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 earlier[4] 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 unexplained, 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 insects[3], 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 Noradrenalingehalt im Gehirn.

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Morphology and DNA Synthesis of Cells Recovering from Thymineless Death in *E. coli* K_{12}

Since its discovery in 1954[1], 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 starvation[2] and the ability of *E. coli* DNA to serve as a template for RNA polymerase is reduced[3]. 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 death[4]. There is however no necessary relationship between viability loss and DNA breakage[5].

Direct observation of the cells shows that they grow in length during thymine starvation and during the pre-division recovery period[6]. 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 K_{12} Thy⁻Arg⁻Thi⁻[7]. 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 (5×10^7 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

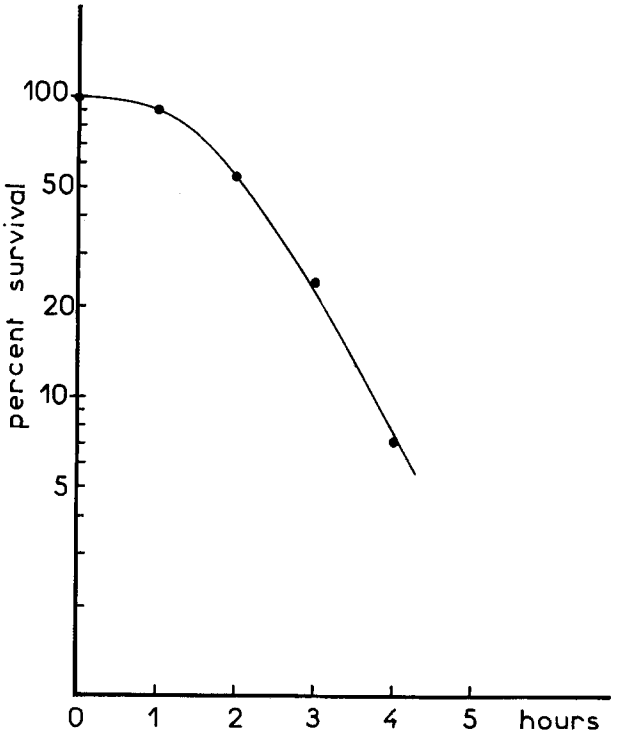

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Fig. 1. Effect of thymine starvation on viability of *E. coli* K_{12} Thy⁻Arg⁻Thi⁻.

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medium in a Petri dish placed under the microscope in a 37 °C room. Observations were made every 15 min to follow the evolution of each cell. Tritiated thymine (20 μCi/ml, specific activity 25 Ci/m*M*) was added either at the time of inoculation or after 3 h of starvation, and aliquots were taken for cell fixation during starvation or recovery. The techniques of light microscope autoradiography described by CARO and VAN TUBERGEN[8] were followed. Ilford L4 emulsion was used and development was made after an 8-day exposure. Photographs were taken at a fixed magnification of ×650.

Results and discussion. The effect of thymine deficiency on the viability of Thy⁻ cells is shown in Figure 1. After 3 h of starvation, the cell viability decreases by 77%. The loss of viability is accompanied by an increase in the cell length. Filaments up to 4 times the size of unstarved cells were found, while there is a decrease in the number of small cells. We then distributed the cells into 4 classes (Table, a). Surviving cells are found among each size group, but there is no strict correlation between cell size and cell survival (Table, b). After 2 h of starvation, intermediate length cells (2c–3c) have a higher percentage of recovery than either long filaments or small cells.

Cell size and cell survival after thymine starvation of *E. coli* K_{12} Thy⁻Arg⁻Thi⁻

Thymine starvation (h)	a) Cells in each group (%)		b) Colony-forming cells in each group (%)
0	c	90	85
	2c	10	90
2	c	55	55
	2c	27	68
	3c	13	75
	4c	5	60
3	c	29	17
	2c	38	30
	3c	20	27
	4c	13	13

4 groups have been distinguished at the time they are plated, according to their length. The unit of length is the size of an unstarved cell, c = 3 μm. 300 bacteria have been observed for each sample.

Some small cells never divide, whereas some filamentous cells (4c or greater) undertake a first division giving rise to a non-viable small cell and a remaining filament which will generate at the second division a small cell which multiplies actively. These results suggest that death is not restricted to filamentous cells and can occur even without any elongation.

