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Newcastle Virus: Conversion of Spherical Forms to Filamentous Forms.

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The filamentous forms of Newcastle virus seen in purified preparations[1] have not been seen in the crude allantoic fluid from infected embryos. This despite the fact that the virus is present in the allantoic fluid in the same concentration which in the purified material shows under the electron microscope great numbers of filamentous or tailed forms. Further work here reported indicates that the virus is roughly spherical in the allantoic fluid.

If allantoic fluid from infected chick embryos is centrifuged at 24,000 r.p.m. for 40 minutes and the pellet from this centrifugation is resuspended in water instead of saline, and this procedure repeated for another washing, the virus then shows a great predominance of roughly spherical forms under the electron microscope (Fig. 1b). This water suspension of virus has full activity (Table I) and remains stable for a number of days in the refrigerator (Table II). An occasional virus particle has a long thin attached tail, but gold shadowing fails to reveal other accessory structures. The virus particles are similar to those in the original allantoic fluid (Fig. 1a), with the exception that untreated preparations of infected allantoic fluid have never shown tailed forms.

If to this purified water suspension a little sodium chloride is added, a conversion from the spherical to tailed or filamentous forms occurs (Fig. 2). In the 3 experiments in which these results have been quantitated, the concentration of saline producing conversion has varied from .07 to .15 Molar. The conversion seems to be a progressive process and in individual experiments the extent of this conversion has varied with the salt concentration. Change of form, however, occurs within a few minutes of adding the saline.

Filamentous forms seen in the saline suspensions may be reconverted to the spherical by transferring them back to a water suspension; this is done by centrifuging at 24,000 r.p.m. and resuspending in distilled water. These again will yield filamentous forms on the addition of saline.

The evidence for this conversion or change in form rests entirely on study of dried preparations with the electron microscope, and as yet we have no proof of the existence of these different forms in suspension.

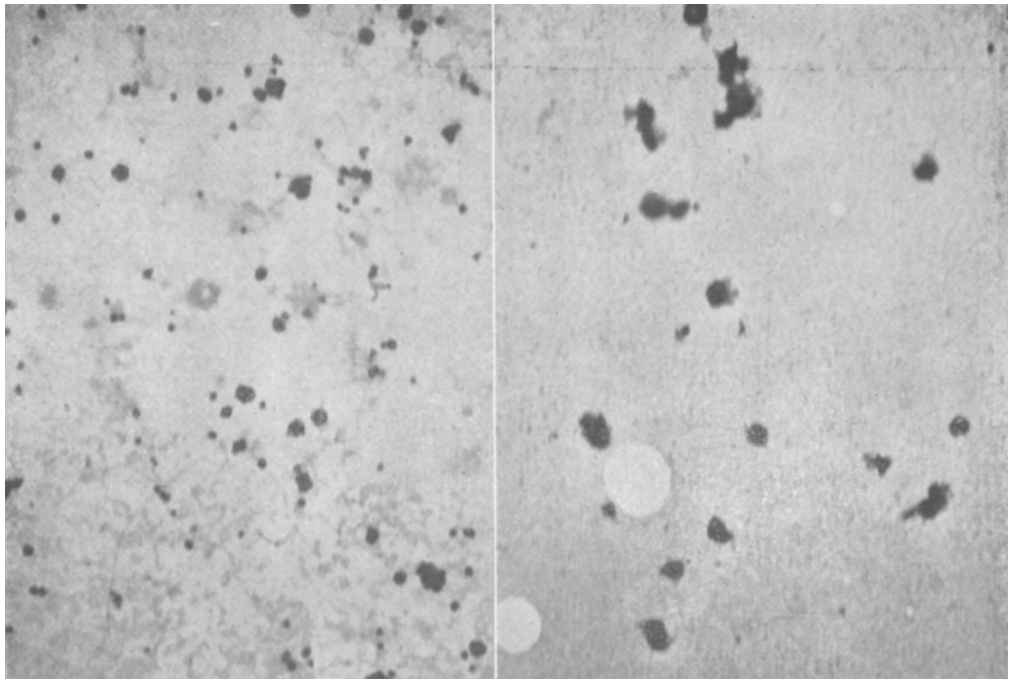
That this conversion is not an artifact, however, is made likely by the following experiments. Dilute solutions (.02%) of formaldehyde progressively inactivate the virus over a period of days. If, an hour or two after formaldehyde has been added, .15 M saline is added, conversion readily takes place. If, however, formaldehyde has been allowed to act for 3 to 5 days before the addition of saline, the stimulus of the saline is no longer able to cause a change in form of most of the particles. Purified preparations in water inactivated by 10^{-3} M or 2×10^{-4} M mustard gas,[2]* or partially inactivated by heating for 10 to 15 minutes at 50°C are also not converted to the filamentous form by the addition of saline. On the other hand, spherical forms which have remained in water without inactivation for as many as 7 or 8 days may be converted to the filamentous form by the addition of saline.

