Boylen *et al.*[2] reported that L-glutamine gave complete protection against abnormalities in chick embryos caused by certain derivatives of TH. The results in Table 1 show that co-treatment with L-glutamine produced no similar effects on seedlings or excised roots of *Linum*.

The work reported here demonstrates that TH generally does not affect the growth of higher plants. Although it is possible that it causes some slight threshold effects, the concentrations of TH required for this are about two orders of magnitude higher than in the case of certain structurally related naturally occurring phthalides.

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E. V. Parups
I. Hoffman

Research Branch,
Canada Department of Agriculture,
Ottawa, Ontario.

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ENTOMOLOGY

Detection of Lipids in the Honeydew of an Aphid

Numerous analyses have been reported concerning the chemical composition of honeydew produced by many homopterous insects, especially members of the family Aphididae. Such compounds as fructose, glucose, sucrose and free amino-acids seem to be universally present in honeydew. Various other mono-, di-, and oligo-saccharides are occasionally found in honeydew. Proteins are apparently absent, although peptides have been reported. Miscellaneous compounds found in honeydew include several organic acids, inorganic ions, sugar alcohols and auxins, such as indolyl-3-acetic acid[1]. With one exception[2], no reports have been located making reference to the presence of lipid material in honeydew, although it is well known that certain lipids (for example, sterols) are of great physiological importance to insects.

Between 150 and 200 mg of honeydew were collected by placing glass plates (which had been sprayed with 'Merthiolate') for 24 h under colonies of *Myzus persicae* (Sulzer) feeding on Chinese cabbage, *Brassica campestris*. The honeydew was washed from the plates with tepid water and extracted from the aqueous solution with chloroform. After evaporation of the solvent under reduced pressure and purifying the crude extract[3], the amount of lipid material present in honeydew on a dry-weight basis was found to be 0·60 per cent. The purified extract was chromatographed on a silicic acid column[4] and the neutral lipid fraction purified by chromatography on a 'Florisil' column[5]. Tentative identification of the lipid classes was made by comparing the emergence of unknowns from the 'Florisil' column with known standards, and by co-chromatography of the 'Florisil' eluates with known compounds on thin-layers of silica-gel *G* in various solvent systems[6]. Free sterols and sterol esters were identified by the *L-B* reaction and by spraying the thin-layer plates with stannous chloride[6]. None of the thin-layer chromatography reported here was quantitated, although visual estimates of the relative amounts of the different classes of lipids were made. No work was carried out on the polar lipids eluted from the silicic acid column.

Using the foregoing methods, the major class of lipids in the honeydew samples was found to be the free fatty-acids. Gas-liquid chromatography of these free fatty-acids[2] revealed that palmitic acid accounted for 44 per cent of the total. Free sterols were readily detectable in honeydew, although sterol esters were absent. Triglycerides were present, but only in minor amounts. Mono- and diglycerides could not be identified with certainty. Hydrocarbons and pigments were abundant and squalene was present in trace amounts. Two major unidentified fractions and numerous minor ones were present in all samples. The major fractions had chromatographic properties similar to hydroxy fatty acids.

The origin of the lipids found in the honeydew is not known, but it is suspected that at least some of them come directly from the host plant. A distinct correlation existed between the physiological condition of the host and the honeydew lipids. Aphids feeding on a senescent plant produced honeydew rich in hydrocarbons and pigments, but containing little or no free sterols; the reverse was true for aphids feeding on young plants.

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Frank E. Strong

Department of Entomology,
University of California,
Davis, California.

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VIROLOGY

Further Observations on the Effect of 6-Aminonicotinamide on Chick Embryo Tissue Cultures infected with Vaccinia and other Viruses

I have recently described the inhibitory effect of 6-aminonicotinamide (6-AN) on the growth of vaccinia virus in the chick embryo fibroblast (CEF) host cell system[1]. When CEF cultures were treated with 6-AN at a dose of 5 μg/ml. for an interval of 8 h at 36° C, it was found that 6-AN rendered the cells refractory to infection with vaccinia virus. This effect increased as the time of exposure to 6-AN lengthened up to 8 or 10 h before virus infection. Evidence was also obtained to show that the inhibitory effect produced by 5 μg/ml. of 6-AN could be reversed by the simultaneous addition of 5–50 μg ml. of nicotinamide, but not if it was added at a later time. The drug did not seem to exert a direct lethal effect on the virus.

