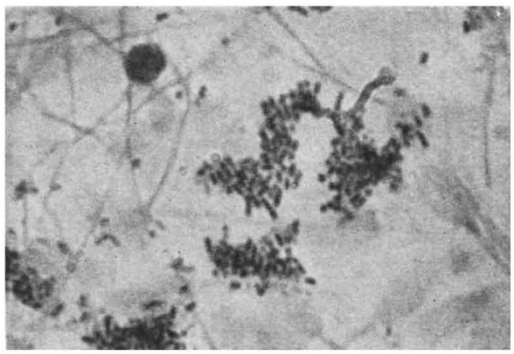

Fig. 1. Prophase chromosomes of spermatocytes II in ejaculated semen 3 weeks after experimental infection (Giemsa stain)

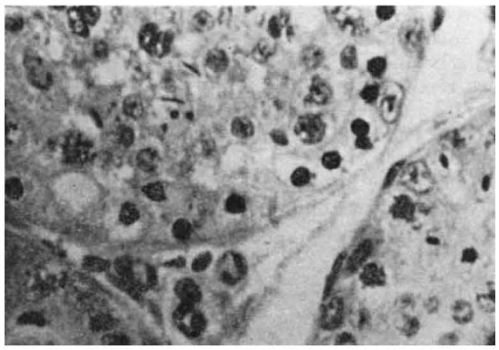

Fig. 2. Pycnosis of the zygotene nuclei (hæmatoxylin and eosin stain)

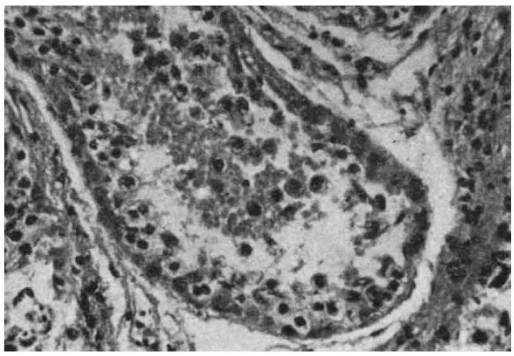

Fig. 3. Desquamation of the germinal epithelium 4 weeks after experimental infection. All types of spermatogenic cells free in the lumen (hæmatoxylin and Congo red stain)

cytes I and II, and by the appearance of loose chromosomes in the ejaculated semen (Fig. 1).

This damaging effect of the virus on the germinal epithelium could be confirmed by histological examination of the testis at slaughter. The degenerative action seems to start in the newly formed spermatozoa as well as in the zygotene nuclei of the first reduction division, resulting in an arrested spermatogenesis (Fig. 2). In the third week after infection the blocked germinal epithelium is eliminated into the lumen of the tubuli seminiferi (Fig. 3). In the experimental bulls normal spermatogenesis was restored within 3 months after infection.

This type of viral infection probably plays an important part in the frequent infertility *e causa ignota* in bulls.

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VIROLOGY

Transmission of Tobacco Necrosis Virus to Tobacco Callus Tissues by Zoospores of *Olpidium brassicae*

Morel[1] infected cultures of plant callus tissue with some, but not all, of the obligate parasites he tested. Thus, with fungi, he succeeded with *Plasmopara viticola* and *Uncinula necator*, but not with several species of rust fungi on callus from susceptible host plants. He also infected tobacco callus with tobacco mosaic virus, but this is difficult and most work with plant viruses in tissue cultures has consequently been done with tissues taken from plants already systemically infected. Unwounded callus tissues are immune from infection by viruses, and although tissues can be infected by pricking with fine needles dipped in concentrated inoculum, the method is not always successful[2]. We have now found that tobacco callus tissue can be readily infected with tobacco necrosis virus with the aid of *Olpidium brassicae*, an obligately parasitic fungus that was known previously only to infect plant roots. Teakle[3,4] showed that *Olpidium* aids the infection of roots by this virus, and we have confirmed this.

