

by GREENBERG and GLICK¹⁰. The interference with the glycolysis by puromycin could, however, be demonstrated in the following way. As can be seen in Table II, steroid output by adrenal slices can also be stimulated by TPN

Table I. Corticosteroid production by adrenal slices of controls and pretreated animals after stimulation by ACTH, 3',5'-AMP, and TPNH

	Untreated	Treated with 5-fluorouracil
Controls	6.1	4.4
ACTH 50 mU	15.8	8.5
3',5'-AMP 3 mg	16.3	7.6
TPN + glucose-6-phosphate 2 mg + 3 mg	10.7	9.3

Table II. Stimulation of steroid production in adrenal slices by various agents in the presence and absence of puromycin

	— Puromycin	+ Puromycin
Controls	2.5	
ACTH	20.0	4.4
Controls	3.0	
Cyclic 3',5'-AMP	16.0	2.7
Controls	3.7	
TPN + glucose-6-phosphate	7.4	7.4
Controls	3.2	
TPN (no glucose-6-phosphate)	6.9	3.2

alone in the absence of glucose-6-phosphate. Apparently the endogenous glycolysis provides enough glucose-6-phosphate for the TPN to act. But in the absence of exogenous glucose-6-phosphate there is a marked difference in the action of TPN in the presence or absence of puromycin. Puromycin, as well as inhibiting the action of ACTH and cyclic 3',5'-AMP, also inhibits the action of TPN alone in the absence of glucose-6-phosphate.

The fact that under the influence of 5-fluorouracil and puromycin the ACTH effect is reduced does not necessarily mean that synthesis of a new protein is required for ACTH action; it could also be due to a depletion of glycogen, in which case the activation of phosphorylase would no longer result in an increased energy supply.

Zusammenfassung. Puromycin und 5-Fluorouracil beeinträchtigen in Nebennierenschnitten der Ratte die durch ACTH und cyclisches 3',5'-Adenosinphosphat stimulierte Corticosteronausscheidung. Die durch TPN plus Glukose-6-phosphat stimulierte Corticosteronausscheidung bleibt unbeeinflusst. Dagegen bewirkt Puromycin bei Zusatz von TPN allein eine Hemmung. Die Wirkung von Puromycin und 5-Fluorouracil dürfte daher eher auf die Beeinflussung des Energiestoffwechsels als auf eine Hemmung der Neubildung induzierter Enzyme zurückzuführen sein.

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¹⁰ L. J. GREENBERG and D. GLICK, J. biol. Chem. 237, 3552 (1962)

The Increase of Frost Resistance of Tomato Plants through Application of 2-Chloroethyl Trimethylammonium Chloride (CCC)

CCC, which is closely related to choline, belongs to the group of plant-regulating substances that retard the growth of plants without injurious effects. This compound is also interesting because of its faculty to build up, in certain plants, a resistance against unfavourable environmental conditions, like dryness¹⁻⁴, excessive salt content⁴⁻⁶ or unsuitable pH⁷.

In this paper the effect of CCC on the germination of tomato seeds at unfavourable temperatures, and also on frost resistance of tomato seedlings, was investigated. The seeds were soaked in aqueous solutions of CCC (10, 100, and 500 ppm) or in water for 24 h at 10°C and then germinated in Petri dishes (100 seeds per dish) at the optimal (25°C) and minimal (12°C) temperature.

The positive effect of CCC on the germination was observed only at the low temperature (12°C) when a concentration of 500 ppm was applied (Table I). The experiments on plant resistance against low temperature were carried out under ordinary conditions during the winter of 1963/64. Control plants as well as those treated with

Table I. Number of germinated seeds in a temperature of 12°C shown in %

Days of germination	Concentration of CCC in ppm				L.S.D. at P = 0.05
	0	10	100	500	
6	9.6	10.5	10.2	14.0 ^a	3.46
10	43.6	44.0	43.8	53.0 ^a	8.39
15	65.0	63.8	63.5	68.6	5.63

^a Significant differences in relation to control.

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² H. A. HALEVY and B. KESSLER, Nature 197, 310 (1963).

³ M. MICHNIWICZ and A. CHROMIŃSKI, II Sympoz. Growth Regul. Toruń (1963), Zeszyty Nauk U.M.K., in press.

⁴ H. E. DAMATY, H. KÜHN, and H. LINSE, Agrochimica 8, 129 (1964).

⁵ P. C. MARTH and J. R. FRANK, J. agr. Food Chem. 9, 359 (1961).

⁶ T. MIYAMOTO, Naturwissenschaften 49, 213 (1962).

⁷ T. MIYAMOTO, Naturwissenschaften 49, 377 (1962).

