Table 1. Color of lean polyvinyl acetate-ultramarine paint when varnished.

Varnish resin	Solvent	Color	Viscosity grade (centipoise)	Refractive index of resin
Experimental polymethacrylate	Cycloparaffins	Light	22	1.48
Experimental polymethacrylate	Cycloparaffins	Dark	8	1.48
AW-2	Cycloparaffins	Dark	1.2	1.52
Dammar	Turpentine	Dark	1.3	1.53
Bakelite polyvinyl acetate AYAB	Toluene	Dark	9	1.467
Bakelite polyvinyl acetate AYAT	Toluene	Light	114	1.467
Polyvinyl alcohol	Water	Light	~400*	1.51

* Value determined in water.

paint did not rub off when it was rubbed with the hand, and yet it was porous to the applied varnishes. When the coat of varnish had dried, the value of the blue was noted simply as "dark" or "light." Table 1 shows that varnishes prepared with resins of high viscosity grade apparently do not form an intimate contact with the paint, with the result that the color appears light irrespective of the refractive index. Practical applications immediately come to mind when one does not wish to darken colors in pastels and porous paints.

The truly outstanding difference between the properties of the traditional picture varnishes, dammar and mastic, and those of many proprietary polymers is not in their refractive indices, but in the viscosity of their solutions. In place of intrinsic viscosity, we have used the viscosity at 20 percent solids by weight in toluene at 70°F as a convenient measure to characterize resins, giving it the name "viscosity grade." A similar measurement has been used to classify chlorinated rubber (*6*). On this scale, dammar, mastic, and resin AW-2 (Badische Anilin und Soda Fabrik) have a viscosity grade between 1.2 and 1.3 centipoises (cp) whereas Bedacryl 122 X (I.C.I.) and Lucite 44 (du Pont) *n*-butyl polymethacrylate have values about 48. Compared with dammar resin, polymers of high viscosity grade resist flow at a relatively low concentration of solids. As the solvent evaporates beyond this point, the film tends to conform to the contours of the paint surface (*7*). In this manner, a varnish formulated with a resin of high viscosity grade tends to be less glossy than the dammar type, which is able to remain fluid, continuing to level itself, until much more of the solvent has departed.

Among museum authorities, interest in refractive index has centered about the appearance of coatings of polyvinyl acetate. The polymer long used in America, Bakelite vinyl resin AYAF (similar to the earlier Vinylite A), is one of relatively high (80 centipoises) viscosity grade. The formulation of Reid, originally presented by Stout and Gettens, (*8*) was tested in our laboratory and found to give poor distinctness-of-image gloss when it was sprayed on window glass, with the spray gun at a distance of 10 to 20 inches from the glass. In a control test, the gun emitted 35 to 70 ml of toluene per minute. Changes in formulation of the solvent markedly altered the gloss. This laboratory has for several years drawn the attention of museums to polyvinyl acetate polymers of 40- and 9-centipoise viscosity grade (*9, 10*).

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10. I am indebted to A. E. A. Werner, Research Laboratory, British Museum, London, for the sample of resin AW-2, and to R. Sneyers, Lab. Central des Musées de Belgique, Brussels, for the sample of Bedacryl 122X.

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Effect of Gibberellin on Cell Division in Hyoscyamus

Recently it has been shown that gibberellin, when it is applied to nonvernalized biennial *Hyoscyamus niger* will cause the formation of an elongate stem (*1*). Since the nonvernalized plant grows as a rosette—that is, without measurable internodes—it was suggested that the gibberellin caused an increase in the number of cells. This report presents direct evidence for an increase in cell division resulting from application of gibberellin to biennial *H. niger* (*2*).

Plants of *H. niger* (biennial, purple-flowered strain) were grown from seed in a greenhouse in which the temperature was not permitted to fall below 20°C and in which the photoperiod was maintained at 18 hours by supplementing natural light with artificial illumination. Crystalline gibberellin (*3*) was dissolved in glass-distilled water to which a small amount of a wetting agent (Tween 20) was added. The desired amount of gibberellin was applied at the base of a leaf as close to the apex as possible by means of a hypodermic syringe.

