

Table 1

Wt. of mother	Wt. of litter	Litter wt. $\times 100$		
		Actual	Maternal wt. Predicted	Deviation
2,718 g	680 g	25	14.2	10.8

Although the large size of the new-born hyrax has often been commented on^{1,6}, there are few records of birth weights. Leitch *et al.*⁷ compared pre-mating weights of young adult females with the total weight of the newly born in 114 species of mammals and found that the larger the species the smaller (relatively) the total weight of offspring. They produced the following equation for the prediction of the weight of the young from the weight of a mother: $N = 0.5408 M^{0.8323}$, where N is the neonatal weight and M the maternal weight. Actual and predicted litter weights are compared with the maternal weight (average for the 6 months before mating) for the *P. johnstoni* female under consideration in Table 1. It will be seen that the actual litter weight is 10.8 per cent (of the maternal weight) greater than predicted, using the foregoing equation. The only wild mammals in the survey of Leitch *et al.* giving a greater positive deviation from the predicted value were two voles (*Microtus oeconomus stimpingii* and *M. arvalis*). The long gestation period (longer than for any other mammal of its size) and high neonatal weight of the hyrax support the palaeontological evidence that the ancestors of the modern hyrax were much larger animals⁸.

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Absence of Growth Effects of Thalidomide on Higher Plants

THE teratogenic effects of thalidomide (TH), α -N-phthalimidoglutarimide, are well recognized and have been recorded for a number of animals². The critical period has been found to correspond to the time of morphogenesis^{2,3}. These drastic effects in animals raised the question of possible effects on plants. Some positive evidence for effects on lower plants was indicated by the work of Boney¹, who noted that high concentrations of TH not only inhibited growth but also induced abnormalities in sporelings of a marine red alga, *Callithamnion tetricum* Ag. Giacomello *et al.*⁴ tested the activity of high concentrations of TH in embryonic vegetal tissues of the common onion, *Allium cepa* L., and reported alterations in cell mitosis as well as the occurrence of chromosomal aberrations.

A number of naturally occurring phthalide substances bearing structural resemblances to TH are known to act as growth inhibitors of plants⁵. These substances, isolated from *Levisticum officinale* Koch, have inhibited germination and growth of *Lepidium*, rhizoid growth of *Marchantia* and germination of pollen of *Impatiens*. The purpose of the present work was to investigate possible growth-inhibitory effects of TH on a number of higher plants. In order to ascertain the true biological action of TH, the massive doses used by the foregoing workers were avoided.

Table 1. EFFECT OF THALIDOMIDE (TH) AND L-GLUTAMINE ON ELONGATION OF ROOTS OR EXCISED ROOTS OF *Linum*

	Controls (mm)	L-Glutamine 6×10^{-3} M (mm)	TH 6×10^{-3} M (mm)	L-Glutamine + TH (mm)	L.S.D. 1%
Roots of seedlings	43.9	43.1	15.5	14.3	2.94
Excised roots	12.3	12.7	6.6	6.2	1.46

Table 2. EFFECT OF THALIDOMIDE (TH) AND INDOLYL-3-ACETIC ACID (IAA) ON THE ELONGATION OF OAT COLEOPTILE (mm)

IAA μ g/ml.	0	0.04	0.04	0.04	0	0	0
TH μ g/ml.	0	0	0.50	2.50	0.55	2.58	12.90
Length of coleoptile	5.93	6.87	6.81	6.99	5.96	6.09	6.03
L.S.D. 1% 0.51							

Tomato, *Lycopersicon esculentum* Mill., barley, *Hordeum vulgare* L., and soybean, *Glycine max* Merr., seeds were germinated in pots containing quartz sand. The seedlings were thinned and triplicate pots were treated with Hoagland's solution containing TH in the following concentrations: (1) 0; (2) 1×10^{-5} M; (3) 1×10^{-4} M; and (4) 1×10^{-3} M. After daily treatments for 15 days the growth effects were observed and compared.

There was no noticeable response to the treatments by soybeans and very little response by barley except for a slight yellowing of leaf tips in the highest treatments. The most concentrated treatments also produced a slight effect in tomato (see Fig. 1); the plants were generally smaller, the leaves were somewhat mottled and leaf margins were curled.

Because of the known effect of TH on animal embryonic tissue, its action was investigated on tomato flowers in all stages of development and on the fruit. Spray-treatment was continued for three consecutive days with a 1:1 water:acetone solution of TH and each plant received 100 mg of TH per day. Regardless of the stage of flower development when treated, TH did not affect fruit development. In addition, the seeds from TH-treated tomato plants germinated normally.

Seeds of *Lepidium* and *Linum* were placed on filter papers in Petri dishes, moistened with several concentrations of aqueous TH and set aside to germinate. For both species the seeds germinated in the same length of time except in the case of the highest concentration of TH (6×10^{-3} M). For the latter it was found that germination was delayed for a period of one day, but the percentage of seeds that germinated was unaffected.

The effect of TH on root growth of flax was tested on seedlings which had previously been germinated in water and afterwards treated with aqueous solutions of TH. After 2 days the following root-lengths (average of 4 replicates) were found: control, 40.4 mm; 1×10^{-4} M, 39.9 mm; 1×10^{-3} M, 38.6 mm; 6×10^{-3} M, 19.2 mm. Since the least significant difference (L.S.D.) at the 1 per cent level was 4.3, the 6×10^{-3} M concentration caused a significant retardation of root elongation.

The effects of TH in inhibiting the elongation of oat coleoptile were checked by co-treatment with indolyl-3-acetic acid which is known to cause such elongation. Even when the relative concentration of TH:indolyl-3-acetic acid was as high as 40:1 such inhibition was not observed (Table 2).



Fig. 1. Effect of thalidomide (TH) on growth of tomato seedlings. From left to right: control, 1×10^{-5} M, 1×10^{-4} M and 1×10^{-3} M of TH

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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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 acid¹. With one exception², 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 extract³, 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 column⁴ and the neutral lipid fraction purified by chromatography on a 'Florisil' column⁵. 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 systems⁶. Free sterols and sterol esters were identified by the *L-B* reaction and by spraying the thin-layer plates with stannous chloride⁶. 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-acids² 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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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 system¹. 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 previously¹. 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