lated virus-transformed brain cells (that is, of virus-transformed astrocytes), and not by neoplastic transformation of subcutaneous cell types by infectious SV40 or polyoma virus associated with the cell inocula. Two additional kinds of evidence support this conclusion. Neither SV40 nor polyoma virus have produced tumors in newborn hamsters within 1 to 4 weeks; and we were unable to recover infectious SV40 from the SV40-transformed cells, even after growing the cells in direct contact with monolayers of primary cultures of African green-monkey kidney cells.

Our observations, therefore, demonstrate that cultured hamster astrocytes are susceptible to neoplastic transformation by both SV40 and polyoma virus. This susceptibility in vitro is in striking contrast to the apparent insusceptibility of astrocytes from newborn hamsters to neoplastic alteration induced by these viruses after intracerebral inoculation. Thus, after intracerebral inoculation into newborn hamsters, SV40 induces ependymomas (*7, 8*) and polyoma virus induces leptomeningeal sarcomas (*9*), but neither virus induces astrocytomas. The factors responsible for the different susceptibility of hamster astrocytes in vitro and in vivo remain undetermined. They may be related to the rapid rate of multiplication observed among astrocytes from newborn hamsters after explantation under the in vitro conditions of this study (*10*). Todaro and Green (*11*) have demonstrated that susceptibility to SV40-induced transformation in a rat cell line requires that the cells undergo at least one mitotic division after SV40 infection; mitotic figures have not been observed among astrocytes *in situ* in brains from newborn hamsters (*10*).

Both the tumor virus used to induce transformation and the developmental age of the cultured astrocytes employed for the transformation affected the degree of histological differentiation that was observed in the astrocytomas that resulted. The SV40-induced fetal astrocytomas were less well differentiated than SV40-induced newborn astrocytomas; and both groups of astrocytomas were less well differentiated than polyoma-induced fetal astrocytomas. However, these differences in histological differentiation were not associated with corresponding differences in the "malignancy" (that is, rate of growth, invasivity, and tendency to metastasize) of the astrocytomas. Thus, although histologically better differentiated, the polyoma-induced astrocytomas were at least as malignant as the histologically less well differentiated SV40-induced astrocytomas.

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12. Supported in part by NIMH career teacher training grant No. MH10677 and in part by NIH grant No. NB06610. I thank Dr. Alfred Pope for helpful discussions and Miss Katherine Rosasco for technical assistance.

15 February 1968

Increases in the Levels of Cytokinins in Bleeding Sap of Vitis vinifera L. after CCC Treatment

Abstract. *Paper-chromatogramed extracts of bleeding sap from grapevines treated with (2-chloroethyl)trimethylammonium chloride (CCC) contained higher levels of substances with cytokinin activity than did untreated plants. With the chromatographic solvent used, CCC was unlikely to interfere with the expression of the cytokinin response.*

Growth retardation by (2-chloroethyl)trimethylammonium chloride (CCC, Cycocel) is normally explained in terms of its effect on the biosynthesis of endogenous gibberellins (*1*). However, the stimulation by this substance of fruit set in *Vitis vinifera* (*2*) is not so readily interpreted on this basis, and it is evident that growth retardants exert other effects in addition to influencing gibberellin biosynthesis (*3*).

A previous report (*4*) described the morphological and cytological effects of CCC on roots of *Vitis vinifera,* and the prevention of these symptoms by gibberellic acid. Because CCC had such a marked effect on the size of the root tips (*4*), it seemed entirely possible that the levels of cytokinins in the bleeding sap (*5*) might be influenced by the treatment. This report describes results of cytokinin assays of bleeding sap from grapevines treated with CCC.

One-year-old plants of *Vitis vinifera* cv. Zante Currant (syn. Black Corinth), grown in a perlite-sand mixture in a greenhouse, were cut back to a single bud near the base of each plant, and when the new shoots from these buds reached a length of 30 cm, the plants were divided into two groups of 12 each. One group was treated three times weekly during April 1967 with 200 ml of a solution of CCC (1000 mg/liter), applied to the growing medium for 3 weeks, during which time the plants received Hoagland's solution once each week. At the termination of treatment, control shoots were 150 cm long, and treated shoots 100 cm. Sap was collected, chromatogramed, and assayed for cytokinin activity with soybean callus, as described in a previous report (*5*).

Figure 1 shows one series of assays from sap obtained between the 32nd and 56th hours after the commencement of collection; 130 ml of sap was collected from control plants and 152 ml from treated plants. In both cases

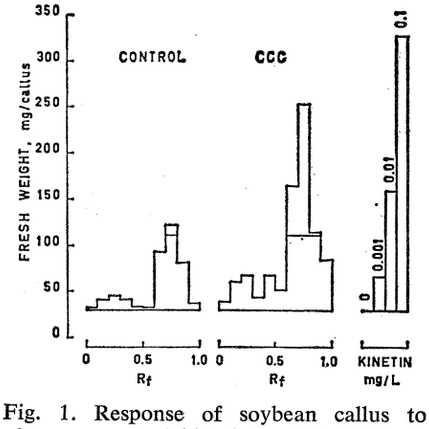

Fig. 1. Response of soybean callus to chromatogramed bleeding sap from Zante Currant vines. (Left) Sap from control plants. (Right) Sap from plants treated with CCC. The chromatographic solvent was a mixture of *n*-butanol, acetic acid, and water (4:1:1). Each assay was the mean of three replicate culture flasks. Calluses were grown at 27°C for 4 weeks. Statistically significant responses ($P = .05$) are shown above the upper horizontal line.

the peak of activity occurred between R_F 0.6 and 0.9, but there was considerably more activity in the sap from treated plants. The difference between treatments was highly significant. In terms of kinetin equivalents, sap from untreated plants contained 3 μg of kinetin per liter; from treated plants, 30 μg of kinetin per liter. Similar results were obtained with earlier collections of sap from the same plants.

