other, although antigenically related strains.

The results indicate that for a constant amount of virus there was a critical level of specific antibodies which separated a state of relative resistance from a state of relative susceptibility. However, the height of titer at the time of inoculation was not always an index of the resistance of the animal to clinical infection. When ferrets which had approximately the same titers of specific antibodies were inoculated with the same amount of the same virus suspension, the animals which had had the more extensive previous experience with influenza virus strains were likewise the more resistant to clinical infection. The more extensive experience apparently endowed those animals with a more active immunological mechanism which enabled them to mobilize more quickly a higher concentration of strain-specific antibodies. In so far as protection against clinical infection is concerned the protective effect of antibodies is not limited to the quantity present at the time of inoculation but is dependent upon the amount made available before the infecting agent has multiplied sufficiently to evoke clinical manifestations.

Summary. Four to 10 weeks following an initial infection with influenza A virus, ferrets were found to be immune to clinical reinfection with that same strain of virus but were susceptible to reinfection with a strain of virus which was antigenically related to but different from the strain used for the original infection.

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Filamentous Forms of Newcastle Virus.

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Relatively few of the animal viruses have been purified and studied under the electron microscope.[1] This preliminary report deals with the causative agent of Newcastle disease of chickens.

Four strains of virus have been studied, 2 isolated in New Jersey, 2 in California. Strain B was isolated from a field case by Dr. F. R. Beaudette and furnished to us in the 1st embryo passage. Strain W was isolated by us from an outbreak of the disease in a flock of White Leghorns at Bound Brook, N. J. Strain Nap is a laboratory strain isolated by Dr. J. R. Beach in California. These 3 strains are identified as Newcastle virus by their behavior in the developing chick embryo, which they kill with specific hemorrhagic lesions of the brain, feather follicles, and entire embryo in 48 to 84 hours,[2] by their ability to agglutinate chicken red blood cells, by the neutralization of these effects by specific antisera against known strains, and by the reproduction of the natural disease in chickens.[3,4] Strain Cg179, also furnished by Dr. Beach, is highly virulent for chickens and is specifically neutralized by antisera in the embryo.

All four of these strains when partially purified from allantoic fluid by 2 cycles of differential ultracentrifugation show filamentous forms which predominate on the screen of the electron microscope. (Fig. 1 and 2); the phagelike structure is not a constant feature of our preparations, but at no time have we failed to demonstrate filamentous forms. That these forms represent active virus is indicated by their presence in concentrates of the allantoic fluid as early as 24 hours after inoculation until 48 to 72 hours when death of the embryo occurs; by their presence at tem-

1 Wyckoff, R. W. G., *Science*, 1946, **104**, 21.

2 Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sci.*, 1942, **20**, 81.

3 Beaudette, F. R., *Cornell Vet.*, 1946, **36**, 105.

4 Beach, J. R., *J. Am. Vet. Med. Assn.*, 1946, **108**, 372.

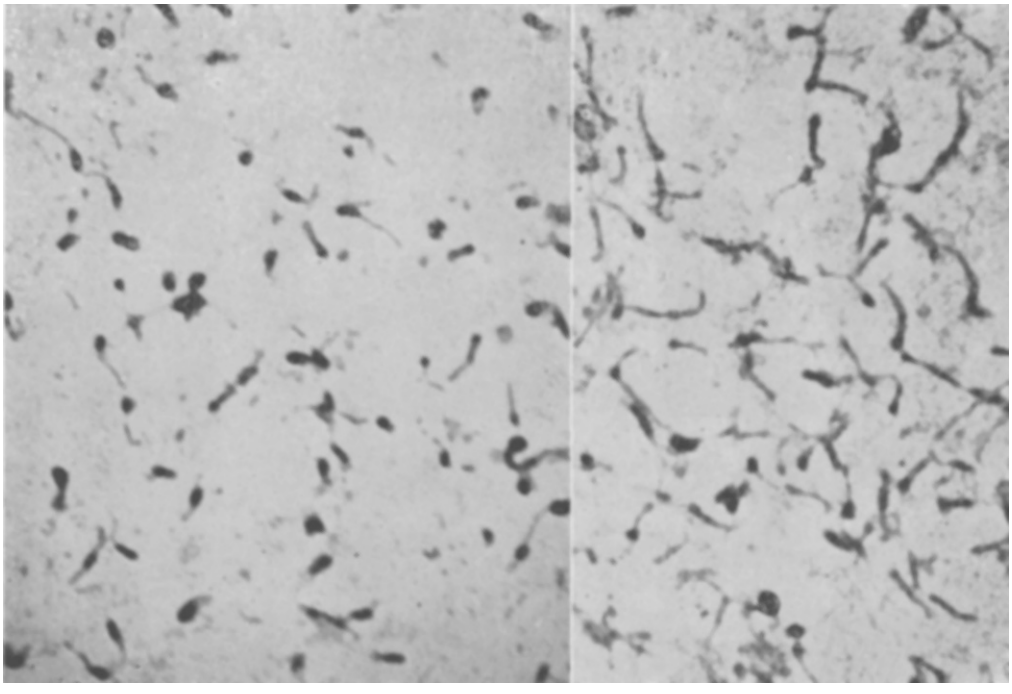

Fig. 1. (Left).
Electron microscope photograph of concentrate of strain B Newcastle virus. 0.01% formaldehyde 2 days. × 17,200.