Summary. Study of Newcastle virus in allantoic fluid shows this virus to have a

1 Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 5.

2 TenBroeck, C., and Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 271.

* Dr. R. M. Herriott kindly treated a preparation with these concentrations of mustard gas.



a b

FIG. 1a. Electronmicrograph of unpurified Newcastle virus in allantoic fluid. × 17,200.
FIG. 1b. Electronmicrograph of Newcastle virus resuspended in distilled water after purification by two cycles of differential centrifugation. × 17,200.

TABLE I.
Purification of Newcastle Virus and Resuspension in Water.
Titer: 50% mortality of 10-day embryos.*

Exp. No.	Original allantoic fluid	Virus resuspended in		
		Water	.15 M NaCl	.15 M sucrose
1	$10^{-8.3}$	$10^{-8.7}$	$10^{-8.5}$	
2	$10^{-9.3}$	$10^{-8.8}$		$10^{-9.3}$

* All titrations are calculated on the basis of resuspension in a volume comparable to that of the original allantoic fluid.

TABLE II.
Stability of Newcastle Virus in Water and Saline at 4°C.
Titer: 50% mortality of 10-day embryos.

Time, day	Water	NaCl			Water (2nd exper.)
		.05 M	.15 M	.3 M	
1	$10^{-9.2}$	$10^{-8.6}$	$10^{-9.0}$	$10^{-7.2}$	$10^{-8.7}$
4		$10^{-8.7}$	$10^{-8.2}$	$10^{-6.0}$	
5					$10^{-8.3}$
8	$10^{-8.7}$				

roughly spherical shape when examined under the electron microscope. Most of the particles maintain this form when purified by ultra-centrifugation and by transfer to water. The addition of .07 to .15 M sodium chloride to the solution converts it from the spherical

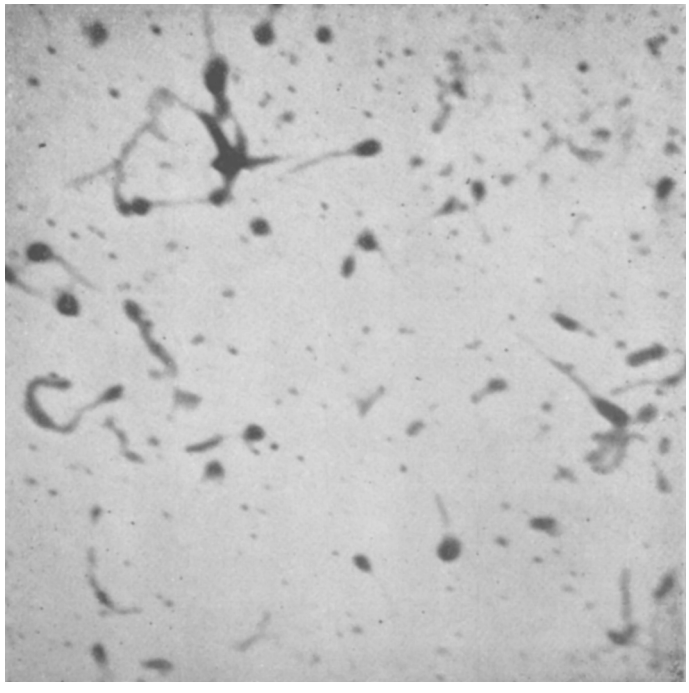


FIG. 2.
Electronmicrograph of same preparation as 1b in .15 M NaCl.
× 17,200.

to the filamentous form previously described. This conversion is prevented by partial inactivation with formaldehyde, mustard gas, or gentle heating.

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Effect of Hexaethyl Tetraphosphate on Choline Esterase *in vitro* and *in vivo*.*

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Hexaethyl tetraphosphate, $C_{12}H_{30}P_4O_{13}$, (HTP), has recently been introduced in this country as an insecticide. It was first used in this capacity by the Germans and was uncovered by technical teams following the close of the European phase of the war. The German scientists who were interrogated stated that it had a nicotine-like action and was used for the control of aphids as a substitute for nicotine. The authors of the present report have been unable to find any reference to its mechanism of action other than its nicotinic effects.

The present study was initiated following observation during routine testing[1] that animals showed symptoms similar to those pro-

* Carried out under a contract with Medical Division, Chemical Corps, U. S. Army.

1 Botkin, A. L., Lipton, M. A., and Mangun, G. H., unpublished data.

2 Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114**, 495.