The effect of chemical vernalization on the average node number in two cucumber varieties (Mean ± Standard error).

Variety	Control *a* (No cold)	Control *b* (Cold treated)	0·001 ppm NAA	0·01 ppm NAA	0·1 ppm NAA	1·0 ppm NAA
Yorkstate	9·2 ± 0·6	5·9 ± 0·2	5·8 ± 0·2	5·9 ± 0·3	5·1 ± 0·1	
Beit-Alpha	7·3 ± 0·4	6·6 ± 0·6	6·6 ± 0·4	6·5 ± 0·5	5·9 ± 0·2	7·6 ± 0·3

The results of an experiment with the cucumber variety Packer are represented graphically in the Figure. Samples of seed, soaked in solutions of NAA varying in concentration from 0·001 to 10 ppm, or in solutions of maleic hydrazide (MH), the concentration of which ranged from 0·01 to 100 ppm, were placed under laboratory conditions for 21 h; they were then maintained at 6° C for 8 days and planted in the field. The mean node number of the 32 to 40 plants subjected to each of the treatments was found. The analysis of variance showed that the least significant difference (LSD) was 1·04 at the 5% level and 1·41 at the 1% level. Whereas NAA solutions of 1·0, 0·1, 0·01 and 0·001 ppm cause a significant reduction in node number, the 0·01 ppm solution of NAA causes a highly significant reduction as compared with control *a* (without cold treatment). In the latter case the reduction was also significant as compared to control *b* (with cold treatment). According to the Figure, control *b* appears to reduce node number but the effect is not statistically significant. Increasing the concentration of the NAA solution from 1·0 to 10·0 ppm delays the appearance of the first pistillate flower. The MH had no appreciable effect on the node number.

THIMANN and LANE[7] regard vernalization as the "prolonged exposure of the seed to its internal auxin supply". This may be interpreted as meaning that in cold-treated soaked seed the auxin retains its potential properties, while its destruction is very slow. Therefore, chemical vernalization seems to produce effects similar to, but stronger than auxin treatment alone.

The effect of chemical vernalization on the number of nodes preceding the first *pistillate* flower in the cucumber is similar to the influence of chemical vernalization on the number of nodes preceding the first perfect flower in peas, as reported by LEOPOLD and GUERNSEY[8]. This raises the question as to whether or not the position of these two different flower types is determined by the same factor.

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The Weizmann Institute of Science Rehovot, January 30, 1956.

Résumé

Il a été trouvé que l'immersion des graines de concombres dans des solutions d'auxines végétales de concentrations déterminées, suivie d'un traitement par le froid, peut amener dans la plante adulte un changement dans les manifestations du sexe. La relation possible entre ce traitement et la vernalisation ordinaire est brièvement discutée.

[7] K. V. THIMANN and R. H. LANE, Amer. J. Bot. *25*, 535 (1938).

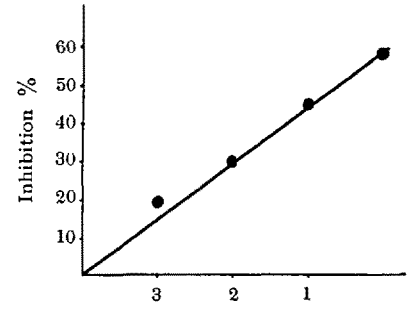
Studies on a Polyphenolase in *Scopolia japonica* III On the Inhibition of DOPA Oxidation

It was previously reported[1] by the author that a polyphenolase prepared from the subterranean stem of *Scopolia japonica* catalyzed the aerobic oxidation of DOPA to form a melanine-like substance, whereas it did not catalyze the oxidation of L-tyrosine[2].

The present report deals with the effects of various inhibitors on the activity of this enzyme preparation.