To correlate cell division with synthesis of DNA in individual cells, we performed autoradiographs of starved cells recovering in the presence of thymine. Tritiated thymine was added to unlabelled 3-h-starved cells during 90 min. The average number of grains was 7 grains per labelled cell; 150 out of 192 cells showed grains i.e. 78% of the population were making new DNA. However, the number of viable cells determined in the culture at the time radioactive material was added, was 23% of its initial value. This indicates that death is not the result of a definite block of DNA synthesis but that cells which resume DNA synthesis do not always recover. Part of this resumed synthesis could be non-conservative[9] suggesting a repair replication.

Autoradiographic grains were found clustered in the center of the cells (Figure 2a). By the time of the first division of one filament, the radioactive material had not yet filled the filament and the small cell attached did not contain any grain (Figure 2b). After further incubation, grains appeared in empty regions along the cell length and the second division took place, giving grain-covered cells (Figure 2c). Autoradiographs extended the direct observation of the dividing filament: the cell issued from the first division, which did not contain any newly synthesized DNA, did not divide. These mini cell-like bacteria[10] might have some of the original DNA. Exponentially grown bacteria were exposed to ^{3}H-thymine and washed free of radioactive label during thymine deficiency. After 3 h of starvation, cold thymine was added to the medium. Samples were removed for autoradiography during starvation and during recovery.

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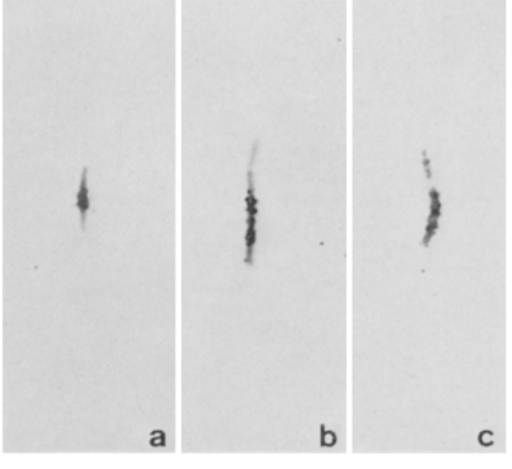

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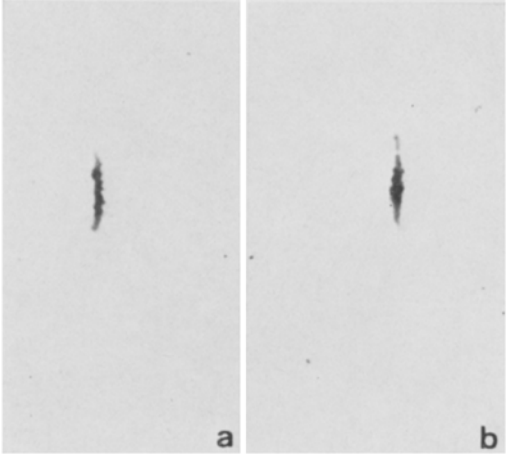

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Fig. 2. Localization of de novo synthesized DNA in cells recovering from thymineless death. Autoradiographs are taken 15 min (a), 45 min (b) and 90 min (c) after addition of ^{3}H-thymine to 3 h-starved cells. ×3000.

Fig. 3. Localization of original DNA in cells recovering from thymineless death. Autoradiographs are taken 0 min (a) and 45 min (b) after addition of cold thymine to 3 h-starved cells prelabelled with ^{3}H-thymine during exponential growth. ×3000.

During the 3 h of starvation, the distribution of radioactive material spread as the cell length increased (Figure 3a). A similar picture was given by electron microscopic observation of *E. coli* 15 T⁻ undergoing thymine deprivation[11]. During recovery, we observed that the number of grains migrating towards the end of the filament is so small that the presence of original DNA in the small cell is uncertain (Figure 3b). Anyway, after this first abortive division an extra cell division is needed to generate a complete living cell, suggesting that damage caused by thymine starvation has been repaired.

Résumé. Dans un milieu dépourvu de thymine, des bactéries (*E. coli*) exigeant la thymine pour leur croissance s'allongent, sans qu'il ait cependant de relation directe entre la longueur atteinte et le taux de mortalité. Toutes les cellules dont la synthèse d'ADN reprend après addition de thymine ne donnent pas obligatoirement de colonies, suggérant ainsi que la mort par carence en thymine n'est pas la conséquence du blocage définitif de la synthèse d'ADN. Pour certains filaments, ce n'est qu'à la seconde division que se forme une cellule contenant de l'ADN néoformé capable de se multiplier activement.