The experiments were repeated with "Cabernet Sauvignon" vines (20 plants per treatment) grown in nutrient culture solutions containing CCC. Again, cytokinin activity at R_F 0.6 to 0.9 was significantly higher ($P = 0.01$) in the sap from treated plants than in that from controls. In this case up to 20-fold differences in activity at R_F 0.6 to 0.9 were recorded.

It is considered that CCC in the sap did not affect the response of the soybean callus to cytokinins in the active fractions. In the solvent system used, CCC migrated to R_F 0.22 to 0.37, as revealed by Dragendorff's reagent (*6*), that is, well behind the active fractions in the sap extracts. Furthermore, CCC at levels up to 500 mg/liter had no significant effect on the growth of callus exposed to 0.5 mg of kinetin per liter.

Further work is needed to establish whether this effect of CCC is directly related to cytokinin synthesis by the root, or whether it is a reflection of greater net cytokinin synthesis by the larger root meristem. The findings might have implications in relation to the known effects of CCC and cytokinins on fruit set in grapes.

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20 February 1968

Snake Food Preference: Innate Intraspecific Geographic Variation

Abstract. *Compared to Florida individuals, a smaller percentage of Massachusetts* Thamnophis sirtalis sirtalis *will accept fish. Both experienced adults and isolated newborn snakes respond similarly; the behavior is innate and correlated with habitat differences between the two localities. Although food preference in* Natrix sipedon *is also innate, geographic variation is not as clearcut.*

A particular snake's preference in food is the result of genetic determination and subsequent environmental modification. Although many isolated studies of the food habits of individual snake species exist, these have mostly been made on the basis of stomach contents in preserved specimens (*1*), or on the food preferences of animals from one locality (*2*). My study, part of an extensive survey of the feeding behavior of American Colubridae (*3*), concerns differences in food preferences between northern and southern *Thamnophis sirtalis sirtalis* and *Natrix sipedon*. Both species occur sympatrically over a wide geographical range (*4*) and were obtained for observation from similar habitats, the swampy margins of streams and periodic marshes. This difference in food preference is present both in adults and inexperienced snakes, and is apparently innate. The fact that this innate preference shows geographic variation is significant and has not previously been demonstrated in vertebrates..

Adult *Thamnophis sirtalis* and *Natrix sipedon*, obtained from the localities given in Table 1, were tested with earthworms, pieces of smelt (*Osmerus mordax*), *Rana pipiens* (tadpoles and adults), *Anolis carolinensis*, miscellaneous small snakes, and newborn mice. Litters subsequently obtained from these snakes were separated from the adults and also tested. After capture or birth the snakes were kept in 20-gallon aquaria lined with paper towels and containing one water dish. The adults were maintained in groups of six, and one litter of offspring was kept in each cage. All animals were without food for 1 to 2 weeks before testing commenced. Food was only brought into the room immediately before feeding. Groups were then offered one food item at a time. If this was not accepted within 5 minutes, another item was substituted. The order of presentation was random. Only if the snakes fed regularly (that is, not less than nine out of ten trials) on an item when tested at weekly intervals (ten repetitions) was this considered a positive response. The juveniles accepting fish have survived for over 2 years in the laboratory.

No snakes accepted lizards or snakes. Five adult *Thamnophis sirtalis* (20 percent) from Massachusetts accepted newborn mice, but snakes from other localities did not do so. As Table 1 shows for both *Natrix sipedon* and *Thamnophis sirtalis* from Florida there was no difference between the responses of experienced adults, captured in the wild, and their newborn offspring, reared in captivity and in isolation from their parents, in preference for fish (χ^2 for *Natrix* adults compared with young = 0.019; $.8 < P < .9$. χ^2 for *Thamnophis* adults compared with young = 0.045; $.8 < P < .9$). Thus the innate choice of newborn snakes parallels the choice of experienced adults.

In Florida these two species differ in their preference for fish (adults $\chi_c^2 = 16.7$; $P < .001$; juveniles $\chi_c^2 = 49.9$; $P < .001$). Except for *Natrix* from Potomac, Maryland, all individuals of both species accepted *Rana pipiens*.

The northern individuals of *Thamnophis sirtalis* show a marked trend away from fish preference (juveniles $\chi_c^2 = 31.2$; $P < .001$; adults $\chi_c^2 = 11.7$; $P < .001$). In *Natrix sipedon*

Table 1. Geographic variation in food preference in *Thamnophis sirtalis* and *Natrix sipedon*. A, adult; NB, newborn.

Locality	Snakes (No.)		Accepting test food (No.)					
			Worms		Fish		Frogs or tadpoles	
	A	NB	A	NB	A	NB	A	NB
			Thamnophis sirtalis					
Everglades, S. Florida	16	64	0	64	15	59	16	64
Potomac, Maryland	2		2		0		2	
Wayland, Massachusetts	4	15	0	15	1	3	4	15
			Natrix sipedon					
Everglades, S. Florida	86	168	0	0	30	57	86	168
Potomac, Maryland	7	20	0	0	4	16	3	20
Great Swamp, New Jersey	1	18	0	0	1	7	1	18