

THE SIZE OF THE VIRUS OF AUJESZKY'S  
DISEASE ("PSEUDO-RABIES", "INFECTIOUS  
BULBAR PARALYSIS", "MAD-ITCH") BY  
ULTRAFILTRATION ANALYSIS

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AUJESZKY (1902), in his paper "Über eine neue Infektionskrankheit bei Haustieren", first described the disease now designated "pseudo-rabies", "infectious bulbar paralysis", or in America "mad-itch". His filtration experiments made with "Nordtmeyer-Berkefeld"<sup>1</sup> candles yielded inactive filtrates. Workers since then have reported conflicting results on the filterability of the infective agent, some (Zwick & Zeller, 1911; Bertarelli & Melli, 1913; Isabolinsky & Patzewitsch, 1912) finding that the virus would not pass through the ordinary types of Berkefeld V, N and W and Chamberland L 3 filter candles, while others (Schmiedhoffer, 1910; Sangiorgi, 1914; Shope, 1931) have succeeded in obtaining virulent bacteria-free filtrates. An analysis of the experimental conditions obtaining in the several cases indicates that the unsuccessful filtration was probably due to early blocking of the candle. This effect can only be overcome by subjecting the crude suspension of infective nervous tissue to preliminary clarification by centrifugation before passing it through the candle, and by the choice of a favourable medium, preferably broth. In the present experiments broth suspensions of brain or lung tissue from rabbits in advanced stages of the disease have been analysed by fractional ultrafiltration through the graded collodion membranes described by Elford (1931), with a view to estimating the size of the virus particles.

EXPERIMENTAL METHODS

*Filtration technique.* The technique of ultrafiltration has been precisely that employed in previous analyses of virus suspensions conducted in this Institute (Elford, 1931; Barnard & Elford, 1931; Galloway & Elford, 1931, etc.). In all filtration experiments positive pressures of nitrogen were used.

*Strains of virus.* Two strains of virus have been employed. The A.P. strain was kindly sent by the late Professor Aujeszky, Budapest, in November 1930,

<sup>1</sup> Nordtmeyer, H. (1891) published a paper entitled "Über Wasserfiltration durch Filter aus gebrannter Infusorienerde" in *Z. Hyg. Infektkr.* **10**, 145.

and the M.I. or "mad-itch" strain was received in 1932 through the courtesy of Dr R. E. Shope, Princeton, U.S.A. Both strains were passaged frequently in young rabbits before and during the course of the filtration experiments. No essential difference has been detected between the behaviour of the two strains in young Dutch or Himalayan rabbits weighing 1100–1500 g. In a limited number of tests the blood from rabbits infected with either strain has been proved infective. Rabbits inoculated subcutaneously, in addition to showing the characteristic syndrome and developing the typical local lesions, have frequently shown intense haemorrhagic lung lesions on autopsy. Similar lung lesions have been seen also in certain rabbits dying after intracerebral inoculation of virus. The incubation period after intracerebral (18–36 hours) or subcutaneous (40–90 hours) inoculation was the same with both strains. Most experiments were made with infective brains from intracerebrally inoculated rabbits, but in two experiments lung tissue, taken from rabbits which had developed intense haemorrhagic lesions after subcutaneous inoculation of virus, was used. Virus was found to be present in high concentration in lungs showing these lesions and in the brains of the intracerebrally inoculated rabbits.

*Preparation of stock filtrates.* A known weight of infective brain or lung tissue to give the required suspension concentration, 5–10 per cent, was ground up with sterile glass powder in Hartley's broth at pH 7.6 and then centrifuged for 10 min. at 2500 r.p.m. The supernatant liquid was filtered through a standardized sand and pulp filter. The relatively clear filtrate thus obtained was filtered through a suitable membrane to furnish a bacteria-free stock filtrate. The stock filtrate, which usually showed a limiting infective dilution of  $10^{-5}$ , was then subjected to analysis with membranes of progressively lower porosities.

*Methods of testing for presence of virus.* Each liquid to be tested was inoculated into two rabbits. One rabbit received a dose of 0.25 c.c. intracerebrally and the other a dose of 4 c.c. subcutaneously. Tests were made on serial tenfold dilutions of the filtrates, and the highest dilution producing typical symptoms and death in one or both of the inoculated rabbits was taken as the virus titre. Surviving rabbits were tested for immunity but none was ever detected.

#### THE FILTRATION END-POINT AND PROBABLE SIZE OF THE VIRUS

The experimental results with the two strains of virus are summarized in Tables I and II. The virus passed through all membranes having porosities greater than  $0.20\mu$ . The end-point of filtration,  $0.20\mu$  on the basis of the method outlined by Elford (1933), indicates the particle size of the virus to be 100–150 m $\mu$  (see *Annual Report, Medical Research Council, 1932–3*; also Galloway, 1936).

Table I. *Filtration of pseudo-rabies virus (A.P. strain)*

Source of virus	A.P.D. of membrane for stock filtrate $\mu$	A.P.D. of membrane for secondary filtrate $\mu$	Amount filtered c.c.	Test of secondary filtrate
Brain	0.95	0.50	10	$10^{-5}$
"	0.95	0.32	10	++
"	0.65	0.30	10	$10^{-3}$
"	*0.50	0.30	10	$10^{-2}$
"	0.50	0.30	10	$10^{-3}$
"	0.50	0.30	10	$10^{-3}$
Lung	*0.50	0.30	10	++
Brain	0.95	0.25	10	$10^{-2}$
"	0.50	0.25	10	$10^{-3}$
"	0.80	0.25	6	++
"	*0.50	0.25	10	++
"	0.90	0.25	10	00
"	*0.50	0.20	10	00
Lung	*0.50	0.20	7	00
Brain	0.80	0.20	6	00
"	0.80	0.16	7	00
"	0.90	0.15	10	00
"	0.95	0.13	10	00
"	0.50	0.13	10	00
"	0.50	0.13	10	00
"	0.50	0.060	10	00

Each stock filtrate had a titre of  $10^{-5}$ .

\* 0.50  $\mu$ . These stock filtrates were obtained after preliminary filtration of the sand and pulp filtrate through a membrane of A.P.D. 0.80–0.90  $\mu$ .

++ = both rabbits inoculated with undiluted filtrate died (no titration made).

00 = neither of two rabbits inoculated with undiluted filtrate showed symptoms.

All rabbits surviving were later tested for immunity but none was detectable.

$10^{-x}$  = limiting infective dilution or titre.

Table II. *Filtration of pseudo-rabies virus (M.I. strain)*

Source of virus	A.P.D. of membrane employed for stock filtrate $\mu$	A.P.D. of membrane for secondary filtrate $\mu$	Amount filtered c.c.	Test of secondary filtrate
Lung	0.90	0.30	10	++
Brain	0.50	0.25	10	$10^{-3}$
"	0.50	0.25	10	++
Lung	0.90	0.20	10	00
Brain	0.50	0.20	10	00
"	0.50	0.13	10	00

Each stock filtrate had a titre of  $10^{-5}$ .

++ = both rabbits inoculated with undiluted filtrate died (no titration made).

00 = neither of two rabbits inoculated with undiluted filtrate showed symptoms.

$10^{-x}$  = limiting infective dilution or titre.

#### SUMMARY AND CONCLUSION

The virus of Aujeszky's disease (pseudo-rabies) has been found to have a particle size of 100–150  $\mu$ . Two strains were employed in these experiments, one that of Aujeszky, and the other Shope's "mad-itch" strain. The results with both strains were identical.

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