In our experiments we used two strains of tobacco necrosis virus[5] (*A* and *D*) and three isolates of *Olpidium*, 2 from England and 1 from Dr. D. S. Teakle in the United States. Methods of culturing the *Olpidium* and preparing zoospore suspensions will be described elsewhere. Normal and conditioned tobacco tissues were grown as already described[2,6]. One day before inoculation the tissues were transferred to small filter paper cups pushed into vials 2 in. × 1 in. containing 5 ml. Hoagland's solution diluted 1/20. The solution just touched the bottom of the paper cup which was used to prevent the callus cells being lost in the liquid. The method of inoculation was to add to each vial 1 ml. of Hoagland's solution containing zoospores and 0·5 ml. containing purified virus. The tissue was left covered with the inoculum overnight and next day enough fluid was pipetted off to expose it again. Four to five days after inoculation, the virus in the tissues and in the fluid beneath them was assayed by infectivity tests. Each crushed tissue and fluid was rubbed over four primary leaves of French bean dusted with 'Carborundum', and the necrotic local lesions produced counted three days later.

All three isolates of *Olpidium* transmitted both strains of the virus to both normal and conditioned callus tissues. However, as the results in Table 1 indicate, isolate 3 seems more effective than the other two, especially in transmitting strain *D*, but further tests will be needed to show whether this is so. The fungus not only aided infection by

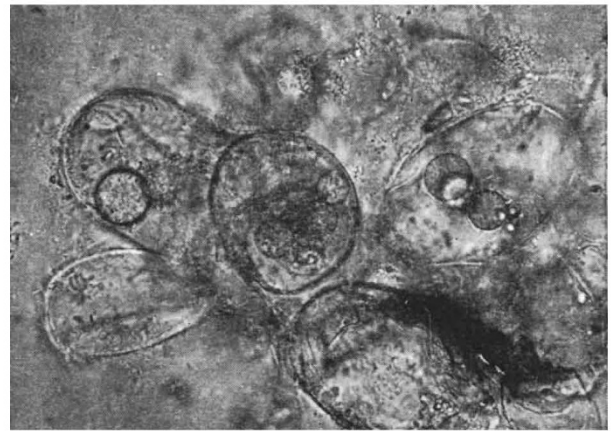

Fig. 1. A group of cells of normal tobacco callus tissue showing sporangia of *Olpidium brassicae* 4 days after inoculation

Table 1. TRANSMISSION OF TOBACCO NECROSIS VIRUS BY THE ZOOSPORES OF *Olpidium brassicae* TO NORMAL TOBACCO TISSUES

Virus strain	Final virus concentration in vial (μg/ml.)	Isolates of *Olpidium* and final concentration of zoospores in vial			
		1	2	3	Without zoospores
		$1{\cdot}8 \times 10^5$/ml.	$1{\cdot}1 \times 10^5$/ml.	$3{\cdot}1 \times 10^5$/ml.	
A	0·06	242/3*	213/5	329/4	3/2
D	0·003	7/0	24/1	422/6	5/3

* Numerator indicates average number of lesions produced per leaf of French bean inoculated with the tissue, and the denominator the average number produced by the fluid in the vial. There were three replicate vials per treatment.

the virus, but also, as shown in Fig. 1, established itself in the callus and formed mature sporangia.

Olpidium appears to transmit tobacco necrosis virus to callus tissues as readily as it does to roots of young lettuce seedlings, with which most of our work has been done, and does so with more dilute inocula than are effective with mechanical inoculation. Infected tissues kept for longer than five days began to decompose and their virus content decreased. This happened because of secondary infection by bacteria, and the method will retain this limitation until *Olpidium* is isolated free from other micro-organisms. We report these preliminary observations because we think they may lead to improved techniques for both the investigation of infection by plant viruses and the examination of the fungus.

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Influence of 5-Fluorodeoxyuridine on the Cell-infective Unit of Adeno Virus in HeLa Cells

WE have already reported that several halogenated pyrimidines including 5-fluorodeoxyuridine (FUDR) did not show a complete inhibition of the multiplication of adeno type 1 virus in the experiments using the $TCID_{50}$ estimating dilution method, while vaccinia virus was clearly inhibited with FUDR and some other halogenated pyrimidines[1]. On the contrary, Flanagan and Ginsberg[2] and Green[3] reported that FUDR showed clear inhibition during the early phase of the multiplication of adeno type 4 or type 2 viruses. Thus, the results previously described by us[1] seemed inconsistent with those obtained by them[2,3]. If the sensitivity of adeno virus towards the inhibitory effect of FUDR is not so clear as vaccinia virus shows, it might be suggested that the metabolic pathway essential for the replication of adeno virus must be a different one from the ways blocked by halogenated pyrimidines. To prove this point, the influence of FUDR on the single growth curve and the cell-infective unit of adeno type 1 virus was investigated.

