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Sedimentation of Poliomyelitis Virus by Means of a Vacuum Ultracentrifuge.*

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This is a brief preliminary report of results indicating that we have been successful in sedimenting poliomyelitis virus, one of the smallest of the ultramicroscopic viruses,[1] from clear aqueous suspensions by means of ultracentrifugation in vacuum. The machine employed is similar in design to that recently described by Bauer and Pickels.[2]

Thus far we have obtained complete results on 2 experiments. In both, the material subjected to ultracentrifugation consisted of a 25% suspension of glycerinated pooled virus cords in physiological saline. The lipoids in these suspensions were removed by ether extraction and the aqueous fraction was centrifuged in an Angle centrifuge at 3000 r.p.m. for at least an hour. In the first experiment the resultant fluid was water-clear to the eye; in the second, very slightly opalescent. Eight cc. of the clear suspension were placed in each of a series of celluloid tubes seated in the rotor.

In the first experiment, the rotor was in motion for a total of 4 hours. Stroboscopic determinations indicated that for 2 hours of this period, the speed was between 27,500 and 30,000 r.p.m. After centrifugation, all of the tubes contained a small membranous type of sediment which could not be completely dispersed by repeated pipetting. The thicker central portion of the sediment presented a pinkish tinge apparently due to sedimentation of hemoglobin present in the original cord suspension.

The supernatant was removed in 2 portions, the upper 6 cc. comprising the "top supernatant," and the pooled bottom 1 cc. portions being the "bottom supernatant." The sediment was resuspended in 6 cc. of saline. Monkeys were injected intracerebrally with 1 cc. quantities of each of these fractions in varying dilutions. The results are presented in Table I.

* These studies were supported by the Mary Hooper Somers Fund for Filterable Virus Research.

1 Clifton, C. E., Schultz, E. W., and Gebhardt, L. P., *J. Bact.*, 1931, **22**, 7; Theiler, M., and Bauer, J. H., *J. Exp. Med.*, 1934, **60**, 767; Elford, W. J., Gallo way, I. A., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 135.

2 Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 50.

TABLE I.

Monkey No.	Inoculum	Dilution	Results
	Top supernatant:		
C 875	1 cc.	1:400	No poliomyelitis
D 4	1 "	1:800	" "
	Bottom supernatant:		
D 46	1 cc.	1:400	" "
C 874	1 "	1:800	" "
C 871	1 "	1:1600	" "
	Sediment suspension:		
C 930	1 cc.	1:2000*	Poliomyelitis 8th day
C 868	1 "	1:4000*	" " "
C 844	1 "	1:6000*	No poliomyelitis

*Dilutions of the original 6 cc. saline suspension.

In the second experiment, the clear aqueous suspension was subjected to 2 successive runs. In the first run the machine operated a total of 6 hours with a maximum speed of 15,000 r.p.m. for 4¾ hours. At the end of this run the tubes showed no sediment. The centrifuging was therefore continued for another 6 hours, with a speed of 30,000 r.p.m. for a period of about 4½ hours. This time a membranous deposit similar to that observed in the first experiment was found in each of the tubes. The sediment was resuspended in saline equivalent in amount to that used in Exp. 1. Monkeys were injected intracerebrally with dilutions of supernatant and dilutions of sediment as given in Table II.

TABLE II.

Monkey No.	Inoculum	Dilution	Results
	Supernatant:		
D 9	1 cc.	undiluted	No poliomyelitis
D 95	1 "	"	" "
C 632	1 "	1:2000	" "
D 103	1 "	1:2000	" "
	Sediment:		
C 749	1 cc.	1:2000*	" "
D 98	1 "	1:2000*	Poliomyelitis 8th day
C 612	1 "	1:3000*	" 22nd "
D 100	1 "	1:3000*	" 24th "
C 618	1 "	1:4000*	" 7th "
D 97	1 "	1:4000*	" 8th "
D 45	1 "	1:5000*	No poliomyelitis
D 102	1 "	1:5000*	" "
D 55	1 "	1:6000*	" "
D 99	1 "	1:6000*	" "

*Dilutions of the original saline suspension of the sediment.

These results indicate that we have been successful in sedimenting the virus of poliomyelitis from an ether extracted, essentially water-

clear aqueous suspension. It is, however, not yet established that the sedimentation is purely the result of centrifugal force acting on the virus aggregates as such, rather than on aggregates larger than the virus to which it may be absorbed. Further experimental work is in progress.

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Studies on Annelid Muscle. II. Observations on Annelid Phosphagen.

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That some annelids exhibit peculiarities in the behavior of their phosphagen complex has been demonstrated by Arnold and Luck[1] and by Needham, Needham, Baldwin and Yudkin.[2] This note extends previous observations.

Early in our work on annelid muscle extracts clarified with basic lead acetate we noted positive Jaffe reactions in the lead-free filtrates. More important was the steady increase in the amount of color produced as the filtrates were evaporated at pH 6.0 at temperatures below 100°C. Thus in *Nereis brandti* muscle extract the total "apparent creatinine" values increased 59% when the volume of the extract was reduced to one-fourth the original. Extracts of *Audouinia spirabranchus* muscle gave smaller increases in color production. That the substance responsible for the positive Jaffe reaction was not creatinine itself was shown by negative Weyl and Salkowski tests. The substance could, however, be precipitated by phosphotungstic acid and recovered in the fraction insoluble in absolute methanol. Arginine phosphotungstate is fairly soluble in this reagent, while creatinine phosphotungstate is but slightly soluble.[3] The methanol insoluble phosphotungstate on removal of the precipitant yielded a filtrate giving positive Jaffe and Sakaguchi reactions; attempts to isolate the substance or substances responsible were not successful.

Determinations of labile phosphate in *Audouinia spirabranchus,*

[1] Arnold, A., and Luck, J. M., *J. Biol. Chem.*, 1933, **99**, 677.

[2] Needham, D. M., Needham, J., Baldwin, E., and Yudkin, J., *Proc. Roy. Soc. London (B)*, 1932, **110**, 260.

[3] Drummond, J. C., *Biochem. J.*, 1918, **12**, 5.