the bladder (Group J).§ At autopsy there were 4 cases in which the data suggest that the rate had been accelerated.‖ This sheds new light on Wolff's observation that in pregnant rabbits nephrectomy causes an increase in the amount of amniotic fluid.[4] Also, it raises again the question as to whether in human pregnancy an excess of amniotic fluid (hydramnios) may result from an abnormal accumulation of metabolites in the maternal blood stream, the metabolites acting in part by inducing an abnormally rapid formation of fetal urine.

§ In 3 additional fetuses in which the bladder was similarly visualized, squeezing it by means of blunt forceps forced its contents into the ureters and the renal pelves without causing any urine to emerge via the urethral outlet (action of sphincters?).

‖ In the other 9 cases, since the bladder was relatively empty, it is assumed that the hole made by puncturing the wall permitted urine to leak out.

[4] Wolff, B., *Arch. f. Gynäk.*, 1904, **71**, 224.

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Isolation of a Macromolecular Constituent with Properties of the Lansing Strain of Poliomyelitis Virus.*

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It has been reported from this laboratory that highly active preparations of the MV strain of poliomyelitis virus could be obtained by differential centrifugation of clarified extracts of infected medullae-cords of rhesus monkeys.[1] Application of the same procedures to brains and spinal cords of cotton rats infected with the Lansing strain resulted in appreciable quantities of high molecular materials which, however, showed only slight activity. Various modifications of the original procedure have been used in attempts to obtain highly active virus. It has been found that if the ether-clarified, aqueous extracts are frozen before they are subjected to ultracentrifugation, virus is obtained which is from 100 to 10,000 times as active on a nitrogen basis as the original clarified extract.

In the modified procedure the ether-clarified extract is prepared as previously described with the exception that filtration through celite is omitted. The slightly turbid solution is stored in 100 ml pyrex centrifuge bottles in a solid-CO_2 chest at from —35° to —60°C for at least 5 days and then allowed to thaw at room temperature. Before the ice present has completely melted, the solution is centrifuged in the cold room at 4°C in an angle centrifuge, and the supernatant liquid is removed; the sediment is extracted in the centrifuge twice with M/15 dipotassium hydrogen phosphate, and the latter extracts combined with the first supernatant liquid. The extracts obtained in this way are crystal clear, and depending on the length of time they have been frozen, contain from 75 to 51% of the nitrogen present before freezing. The appreciable amount of nitrogen precipitated is protein in nature, and as the specific activity of the extracts after prolonged freezing is the same or greater than before freezing, it appears that normal protein rather than virus is denatured during this treatment. The results of a typical experiment showing the effect of freezing for various lengths of time on the yield of soluble nitrogen and on specific activity are shown in Table I. Activity is expressed as the 50% infective dose ($I.D._{50}$) in young cotton rats and is calculated by the method of Reed and Muench.[2] The 50% infective dose is defined

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] Loring, H. S., and Schwerdt, C. E., *J. Exp. Med.*, 1942, **75**, 395.

TABLE I.
Effect of Freezing on Soluble Nitrogen and Specific Activity of Extracts.

Exp No.	Days frozen	Soluble N. in extract	Specific activity*	
			Before freezing	After freezing
		%	g	g
73C	4	75	$10^{-6.5}$	$10^{-5.9}$
"	45	53		$10^{-7.9}$
"	65	51		$10^{-7.3}$
"	120	53		$10^{-7.2}$

* Expressed as g nitrogen which produces poliomyelitis in 50% of young cotton rats as calculated by method of Reed and Muench.[2]

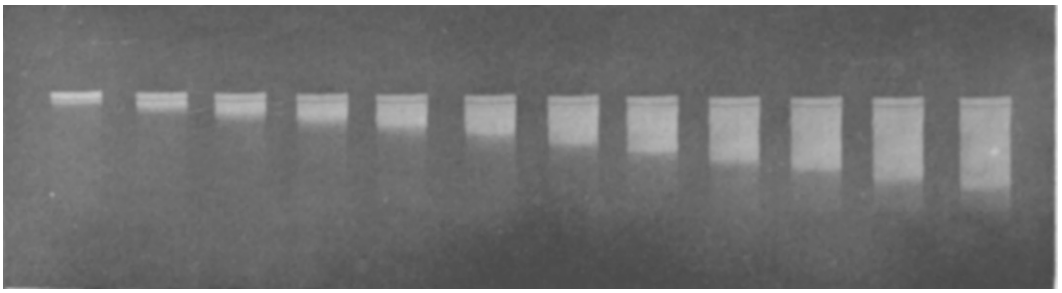

Fig. 1.
Sedimentation diagram of purified Lansing virus. (Speed 813 rps; interval between photographs, 2 min.; distance from center of rotor to top of cell, 8.04 mm; magnification of photograph, 5.07; temperature, 13.6°C).

as the amount of virus nitrogen which produces definite signs of poliomyelitis within 2 weeks in 50% of cotton rats from 4 to 6 weeks old injected intracerebrally with 0.05 ml of inoculum in 0.10 M acetate buffer at pH 4.