The effect of FUDR on the single growth curve of adeno type 1 virus in HeLa cells was investigated as follows: A single sheet of HeLa cells was washed with phosphate-buffered saline and infected with adeno type 1 virus at a rate of one infectious unit per cell. After the incubation at 37° C for 2 h, the sheet was re-washed three times with phosphate-buffered saline, and then 1·0 ml. of the maintenance medium (Eagle's basal medium supplemented with 15 per cent horse serum) containing 10^{-4} M of FUDR in a final concentration was added into the tubes. For the control group, the same amount of the maintenance medium without FUDR was added. At various intervals after the viral inoculation, the medium was removed from the tubes, and the amount of cell-associated virus was determined after the incubation at 37° C for 21 days by

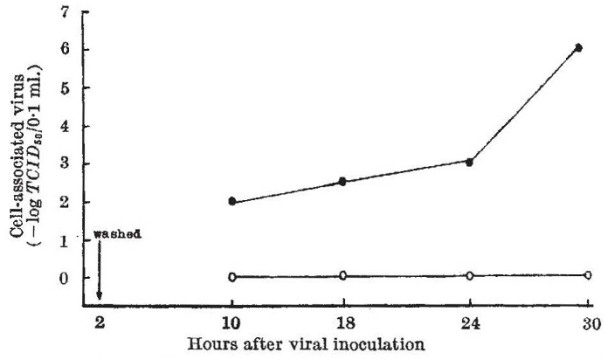

Fig. 1. Effect of 5-fluorodeoxyuridine on the single growth curve of adeno type 1 virus in HeLa cells. ●, Control group inoculated at a rate of 1 infectious unit per cell; ○, group treated with FUDR inoculated at a rate of 1 infectious unit per cell

using the end-point estimating dilution method. In the group treated with FUDR, the cell-associated virus to be estimated by the dilution technique could not be seen, while the clear increase of viral amount was observed in the control group (Fig. 1).

Next, the influence of FUDR on the cell-infective unit of adeno type 1 virus was investigated by using fluorescent antibody technique. The complete single sheet of the HeLa was placed on a coverslip of 38 mm × 11 mm in a Petri dish, 45 mm in diameter, and then 2·7 ml. maintenance medium (*tris* buffered *LE* medium supplemented with 5 per cent horse serum) and 0·3 ml. of the various dilutions of adeno type 1 virus were inoculated into each dish. After incubation at 37° C for 2 h, the medium was removed and 3·0 ml. of the maintenance medium containing 10^{-4} M of FUDR final concentration was added into each of the dishes of the treated group, while for the control the same amount of the maintenance medium without FUDR was added. After incubation at 37° C for 28 h, the coverslips of one of the treated groups and the control group were stained with double staining method of Smith *et al.*[4]. In another of the treated group, the maintenance medium was removed 28 h after the viral inoculation, and the same amount of fresh maintenance medium without FUDR was added to the group. The tubes of this group were further incubated for 18 h, and then the coverslips of this group were stained as described here. Cell-infective unit was estimated with Philipson's method[5]. The cell-infective unit of the control group was $7{\cdot}7 \times 10^8$ per 1 ml., while those of the first and second treated groups were $4{\cdot}9 \times 10^4$ per 1 ml. and $2{\cdot}9 \times 10^6$ per ml., respectively.

These results suggest that the multiplication of adeno virus is sensitive towards the inhibitory effect of FUDR when the low input multiplicity of the virus was used and the cell-associated virus was determined within the first growth-cycle after viral inoculation. This suppression is not the complete inhibition of the multiplication of adeno virus but is only the delay of the maturation, because the cell-infective unit of the group treated with FUDR showed an increase after removing FUDR from the culture. If the inhibition with FUDR is the complete suppression of the multiplication of adeno virus, the increase of cell-infective unit will never occur even after removing FUDR as can be seen in the second treated group.

Green[6] suggested that the character of the incorporation of ^{14}C-thymidine into the cell-free extract of *KB* cells infected with adeno virus was different from that of the cell-free extract of the cell infected with vaccinia virus. Salzman[7] also suggested, from the remarkable inhibition of the multiplication of vaccinia virus with FUDR, that the process of thymidylic acid synthesis, which is blocked with FUDR, must be essential, and one of the most important pathways for the replication of vaccinia virus. The results presented here suggest that this pathway blocked