The apical region of the shoot (rosette) was prepared for microscopic examination by fixation in formalin, acetic acid, and 50-percent ethanol (5/5/90); dehydration in tertiary butanol; imbedding in Tissuemat (mp 56°C); longitudinal sectioning at 6 μ; and staining with safranin and fast green according to the procedures outlined by Johansen (*4*). Generally, ten sections per plant, comprising a median slice of 60-μ thickness through the apical region, were examined. Certain restrictions were imposed on this research; only meta-, ana-, and telophase division figures were counted, and only the shoot apex proper and that part of the subapical region which is enclosed by the provascular tissue were examined (see Fig. 1, top). During the period in which observations were made, there was no measurable change in shape and total volume of the subapical region; hence, the cell division counts refer to the same tissue volume.

In one experiment, plants were treated with 0.2 ml of a 25 mg/lit solution of gibberellin (equivalent to a dose of 5 μg per plant) while they were receiving continuous illumination. Illumination was supplied by fluorescent tubes (warm and cool white) yielding approximately 500 ft-ca at plant height, at 23 ± 2°C. In two other experiments, checking the 24-hour point, the plants were treated with gibberellin as described while maintained in greenhouse conditions (varying temperatures, 18-hour photoperiod). Since the results were substantially the same for all three experiments, the data are grouped together.

As can be seen from Table 1, there was no significant difference between the treated and control plants with respect to the number of division figures in the apical region. However, beginning between 12 and 18 hours after treatment, the number of divisions in the subapical region underwent a striking increase. In this region, a distinction was made between "longitudinal" and "transverse" division figures—that is, division figures the spindle axes of which form an angle of 0 to 45 deg or of 45 to 135 deg with the longitudinal axis of the plant (see Fig. 1, bottom). Table 1 shows that the increase relates mainly to the longitudinal ones; the differences in transverse

division figures between treated and control plants were not significant at the 1-percent level. This effect extended over the uppermost 650 to 800 μ of the subapical region; below this distance, the number of mitotic figures drops rapidly to zero.

The increase of stem growth by gibberellin, the first gibberellin effect observed, and demonstrated in a great number of plants (*5*), is based on an increase in cell length, although in some cases a certain increase in cell number has been considered as probable (*6*). Our results show directly that gibberellin causes a great increase in the number of cell divisions in the subapical region of nonvernalized biennial *H. niger* rosettes, thus proving that gibberellin may function as a regulator not only of cell elongation, but also of cell division.

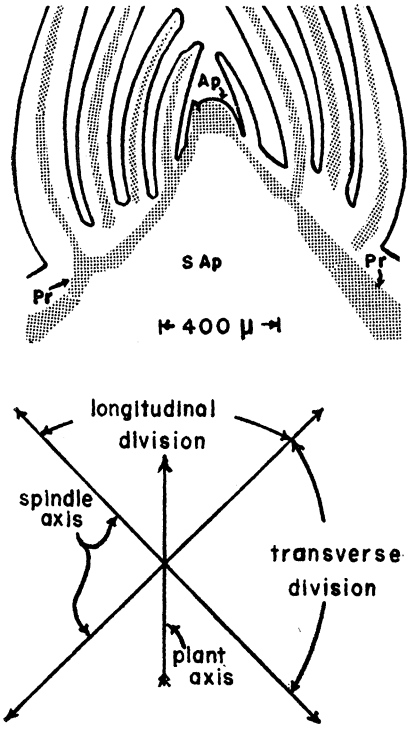

Fig. 1. (Top) Semidiagrammatic drawing of the shoot of nonvernalized, biennial *Hyoscyamus* plant. Ap, apical region; SAp, subapical region; Pr, provascular tissue. (Bottom) Sketch showing the basis for selection of longitudinal and transverse cell division figures.