Details of techniques, including the propagation of vaccinia virus, preparation of CEF cultures and a simple fluid-overlay plaque assay technique employed throughout the experiment have been described previously[1]. Influenza *A* virus (strain 'MEL') and influenza *B* virus (strain '*GL*') were grown in the allantoic cavity of embryonated eggs. The strain of herpes simplex virus was one originally isolated in primary human amnion cell cultures derived from a recent case of fatal encephalitis and was propagated in HA-FL cells. Sindbis virus was grown in CEF cultures.

6-AN when diluted in Hanks's balanced salt solution (BSS) to yield a 5 μg/ml. concentration was introduced to CEF cultures grown in 19 × 25 mm Leighton tubes for periods of 6, 4, 3, 2 and 1 h before infection with vaccinia virus; and simultaneously with addition of virus; and 6, 4, 3, 2 and 1 h after addition of virus. 6-AN was also added to cultures 6 and 8 h after virus adsorption up to termination of the experiment. The cultures were washed three times with BSS before and after the addition of 6-AN

Table 1. Effect of Addition of 6-AN at Time Intervals, on Vaccinia Growth on CEF Cultures

Time before infection (h)					Infection (h) *	Time after infection (h)						Virus titre at 20 h P.F.U./ml.	% of control
−6	−4	−3	−2	−1	0 1·5	1	2	3	4	6	8		
M	M	M	M	M	Vaccinia	M	M	M	M	M	M	M	$1·3 \times 10^6$	100·0
6-AN	6-AN	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	$1·0 \times 10^4$	0·77
M	6-AN	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	$1·5 \times 10^4$	1·1
M	M	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	$2·0 \times 10^4$	1·5
M	M	M	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	$2·6 \times 10^4$	2·0
M	M	M	M	6-AN	Vaccinia	M	M	M	M	M	M	M	$1·0 \times 10^4$	7·7
M	M	M	M	M	Vaccinia + 6-AN	M	M	M	M	M	M	M	$2·0 \times 10^5$	15·4
M	M	M	M	M	Vaccinia	6-AN	M	M	M	M	M	M	$2·0 \times 10^5$	15·4
M	M	M	M	M	Vaccinia	6-AN	6-AN	M	M	M	M	M	$2·9 \times 10^4$	2·2
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	M	M	M	M	$2·5 \times 10^4$	1·9
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	6-AN	M	M	M	$1·6 \times 10^4$	1·2
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	6-AN	6-AN	M	M	$1·5 \times 10^4$	1·2
M	M	M	M	M	Vaccinia	M	M	M	M	6-AN	6-AN	6-AN	$9·1 \times 10^5$	70·0
M	M	M	M	M	Vaccinia	M	M	M	M	M	6-AN	6-AN	$1·1 \times 10^6$	84·6

* Immediately after exposure to virus for 1·5 h with a virus input of 2-10 P.F.U./cell at 36° C, the cell cultures were washed and re-fed with the corresponding medium. Samples were taken from each group and assayed for the amount of virus adsorbed. Results showed that $1·0–1·2 \times 10^5$ P.F.U./ml. of virus had been adsorbed per culture.
M = Hanks's BSS.

at each time interval as well as after virus adsorption. At the end of the experiments, the corresponding cultures were collected. Infectivity was determined by plaque-forming units (P.F.U.) on CEF cultures set up in screw-capped tubes, using a fluid-overlay plaque assay technique. Table 1 demonstrates that the 6-AN does not affect virus adsorption, or prevent the release of virus. It is interesting to note that 6-AN displays only a slight difference in the extent of inhibition on growth of vaccinia virus, in the CEF cultures, when the latter were exposed to 6-AN for the corresponding period of time before and after a period of 1·5 h of virus adsorption. 6-AN appears to have no marked demonstrable effect when added 6–8 h after virus adsorption.

