mining the role of trace elements in growth and fruiting.[7] The optimal ratios of essential elements, such as N: P, could be investigated. Information on the cause of shedding might be obtained.[8] The technic also can be used in studies of plant metabolism.[9]

Thus, this procedure, as a useful method in determining the physiological requirements of the cotton plant, may ultimately be helpful in improving the yield of lint and seed. In the present plight of cotton agriculture, any means for reducing the cost of raising cotton merits especial attention. Lower production expenses, and hence economically sound decreases in price, would stimulate domestic uses and the export trade.[10]

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PHYSICO-CHEMICAL PROPERTIES OF THE ROUS CHICKEN TUMOR AGENT[1]

THE agent causing Rous chicken tumor I may be separated from cell-free tumor extracts by high-speed centrifugation (Ledingham and Gye, McIntosh, Elford and Andrewes, Amies, Claude, Pollard). The purified material serving for the present experiments was prepared essentially according to Claude[2] with the aid of an air-driven ultracentrifuge.[3] The nitrogen content was 8.9 per cent. The material is precipitated from its strongly opalescent solutions by half-saturation with ammonium sulfate and by protamine. It contains thymonucleic acid. Even after four sedimentations in the high-speed centrifuge the material gives a positive hemochromogen reaction. Amounts of 10^{-7} to 10^{-9} gm produced typical sarcomas in chickens. *In vitro* the material exhibited cytochrome oxidase and catalase activity (unpublished experiments with Mr. J. L. Melnick). It remains to be decided whether these enzymatic activities are intrinsic properties of the tumor-producing agent or whether they are due to associated substances.

The solutions of the purified agent show no appreciable flow double refraction. Their relative viscosity is 1.3 in 1 per cent. solutions after complete removal of the highly viscous mucin material present in crude tumor extracts. Measurements by the falling drop method of Barbour and Hamilton,[4] for which we are indebted to Mr. P. H. Barbour, Jr., indicate a density of 1.23. Preparations purified by four alternate high-speed (30,000 r.p.m.) and low-speed (2,600 r.p.m.) centrifuge runs, when examined in the analytical ultracentrifuge by the light-absorption method, yielded a mean sedimentation constant $s_{20} = 550 \times 10^{-13}$ cm dynes^{-1} sec^{-1} (Fig. 1).

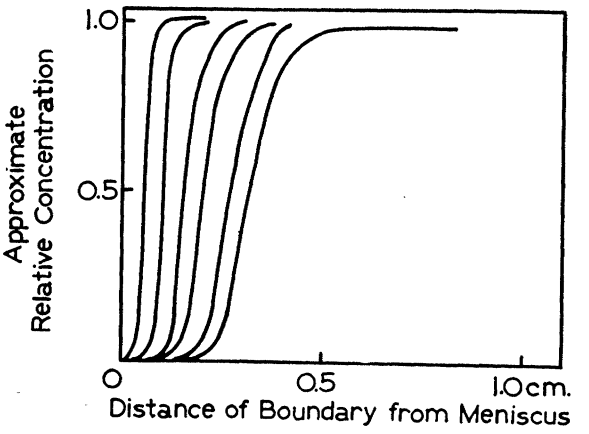

FIG. 1. Tracings of microphotometer curves obtained from sedimentation photographs of purified Rous chicken tumor I agent. Concentration of material, 1.08 per cent., in 0.005 M. phosphate, pH 7.3; mean gravitational force, 3,500 g (7,200 r.p.m.); intervals between exposures, 6 min.; length of exposure time, 5 sec. (Eastman positive film); $\lambda = 2{,}480 - 3{,}600$ Å (high-pressure mercury arc, bromine filter); photographic magnification, 1.5; magnification ratio during recording, 1: 6.

The macromolecular material prepared from normal chick embryos according to Claude[5] shares many physico-chemical properties with the Rous chicken tumor I agent. The average sedimentation rate of two purified preparations was found to be $s_{20} = 532 \times 10^{-13}$ cm dynes^{-1} sec^{-1}.

While only one boundary could be observed upon sedimentation of the preparations from chicken tumor I and normal chick embryos the spreading of the boundary in the course of the runs indicated a relatively low homogeneity of the materials. The figures given above represent the sedimentation rates of the particles of average size present in populations of particles of somewhat differing dimensions. The more extensively purified preparations were found to be free

[7] Although the unglazed pots were washed, it is possible that traces of an essential element or elements were slowly leached from them. Growth experiments in glass containers would be more conclusive.

[8] According to Brown, "Even where there are no insects to bother, more than half the flowers that open fail to make bolls that mature." H. B. Brown, "Cotton," McGraw-Hill Book Co., New York, 1938, p. 124).

[9] The root system is easily obtained for analysis by washing away the coarse sand in a stream of water. According to macroscopic observation, no particularly fine root hairs develop. The availability of water and nutrients apparently renders them unnecessary.

[10] It is necessary to give credit to the Cotton Research Foundation (see SCIENCE, 87: 87, 1938). Since this paper was submitted we have learned that sand culture of cotton has been practiced for some time at several southern agricultural experiment stations.

[1] This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

[2] A. Claude, *Amer. Jour. of Cancer*, 30: 742, 1937; SCIENCE, 87: 467, 1938.

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

[4] H. G. Barbour and W. F. Hamilton, *Jour. Biol. Chem.*, 69: 625, 1926.

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

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.

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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.