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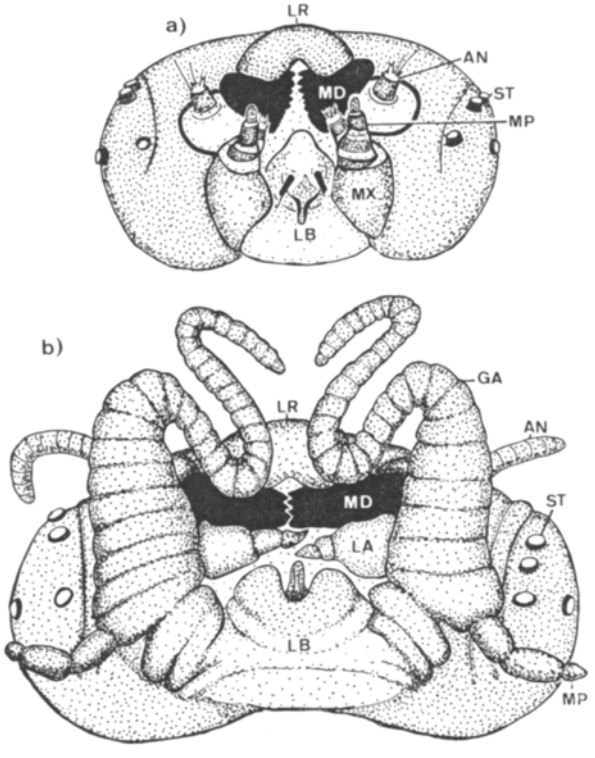
Homoeötic Transdetermination Caused by Juvenoid in Larvae of *Pieris brassicae* L.[1]

In the course of normal development of insects, all cells become 'determined', i.e. committed to form a given pattern, organ or part of it. During this process the cells or organs change from a stage of high morphogenetic potency, when they are still capable of 'regulation', to the 'mosaic' state with a narrowly restricted morphogenetic potency. Determination takes place in an orderly sequence, and long before the growth and differentiation processes put the morphogenetic plan into effect, some cells being determined at an early, others at a later stage of ontogenesis. However, determination is not always absolute. Under experimental conditions, transdetermination is possible; i.e. one cell type can be transformed into another[2,3]. A familiar example of transdetermination is homoeösis or 'heteromorphous' regeneration, i.e. the replacement of an appendage by one belonging to another region of the body, e.g. regeneration of a leg in the place of a burnt or amputated antenna[4,5]. As WIGGLESWORTH[6] points out, transdetermination is a natural phenomenon at metamorphosis of the Hemimetabola, when the epidermal cells lay down a different cuticle and form different structures in the absence of juvenile hormone[7]. It is commonly believed that the transdetermination of a cell consists in the activation of new sets of genes.

Experiments on the action of juvenoids applied topically to last instar larvae of *Pieris brassicae* have been described in a recent publication[8]. In the course of those experiments a large number of treated larvae underwent an abortive supernumerary larval moult[9]. These insects were not able to ecdyse the old head capsule properly. However, it could be removed with forceps.

The head appendages of all individuals had more or less differentiated in the pupal/adult direction, as shown in Figure 1b. Though the mandibles had maintained their larval character, the antennae were much longer than the larval antennae and of different shape (compare with Figure 1a). The most characteristic feature of these



[1] Ro 20-3600 = isomere mixture of 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)-phenoxy]-2-nonene. The author thanks Hoffmann-La Roche Ltd., Basle for a sample of the juvenoid.
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[9] For details concerning rearing of insects and application of juvenoid the reader is referred to the afore mentioned paper[8].

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Fig. 1. a) Head and mouth parts of normal 5th instar larva of *Pieris brassicae*. b) Same of abnormal 6th instar larva with large maxillae (long galeae and relatively large laciniae and palpi) and elongated antennae. Magnification in a) and b) is the same. AN, antennae; GA, galea; LA, lacinia; LB, labium; LR, labrum; MD, mandible; MP, maxillary palpus; MX, maxilla; ST, stemmata (larval eyes).