17075 P. Formation of Filamentous Forms of Newcastle Disease Virus in Hypertonic Concentration of Sodium Chloride.*

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The shape of the virus of Newcastle disease of chickens is filamentous when prepared from a saline suspension[1-3] and roughly spherical when prepared from allantoic fluid or water.[4] If the virus is partially inactivated while in water, it does not change shape when the saline is brought to .15M. This concentration of salt is sufficient to cause a change in the form of the active virus when such a suspension is dried on an electron microscope screen.[5]

Two recent chance observations added further qualifications to the system, and with subsequent experiments further indicate that the change in shape takes place in solution. A hypertonic concentration of saline is, however, needed to cause the change in shape, al-

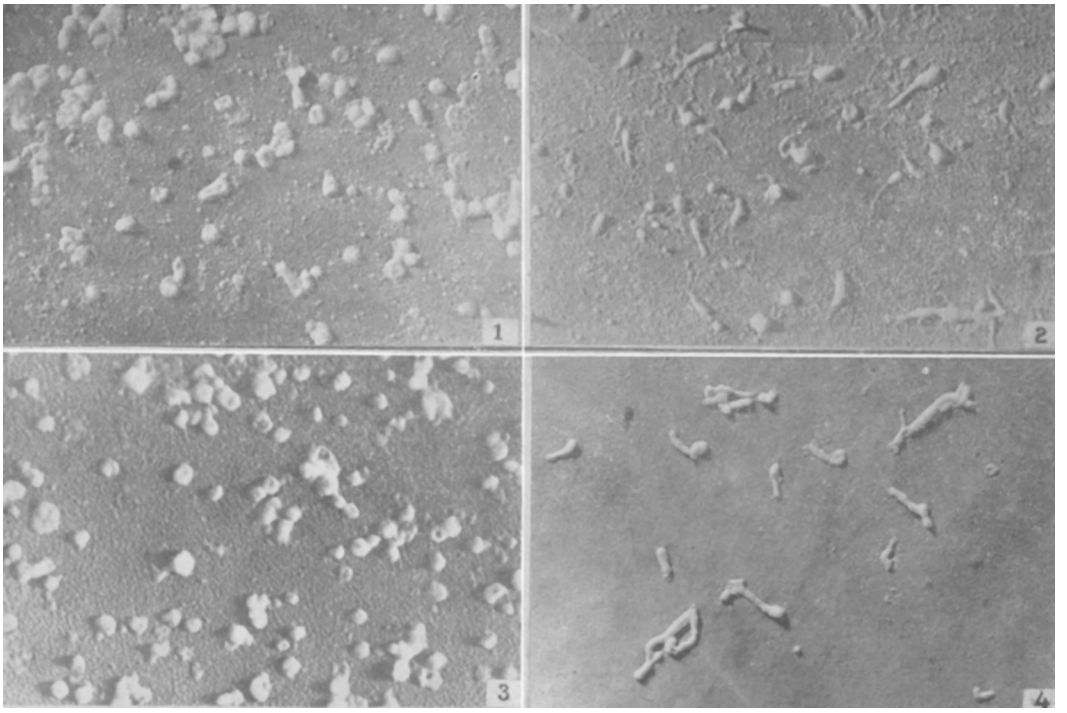

FIG. 1. Fixed in .15 M. saline by exposure to osmic acid before drying on electron microscope screen.

FIG. 2. Dried from similar suspension without fixation.

FIG. 3. Exposed to 3% saline by 2 centrifugation-washing cycles. Put back in water before preparation of screen.

FIG. 4. Same as 3, except fixed in osmic acid while in 3% saline.

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1 Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **64**, 135.

2 Cunha, R., Weil, M. L., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, T. W., *J. Immunol.*, 1947, **55**, 69.

3 Elford, W. J., Smiles, J., Chu, C. M., and Dudgon, J. A., *Biochem. J.*, 1947, **41**, XXV.

4 Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 135.

5 Bang, F. B., *J. Exp. Med.*, 1948, **88**, 251.

though there is no detectable loss of activity (red cell agglutinating or embryo infectivity) of the virus.

If a 2% osmic acid solution was added to the .15M saline suspension of the virus no change in shape was detected (Fig. 1). It remained spherical. If the same .15M saline suspension of virus was dried slowly in the cold room at 4°C instead of at room temperature, again filamentous forms were not detected. This is in contrast to drying at room temperature (Fig. 2). This made it likely that a change in shape was taking place during the drying process. When we combined this idea with the assumption that osmic acid fixed the virus in the form in which it existed in solution we were led to the following experiments.