Fig. 2. (Right).
Concentrate prepared immediately after centrifugation and resuspension in buffered saline. × 17,200.

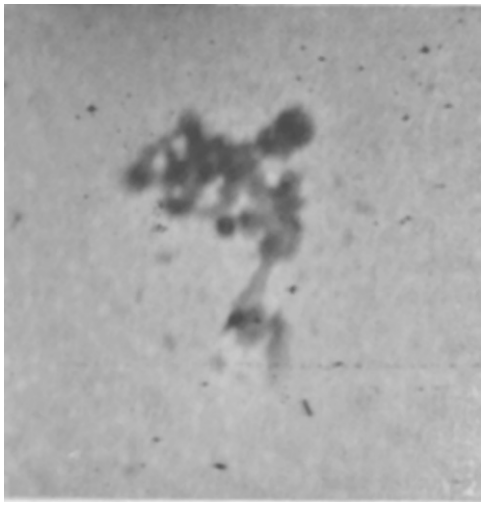

FIG. 3.
Effect of immune sera. Screen prepared 2 hr after the addition of antisera. Control sera failed to agglutinate virus. × 17,200.

peratures of incubation of the inoculated embryo from 35° to 39°C; by the stability of the virus during centrifugation (see Table I); and by the facts that calculations of size and weight of the virus by electronscope micrography and by light scattering measurements[5] yield a size of about 115 mμ and a molecular weight of 450 million,* which on the basis of demonstrated infectivity for the embryo (Table I) indicates that 10 or less particles are able to infect the embryo; that concentrates of the virus may be specifically precipitated by antisera and electron microscope pictures show the filamentous forms to be clumped by these antisera; and that the sharpness of outline of these forms is gradually lost as the suspension stands and as chick embryo in-

[5] Oster, G., *Science*, 1946, **103**, 306.

* We are indebted to Dr. R. M. Herriott for these determinations.

TABLE I.
Purification of Newcastle Virus by Ultracentrifugation.

	Original material			1st supernatant		
	Titer by			Titer by		
Exp. No.	Embryo inoculation	Chicken r.b.c. agglutination	N mg/cc	Embryo inoculation	Chicken r.b.c. agglutination	N mg/cc
1	$10^{9.5}$	1/4000	.48	$10^{6.0}$	1/20	.47
2	$10^{8.5}$	1/800		$10^{6.7}$	1/10	
3	$10^{8.7}$	1/400		$10^{6.3}$	1/10	
4	$10^{8.6}$	1/1600			Undiluted	
5	$10^{8.3}$	1/3200			1/16	
6	$10^{8.7}$	1/800			Undiluted	

	Final concentrate			
	Titer by			
Exp. No.	Embryo inoculation	Chicken r.b.c. agglutination	N mg/cc	Titer/ g protein
1	$10^{10.3}$	1/4000	.014	$10^{15.4}$
2	$10^{8.7}$	1/400	.007	$10^{14.1}$
3	$10^{9.5}$	1/400	.01	$10^{14.8}$
4	$10^{9.0}$	1/800	.008	$10^{14.4}$
5	$10^{9.0}$	1/3200	.004	$10^{14.7}$
6	$10^{8.6}$	1/800	.0036	$10^{14.4}$
			Average	$10^{14.6}$

Virus was titered by calculating the 50% end point mortality after inoculation of 10-11 day chorioallantoic membranes. Since by Avagadro's law a gram of a substance with a molecular weight of 450 million would contain 1.3×10^{15} particles, and since the average preparation here titered to $10^{14.6}$ or 4×10^{14}, there is a three-fold difference between actual and possible end points. The crudeness of the method of titration does not warrant further calculation.

fectivity is lost.

We have not seen these forms in preparations of allantoic fluid containing the same concentration of virus before purification and resuspension in phosphate buffered saline solution. Whether this is due to a masking of form by the allantoic fluid in which the virus exists or whether it is due to a change of shape on transfer into a new medium is undetermined as yet.

Summary. Studies on purified preparations of Newcastle virus of chickens demonstrate that this virus may be filamentous or stringy in form, sometimes with a large head, approaching the tailed forms of the bacteriophages or bacterial viruses.