## Infectivity of Influenza Virus Filaments

Although the presence of filamentary forms in preparations of influenza virus was first noted in 1946 [1], the finding remained one of academic interest until the demonstration by Chu, Dawson and Elford in 1949 [2] that recently isolated influenza *A* strains contain a high proportion of filaments. These forms have since been shown to possess many of the biological characteristics of spherical influenza virus particles[3,4]; but the question of the number of filaments needed to induce infection was not considered until recently. The main contribution in this direction has been the pioneer work of Donald and Isaacs[5], who used quantitative counting techniques. They, however, did not claim any differences in the relative infectivity of virus filaments and spheres. Valentine and Isaacs[6] have since suggested that, owing to the tendency of filaments to break up during adsorption on red cells, the actual counts of Donald and Isaacs[5] are over-estimates of the number of filaments initially present, and hence there is an increased probability that filaments are infectious. In connexion with nucleic acid studies on filaments, we have used a strain of filamentary virus (Ryan) that has a mean ratio of 50 per cent egg infectivity dose/agglutinating dose of the same order as that given by strains which exist almost exclusively as spheres. Using the counts of Donald and Isaacs[5] together with our 50 per cent egg infectivity dose/agglutinating dose value, it can be calculated that, for this strain of virus, fewer filaments than spheres are necessary to induce infection in the embryonated egg.

The *A* strain Ryan was isolated from the 1954 influenza epidemic in Melbourne. It has been passaged sixteen times at limit dilution in eggs. Following inoculation of 0·05 ml. at a $10^{-2}$ dilution into 10-day embryonated eggs, the allantoic fluid was harvested after 72 hr. incubation at 35° C.; the classical *A* strain *PR*8 was grown and all hæmagglutinin and infectivity determinations carried out as described previously[7]. These techniques are similar to those used by Donald and Isaacs[5]. In nineteen experiments, the mean ratio ($\log_{10}$) 50 per cent egg infectivity dose/agglutinating dose value for Ryan was $6{\cdot}0 \pm 0{\cdot}5$; in twelve experiments, for *PR*8, $6{\cdot}0 \pm 0{\cdot}3$. The average proportion of filaments in Ryan allantoic fluid is 30 per cent; their morphology is similar to that of filaments in *A*/Persian Gulf/2/52 [5] and upon fragmentation there is an increase in hæmagglutinin. Donald and Isaacs[5] have shown that the ratio ($\log_{10}$) of particles to agglutinating dose for non-filamentary strains is $7{\cdot}2 \pm 0{\cdot}13$ (that is, $16 \times 10^6$ particles = 1 agglutinating dose) while for a strain in which the proportion of spheres to filaments is approximately 50 : 50, the ratio ($\log_{10}$) is $6{\cdot}6 \pm 0{\cdot}08$ (that is, $4 \times 10^6$ particles = 1 agglutinating dose). In the latter case, the contribution due to the spheres is $2 \times 10^6$ particles, which is $\frac{1}{8}$ of the agglutinating dose given above for *PR*8. Hence that due to the $2 \times 10^6$ filaments is $\frac{7}{8}$ agglutinating dose. Thus in a preparation containing filaments only, $2{\cdot}3 \times 10^6$ units = 1 agglutinating dose. From this, it can be calculated that in a preparation containing 30 per cent filaments, $6{\cdot}1 \times 10^6$ particles = 1 agglutinating dose, that is, $10^{6{\cdot}78}$.

The ratio ($\log_{10}$) particles/agglutinating dose for *PR*8 = 7·2; for Ryan = 6·78. The ratio ($\log_{10}$) 50 per cent egg infectivity dose/agglutinating dose for *PR*8 = 6·0; for Ryan = 6·0 Therefore, the ratio ($\log_{10}$) particles/50 per cent egg infectivity dose for *PR*8 = 1·2; for Ryan = 0·78. The number of particles/50 per cent egg infectivity dose for *PR*8 = 16; for Ryan = 6·1.

Of this figure 6·1 for Ryan, 70 per cent or approximately four particles are spheres and contribute a quarter of the 50 per cent egg infectivity dose. Therefore, two or three filaments are equivalent to one 50 per cent infectivity dose. In view of the finding[3,4,6] that filaments break down during manipulation, it appears possible that one filamentary particle as present in unharvested allantoic fluid may induce infection.

This calculation assumes that the spheres present in Ryan preparations have the same relative infectivity as *PR*8 spheres. Two observations support this. (1) The value of the ratio 50 per cent egg infectivity dose/agglutinating dose for spheres isolated by filtration from *A*/Persian Gulf/2/52 was not appreciably different from that of the allantoic fluid preparation[5]. (2) In three preparations the value of the ratio ($\log_{10}$) 50 per cent egg infectivity dose/agglutinating dose of a strain of Ryan, derived from the parent strain by passage at low dilution and containing very few filaments[4], ranged from 6·2 to 5·9.

The finding that considerably fewer filaments than spheres are needed to induce infection has a number of important implications. These will be discussed in conjunction with the results of the nucleic acid studies in a paper to be published elsewhere.

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## Structure of Molluscan Tropomyosin

Certain molluscan smooth muscles contain protein filaments with a regular and characteristic fine structure[1–3] to which the name paramyosin has been given[4]. Neither the chemical nature nor the function of these filaments is understood, and attention is once more drawn to these problems by the recent discovery in the same muscles of an asymmetric globulin[5,8] precipitating in the form of needle-shaped 'crystals'. This was found[6–8] to have the amino-acid pattern of a tropomyosin, and the large amounts present in slow lamellibranch adductor muscles suggested that it might be responsible for the paramyosin structure, an inference supported by some preliminary studies of the X-ray diffraction pattern[5,7]. These 'crystals' have now been studied by electron microscopy.

The protein was usually made from the white part of the adductor muscle of the oyster (*Gryphaea angulata*), but similar results were obtained with the equivalent muscle of *Pinna nobilis*. Fresh muscles were homogenized in 0·04 *M* potassium chloride buffered at