The subterranean stems of plants were washed and peeled. About 30 g of this material was blended with 150 ml of cold phosphate buffer (M/30). It was filtered through gauze, centrifuged for 30 min at 4000 rpm and the sediment discarded. The supernatant liquid (I) was either assayed directly for polyphenolase or was partially purified (II) by acetone precipitation and dialysis in the cold for 24 h against distilled water.

Fig. 1.–Inhibition of DOPA oxidation by thiosulphate.



–log [thiosulphate].
Final concentration of DOPA is $1{\cdot}25 \times 10^{-3}$ Mol.

The effects of various inhibitors upon the oxidation of DOPA are listed in the Table. It has been shown previously[3] that the inhibition of polyphenoloxidase by phenylthiourea is typical, but that cytochrome oxidase activity is little inhibited by this reagent. According to the present experiment, the oxidation of DOPA by the polyphenolase is much inhibited by this substance at a lower concentration.

The inhibitory effect of sodium diethyldithiocarbamate (which is a precipitant almost specific of copper) was more pronounced than with cyanide. On the other hand, carbon monoxide with 5% oxygen mixtures caused 73% inhibition, most of which was non-reversible by light, and this is characteristic of polyphenoloxidases[4]. This enzyme, therefore, is probably concerned with the copper.

[1] Y. SUZUKI, Bot. Mag., Tokyo *68*, 227 (1955).
[2] Y. SUZUKI, Bot. Mag., Tokyo (in press).
[3] K. P. DUBOIS and W. F. ERWAY, J. biol. Chem. *165*, 711 (1946).
[4] O. WARBURG, *Schwermetalle als Wirkungsgruppen von Fermenten* (Berlin 1949).

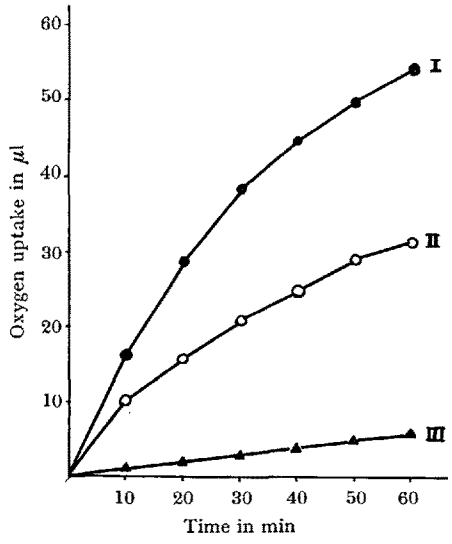
Inhibition of DOPA oxidation by various substances.

Inhibitors	Final concentration (Mol)	Inhibition % (in 60 min)	
Sodium diethyldithiocarbamate	$0{\cdot}25 \times 10^{-2}$	96	Enzyme (I)
Cyanide	$0{\cdot}25 \times 10^{-2}$	38	DOPA conc.,
Phenylthiourea	Saturate/4	84	$0{\cdot}125 \times 10^{-2} M$
95% CO with 5% O_2	in light	73	
p-Nitrophenol	$0{\cdot}5 \times 10^{-3}$	10	
Phenylthiourea	Saturate/4	95	Enzyme (II)
Salicylaldoxime	$0{\cdot}25 \times 10^{-2}$	20	DOPA conc.,
p-Nitrophenol	Saturate/4	31	$0{\cdot}125 \times 10^{-3} M$
8-Oxyquinoline	Saturate/4	20	

The activity of the enzyme was determined by measuring oxygen uptake in the Warburg apparatus at 35° C and pH 7·3, taking 1·0 ml of the enzyme preparation, 1·0 ml of inhibitor and others in the main chamber; 0·5 ml of DL-dihydroxyphenylalanine solution in the sidearm of a flask, the total volume of liquids being made to 4·0 ml. The center well contained 0·3 ml of 10% KOH; the gas phase was air.

Salicylaldoxime[5], *p*-nitrophenol[6] and 8-oxyquinoline[7] are known as sensitive reagents against some copper enzymes which are responsible for the terminal step in the biological oxidation, but inhibition by these inhibitors is not very extensive.