Virus freshly harvested from allantoic fluid was centrifuged at about 15,000 g for 40 minutes in an angle centrifuge. It was then resuspended in varying concentrations of saline (Table I). It was washed again by a repeated centrifugation and resuspension in the same concentration of saline. All of the preparations were then "fixed" by exposure to the osmic acid in solution. They were then transferred back to water by a third centrifugation. All of the preparations for electron microscopy were then similarly prepared by drying a drop of this final water suspension on the usual formvar screen, washing, and shadowing with chromium at angle of 4 to 1.

Figs. 3 and 4 show the change in shape which has then been detected in four separate experiments with 2 strains of the virus. We have not as yet determined the concentration of saline at which the change in shape will occur or the quantitative effect of different concentrations on different morphologies, but have shown that a marked change in shape occurs at 2% saline which is 2.5 the normal concentration of saline. This despite the lack of any evidence that the embryo infectivity was detectably reduced by placing the virus in even four per cent saline. Furthermore, the rate of loss of infectivity of the virus on remaining in the ice box was the same in different concentrations of saline.

TABLE I.
Effect of Concentration of Saline on Infectivity of Suspension for Embryos and Red Cell Agglutinin.

Exp.	Conc. of saline	.8		2%		3%		4%		Untreated
		Embryo infect.	Red cell titer	Embryo infect.	Red cell titer	Embryo infect.	Red cell titer	Embryo infect.	Red cell titer	Embryo infect.
1	Immediate	9.0		8.0		9.4		8.8		8.5
								8.8*		
	9 days at 4°C	7.5						7.6		
			1280		1280		640		640	
2	Immediate				2560		1280		640	
	7 days at 4°C	7.8		8.3				7.4		

* Duplicate titration.
All titrations were carried out as in previous studies.[6]

[6] Bang, F. B., *J. Exp. Med.*, 1948, **88**, 233.

Discussion. Since the original description of filamentous and sperm shaped virus particles[1-3] as contrasted with the spherical ones present in allantoic fluid and water we have been puzzled about the shape of the virus under physiological conditions. This is of practical interest in any attempt to study the virus in cells. Two things now seem to be fairly clear. First, the virus is probably spherical when present in solutions of physiological concentrations. This fully explains our previous failure to detect a change in shape by direct measurements of viscosity, light scattering and double refraction of flow.[5] However, it also seems clear that a change in shape will occur in hypertonic salt solutions, for elimination of the salt from the solution returns the virus to its previous shape if it has not been fixed by osmic acid.† To the following extent then the filamentous and sperm shaped virus particles are artifacts. They are formed by the increasing hypertonicity of the salt solution when it dries on the formvar screen. They are, however, real in that they probably exist in free solutions of somewhat hypertonic concentrations. They are at least roughly as active as the spherical forms, and do not lose activity any more rapidly than the spherical forms in water or physiological saline.

† Recent electron microscope observations by Elford *et al.*, *Brit. J. Exp. Path.*, 1948, **29**, 590, also indicate the presence of an osmotic membrane around the virus of Newcastle disease. We are in essential agreement with their results.

Summary. In 4 separate experiments it has been shown that the virus of Newcastle disease of chickens probably has a spherical shape when in water or .8% saline. If the concentration of saline is increased to two per cent or more the virus becomes filamentous. It may be fixed with osmic acid at this concentration and will then retain its shape when placed in water. It reverts to the spherical form when replaced in water without previous fixation. No loss of infectivity was detected during these changes in concentration of saline.

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17076. Effect of Acute Bradycardia on Pulmonary Vascular Pressures in Anesthetized Dogs.*

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Pulmonary edema, congestion and hemorrhage were reported in guinea pigs subjected to increased intracranial pressure; however, bilateral cervical vagotomy prior to elevation of intracranial tension resulted in a significantly lesser degree of pulmonary pathology.[1] Luisada and Sarnoff[2] employed massive, rapid venous infusion in dogs simultaneously with vagal stimulation and concluded "electrical stimulation of either the cardiac end of the cut vagi or the intact nerves favors pulmonary edema by causing extreme bradycardia".

The present authors[3] have reported bradycardia, decreased cardiac output, a significant rise in pulmonary venous pressure and pulmonary edema in dogs subjected to increased intracranial pressure. Intravenous administration of atropine after induction of the changes listed above as following maintained elevation of the intracranial pressure, reversed those changes, and significant pulmonary edema did not occur.

Methods. Mongrel dogs were anesthetized

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1 Campbell, G. S., and Visscher, M. B., *Am. J. Physiol.*, in press.

2 Luisada, A. A., and Sarnoff, S. J., *Am. Heart J.*, 1946, **31**, 282.

3 Campbell, G. S., Haddy, F. J., Adams, W. L., and Visscher, M. B., *Am. J. Physiol.*, in press.