

betrifft, in gleichaltrigen Wirten deutliche Mehrleistungen hervor. Ähnliche Regulationsleistungen der Spermathekananlage wurden auch schon nach sagittaler Zweiteilung von *Drosophila*-Genitalscheiben beobachtet⁶. Über Entwicklungsleistungen fragmentierter und kultivierter^{7,8}

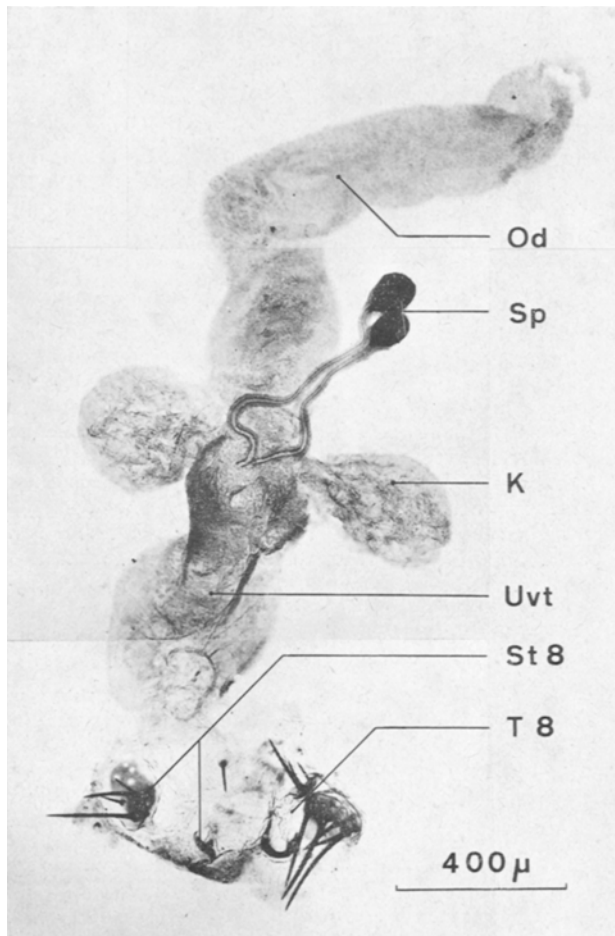


Fig. 4. Metamorphosiertes Implantat einer weiblichen Nebengenitalscheibe von *Musca domestica*. Bezeichnungen wie in Figur 2. Färbung: Karminessigsäure.

Nebengenitalscheiben werden erst Untersuchungen, die zur Zeit noch im Gange sind, Auskunft geben können.

Im Gegensatz zur Entwicklung im weiblichen Tier geht der vollständige innere und äussere männliche Genitalapparat aus der medianen Genitalscheibe hervor. Männliche Nebengenitalscheiben liefern als Implantat bei *Musca* und *Phormia* harte, stark pigmentierte Chitintteile, mit kräftigen Borsten und feinen Trichomen besetzt. Auf Grund der Lage der Nebengenitalscheiben und ihrer beim Weibchen eindeutigen Zugehörigkeit zum 8. Segment liegt der Schluss nahe, dass die erwähnten Strukturen dem 8. Abdominalsegment zuzuordnen sind. Minderleistungen von männlichen Larven, denen eine Nebengenitalscheibe exstirpiert wurde, zeigen ebenfalls klar, dass ihre Derivate am Aufbau des Syntergums 7+8 beteiligt sind.

Summary. In *Drosophila*, both sexes develop their whole genital and anal apparatus from one single median genital disc. Larvae of *Musca domestica* and *Phormia regina* are equipped with 3 post-abdominal discs. In females, the median genital disc develops into the external analia, the hind gut and the parovaria. The 2 lateral genital discs together yield the chitinized structures of the 8th abdominal segment and the whole internal genital apparatus except the parovaria and the gonads. When transplanted into larval hosts of the same age as the donors, each lateral genital disc gives rise to an almost complete internal genital apparatus, i.e. they show a regulative capacity without previously being cultured. The entire male genital and anal apparatus develops from the median genital disc. The lateral genital discs of the male, as revealed by transplantation and exstirpation, seem to take part in the development of the syntergite 7+8.

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⁶ E. HADORN und P. S. CHEN, Rev. Suisse Zool. 63, 268 (1956).

⁷ E. HADORN, Devl. Biol. 7, 617 (1963).

⁸ E. HADORN, Devl. Biol. 13, 424 (1966).

Induction of Thermosensitivity and Salt Sensitivity in Wheat Roots (*Triticum aestivum*) and the Effect of Kinetin

Heat sensitivity has been studied in many organisms and it is known that it can be induced by heat shock, UV-irradiation, and certain other treatments¹⁻³. In the present study roots of wheat (*Triticum aestivum* cv. 'Svenno vårvete' from Weibull, Sweden) were used. The seeds were germinated at 25°C in the dark. After 2 days the developing seedlings were placed with their roots in containers, each holding 250 ml liquid nutrient medium and 19 wheat plants. The medium contained $10^{-3}M$ $Ca(NO_3)_2$, $10^{-3}M$ KNO_3 , $10^{-3}M$ KH_2PO_4 , $5 \times 10^{-4}M$ $MgSO_4 \cdot 7H_2O$, $5 \times 10^{-5}M$ Na_2HPO_4 and $10^{-4}M$ Fe (EDTA) in deionized water.