Fig. 2.–Inhibition of DOPA oxidation by thioglycolate.



Curve I. DOPA only (final concentration $1{\cdot}25 \times 10^{-3}$ Mol).
Curve II. DOPA plus thioglycolate (final concentration $0{\cdot}25 \times 10^{-3}$ Mol).
Curve III. DOPA plus thioglycolate (final concentration $0{\cdot}25 \times 10^{-2}$ Mol).

According to FLESCH[8] the formation of melanine in the animal is inhibited by sulfhydryl compounds, the extent of inhibition being dependent on the concentration of the inhibitor.

[5] F. KUBOWITZ, Biochem. Z. *292*, 221 (1937).
[6] J. BONNER and S. G. WILDMAN, Arch. Biochem. *10*, 497 (1946).
[7] E. STOTZ, C. J. HARRER, and C. G. KING, J. biol. Chem. *119*, 511 (1937).
[8] P. FLESCH, Proc. Soc. exper. Biol. Med. *70*, 136 (1949).

In the present experiment the oxidation of DOPA by the polyphenolase (I) was inhibited by thiosulphate and thioglycolate. The former is the simplest of sulfhydryl compounds[9]. Its inhibitory effect, as is shown in Figure 1, supports the conclusion by FLESCH. The effects of thioglycolate are shown in Figure 2.

Copper ions are known to be sensitive to thiol enzymes. The inhibition by thiosulphate and thioglycolate, therefore, might be concerned with the prosthetic group of the polyphenolase.

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Zusammenfassung

An einer aus *Scopolia japonica* isolierten Polyphenolase wurde die Wirkung verschiedener Hemmstoffe studiert. Cyanid und Salicylaldoxim wirkten relativ schwach, Diäthyldithiocarbamat, Phenylthioharnstoff und Kohlenoxyd hemmen die Oxydation stark. Der Ablauf der Hemmwirkungen von Thiosulfat und Thioglycolat wird kurvenmässig dargestellt.

[9] J. HIRAIDE, *Progressive Study of SH Group* (in Japanese) (Tokyo 1954).

Further Data on the Evaluation of Platelet Ac-globulin and its Plasmatic Origin

In the past few years, many authors have described a platelet accelerator factor which has been called Platelet Ac-globulin (or factor I). The action of this accelerator is similar to that of Plasma Factor V, and accelerates the speed of transformation of prothrombin and thrombin. Such a factor, according to some authors[1], is present in the platelets in a form which is already active, and acts in a way similar to the Serum Ac-globulin (or factor VI). Also, considering some of the physical chemical characteristics, there is an analogy between Platelet Ac-globulin and Plasma Ac-globulin. It is destroyed by heat at 56° C for 30 min, and is not dialyzed. It has been prepared in concentrated form and it has been shown to act like a protein. The activity of this accelerator has been established to be less than that of the Plasma Ac-globulin, and even though qualitatively equal, it seems that it is quantitatively equivalent to $^1/_8$–$^1/_{16}$ of the accelerator activity of Plasma Ac-globulin: such values vary considerably according to different authors. According to OWREN, the platelets possess only 6% of the accelerator activity of plasma. Recent researches[2] suggest that the accelerator factor of the platelets is adsorbed Plasma Ac-globulin (or factor V). The platelets of a para-haemophilic patient do not contain accelerators, but can acquire such an activity after having been in contact with normal plasma. Trypsin destroys almost completely Platelet Factor I, without altering platelets. If the platelets thus treated are placed in contact with a normal plasma, they recover all of the lost activity.

[1] A. G. WARE, J. L. FAHEY, and W. H. SEEGERS, Amer. J. Physiol. *154*, 140 (1948).
[2] P. HJORT, S. I. RAPAPORT, and P. A. OWREN, Blood *10*, 1139 (1955).