money the research committee will have available each year for the encouragement of research in Virginia.

The officers elected were the following: Major W. Catesby Jones, *President;* Dr. Robert F. Smart, *President-Elect;* Dr. E. C. L. Miller, *Secretary-Treasurer;* Dr. Sidney S. Negus, *Assistant Secretary.*

E. C. L. MILLER,
Secretary

SPECIAL ARTICLES

ISOLATION AND CHARACTERIZATION OF INFLUENZA VIRUS B (LEE STRAIN)[1]

THE influenza virus A (PR8 strain) has been isolated from virus-infected chick embryo chorio-allantoic fluid in preparations of high homogeneity with respect to particle kind.[2] The particles, which behaved as the virus and which consisted of a lipoprotein complex containing nucleic acid of the desoxypentose type, were revealed in the electron microscope as ovoid or kidney-shaped images of variable size corresponding to particles of 77.6 mμ average diameter. Sedimentation velocity diagrams showed a single slightly diffuse boundary. The sedimentation constant was $S_{20°} = 724 \times 10^{-13}$, which with the assumption of a spherical shape for the particles and a density of 1.2,[3] gave a value of 80 mμ for the particle diameter. In the present paper are described briefly the results of similar studies of the Lee strain of the influenza virus B.

The influenza virus B was cultivated in the chorio-allantoic sac of 11-day-old chick embryos. After 42 to 48 hours' incubation at 37° C. following inoculation with the virus, the chorio-allantoic fluid was drawn off, cleared of aggregates by angle centrifugation, dialyzed 24 hours at 2–5° C. against flowing Ringer-calcium chloride solution,[2] and cleared again by angle centrifugation. The fluid was adsorbed twice with adult chicken red blood cells from which the virus was eluted in one fourth the original volume for 2.5 hours at room temperature (24 to 28° C.). The eluate was twice adsorbed in like manner and elution carried out as before. The second eluate was then ultracentrifuged at 27,000 g for one hour, and the resulting pellets were dissolved at 150 times concentration with respect to volume. The pellet suspension was spun at 11,000 g for 2 minutes to remove aggregated material.

In this process, practically all the red blood cell agglutinating capacity and the infectious properties of the chorio-allantoic fluid were carried into the second eluate. Ultracentrifugation of the eluate at 27,000 g for 1 hour resulted in sedimentation of about 90 per cent. of these biological characters. The low-speed centrifugation at 11,000 g was associated with a further small loss of the red blood cell agglutinating capacity and infectivity, leaving about 40 to 70 per cent. of these properties in the clarified concentrates. The concentrates reacted specifically in complement fixation and precipitation reactions with the sera of ferrets and rabbits immunized with mouse lung infected with this strain of the virus.

Electron micrographs of the purified virus concentrates showed rounded or ovoid images of variable size indicating an average particle diameter of 98 mμ. Exceedingly little extraneous material was present. Definite differentiation of structure within the individual particles was indicated by an approximately centrally placed area of relatively high density. The chorio-allantoic fluid and the inactive fractions—the supernate after adsorption with red blood cells and the supernate of ultracentrifugation—contained large numbers of amorphous particles of all sizes, but many especially of about 40 mμ to approximately the size of the virus particle. Particles of this range of diameter were not seen in chorio-allantoic fluid from normal embryos or in the fluid or comparable fractions of it from embryos infected with influenza virus A.[2]

Sedimentation velocity diagrams showed a single slightly diffuse boundary corresponding to a sedimentation constant of $S_{20°} = 832 \times 10^{-13}$. From the sedimentation constant and the specific volume, 0.865, determined by pyknometer measurement, assuming a spherical shape, the calculated particle diameter was 100 mμ.

Suspensions of the concentrated material were opalescent and bluish. Positive biuret, ninhydrin and Millon tests were obtained. The glyoxylic acid and Molisch tests were negative. A positive reaction was seen in Bial's reagent after hydrolysis of the material with 10 per cent. sulfuric acid. This and a weakly positive Dische diphenylamine reaction indicate the presence of desoxypentose. The nitrogen content of the material dehydrated over P_2O_5 varied from 9.6 to 10.4 per cent.