The purified virus is obtained by subjecting the clear extracts to 3 or 4 cycles of ultracentrifugal purification as previously described. When tested for specific activity, samples from different experiments have given values for $I.D._{50}$ of from $10^{-10.1}$ to $10^{-8.3}$ g of virus nitrogen. The purified material, which is present after the third ultracentrifugation as an amber-colored gel, dissolves in water to form opaque, slightly yellow-colored solutions, which have failed to show any evidence of streaming birefringence at concentrations where this phenomenon is readily detected with tobacco mosaic virus. Similarly, the sedimented gel is completely isotropic when viewed between crossed Nicol prisms in contrast to similar gels of tobacco mosaic virus which, as is well known, are highly birefringent. Solutions containing 0.2 mg of virus nitrogen per ml give a faint Millon's test for protein and a positive carbohydrate test with the orcinol-sulfuric acid reagent.[3]

The yield for third cycle virus has been from 0.1 to 0.2 mg of virus nitrogen[4] per 100 g of pooled brain and spinal cord. When the same purification procedures are applied to extracts of normal cotton rat brain and spinal cord, the percentage yields of macromolecular nitrogen after the extracts have been frozen for 5 and 10 days are of the order of 0.02 mg and 0.01 mg respectively. If the normal protein is present in the infectious extracts to the same extent it is found in normal tissue, it is evident that the maximum amount of normal macromolecular impurity present in the purified virus is of the order of 5 to 20%.

Examination of the virus samples for homogeneity in the McBain and Lewis 37 mm

[2] Reed, L. J., and Muench, H., *Am. J. Hygiene*, 1938, **27**, 493.

[3] Tillmans, T., and Phillipi, K., *Biochem. Z.*, 1929, **215**, 36.

[4] Tompkins, E. R., and Kirk, P. L., *J. Biol. Chem.*, 1942, **142**, 477.

transparent ultracentrifuge[5] have shown them to be relatively monodisperse as shown in Fig. 1 and to have a sedimentation rate of 83.5 ± 7.35 (average of 20 sedimentation runs on 5 preparations).

Conclusions. The results outlined above including (a) the presence in the purified samples of a uniform and high specific activity, (b) the demonstration that normal macromolecular constituents are largely eliminated by the purification procedures, and (c) the fact that the samples are relatively monodisperse in the transparent ultracentrifuge provide strong evidence that the preparations consist of essentially pure Lansing virus.

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5 McBain, J. W., and Lewis, A. H., *J. Physical Chem.*, 1939, **43**, 1197.

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Electron Microscopy of Purified Lansing Virus.*

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Eleven different samples of purified Lansing virus prepared as described in the preceding paper have been examined in the Stanford election microscope.[1] The preparations were obtained from extracts that had been frozen from 4 to 26 days and gave specific activities, $I.D._{50}$, from $10^{-10.1}$ to $10^{-8.3}$ g of virus nitrogen. Mounts were prepared by the usual technic of applying a small drop of dilute solution (10^{-8} to 10^{-7} g of virus nitrogen per ml) to the collodion film and allowing the solution to dry or by washing away the remainder of the drop with distilled water after 2 to 5 minutes. The mount was then either introduced directly in the microscope or it was treated by the gold shadowing technic of Williams and Wyckoff[2] before placing in the microscope.

Although all the micrographs have not yielded uniform or conclusive results, they have in general shown the presence of spherical or possibly slightly asymmetrical particles ranging in size from 12 to 34 mμ. Examples of micrographs obtained when the virus was examined without gold shadowing as well as of those obtained by the gold-shadowing technic are shown in Fig. 1a and 1b. Fig. 1a was obtained from an extract that had been frozen for 26 days and purified by 2 cycles of ultracentrifugal purification and Fig. 1b from an extract frozen for 4 days and purified by 3 cycles of ultracentrifugal purification, the specific activities being $I.D._{50} = 10^{-9.1}$ in both cases. Fig. 1a was obtained by the usual technic and Fig. 1b after gold shadowing.

In Fig. 1a there is some indication of slightly asymmetric particles with an average width of about 16 mμ and an average length of about 31 mμ or an average particle diameter of 23 mμ. In Fig. 1b this is not so apparent and the range of particle size as measured by shadow width is from about 15 to 34 mμ with an average value for particle diameter of 25 mμ. The slightly lower value for the non-shadowed specimen can probably be explained by the relatively low contrast between particles and background with the accompanying lack of definition in the particle image. Under these conditions the measured dimension is the greatest distance within the particle image of points of appreciable pho-

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1 Marton, L., *J. Applied Physics*, 1945, **16**, 131.

2 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.