CCC were examined at different stages of their development.

The presowing treatment of seeds with CCC did not have any influence on the frost resistance of tomato seedlings. In the other experiments the seeds were sown in soil in a greenhouse, where they were grown until cotyledons developed. Then the plants were transplanted to Knop's nutrient solution, without and with CCC addition. The seedlings were kept in these conditions until they reached an adequate stage of growth, and then they were exposed to low temperature. After cold treatment the plants were transferred for 3-4 h to a cold room

(6-8°C) and subsequently brought back to the greenhouse. Two days later the percentage of the frozen plants in the particular variants was established.

After some preliminary experiments a concentration of 100 and 200 ppm of CCC was chosen as the most effective. Altogether 9 experiments were made: 3 for every phase of growth of the investigated plants. The experiments were repeated 4 times and for each variant 10 plants were used. The results of all experiments were in conformity. Tomato seedlings treated with CCC showed the typical feature (short and thick stems) of plants grown under the influence of growth retardants. The number of plants killed by frost was considerably lower in the variant treated with CCC, especially at the concentration of 200 ppm (Table II). All differences in relation to control were statistically significant even at $P = 0.001$. A more significant effect of CCC treatment was obtained with tomato plants in a later stage of their growth, also by a prolonged action of this compound. On the basis of these experiments, the conclusion might be drawn that CCC effects in tomatoes an increase of their resistance against low temperature.

Table II. Number of frozen plants quoted in %

Growth stages	Temperature and its duration	Concentration CCC in ppm			L.S.D. at	
		0	100	200	$P = 0.01$	$P = 0.001$
Cotyledon	- 4 to 8°C 1 h	93.0	55.0	27.5	24.8	36.5
1st pair of leaves	- 4°C 6 h	97.5	27.5	5.0	12.1	17.8
2nd pair of leaves	- 2°C 12 h	92.5	27.5	0	30.15	44.3
	- 10°C 20 min	92.5	52.5	25.0	32.9	48.4

All differences in relation to control are significant.

Zusammenfassung. Tomatensamen in CCC-Lösung (Konzentration 500 ppm) ausgekeimt, zeigten aktivierten Keimungsprozess bei niedriger Temperatur. Tomatenkeimlinge in Knops Nährlösung mit Zugabe von CCC (100 und 200 ppm) zeigten wesentlich erhöhte Resistenz gegen niedrige Temperaturen.

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Influence of an Adrenergic β -Receptor Blocking Agent on the Effect of Various Hypotensive Agents in the Hypertensive Rat

PRICHARD¹ and PRICHARD and GILLAM² showed that adrenergic β -receptor blocking agents have a hypotensive effect in man¹. We attempted to reproduce this effect in renal hypertensive rats and also investigated the influence of pronethalol on the anti-hypertensive effect of various drugs.

Renal hypertensive (Goldblatt) rats with stable systolic blood pressure levels above 160 mm Hg were used as test animals. Blood pressure was measured twice daily (2 h [acute effect] and 24 h [prolonged effect] after preceding injection) in light ether anaesthesia by tail plethysmography³.

Hydralazine (Apresolin, CIBA), guanethidine (Ismelin, CIBA), and DL- α -methyl-DOPA were used as hypotensive agents, and pronethalol as an adrenergic β -blocker. The treatment schedule used is given in the Table.

The mean of the blood pressures measured on the 3rd and 4th days of treatment was used to evaluate the effects of the various treatments. At this time, all hypotensive drugs produced a maximal response. The average of two daily blood pressure measurements was used in order to exclude the effect of daily variations in blood pressure.

All hypotensive agents tested showed marked and constant antihypertensive effects. The blood pressure levels

measured 24 h after the preceding injection on the 3rd and 4th day of treatment were reduced considerably from the initial control values. Pronethalol had a slight prolonged hypotensive action. The daily dose of hydralazine (blood pressure measurement 2 h after administration = acute effect) caused a slight additional decrease in blood pressure as compared to the values measured immediately before injection.

Pronethalol produced an increase in blood pressure measured after 2 h when injected simultaneously with guanethidine or the lower dose of hydralazine (Table). The effect of α -methyl-DOPA 2 h after injection was not influenced. Simultaneous injection of pronethalol and a hypotensive agent did not influence the prolonged anti-hypertensive effect of these drugs.

The results obtained with a higher dose of hydralazine indicate that, although reduction of blood pressure is not significantly greater, inhibition of the hypotensive effect is less complete. It is therefore possible that the hypotensive effect of lower doses of α -methyl-DOPA could also be antagonized by pronethalol. An inhibition of the action of hydralazine and guanethidine by pronethalol was seen only 2 h after injection. No influence on the prolonged

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