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.

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.

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 prolongated 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.

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

Prichard[1] and Prichard and Gillam[2] showed that adrenergic β-receptor blocking agents have a hypotensive effect in man[1]. 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 plethysmography[3].

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 antihypertensive 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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Influence of pronethalol on the effect of various hypotensive agents in the renal hypertensive rat

Treatment mg/kg·day		n	Control value mm Hg	Mean blood pressure values (mm Hg) on 3rd and 4th day of treatment	
				Acute effect measured 2 h after last injection	Prolonged effect measured 24 h after last injection
Saline control		4	195 ± 5	186 ± 4	187 ± 2
Pronethalol	10 s.c.	8	193 ± 2	186 ± 4	173 ± 4
Hydralazine	6 p.o.	7	198 ± 7	133 ± 8	152 ± 9
Hydralazine + Pronethalol	6 p.o. 10 s.c.	8	195 ± 6	186 ± 5	160 ± 7
Hydralazine	15 p.o.	7	192 ± 6	113 ± 10	151 ± 11
Hydralazine + Pronethalol	15 p.o. 10 s.c.	7	194 ± 6	154 ± 11	148 ± 10
Guanethidine	6 s.c.	7	198 ± 6	122 ± 5	133 ± 7
Guanethidine + Pronethalol	6 s.c. 10 s.c.	6	195 ± 7	167 ± 5	132 ± 4
α-Methyl-DOPA	300 p.o	13	188 ± 5	143 ± 3	152 ± 3
α-Methyl-DOPA + Pronethalol	300 p.o. 10 s.c.	7	191 ± 7	146 ± 7	148 ± 7

Treatment daily for 4 days. Blood pressure measurement twice daily, immediately before and 2 h after treatment, by tail plethysmography. Values given are means ± SE.

action was found. This may be due to the relatively short duration of action of pronethalol. It is possible that pronethalol, given in higher doses and at shorter intervals, could inhibit the prolonged as well as the acute hypotensive effects.

Hydralazine and guanethidine produce – as far as is known – a reduction in peripheral resistance and a fall in blood pressure by different mechanisms[4–6]. The inhibition of their hypotensive effects by pronethalol indicates, however, that they probably have some common peripheral or central mechanism of action. It is, as yet, not possible to decide between these two possibilities.

Although preliminary experiments with other β-receptor blocking agents show the same results, the inhibition of the effects of hypotensive drugs by pronethalol may not be related to its β-receptor blocking activity.

Zusammenfassung. Die hypotensiven Wirkungen von Hydralazin und Guanethidin an renal hypertonischen Ratten konnten für kurze Zeit durch Pronethalol antagonisiert werden. Die Wirkung von α-Methyl-DOPA wurde nicht beeinflusst.

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PRO EXPERIMENTIS

A Rapid Technique for Characterizing Metal-Protein Complexes by pH-Metry

The pH-metric method has been successfully used to study the hydrogen ion binding capacities of various proteins. The technique has, however, not been employed so far to investigate metal-protein interaction. In this communication the results of such studies on cobalt and nickel complexes of transfusion gelatin are reported.

MALIK et al.[1] have found that the equation[2]:

$$\mathrm{pH} - \log \frac{v_i}{n_i - v_i} = (\mathrm{pK}_{int})_i - 0.868\, Z\, W$$

which represents a titration curve in absence of metal ion for globular protein may also be applied to collagen type of protein. Since, in the presence of metal ions, a fraction of the acidic groups will be removed from participation in hydrogen ion equilibria by metal binding, it is possible to calculate the binding data directly from the difference in hydrogen ion titration curves in the presence and absence of metal ion, just as in the case of BJERRUM's[3] method.

Solutions of cobalt and nickel were prepared by dissolving chemically pure sample (A.R.) of the chloride salts in

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