LETTERS TO THE EDITORS

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Filamentous Forms of Influenza Virus

THERE is good evidence that the filamentous forms characteristic of many recently isolated strains of influenza A are infective units[1]; but their significance in relation to the process of influenza virus replication is by no means clear. It has been suggested that filaments represent the initial form in which the infective phase of the virus is produced, spheres being formed secondarily[2]. Others have suggested that the filament is a by-product, as it were, of the process by which new infective particles are produced at the free surface of the allantoic cell[3].

Although it has been known since 1949[4] that the filaments are clearly visible by dark-ground illumination at a magnification of 500 or even less, very little use has been made of this method of observation. It has, however, a number of advantages over the conventional use of the electron microscope for such work. A simple, and for most purposes sufficiently accurate, method of comparing concentrations of filaments is as follows. A standard volume, 20 c.mm., is placed on a clean slide and covered carefully with a coverglass of standard size ($\frac{7}{8}$-in. circle). The preparation is put into a moist chamber for 15 min. or longer to allow the great majority of the filaments to attach themselves to one or other glass surface. Using a standard area of field with an 'Ehrlich' eyepiece and a 47 × objective, the number of filaments on the two glass surfaces plus the few seen in moving from one focal level to the other can be readily counted. To a reasonable approximation the number of filaments per field area X is equivalent to $X \times 10^7$ filaments per c.c., and with the highly filamentous strains Oc. Is., *PERS* and *RYAN*, X is of the same order as the hæmagglutinin titre. For convenient counting it is necessary to dilute the average infected fluid 1 : 10 or 1 : 20.

This method has been used in an exploratory series of investigations for finding agents capable of destroying the filaments as estimated by the reduction in the numbers of visible forms. The interesting result has emerged that most agents capable of hæmolysing red cells will destroy virus filaments and show a threshold at about the same level of concentration. Hypotonic salt solutions are destructive at concentrations of 0·3 per cent sodium chloride or lower. At or near threshold concentrations, distorted forms with angulation and a beaded appearance may be seen; at lower concentrations forms recognizable as filaments become extremely rare. Disintegration is more rapid and complete at *p*H 6 than at *p*H 8.

As representative hæmolytic agents saponin, cetyltrimethylammonium bromide and sodium lauryl sulphate were used. They were completely destructive at concentrations of 0·01 per cent, but had virtually no effect at a concentration of 0·001 per cent. The end point of hæmolysis of 1 per cent fowl cells also lay between these concentrations. Saponin was less active in the sense that few filaments were completely destroyed. Obvious evidence of damage was produced to a concentration of 0·003 per cent in the form of distortion, beading and, particularly around 0·01 per cent, very firm adsorption to glass surfaces. The damage was confirmed in electronmicrographs made for me by Dr. Heather Donald.

Shaking with ether or chloroform destroys the filaments. Using the normal procedure of shaking infective fluid with 20 per cent of freshly redistilled ether and leaving overnight in the refrigerator, virtually all filaments were destroyed. The hæmagglutinin titre showed a moderate fall when tested with fowl cells; but a rise with human or guinea pig cells. Untreated, the strain Oc. Is. gave fowl-to-human hæmagglutinin titres of 600/700, after treatment with ether 300/3,200. Of non-filamentous strains similarly treated, only *PR*8 showed a change of this type from 500/700 to 130/600. Infectivity titrations showed no reduction beyond 0·5 $\log_{10}$ when treatment was with redistilled ether on allantoic fluid buffered with phosphate at *p*H 7·0. The same lack of effect on infectivity was seen when filaments were broken down with water lightly buffered at *p*H 7·0 or 8·0.

The results suggest that the filaments have qualities resembling those of the host cell surface, and that their formation is a result of an interaction of virus components with the surface of the cell in which virus has multiplied. Failure to influence infectivity by filament destruction may mean that their infectivity is limited to the spherical units that often appear to be incorporated in the filaments.

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[3] Hotz, G., and Schafer, W., *Z. Naturforsch.*, **10**, 1 (1955).
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Reactivation of Partially Degraded Tobacco Mosaic Virus

RECENT infectivity studies with partially degraded tobacco mosaic virus have led to the inference that the virus particles can remain active after some of the protein has been experimentally removed so as to expose portions of the ribonucleic acid core[1,2]. In one of these studies[2] it was found that, upon heating the virus briefly with a detergent, the infectivity was reduced from that in the original sample by a factor of 4–10; most of the activity then remaining was found to reside in partially degraded virus particles, as judged by the fact that their infectivity could be greatly reduced by digestion with crystalline pancreatic ribonuclease.

While there is as yet no clear understanding of the separate roles of virus protein and ribonucleic acid in the process of infection, it appears that the structural integrity of the ribonucleic acid is essential for activity[2], whereas the protein coat need not be intact. It may then be asked whether the mere removal of part of the protein has any direct effect on infectivity. Such an effect is not necessarily indicated by the partial inactivation occurring in the heat-detergent treatment, since the treatment may disrupt the ribonucleic acid as well as the protein in some of the particles. In order to determine whether part of the heat-detergent inactivation might be directly due to the removal of protein, an attempt has been made to reverse this inactivation by replacing the protein removed in the treatment. That a replacement of protein might be possible was suggested by the work of Fraenkel-Conrat and Williams[3], who showed that the purified protein and