are synthesized in steps, and each step occupies only a small fraction of the time required for the first signs of cell division (220 min). Analysis of acrylamide-gel electrophoretic patterns of cell extracts at various times during outgrowth showed that different classes of proteins were synthesized at each time interval[8]. Similarly, sequential synthesis of three enzymes during synchronous division of vegetative cells following outgrowth has been observed[8].

Two conclusions are apparent from these observations. First, the conversion of a dormant spore to a vegetative cell involves two distinct stages, germination and outgrowth. Germination does not involve protein synthesis but leads to an activation of enzymes in the spore, whereas outgrowth is ordered. During this period *m*RNA has a half-life of a few minutes. Therefore, the sequential synthesis of proteins must reflect differences in the time of transcription of corresponding portions of the genome.

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VIROLOGY

Nucleic Acid of Infectious Laryngotracheitis Virus

THERE is now considerable evidence that halogenated derivatives of the nucleoside 2′-deoxyuridine inhibit the growth of viruses that have DNA as their nucleic acid constituent but not of those containing RNA[1,2]. Inhibition occurs at a stage of nucleotide assembly and the thymidine analogue 5-iodo-2′-deoxyuridine (IUdR) has been shown to be incorporated into the DNA of vaccinia virus[3]. For cells infected with herpes simplex, this inhibition is partly reversible when thymidine is added together with the drug[4].

Close similarities in the structure and development of the virus particles of infectious laryngotracheitis (ILT) and those of the herpes group of viruses were noted in a previous report[5]. A more recent study[6] has demonstrated marked similarities in the ether sensitivity and fine structure of these particles and in the type of cytopathic effect produced when they are propagated in cultured chicken embryo kidney (CEK) cells. While these observations suggest a close resemblance between members of the herpes virus group and ILT virus, they are not sufficient to allow chemical similarities to be presumed. In an effort, therefore, to obtain information as to the type of nucleic acid which is present in ILT virus, experiments were performed to determine the effects of IUdR on the growth of ILT and herpes simplex virus (HS) in CEK monolayer cultures.

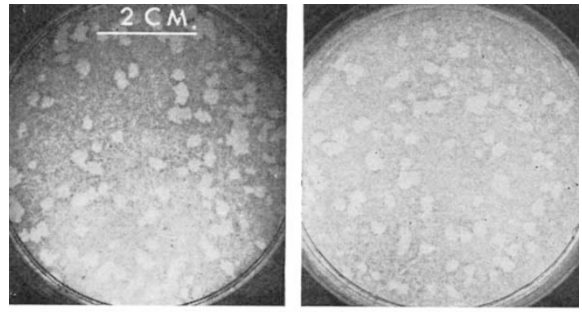

Fig. 1. Plaques produced in CEK monolayers by (*a*) ILT and (*b*) HS

A plaque system previously described for ILT[7] was used for the assay of both viruses. Modifications to this method included the use of a growth medium described elsewhere[8] for chicken fibroblasts and 6-cm plastic Petri dishes specially coated for use in cell culture (Falcon Plastics, Baltimore Biological Coy., Md., U.S.A.). The cultures were stained with neutral red diluted 1:10,000 in phosphate buffered saline (PBS), 5 days after inoculation with ILT, and 7 days after inoculation with HS. Plaques were counted the day after the addition of stain. The morphology of plaques produced by both viruses was similar, but those due to ILT appeared sooner. Plaques produced by both types of virus are shown in Fig. 1.

In the growth experiments the *HFEM* strain of HS was used and was obtained as yolk-sac propagated material from Mr. I. Jack of the Royal Children's Hospital, Melbourne. It was passaged twice through CEK and gave a titre of $1{\cdot}57 \times 10^7$ plaque-forming units (P.F.U.) per ml. A lyophilized preparation of the Queensland strain of ILT was used, an egg-propagated strain of moderate virulence with a plaque titre of approximately 10^6 P.F.U. per ml.

