Table 1. The effects of L-phenylalanine injection on in vitro protein synthesis. Animals were killed 1 hour after an intraperitoneal injection of either 0.85 percent NaCl or L-phenylalanine solution. The incorporation of L-[^{3}H]lysine into protein was determined. Tissues from ten animals of each group were pooled to provide the cellular fractions; dpm, disintegrations per minute.

Source of microsomes	Source of *p*H 5 fraction	7-Day-old rats		4-Week-old rats	
		Protein specific activity (dpm/mg of protein)	Percent of control	Protein specific activity (dpm/mg of protein)	Percent of control
		Cerebral cortex			
Control	Control	31501		14734	
Control	Phenylalanine	33387	106	18226	124
Phenylalanine	Control	19703	62	15649	106
Phenylalanine	Phenylalanine	19738	63	16732	114
		Liver			
Control	Control	25253		18013	
Control	Phenylalanine	26600	105	16557	92
Phenylalanine	Control	26382	104	17723	98
Phenylalanine	Phenylalanine	27891	110	18783	104

Table 2. The effects of a single injection of L-phenylalanine on tissue levels of phenylalanine and tryptophan in the rat. Injections were given according to the procedure described in the legend to Table 1. Each value represents the mean value from five rats. Values are expressed in milligrams per 100 grams of tissue.

Tissue	Group	Phenylalanine		Tryptophan	
		7-Day-old rats	4-Week-old rats	7-Day-old rats	4-Week-old rats
Brain	Control	0.90	1.38	0.55	0.32
Brain	Phenylalanine	14.96*	7.50*	.31†	.16*
Liver	Control	2.00	2.24	.81	.66
Liver	Phenylalanine	33.08*	18.77*	.90	.70

* Difference from control significant, $P < .001$. † Difference from control significant, $P < .01$.

sisted almost entirely of large aggregates, with small oligosome peaks. Injection of L-phenylalanine caused pronounced disaggregation of polyribosomes (Fig. 1). Liver polyribosomes were not affected by injection of phenylalanine. In 4-week-old rats, however, phenylalanine injection did not cause disaggregation of brain polyribosomes (Fig. 1). Large polyribosomes are more active in protein synthesis than are monosomes (*12*); thus the finding of polyribosome disaggregation is consistent with impaired protein synthesis.

Phenylalanine and tryptophan levels were determined 1 hour after injection of phenylalanine. A decreased level of tryptophan was found in brain, whereas tryptophan levels in liver were not significantly altered by phenylalanine injection (Table 2). Hepatic levels of phenylalanine were found to be greater than those of brain, after the injection of phenylalanine, but no impairment of in vitro protein synthesis nor disaggregation of liver polysomes was found. This indicates that high levels of phenylalanine per se are not responsible for the inhibition of brain protein synthesis in phenylalanine-injected rats. Brain tryptophan levels are lowered by phenylalanine injection, while hepatic levels of tryptophan are unchanged, which suggests a mechanism of polysome disaggregation involving tryptophan depletion, such as has been reported to take place in the livers of fasting mice (*8*). Tryptophan depletion also takes place in the brains of 4-week-old rats following phenylalanine injection, but no polysome disaggregation follows (Fig. 1). This may indicate that free polysomes, which predominate in 7-day-old rat brain (*13*), are more easily disaggregated than are membrane-bound polysomes, which predominate in 4-week-old rat brains. A recent report of Sarma *et al.* (*14*) indicates that free, but not bound, polysomes of liver are disaggregated by actinomycin D, providing a basis for this hypothesis.

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Hydroxyurea: Suppression of Two-Stage Carcinogenesis in Mouse Skin

Abstract. *Hydroxyurea, a selective cytotoxic agent for cells in DNA synthesis, injected intraperitoneally at 24 and 48 hours after the first painting with 1 percent croton oil, significantly reduced the tumor yield in the two-stage chemical carcinogenesis in mouse skin. A comparable group of mice receiving hydroxyurea only once at 24 hours had a tumor induction similar to that in controls.*

Chemical carcinogenesis in mouse skin can be a two-stage process: initiation and promotion. A subthreshold dose of a carcinogenic hydrocarbon can initiate the tumor which then develops upon repeated application of a promoter (*1, 2*). Croton oil has been used extensively as a promoting agent for the study of the mechanism of skin carcinogenesis (*3*). After one application of croton oil to mouse skin, the rate of uptake of tritiated thymidine in the epidermal basal cells is elevated with a maximum at 22 to 24

