solar time by remaining oriented in the "trained" direction throughout the day (*3*). It has been proposed that the sun-orientation rhythm is regulated by an internal rhythm, or inner time sense. The rhythmic fluctuations in the light-shock reaction in phase with the daily light-dark cycle could be a manifestation of this capacity. Such clear-cut behavior rhythms of fishes could be used in further investigations of the so-called time sense (*4*).

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Gravity Factor for Auxin Transport

Abstract. The elongating internodes of the axis of a vigorous dicotyledonous plant develop strong curvatures if the tropistic effect of gravity is eliminated on a clinostat. Similar curvatures are produced by unbalancing either the supply of auxin or its transport paths, but only in the absence of unidirectional gravity as a distributive force.

The corrective growth response in the axis of a plant after its placement in a nonvertical position is known to be mediated by an imbalance of auxin induced by gravity (*1*), yet no experimental evidence has been offered to support the possibility that the vertical position of the axis is maintained by a similar effect of gravity on transport of its growth regulators. In 1952 Söding (*2*) made the broad statement that such transport is not influenced by gravity, but Snow (*3*) noted that "phenomena of polarity make it probable that gravity does affect longitudinal transport of auxin."

When such 19th-century workers as Sachs and Pfeffer were using horizontal clinostats in their studies of plant form and movements, they had no knowledge of auxins. Asymmetric growth of the primary axis was never reported. Authors of current textbooks appear to be guided by Sachs's observation (*4*, p. 256) that the axis of the plant continued to grow "in the direction in which it was placed."

From a continuous series of tests started in 1958, using over 20 species of dicotyledonous plants on horizontal clinostats turning at rates from 1/240 to 1 revolution per minute, about 96 percent of plants with active apical meristems have been found to show pronounced growth curvatures in the elongating internodes of their major axes, in the region of expanding leaves and regardless of their phyllotaxy. Strong curvatures develop within a day or two, depending on the growth rate rather than on age or size of the plant. They exhibit minor fluctuations and vary from approximately 45° to 180°, as illustrated in Fig. 1. The rate and degree of curvature are independent of the intensity or period of illumination and of the speed of rotation.The tests were made with vigorous plants rooted mostly in plastic pots of loam soil, watered normally and tested in a temperature range from 17° to about 25°C.

The direction of the curvature of an undisturbed axis is unpredictable unless the stalk of the plant leans perceptibly to one side; in these cases the growth curvatures of the tip will be in that direction. Adjoining internodes commonly develop their curvatures in the same plane, but exceptions occur in a few species, with resultant twisting effects. A predictable curvature can be produced by removing the leaves from one side of a growth zone, as illustrated with the stock plant in Fig. 2. The growing tip bends toward the defoliated side but only when the plant is rotated on the clinostat. The force of gravity seems to equalize some material supplied by the leaves, even to the extent of straightening the curvature in less than 24 hours if the plant is returned to the vertical position before the axial tissues have matured. Possible differences in the nutrition, auxin supplies, and water relations within leafy plants are thus regularly equalized unless the effects of gravity are eliminated on a clinostat.

A predictable curvature that appears only if the plant is turning on a clinostat can be produced if a film of lanolin containing 1 percent triiodobenzoic acid (TIBA) is applied to one side of the growth zone of an intact plant such as tomato or sunflower. The growth curvature is always toward the triiodobenzoic acid, which is known to interrupt the transport of auxin in tissues affected by it (*5*). Return of the plant to the vertical position results in straightening the curvature if a minimal amount of triiodobenzoic acid has been applied. This curvature is clearly due to more endogenous auxin moving down the