Of these two-day-old plants only the roots were exposed to heat shock and various other treatments. The heat

shock was performed by placing the root systems for 2 min in another container with distilled water, the temperature of which was above 44°C, in most experiments thermostatically set to 45°C. Immediately after the heat shock the plants were transferred to a third container with a fresh medium, the temperature of which was also thermostatically controlled, within a range of $\pm 0.1^\circ C$.

¹ N. FRIES and I. SÖDERSTRÖM, Expl. Cell Res. 32, 199 (1963).

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The temperature of the root medium before and after the heat shock was varied in the different experiments as described below. The shoots of the plants were maintained in continuous white light (1600–2000 lux) and at approximately the same temperature as the roots, except for the heat shock. 5 days after the heat shock the number of living and dead roots was scored. Since each plant possessed 3 roots each series comprised 57 roots. In this context a dead root always means a root with a dead apical meristem. It is therefore incapable of further apical growth, but still able to produce laterals, for instance.

Untreated roots grew equally well at 25°C (optimal) as at 35°C (supraoptimal temperature). The mode of response of the heat-shocked roots depended both on the temperature during the shock and the following incubation temperature. As shown in Table I all roots survived

Table I. The effect of the heat shock temperature on the survival of wheat roots at 2 different, subsequent incubation temperatures

Incubation temperature after treatment	Surviving roots (%)					
	No heat shock	Heat shock at				
		44.5°C	45.0°C	45.5°C	46.0°C	46.5°C
25°C	100	100	100	55	20	0
35°C	100	100	0	0	0	0

Pretreatment temperature 25°C. The figures represent average values from 3 experiments.

Table II. The effect of heat shock on the survival of wheat roots at 2 different, subsequent incubation temperatures if a period at 12°C was inserted immediately *before* or *after* the heat shock

Incubation temperature after treatment	Living roots (%)			
	20 h at 12°C <i>Before</i> heat shock		5 h at 12°C <i>After</i> heat shock	
	Heat shock 0 min	Heat shock 2 min	Heat shock 0 min	Heat shock 2 min
25°C	100	45	100	100
35°C	100	0	100	98

a shock temperature of 44.5°C, whereas very few survived a shock temperature of 46°C. Roots that had been shocked at the intermediate temperature of 45°C, however, survived if they were incubated afterwards at 25°C, but not if they were incubated at 35°C. Thus, the heat shock had sensitized the roots to a subsequent supraoptimal incubation temperature.

The heat-shocked roots were even able to survive at 35°C, however, if they were given a 5 h period of incubation at 12°C immediately after the heat shock. By putting this period before the heat shock, the temperature sensitivity of the roots seemed rather to increase (Table II).

Several different additions to the nutrient medium were tested in order to see whether they affected the mode of response of the roots. A striking effect of kinetin was observed (Table III). At a concentration of $10^{-6}M$, or higher, kinetin saved all the heat-shocked roots from being killed at 35°C. If the roots had been kept cold before the heat shock, the addition of kinetin had no protective effect, however. Instead, $10^{-4}M$ kinetin induced an increased thermosensitivity in these roots.

A heat shock produced a sensitization not only to supraoptimal incubation temperatures, but also to an increased salt concentration (Table IV).

The results reported above thus show that wheat roots can be sensitized to a supraoptimal incubation temperature in the same way as has been earlier demonstrated with several other types of organisms. In roots, however, in contrast to e.g. micro-organisms this effect of the heat shock could be at least partly alleviated by kinetin. This was shown earlier for leaves⁴ and may be a common characteristic of seed plants.

Since the biochemical and structural changes produced in the cell by heat shock are still very little known, it is hard to suggest a satisfactory explanation of the protective effect of kinetin. In onion root cells it has been demonstrated^{5,6}, however, that the endoplasmic reticulum undergoes certain drastic modifications when the cells are exposed to kinetin. An increase in the RNA content of the cells could also be observed. After a few hours a structural reorganisation of the reticulum occurred.

⁴ K. MOTHES, *Naturwissenschaften* 47, 337 (1960).

⁵ W. A. JENSEN, E. G. POLLOCK and P. HEALEY, *Expl. Cell Res.* 33, 523 (1964).

⁶ M. A. HAYAT and F. M. SALAMA, in 16th Int. Congr. Electron Microscopy, Kyoto 1966, p. 325.