Cell monolayers, washed with PBS, were inoculated with 1·0-ml. amounts of each virus and allowed to adsorb for 1 h at 37° C, in a humidified incubator gassed with 5 per cent CO_2. Unadsorbed virus was then removed by rinsing 3 times with PBS. Five ml. of a maintenance medium, which differed from the growth medium in that it contained only 2 per cent calf serum, was added. Concentrations of IUdR of 0, 10, 100, or 1,000 γ/ml. were included in the medium, the latter concentration being the threshold of toxicity for established monolayers of other cells[9]. All cultures were then returned to the incubator.

Whole cultures containing each drug-level were collected 24, 48 or 72 h after infection. Cells were removed from the culture dish with a rubber 'policeman' and, after pipetting several times, the cell clumps were treated for 1 min by a Mullard ultrasonic generator—a procedure found to break up cells effectively and to release more virus than could be obtained by freezing and thawing. Cultures containing no drug were treated in a similar fashion immediately after

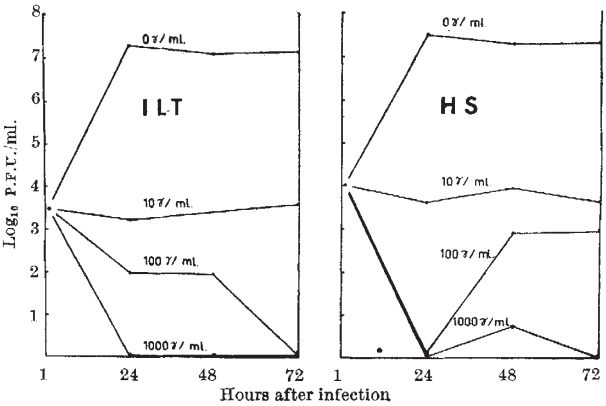

Fig. 2. The growth curves of ILT and HS in CEK monolayers in the presence of varying concentrations of IUdR

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adsorption and washing, to provide an estimate of the 'background' level of virus associated with cells during the eclipse phase of the growth cycle. All samples were snap frozen and stored at dry ice-box temperatures until assays could be carried out.

Results of one experiment are shown in Fig. 2. This indicates that considerable inhibition of both viruses occurred at all concentrations of the analogue studied. The recovery of HS at higher drug concentrations after treatment for 24 h may have been due to the survival of IUdR resistant mutants[9] of HS and their subsequent replication in later cycles of growth.

The inhibition of both viruses by IUdR, when grown under identical conditions, indicates that the nucleic acid of ILT is DNA, and provides further evidence for the inclusion of this virus within the herpes virus group.

Note added in proof. Since this letter was submitted for publication, evidence has come to hand that some halogenated derivatives of 2′-deoxyuridine, notably 5-bromo-2′-deoxyuridine, inhibit the multiplication of certain RNA viruses which require the participation of DNA at an early stage of multiplication (Bader, *Virology*, **22**, 462; 1964; Thormar, *ibid.*, **26**, 36; 1965). However, in conjunction with other reports of similarities between ILT and HS, a virus known to contain DNA, the parallel inhibition of both viruses by IUdR must be regarded as further evidence that the nucleic acid of ILT is DNA.

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SOIL SCIENCE

Measurement of Exchangeable Aluminium in Acid Soils

Many acid soils are known to contain exchangeable aluminium, but no satisfactory quantitative method for its determination has hitherto been available. This has proved to be a handicap in investigations of the degree of saturation with metal cations, suspected toxicity of adsorbed aluminium to crop plants as well as other soil properties influenced by exchangeable aluminium.

Neutral solutions of various salts will displace a definite amount of aluminium under standard experimental conditions. The quantity of aluminium released in this way may exceed the cation exchange capacity of the soil, so that some way must be found for distinguishing between the exchangeable and non-exchangeable components present in the extract. A technique for estimating this latter function is proposed here.