Single-cycle growth curve experiments were performed in 6-AN-treated (5 μg/ml. in BSS for 8 h at 36° C) and untreated CEF cultures. The cultures were infected with stock vaccinia virus with an input multiplicity of 2–10 P.F.U./cell. After 2 h adsorption, the cultures were washed three times and re-fed with BSS. At time intervals, two cultures were removed from the incubator and the content of intracellular virus was measured by P.F.U. as here. The data, as shown in Fig. 1, reveal that the vaccinia virus multiplies at different rates in the two types of cultures. It would seem that in treated cultures the eclipse phase was not as marked as that observed in untreated control cultures. This result suggests that only a small proportion of the adsorbed virus may participate in the eclipse period and so replicate in the treated cultures. By using fluorescence microscopy and acridine orange staining techniques[2], it was also demonstrated that the development of vaccinia virus intracytoplasmic inclusions was markedly suppressed. The mode of action of 6-AN on the growth of vaccinia virus in CEF cultures is obscure and awaits further investigation.

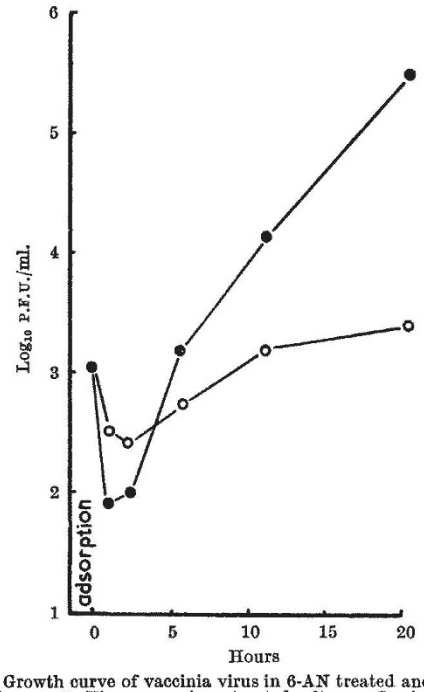

Fig. 1. Growth curve of vaccinia virus in 6-AN treated and untreated CEF cultures. ●, Virus grown in untreated cultures; ○, virus grown in treated cultures

Additional investigations on the effect of 6-AN on other viruses in CEF cultures indicated that 6-AN also exerted a delaying effect on the growth of influenza *B* virus but not the influenza *A* virus. Cultures were observed microscopically for cytopathic effect and assayed by standard haemagglutination procedures. Similarly, no evidence of significant inhibitory effect was revealed in the case of herpes simplex and Sindbis viruses.

Spencer H. S. Lee

Department of Bacteriology,
Dalhousie University,
Halifax, Nova Scotia.

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Relationship between Guanidine-dependence and Neurovirulence of Poliovirus

Guanidine-resistance[1] and guanidine-dependence can be induced *in vitro* in poliovirus[2,3]. We have recently demonstrated that both characters can be easily conferred and withdrawn in *in vitro* experiments[4,5]. Moreover, we have demonstrated that guanidine-dependent viruses are not neurovirulent for monkeys[6]. In the present report we show that neurovirulence (of the type producing paralysis) for monkeys and guanidine-dependence *in vitro* are two strictly related viral properties.

Indeed, although guanidine-dependent poliovirus is not neurovirulent for monkeys, it reacquires this character when guanidine-dependence is reversed. The techniques used are described in ref. 7. A strain of virulent Mahoney poliovirus isolated from the spinal cord of a monkey was cloned twice and was made guanidine-dependent *in vitro* by sub-culturing it in the presence of increasing concentrations of guanidine. When this strain had acquired a high degree of guanidine-dependence, it was sub-cultured in decreasing concentrations of guanidine and finally in the absence of guanidine. The original viral strain and the two strains obtained with the foregoing procedures were injected intramuscularly into cercopithecus velvet monkeys at a dosage of 10^8 cytopathic units (C.P.U.) per animal. The infected monkeys were observed for signs of paralysis up to 100 days.