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[2] A. R. Taylor, D. G. Sharp, D. Beard, J. W. Beard, J. H. Dingle and A. E. Feller, *Jour. Immunol.*, in press.

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In preliminary chemical studies, fractionation by the procedures of Kirk[4] and Kirk, Page and Van Slyke[5] showed that about 20 per cent. of the material were soluble in petroleum ether. Of this, 5.7 per cent. were in the form of neutral fat, 10.2 per cent. were phospholipid and 5.1 per cent. cholesterol. The alcohol-ether insoluble fraction constituted about 65 per cent. of the material and contained phosphorus to the extent of 0.27 per cent. of the whole virus. If all this phosphorus were present in nucleic acid, the whole virus complex would contain about 3 per cent. nucleic acid.

The yield of the purified material in all experiments averaged about 2 mg per 100 cc of chorio-allantoic fluid. On the basis of nitrogen precipitable with trichloracetic acid the degree of concentration for practical conditions was about 20 times and on the basis of volume it was 150 times. The specific 50 per cent. point infectivity for chick embryos varied from $10^{-11.2}$ to $10^{-12.1}$ grams per 0.1 cc inoculum with an average of $10^{-11.4}$ grams corresponding to 6,600 particles. The quantity of virus giving the 2_+ red blood cell agglutination end point was $10^{-6.5}$ grams.

The findings indicate that the influenza virus B is a relatively large particulate complex consisting of lipoprotein with which is associated nucleic acid of the desoxypentose type. From electron micrography, the average diameter of the spherical or ovoid bodies was 98 mμ. The diameter calculated from the sedimentation constant, $S_{20^\circ} = 832 \times 10^{-13}$, the specific volume, 0.865, and an assumed spherical shape was 100 mμ. The influenza virus B (Lee strain) thus appears to be significantly larger than the influenza virus A (PR8 strain).

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[5] E. Kirk, I. H. Page and D. D. Van Slyke, *Jour. Biol. Chem.*, 106: 203, 1934.
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THE ETHER SOLUBLE FRACTION OF NAVY BEANS AND THE DIGESTION OF STARCH

DURING the course of experiments dealing with the digestibility of dried navy beans it was observed that the oil of these beans retards the digestion of soluble starch by pancreatic amylase *in vitro*. The degree of this action appears to be of sufficient magnitude to warrant detailed study and comparison with other fats. It was also found that the retarding influence of the bean oil can be overcome to a large degree by a treatment with yeast. The object of this preliminary note is to briefly describe some of these findings.

Oil having these interfering properties can be readily obtained by extracting finely ground navy beans with ether for about one week. The total fat-soluble fraction when added to soluble starch in the same concentration in which it occurs in the beans retards the digestion of the starch more than some of the other edible fats. The amount of pancreatic amylase which completely digests 50 cc of a 1 per cent. solution of untreated soluble starch at pH 7 in less than thirty minutes *in vitro* leaves the treated starch incompletely digested after forty-eight hours.

Starch impregnated with the same amount of butter, lard or olive oil shows a negative starch-iodine test at about the same time as the untreated control. These findings are summarized in Table I. The oil which is

TABLE I

Fat added to starch in concentration of 1.5 per cent.	Starch-iodine test	
	½ hour digestion	48 hours digestion
bean oil	strongly positive	strongly positive
lard	negative	negative
butter	negative	negative
olive oil	negative	negative

quickly extracted from coarsely ground beans instead of allowing the usual period of one week for extraction does not retard the digestion. The difference in digestibility can not be attributed to the action of the ether on the oil.

The amount of starch which remains undigested in the presence of the bean oil varies with the age of the starch-oil preparation. In fresh preparations about half of it is undigested after two hours under the above conditions. As the age of the preparation increases the starch becomes less digestible until the inhibition is essentially complete in those preparations which have stood several months. A similar preparation of starch and olive oil becomes less digestible after standing for an equal period but is considerably more digestible than the starch impregnated with bean