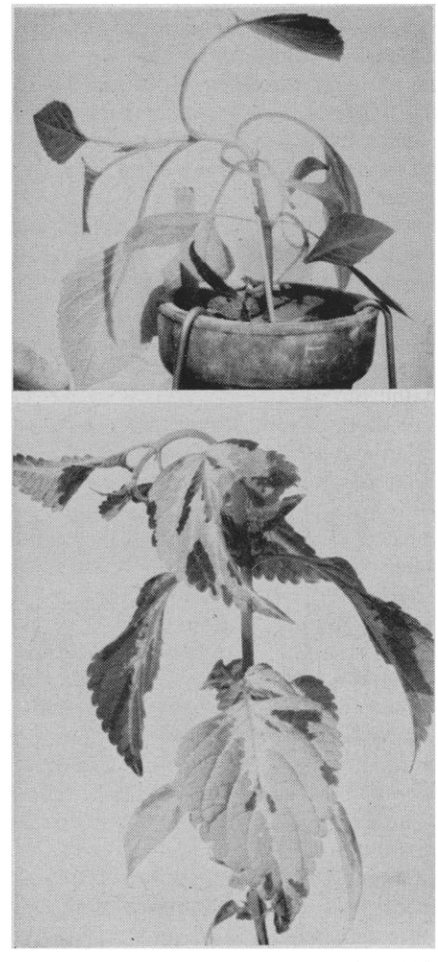

Fig. 1. (Top) *Dahlia pinnata* with 90° bend in axis after 3 days on clinostat. (Bottom) Strong curvature in tip of *Coleus blumei* after 24 hours on clinostat.

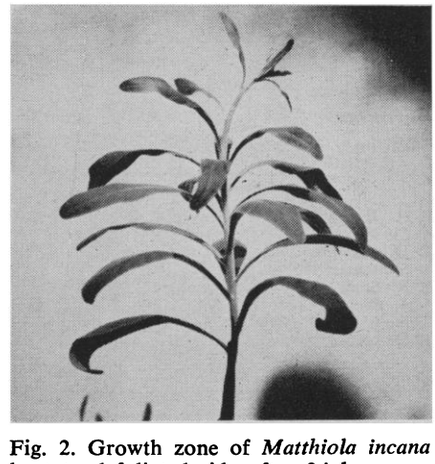

Fig. 2. Growth zone of *Matthiola incana* bent to defoliated side after 24 hours on clinostat.

convex side than down the concave side of the bending axis.

Axial curvature by residual auxin in entirely defoliated and disbudded stems, turned as usual on the clinostat, has been observed in a few tests with tomatoes and seedling *Coleus blumei*. For all other such defoliated stems, curvatures in the growing internodes are producible by supplying indoleacetic acid in solution or emulsion form in place of the terminal bud. Solutions of the order of $10^{-4}M$ have produced curvatures in excess of 90° in 3 days, with the growth curvatures straightened in the immature tissues by return of the plant to the erect position for a few days. Substitution of a layer of lanolin containing 1 percent indoleacetic acid for the terminal bud of a similar straight axis of sunflower, geranium, or coleus, applied uniformly over the freshly cut surface at the tip, also produces a stem curvature that varies in degree with the rate of growth of the apical tissues, just as in an axis with leaves and terminal bud. Thus with a symmetrical supply of indoleacetic acid to the cut end of the stem, the growth is still asymmetrical without the normal action of gravity on the transport system.

The results of all these tests and observations are consistent with the hypothesis that gravity is a factor in maintaining an even distribution of one or more of the auxins within the growth zone of the plant's axis. In the absence of an effective gravitational force, more auxin enters one sector of a growing internode than other sectors. Differences in transport rate are probably more important than possible differences in auxin supply from the young leaves. The effect of gravity on the erect stem is to equalize any such differences in auxin content of the growing tissues, presumably by the same mechanism by which a nonvertical axis is caused to assume the erect position (*6*).

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20 April 1962

Dialyzable Cofactor in Nerve Growth Promoting Protein from Mouse Salivary Glands

Abstract. Cohen's method for preparing the nerve growth factor from mouse submaxillary glands was followed to the last ammonium sulfate fraction. Further purification was accomplished with carboxymethyl- and diethylaminoethyl- column chromatography. Three peaks were obtained for each column; the third peak obtained with diethylaminoethyl cellulose was the most active. Electrophoresis of this active fraction produced one anodal and two cathodal bands. Each band was inactive for nerve outgrowth. However combinations of the cathodal bands produced 3+ growth. One cathodal band was not dialyzable; the other was dialyzable and negative for ultraviolet absorption at 280 millimicrons.

