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.

B. KASSANIS
I. MACFARLANE

Rothamsted Experimental Station,
Harpenden, Herts.

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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

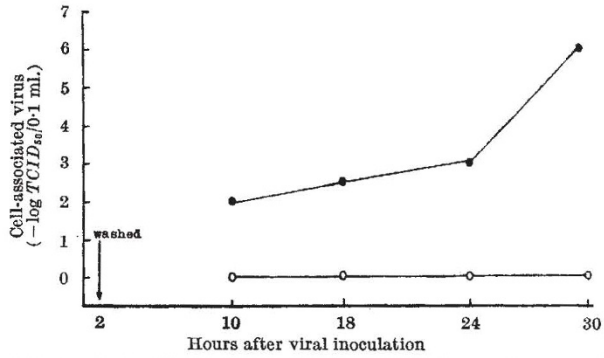

Fig. 1. Effect of 5-fluorodeoxyuridine on the single growth curve of adeno type 1 virus in HeLa cells. ●, Control group inoculated at a rate of 1 infectious unit per cell; ○, group treated with FUDR inoculated at a rate of 1 infectious unit per cell

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

with FUDR must participate in the replication mechanism of adeno virus, but should not play so great a part for the replication of the virus.

Yoshiko Seto
Shigeshi Toyoshima
Takeo Ueda

Pharmaceutical Institute,
School of Medicine,
Keio University,
Shinanomachi, Shinjukuku, Tokyo.

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PSYCHOLOGY

Lateralization of Learning of Colour and Brightness Discriminations following Brain Bisection

Several investigations dealing with interocular transfer of learning of visual discriminations in split-brain cats and monkeys support the general conclusion that brain bisection including the optic chiasm and posterior corpus callosum restricts the learning and memory of the discriminations to the trained hemisphere. These tasks cannot be correctly performed through the other eye until it has received comparable training, in contrast to the immediate interocular transfer usually shown by normal or chiasm-sectioned controls[1–3]. This generalization has been qualified by more recent studies indicating that transfer of learning of brightness[4,5] and pattern[6] discriminations in split-brain cats, and of brightness and colour discriminations in split-brain monkeys[7], does occur under certain conditions. Transfer of colour and brightness learning in split-brain monkeys would, therefore, seem to be dependent on the conditions used. It appeared advisable, in reference to future studies on vision, to see if transfer of these tasks takes place under the conditions at present being used in this laboratory.

Nine monkeys (8 *Macaca nemestrina* and 1 *M. mulatta*) were tested for transfer of learning on brightness (grey/white, relative intensity difference = 1 log unit) and colour (red/indigo, green/yellow, blue/orange) discriminations. Seven cases had the optic chiasm, anterior commissure, and corpus callosum sectioned; one control case had the chiasm alone sectioned, and one was normal. Histological verification of the surgery has been obtained for two of the experimental cases (fourth and last experimental animals in Table 1).

Table 1. Trials to Criterion for Each Eye

	Red/Indigo		Green/Yellow		Blue/Orange		Grey/White	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Normal	280	0	80	0			200	0
Chiasm	1,520	0	1,280	80	200	0	300	80
Split	1,320	1,200	480	680			680	800
	60	70	400	80	360	360	1,440	320
			130	150	160	80		
	120	200	200	200	240	80	600*	800
	60	260	160	30	80*	40	80	160
	100	40	70	170			320	240
	880	240			480*	240	360†	560

* Train contralateral eye-hand; change eyes and test ipsilateral eye-hand.
† Train ipsilateral eye-hand; change eyes and test contralateral eye-hand.

The stimulus-pairs were projected side-by-side on to two translucent screens, $1\frac{1}{2}$ in. × $1\frac{1}{2}$ in., which the monkey could push to indicate his choice. The possibility of cues arising from brightness differences was controlled in the blue/orange task by unpredictably altering gross intensity differences during training and testing. The animals were kept in their home cages, each of which was equipped with a training compartment. Sliding panels restricted the eye and hand use to specific combinations. The trials were programmed, rewarded, and recorded by an automated system. All animals had previously learned at least one black/white pattern discrimination, trained with this apparatus, and had shown no evidence of interocular transfer. As a rule, one contralateral (intrahemispheric) eye-hand pair was used during the initial training, then the opposite contralateral pair was tested and trained to criterion if transfer was not immediate. This procedure was varied in four cases by training or testing an ipsilateral combination (Table 1). For the longer learning curves the trials were grouped in blocks of 40 and for the shorter ones in blocks of 10. Criterion was arbitrarily set at 90 per cent correct responses during 40 consecutive trials.

The results in Table 1 show that after reaching criterion with the first eye the normal monkey immediately performed colour and brightness discriminations correctly with the untrained eye. The chiasm-sectioned animal showed full transfer for two of the tasks, although some retraining through the second eye was required for the other two problems. In the split-brain monkeys, on the other hand, immediate transfer of learning to the second eye was never seen and retraining through the second eye was always required. For the cases trained and tested with contralateral eye-hand combinations, the average number of correct responses on the first 40 trials, with the first and second eye, respectively, was 18·7 and 19·8 (colour) and 16·2 and 14·0 (brightness). Relearning with the second eye showed median savings of 0 per cent for the colour task and 3·5 per cent for the brightness problem. It should be noted, however, that with a criterion of 85 per cent instead of 90 per cent correct responses, the median savings were 20 and 6·5 per cent respectively, the former being significantly greater than zero (sign test, $\alpha = 0{\cdot}01$). Therefore, the possibility of some savings should not be definitely excluded. The scores of the four cases involving an ipsilateral eye-hand combination suggest that interocular transfer is not increased when both eyes are tested with the same hand.

The results recorded here support the earlier work of Sperry[8] and Downer[9] in which learning of discriminations involving coloured and black or white objects failed to transfer interocularly in split-brain monkeys. The immediate transfer of colour and brightness discrimination-learning reported by Trevarthen[7] remains puzzling. The main difference between his experimental conditions and ours is his use of simultaneous training of contradictory tasks to the two hemispheres, which would appear, at first glance, to hinder rather than induce interocular transfer.

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C. R. Hamilton
M. S. Gazzaniga

Division of Biology,
California Institute of Technology,
Pasadena, California.

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