Exchangeable aluminium is displaced with difficulty by other cations, but it can be effectively removed by a long series of successive extractions. If it is assumed that the amount of non-exchangeable aluminium dissolved during each extraction in the series is constant, then the sum of the contributions from this source can be subtracted from the total extractable aluminium to give an estimate of exchangeable aluminium. For this approach to be successful, experimental conditions with respect to soil-to-solution ratio, temperature and period of contact must be kept constant during extraction.

In accordance with a Nernst type distribution, it may be expected that with each successive extraction, a steadily decreasing amount of exchangeable aluminium will be released together with a constant quantity of non-exchangeable aluminium. The Nernst curve should thus become linear if continued beyond the point at which all exchangeable aluminium has been removed.

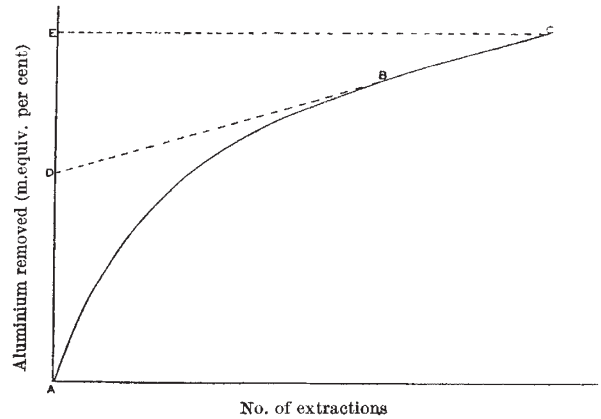

Fig. 1. Characteristic curve for the progressive extraction of aluminium from acid soils

In Fig. 1, the curve AB represents the progressive removal of both forms of aluminium and the linear part BC that of non-exchangeable aluminium only. The amount of the latter removed per extraction will determine the slope of BC which will also depend largely on the solubility of the solid phase aluminium compounds. When BC is extrapolated, AD will represent exchangeable and DE non-exchangeable aluminium.

The experimental procedure is as follows. Place 5 g air-dry soil in a tared 100-ml. centrifuge tube and add 50 ml. extracting solution adjusted to the field pH of the soil; shake for exactly 2 min in a reciprocating shaker. Centrifuge and decant the clear supernatant into a suitable container. Re-weigh the tube so as to obtain the weight of the occluded solution; add a further 50 ml. of extractant, shake, centrifuge and decant. This procedure is repeated 24 times, decanting into a separate container each time. Determine the aluminium present in each extract and plot the cumulative data as shown in Fig. 1.

Exchangeable aluminium measured by this technique appears to be a characteristic of the particular soil and is reasonably constant irrespective of the nature of the extractant as shown in Table 1.

Table 1. Exchangeable and Non-exchangeable Aluminium extracted by Different Salt Solutions

Solution	Aluminium removed (m.equiv. per cent)		
	Total aluminium	Non-exchangeable aluminium*	Exchangeable aluminium
0·2 N KCl	5·7	0·075	3·9
0·2 N NH_4Cl	6·0	0·079	4·0
0·2 N $CaCl_2$	5·4	0·074	3·6
0·2 N NH_4NO_3	6·0	0·087	3·8
N NaCl	6·1	0·090	3·8

* Per extraction.
Aluminium was determined by the method of Frink and Peech[1].

The sample used was from the A_1 horizon of a highly weathered acid ferrallitic soil with a pH value of 4·5 in water and 3·8 in N potassium chloride. It has a cation exchange capacity of 6·0 m.equiv. per cent with a base saturation of 20 per cent (excluding aluminium). The five extracting solutions were all adjusted to pH 4·0, which was regarded as the field pH value of the soil. Twenty-four separate aluminium determinations are required for each estimate of exchangeable aluminium, so that differences between the five extractants could be due to experimental error. Exchangeable hydrogen probably accounts for the difference between cation exchange capacity and total bases including exchangeable aluminium.

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