Table III. The effect of kinetin on the sensitivity of heat-shocked wheat roots to a supraoptimal incubation temperature

Incubation temperature between germination and heat shock	Incubation temperature after heat shock	Surviving roots (%)									
		No kinetin		$10^{-7}M$ kinetin		$10^{-6}M$ kinetin		$10^{-5}M$ kinetin		$10^{-4}M$ kinetin	
		No heat shock	Heat shock	No heat shock	Heat shock	No heat shock	Heat shock	No heat shock	Heat shock	No heat shock	Heat shock
First 25°C, then, during 16 h before heat shock 12°C	25°C	100	55	100	10	100	55	100	0	100	0
	35°C	100	0	100	0	100	0	100	0	0	0
25°C	25°C	100	100	100	100	100	100	100	100	100	100
	35°C	100	0	100	80	100	100	100	100	100	80

The kinetin concentration of the medium was the same in each series from the beginning to the end of the experiment.

Table IV. Sensitivity of heat-shocked wheat roots to high concentrations of sodium chloride

Heat shock	Living roots (%) NaCl added (%)				
	0.5	0.9	1.0	1.2	1.4
No	100	100	100	100	60
Yes	100	100	65	5	0

After the heat shock (or in the control series: no shock) the roots were placed for 5 min in the nutrient solution with NaCl added. Before and after the treatment with heat shock and NaCl the plants were incubated at 25°C.

These observations may indicate that the influence of kinetin on the sensitivity of cells to heat shock depends on how long the kinetin treatment has lasted and on the intensity of the metabolism of the cell. This may explain the observations reported here, that kinetin under certain conditions protects against and under others sensitizes to a supraoptimal environmental temperature.

Zusammenfassung. Die Hitzesensibilisierung von Weizenwurzelmeristemen kann durch Kältebehandlung oder Kinetinzugaben zumindest teilweise rückgängig gemacht werden.

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The Effects of Nicotinamide on Heat Resistance of Spores of *Bacillus cereus* T

Heat resistance is one of the most important characteristics of the bacterial endospore. Since the discovery of dipicolinic acid (DPA) in the bacterial endospores¹, evidence has been steadily accumulated to implicate DPA in the heat resistance of the bacterial endospores. The protection of some enzymes and proteins by DPA against heat denaturation²⁻⁴ has also been demonstrated. Indirect evidence has also been adduced through the production of DPA deficient spores sensitive to heat by using imbalanced media⁵, specific inhibitors⁶ and DPA less mutants⁷. In the latter two cases the effects could be reversed by exogenous DPA.

Recently UPRETI et al.⁸ have shown that picolinamide specifically inhibits DPA synthesis leading to the production of DPA deficient heat sensitive spores. The effect of picolinamide cannot be reversed by zinc. DPA was shown to activate the soluble reduced diphosphopyridine nucleotide (DPNH) oxidase of spores by electron accepting mechanism⁹ and also to protect it against heat inactivation². It was thought that picolinamide may be interfering with the role of DPA as an electron acceptor in the spore. Therefore a study of the effects of nicotinamide was undertaken.

Materials and methods. *Bacillus cereus* strain T was used throughout these investigations. The active culture technique was used and the organism was allowed to grow and sporulate in a glucose yeast extract-salts medium (G medium). Total viable counts (TVC) were made by plating suitable dilutions on nutrient agar. Octyl alcohol kills vegetative cells and germinated spores of

this organism. Octyl alcohol stable counts (OSC) were made by using octyl alcohol saturated water for dilution in making plate counts. Heat stable counts (HSC) were made by plating suitable dilutions, after first heating the suspension at 80°C for 30 min. The OSC gives the total number of spores while the HSC gives the number of heat resistant spores in the culture.

Results and discussion. The effects of nicotinamide and nicotinic acid on heat stability are given in Table I. The effects of exogenous DPA on nicotinamide induced heat sensitivity are given in Table II. The results show that whereas nicotinic acid has no effect, nicotinamide

Table II. Effect of DPA on the production of heat sensitive spores of *Bacillus cereus* T in the presence of nicotinamide

Addition to G medium	After 30 h incubation		
	OSC (ml)	HSC (ml)	HSC (%)
None (Control)	2.1×10^8	2.2×10^8	100
Nicotinamide (2 mg/ml)	1.9×10^8	5.0×10^5	0.26
DPA (0.5 mg/ml)	2.0×10^8	2.2×10^8	100
Nicotinamide (2 mg/ml) + DPA (0.5 mg/ml)	2.0×10^8	5.5×10^5	0.27

Table I. Effect of nicotinamide and nicotinic acid on heat stability of spores of *Bacillus cereus* T

Additions to G medium	After 30 h incubation ^a			
	TVC (ml)	OSC (ml)	HSC (ml)	HSC (%)
None (Control)	2.0×10^8	2.1×10^8	2.1×10^8	100
Nicotinamide (2 mg/ml)	2.1×10^8	2.2×10^8	6.0×10^5	0.27
Nicotinic acid (2 mg/ml)	1.0×10^8	1.0×10^8	1.0×10^8	100

^a Incubated on a rotary shaker at 30°C ($\pm 1^\circ$ C).

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¹⁰ H. O. HALVORSON, *J. appl. Bact.* **20**, 305 (1957).