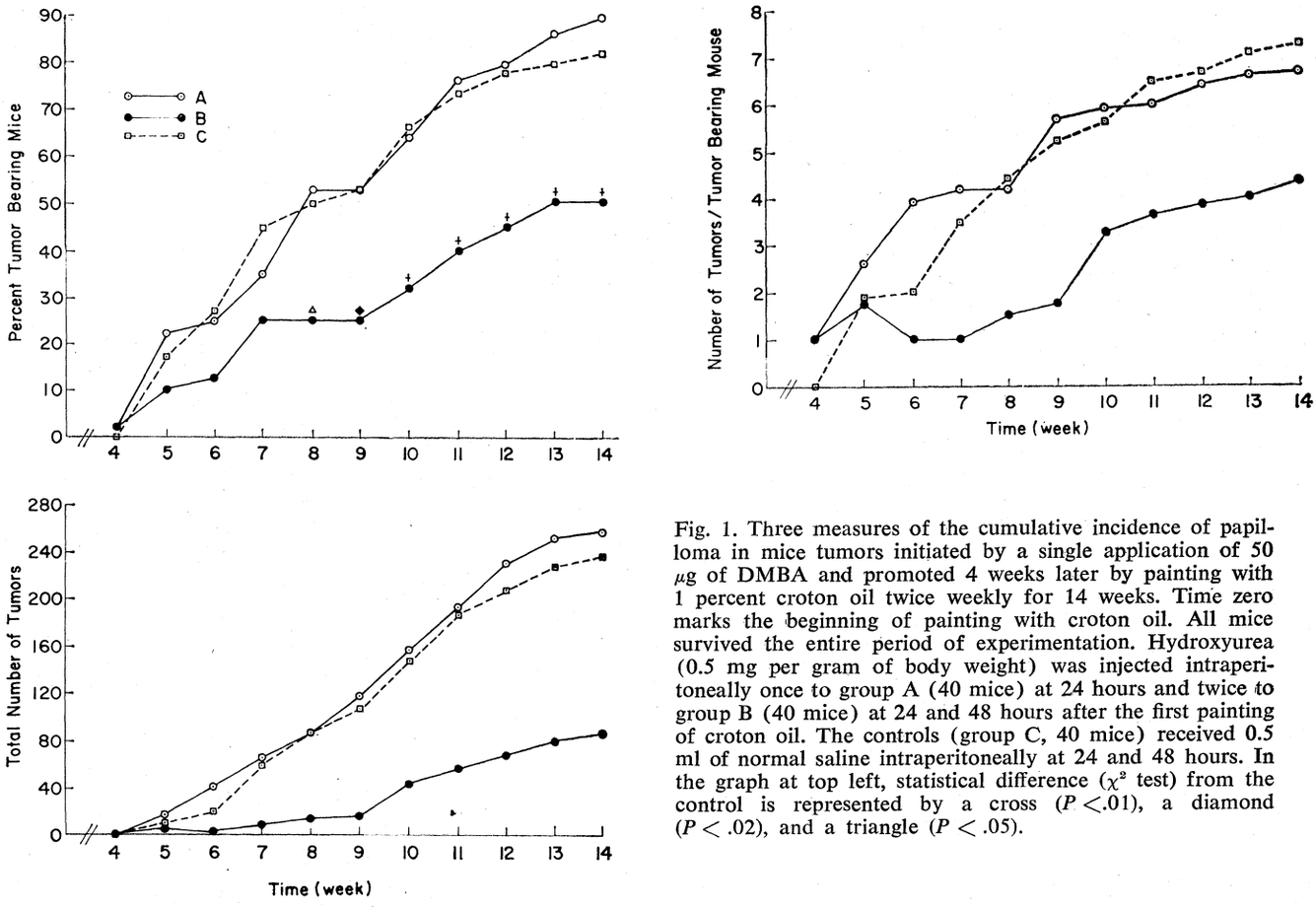

Fig. 1. Three measures of the cumulative incidence of papilloma in mice tumors initiated by a single application of 50 μg of DMBA and promoted 4 weeks later by painting with 1 percent croton oil twice weekly for 14 weeks. Time zero marks the beginning of painting with croton oil. All mice survived the entire period of experimentation. Hydroxyurea (0.5 mg per gram of body weight) was injected intraperitoneally once to group A (40 mice) at 24 hours and twice to group B (40 mice) at 24 and 48 hours after the first painting of croton oil. The controls (group C, 40 mice) received 0.5 ml of normal saline intraperitoneally at 24 and 48 hours. In the graph at top left, statistical difference (χ^2 test) from the control is represented by a cross ($P < .01$), a diamond ($P < .02$), and a triangle ($P < .05$).

hours (*4*). Though the mechanism of action of croton oil is obscure (*5*), its ability to induce cell proliferation and sustained hyperplasia may constitute one of the biological events leading to tumor development (*6*).

