

reversed by pressure. Ethylene at 12 lb inch⁻² (80×10^4 nl ml⁻¹) stimulated growth just as 100 nl ml⁻¹. Nitrous oxide at 80 lb inch⁻² (ref.¹⁴) was without effect and the application of 90 atmospheres of pressure merely caused the usual growth stimulation.

In conclusion it seems that growth effects caused in plants by ethylene are the result of a different mode of action from that involved in animal anaesthesia. If ethylene acts upon membranes in plants, it seems more likely that it interacts with protein receptors^{15,16} rather than with the whole lipid phase of the membrane^{16,17}.

Résumé. Nous avons tenté d'inverser les effets de l'éthylène sur la croissance des plantes par une pression accrue, ainsi qu'il est possible de le faire lorsque l'éthylène agit comme anesthésique chez les animaux. Nous n'avons découvert aucun renversement, ce qui implique une différence fondamentale entre la mode d'action de l'éthylène chez les animaux et chez les plantes.

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Dopamine and Noradrenaline in the Salivary Glands and Brain of the Tick, *Boophilus microplus*: Effect of Reserpine

Salivary secretion has been recognized as the major route of water excretion in the female cattle tick, *Boophilus microplus*¹. The mechanism by which salivary secretion is controlled is therefore of some interest to those concerned with the design of acaricides. Recent studies have revealed that the salivary glands of ixodid ticks are innervated by nerves containing dense core granules². It is being increasingly realized that, although cholinergic agonists such as pilocarpine cause salivary secretion in ticks¹ and insects³, these agents act indirectly³⁻⁵. It has, for example, been shown that while a number of organophosphorus insecticides cause salivary secretion in *B. microplus*, secretion can be blocked by pre-treatment with the catecholamine-depleting drug reserpine⁴. Adrenergic agonists are also known to stimulate tick salivary gland secretion both in vivo and in vitro^{4,5}. There is now good evidence that dopamine is the transmitter at a

number of insect salivary glands^{3,6-9}. It was therefore of interest to ascertain whether a catecholamine was present in the salivary glands of the tick as well.

Fed ticks (*Boophilus microplus Canestrini*) were obtained from the Wellcome Research Laboratories, Beckhamstead, England. Reserpinized ticks received 10 µl of physiological solution containing 10 mg/ml reserpine (Sigma Chemical Co.). Controls received physiological solution only. After 14 h, salivary glands and brains were dissected out, weighed and frozen on dry ice. Tissue from at least 7 ticks was required for each assay. The frozen tissues were then assayed for dopamine and noradrenaline by an enzymatic-radiochemical technique¹⁰ in which bovine liver catechol-*o*-methyltransferase is used to catalyse ³H-methyl transfer from ³H-S-adenosyl-methionine to dopamine and noradrenaline. The 3-methoxytyramine and normetanephrine formed are separated from the reaction mixture by organic extraction and from each other by paper chromatography. ³H-3-methoxytyramine and ³H-normetanephrine are eluted from the chromatogram and the radioactivity determined by liquid scintillation counting. Dopamine and noradrenaline are quantitated by comparison with standards carried through the above procedure. The results for tick salivary glands and brain are summarized in the Table.

Salivary glands and brain contain both dopamine and noradrenaline. Dopamine is the major catecholamine in the salivary glands of the tick as in other invertebrates,

Dopamine and noradrenaline in the salivary glands and brain of the tick. Effect of reserpine

| | Salivary glands | | |
|---------------|------------------------------|-----------------------|--------------|
| | Control | Reserpinized | Decrease (%) |
| Dopamine | 0.74 ± 0.04 (5) ^a | 0.18 (4) ^b | 76 ° |
| Noradrenaline | 0.45 ± 0.11 (3) | 0.07 ± 0.00 (3) | 85 ° |
| | Brain | | |
| | Control | Reserpinized | Decrease (%) |
| Dopamine | 0.32 ± 0.05 (4) | 0.21 ± 0.11 (3) | 34 N.S. |
| Noradrenaline | 0.36 ± 0.21 (4) | 0.22 ± 0.04 (3) | 39 N.S. |

^a Values shown are means (µg/g ± S.E.M., number of determinations in brackets) of determinations on salivary glands from at least 7 ticks. ^b 2 of the 4 determinations were below the detection level of the assay (0.11 µg/g). ° *p* < 0.05; N.S., not significant.