Evidence for the existence of a nerve growth factor, specific for spinal (*1, 2*) and sympathetic ganglia (*3*), was first obtained from mouse sarcomas which were grown as intraembryonic grafts in chick embryos. Tumor fragments were transplanted to the flank of 2½-day-old chick embryos. After 9 to 14 days of incubation, the spinal and sympathetic ganglia adjacent to the grafts were found to be 1.5 to 6 times larger than comparable controls (*3, 4*). Neural centers in the spinal cord and parasympathetic centers failed to respond. Cell counts of comparable square areas and reconstructions of serial sections provided conclusive evidence that this increase in size of the ganglia was the result of neuron hypertrophy and hyperplasia (*2, 3*). This growth response was then also elicited from sarcoma fragments grown as allantoic grafts (*5*). It was postulated that a humoral agent was being transmitted to the embryo, possibly by way of the blood (*5*). In spinal or sympathetic ganglia grown as explants in hanging drop cultures supplemented with cell-free sarcoma derivatives (microsomal, nucleoprotein, protein fractions), a dense outgrowth of neurites appeared during 24 hours (*6*). These observations led to the concept of a nerve growth factor as an entity which has in part been characterized (*7*).

This nerve growth factor has since been shown to be present in a wide variety of biological materials (*8*) and has been isolated as a protein fraction from each of the following: mouse sarcoma 180, snake venoms, mouse submaxillary salivary glands (*9–12*) and axial structures of 8- to 9-day-old chick embryos (*8*). Cohen and Levi-Montalcini have shown that the submaxillary gland from the adult male mouse is by far the richest source (*9, 10*). Rabbit antiserum to this protein inhibited nerve outgrowth from ganglion explants in vitro and produced nearly total destruction of sympathetic ganglia in newborn mice, rats, rabbits, and cats (*13*).

Ultraviolet absorption ratios (for absorption at wavelengths of 280 and 260 mμ) for purified proteins from mouse sarcoma 180 (*11*), from snake venom (*12*), and from mouse submaxillary glands (*9*) were reported as 1:25, 1:30, and 1:53, respectively. The molecular weight of the protein fraction from sarcoma 180 was not determined. Molecular weights, as determined with the Spinco analytical ultracentrifuge, were of the order of 20,000 for the venom protein (*12*) and 44,000 for the mouse submaxillary gland protein (*9*). The purpose of the present study was to obtain further chemical information on the nature of the factor as isolated from the submaxillary gland of the adult male mouse.

We have followed the procedure of isolation and partial purification through the last ammonium sulfate precipitation as described previously (*9*). Briefly, the steps up to this point included homogenization, centrifugation, streptomycin sulfate precipitation, two ethanol precipitations, and ammonium sulfate precipitation in three steps. The fraction precipitating at 75 to 85 percent ammonium sulfate sat-

Table 1. Quantitative amino acid analysis of electrophoretic band A. Residues, on basis of arginine as 1 (corrected to nearest whole numbers, taking into account destruction during hydrolysis, and multiplied by 2). Total nitrogen (by micro-Kjeldahl method) added to column, 0.0934 mg. Total nitrogen recovered (not counting tryptophan and ammonia), 0.0858 mg. Total weight of residues, 8600.

Amino acids	Micro-moles	Resi-dues	N eluted (μg)
Arginine	0.159	1.00	8.90
Lysine	.339	2.12	9.54
Histidine	.165	1.03	6.84
Cysteic	.155	0.98	2.15
Aspartic	.617	3.95	8.53
Threonine	.307	1.95	4.27
Serine	.389	2.44	4.44
Glutamic	.473	2.99	7.23
Proline	.357	2.25	5.01
Glycine	.453	2.87	6.32
Alanine	.280	1.78	3.92
Valine	.276	1.75	3.86
Isoleucine	.184	1.10	2.56
Leucine	.487	3.04	6.80
Ø-Alanine	.136	0.85	1.90
Tyrosine	.173	1.05	2.44
Methionine sulfone	.065	0.50	1.15
Sulfoxide	.022		