Hydroxyurea inhibits DNA synthesis in mammalian cells in vivo (*7*) and in vitro (*8*) and is selectively lethal to cells synthesizing DNA in vivo (*9, 10*) and in vitro (*8*). We now report that promotion of mouse skin tumor can be suppressed by the use of hydroxyurea.

To the shaved backs of 120 female Swiss mice (6 to 7 weeks old) in the second telogenic hair cycle we applied 50 μg of 9,10-dimethyl-1,2-benzanthracene (DMBA) in 50 μl of acetone to initiate tumor formation. Four weeks later the backs of the mice were painted once with 1 percent croton oil in acetone, and the mice were divided at random into three groups of 40 animals each. Hydroxyurea (0.5 mg per gram of body weight) was given intraperitoneally to group A at 24 hours and to group B at 24 hours and again at 48 hours. Animals in group C were given an intraperitoneal injection of 0.5 ml of normal saline at 24 and 48 hours to serve as controls. Four days after the first application of croton oil, all three groups of mice were painted twice weekly with 1 percent croton oil for 14 weeks. The papilloma yield was checked weekly. Papillomas larger than 1 mm in diameter were recorded.

Hydroxyurea given at 24 and 48 hours after the first painting of croton oil (group B) significantly reduced the tumor yield as compared to the controls (group C) (Fig. 1). However, mice receiving an injection of hydroxyurea only at 24 hours after the first application of croton oil (group A) had a tumor yield that did not differ significantly from that of the controls.

A single subthreshold application of a hydrocarbon carcinogen on mouse skin leads to the production of potential tumor-developing cells. These transformed cells may remain dormant for more than a year, and promotion will cause expression of the tumor-developing potential (*5*).

Although croton oil induces increased rates of tritiated thymidine uptake in mouse epidermis (*4*), the potential tumor cells apparently begin to synthesize DNA more than 24 hours after being stimulated by croton oil. Thus, hydroxyurea given 24 hours later was unable to curtail the tumor yield in group A. This finding agrees with the observation of Gelfant (*11*) who reported that a specific stimulus would activate two subpopulations of cells in mouse epidermis: G_1 cells which undergo DNA synthesis almost immediately and G_2 cells which have to traverse certain cellular events before duplicating DNA. Hydroxyurea does not interfere with protein and RNA syntheses in vivo, though it blocks mouse epidermal cells from going into DNA synthesis (*12, 13*). Thus, administration of hydroxyurea at 24 hours in group B may have had a synchronizing effect on the late DNA replicating potential tumor cells, subjecting them to the cytotoxic action of hydroxyurea given at 48 hours.

Since hydroxyurea is rapidly metabolized and excreted (*9, 14*) and apparently causes no long-lasting injury to the tissues (*9, 10, 13, 15*), it can be used to kill a selected population of cells in DNA synthesis.

Evidence of two-stage mechanism of carcinogenesis has also been found for liver, lung, bladder, gastrointestinal tract, reticuloendothelial system, subcutaneous tissues, skeletal muscle, thy-

roid, and mammary gland (*2*, *16*). Our report could serve as a model for the use of hydroxyurea in experimental carcinogenesis.

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Dodder Weevils in Simultaneous Association with Parasitic Plants and Their Hosts

Abstract. *The weevil* Smicronyx quadrifer (*broad sense*) *is restricted in host preference to the parasitic dodders* (Cuscuta *species*) *during its adult life, but its larvae consistently move from dodder stems into living stems of certain hosts of dodders, where they feed, grow to maturity, and undergo metamorphosis. Such associations, involving three interacting organisms, are very unusual among phytophagous insects.*