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but the presence of noradrenaline is interesting as this catecholamine has not been demonstrated in insect salivary glands^{9,11}. Pre-treatment of ticks with reserpine resulted in large decreases in salivary gland dopamine and noradrenaline levels while there was no significant effect on catecholamine levels in the brain. These results add weight for the model for insecticide-induced salivary secretion proposed earlier⁴ in which cholinergic agonists act indirectly to activate the adrenergic salivary gland nerves. In this model reserpine acts directly on the salivary gland nerves to deplete them of transmitter. Our results confirm that reserpine greatly reduces the catecholamine-content of the salivary glands. Thus although the lack of effect of reserpine on brain catecholamines is un-

explained, the results strongly suggest that catecholamines play a role in the regulation of salivary secretion in the ixodid ticks.

Furthermore, the fact that a catecholamine appears to be the transmitter at the salivary glands of ticks as well as at insect salivary glands^{3,6-9} suggests that catecholamines may be the transmitters at many arthropod salivary glands. Since cholinergic agonists induce salivary secretion in both ticks^{1,4} and insects³, the basic neuronal pathways involved may also be similar. Further work is underway to ascertain which of the catecholamines is the actual transmitter regulating the secretion of saliva.

Zusammenfassung. Dopamin und Noradrenalin wurden in den Speicheldrüsen und im Gehirn der gefütterten Zecken *Boophilus microplus* gefunden. Vorbehandlung der Zecken mit Reserpin führt zu einer starken Reduktion dieser Katecholamine in den Drüsen, hat aber keinen bedeutenden Einfluss auf den Dopamin- oder Noradrenalin Gehalt im Gehirn.

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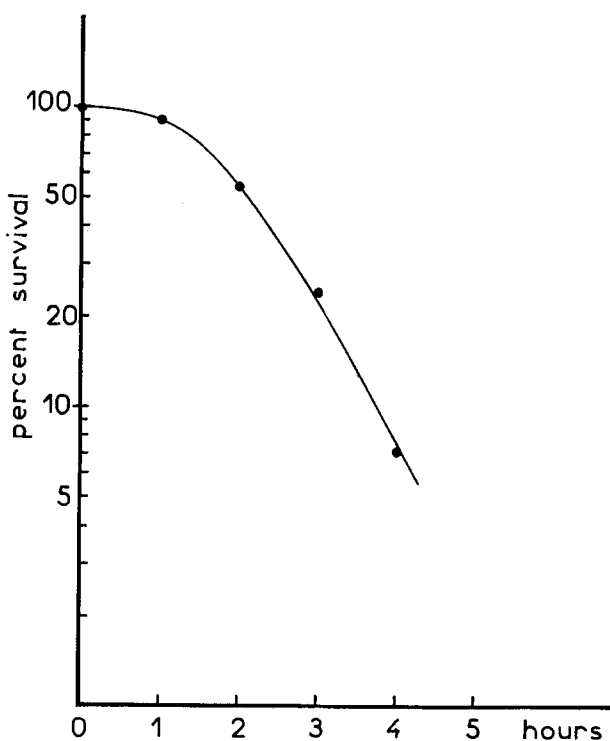
Morphology and DNA Synthesis of Cells Recovering from Thymineless Death in *E. coli* K₁₂

Since its discovery in 1954¹, thymineless death in thymine-requiring bacteria has been studied extensively. In the absence of thymine, cytoplasmic syntheses proceed although DNA synthesis has stopped. Some experiments suggest that the DNA of starved cells is altered: the transforming activity of *B. subtilis* DNA decreases during starvation² and the ability of *E. coli* DNA to serve as a template for RNA polymerase is

reduced³. This might be related to some changes in the DNA, such as breaks in the molecules. Indeed, single-strand breaks have been found in relation with a stimulation of a nuclease in *E. coli* undergoing thymineless death⁴. There is however no necessary relationship between viability loss and DNA breakage⁵.

Direct observation of the cells shows that they grow in length during thymine starvation and during the predivision recovery period⁶. We then studied the incidence of cell size on cell survival and we have observed newly synthesized DNA in individual starved cells after addition of radioactive thymine by means of light microscope autoradiography.

Materials and methods. Experiments were performed on *E. coli* nonlysogenic K₁₂ Thy-Arg-Thi⁻. Bacteria were inoculated in phosphate salt minimal medium supplemented with thymine (2 µg/ml), thiamine (1 µg/ml) and arginine (30 µg/ml). Cells grown exponentially (5 × 10⁷ cells/ml) were centrifuged, washed and resuspended in the same medium without thymine. Samples were taken at different times to determine the number of viable bacteria and to make microscopic observations of individual cells. Cells were spread onto complete solid



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Fig. 1. Effect of thymine starvation on viability of *E. coli* K₁₂ Thy-Arg-Thi⁻.