It is conceivable that the effect of gibberellin on cell division is an indirect one, the division being induced by accelerated cell elongation. Dividing cells in the subapical region of *H. niger* are indeed 1.5 times as long as nondividing ones. However, this is equally true of treated and untreated plants, and the length of cells is not increased even after 9 days of daily gibberellin applications, although their shape is then quite different. It thus seems that increased cell elongation follows rather than precedes the effect of gibberellin on cell division.

The data presented in Table 1 further indicate a directional influence of gibberellin on cell division, longitudinal division figures being increased to a much greater extent than transverse ones. The factors responsible for this effect require further investigation.

It will also be necessary to check in further experiments whether, as suggested by the ones reported here, gibberellin fails to increase cell division in the apical region of the *H. niger* shoot. If this situation is generally true, it is suggestive of the hypothesis of Buvat (*7*), according to which the extreme apical region of the shoot meristem does not participate in vegetative growth, the actual meristematic activity being localized below that region. This area of meristematic activity, called the *méristème medullaire,* corresponds to the lower part of the apical and the upper part of the subapical region of *H. niger.* However, Buvat's theory goes further and describes the *méristème medullaire* and not the extreme apex as the origin of the initial cells of the entire stem (*7*, see particularly Fig. 12), whereas our results by no means exclude the apex as the primary source.

Table 1. Effect of gibberellin on cell division in biennial *H. niger*. All figures represent the number of division figures per 60-μ tissue slice. The errors quoted are the standard errors of the mean.

Hours after application of gibberellin	Apical region	Subapical region		Number of plants
		Longitudinal division figures	Transverse division figures	
		Controls		
0	12 ± 2	2 ± 1	3 ± 1	5
6	12 ± 1	3 ± 1	4 ± 1	4
12	15 ± 2	1 ± 0.5	4 ± 0.5	5
18	15 ± 3	1 ± 1	3 ± 1	5
24	9 ± 3	3 ± 0.5*	8 ± 2	10
		5 μg *of gibberellin*		
6	8 ± 1	3 ± 1	6 ± 1	5
12	13 ± 2	3 ± 2	6 ± 2	5
18	11 ± 1	12 ± 4	11 ± 2	5
24	12 ± 1	29 ± 3*	10 ± 2	9

* The difference is significant at the 1-percent level using the *t* test for unpaired samples in which the variances for the parent populations are unequal; described by Stearman (*8*, pp. 182 and 183).

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Proposed Mechanism of Action of Hydrolytic Enzymes

Certain hydrolytic enzymes, notably chymotrypsin, trypsin, and acetylcholinesterase have been shown to react with reagents such as diisopropylfluorophosphate to yield inactive proteins which contain single diisopropylphosphate residues (*1, 2*). The inactive protein is relatively stable in water but may be converted into the active enzyme by treatment with a variety of nucleophilic reagents (*3*). Chemical or enzymatic hydrolysis of the inhibited protein has invariably shown the dialkylphosphate residue to be attached at the hydroxyl group of a single serine residue (*4*).

Recently, singly substituted, inactive, acyl derivatives have been successfully prepared by reaction of delta-chymotrypsin with compounds such as *p*-nitrophenyl acetate, acetic anhydride, and benzoyl chloride (*5*). These proteins appear to be very similar to the dialkylphosphate-substituted enzymes except that they are more readily reactivated by water. These facts, together with the close parallel of the *p*H dependence of acylation (*5, 6*), normal hydrolytic activity (*7*), and dialkylphosphorylation (*1*), have led to the conclusion that these reactions are closely related and involve directly at least a portion of the active center of the enzymes (*3*). Dixon and Neurath (*5*) have also shown that the *pK'* for acylation and deacylation of delta-chymotrypsin are different, being about 6.2 and 6.8, respectively. The latter value is very close to that reported