Dodders (*Cuscuta* spp.) are vinelike, yellow or orange parasitic plants that grow attached to a wide variety of other plants in many parts of the world (*1*). In turn, they have some permanent insect associates (or parasites), including aphids (*2*), a small fly (*3*), a moth (*3*), and weevils of the genus *Smicronyx* (*4*). The weevils have been found developing in dodder stem galls and seed capsules in North and South America, Europe, Asia, and North Africa (*4*), and a few Old World species are associated with parasitic plants of the genera *Orobanche* (*5*) and *Striga* (*6*). In North America, larvae of several species of *Smicronyx* feed in the seeds of certain Compositae, such as *Ambrosia* spp. and *Helianthus* spp., and a few unusual records indicate that stems of Compositae have been attacked, when infested with dodder, by larvae of *Smicronyx sculpticollis* Casey, a species associated with dodder (*4*). I now report a very unusual host relationship in which *Smicronyx quadrifer* Casey (broad sense) (*7*) is consistently associated with dodder (*Cuscuta gronovii*, *C.* sp., probably *pentagona*) (*8*) and certain hosts of the dodder. This unusual insect-plant association involves three, rather than the usual two, interacting organisms, and it may be significant to other studies involving dodders, their hosts, and other organisms (see *2*, *9*).

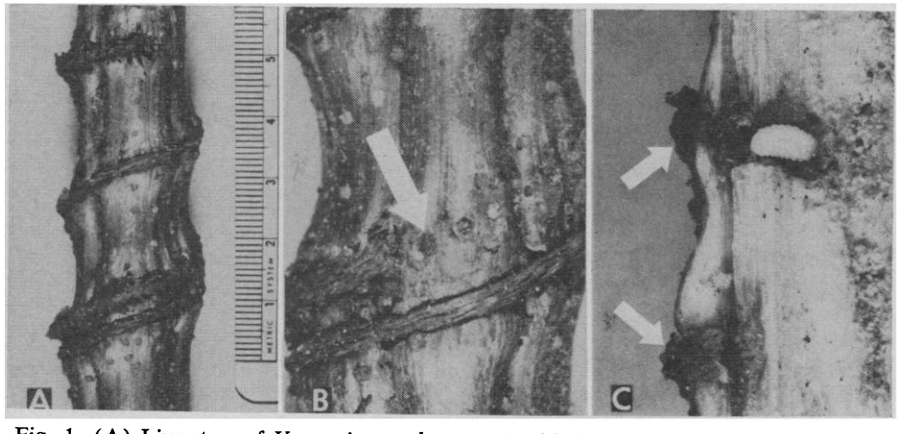

Fig. 1. (A) Live stem of *Vernonia noveboracensis* with dry remains of dodder attached; scale is marked in centimeters. (B) Enlarged view of the stem with part of the dodder removed, showing position of larval entry hole (now filled with excrement), made through dodder attachment (arrow). (C) Longitudinal section of stem, showing partly grown *Smicronyx* larva burrowing inward from tunnel under dodder (arrows indicate dodder).

During the summer of 1968, I investigated the dodder-infested stems (Fig. 1A) of seven perennial species of Compositae, identified as *Vernonia noveboracensis, Eupatorium rugosum, E. perfoliatum, E. fistulosum, Aster puniceus, Artemisia vulgaris,* and *Solidago* sp. (*8*), growing near the Chesapeake and Ohio Canal in Washington, D.C. and Maryland. Many of these stems contained weevil larvae identifiable as *Smicronyx* sp., burrowing in and near the areas in which the dodder was attached. The pattern of excrement-filled larval tunnels indicated that the larvae had mined out short sections of dodder stem, then burrowed through the sucker-like haustoria into the stems of the dodder hosts (Fig. 1B). They usually had tunneled just beneath the dodder before they chewed their way inward (Fig. 1C) and formed pupal cells in the cortex or in the pith of the hosts. Dry stems remaining from the previous summer showed the same pattern of tunnels and yielded the remains of a few adult *Smicronyx quadrifer*. Dodder-infested plants representing seven families other than Compositae were growing in the same areas, but I found no *Smicronyx* larvae in their stems.

In the succeeding fall and winter approximately 170 adult *S. quadrifer* and 1 *S. sculpticollis* were treated from 110 stems (15 to 22 cm long) representing all seven plant species in which larvae had been found. Concurrent rearings of larvae from dodder stems and fruit stripped from the composite stems or from plants growing in the same areas produced 72 adults representing five species of *Smicronyx*, including *S. sculpticollis*, but no *S. quadrifer*. Thus, the larvae of the latter species cannot, or normally do not, reach maturity in the tissues of dodder alone, although that