by Stanley,³ and, in general, to bring about molecular sedimentation, at appreciable rate, of protein particles from about 10⁶ molecular weight upwards. As examples of such materials, the sedimentation of earthworm hemoglobin and of Stanley's crystalline ³ W. M. Stanley, in Handb. d. Virusforschg., p. 538. Wien, 1938.

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 $^{^2}$ The writer wishes to thank Professor J. W. Williams for his hospitality and his encouraging interest in this work. \cdot

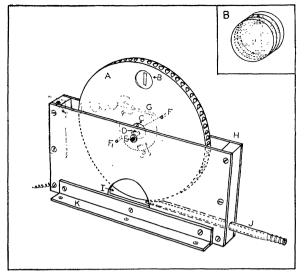
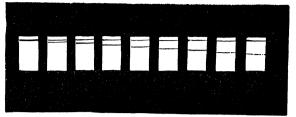


FIG. 1. Air-turbine Ultracentrifuge with Plastic Rotor. A, Lucite rotor, 0.5 inches thick, 6 inches diameter, with flutings milled into the periphery; B, analytical fluid cell (see below), inserted in cylindrical cell hole; C, brass disc, connected with similar disc on other side of rotor by brass bushing and screws; D, axle, made from 3/16 inches thick drill rod, fastened to C and turned down and surfacehardened at ends to fit E; E, Torrington needle bearing, 3/16 inches, mounted in casing, H, and carefully aligned with bearing on opposite side, F, F_1 , brass contacts, inserted in rotor surface; G, contact brush, made from spring bronze, insulated from casing H, adjustable in position; H, centrifuge casing, made from sheet brass; I, semi-circular opening in casing, H, to permit free escape of expanded driving air; J, air-jet, 7/32 inches lumen, trumpet-shaped at inlet end and conforming with rotor shape at outlet end; K, angle for mounting on wooden base. Insert B, analytical fluid cell, made by cementing, with Lucite cement, two outer discs of colorless Plexiglas resin to central disc of red Plexiglas into which a sector-shaped opening of 12 mm. height and 3 mm. depth has been cut, connected with periphery by narrow drill hole, through which the solution under study is introduced with a hypodermic syringe. When in use, the cell is inserted into cell hole in rotor with the drill hole pointing towards the rotor center and the broad base of the sector pointing towards the periphery. During operation, the centrifuge is covered by a steel guard, made from 0.5 inch thick boiler plate by welding, equipped with openings opposite the cell holes and slots near the base to permit escape of air stream.

tobacco mosaic virus protein,⁴ with sedimentation constants of $s_{20} = 60 \times 10^{-13}$ and 175×10^{-13} and molecular weights of 3×10^6 and 40×10^6 , respectively, has been photographically recorded (Fig. 2), employing the 6-inch Lucite rotor.

The definition of the sedimenting boundaries, as

⁴ The writer is indebted to Dr. W. M. Stanley for a sample of this material.



0 10 20 30 40 50 60 70 80 min. FIG. 2. Sedimentation Diagram of Stanley's Crystalline Tobacco Mosaic Virus Protein obtained with 6-inch Plastic Airturbine. 1 per cent. virus solution; 9,000 r.p.m. (5430 \times g.); Toepler schlieren band method; 20 sec. exposures on Eastman contrast lantern slide plate; light source, Mazda 200 watt projection lamp; schlieren lens, Kodak projection lens, F=4 inches; camera lens, Kodak anastigmatic lens, F=4 inches.

exemplified in Fig. 2, the regular rate of sedimentation during the individual intervals, and the values of the sedimentation constants obtained for the virus with this centrifuge $(s_{20} = 157 \text{ and } 161 \times 10^{-13})$ as compared with that determined in our Beams ultracentrifuge $(s_{20} = 175 \times 10^{-13})$ for the same preparation, may be regarded as evidence that sedimentation in the plastic rotor proceeds essentially undisturbed by mechanical vibration or thermal convection currents.

The plastic rotors may be adapted to use in *centrifuge microscopes* as well as in *analytical ultracentrifuges*. The contact arrangement indicated in Fig. 1 (parts F and G) has been used to synchronize a stroboscopic light source (*e.g.*, Strobotak of the General Radio Company) with the rotor and to examine living cells during centrifuging with a low-power microscope.⁵ In this manner, the stratification of Arbacia eggs has been observed with as yet not wholly satisfactory results.

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⁵ The author is indebted by Professor N. E. Harvey for valuable advice and the loan of a Strobotak lamp.

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