observed as evaluated by the serum dopamine-beta-hydroxylase activity in man[7]. Nevertheless, the serum level of this enzyme has not been generally accepted as a measure of the peripheral sympathetic activity.

Experiments with repeated ECS show an evidence of diminished response of the peripheral sympathetic nervous system to repetition of ECS in man, but some other factors (degradation and/or depletion of catecholamines) or a specific reaction in schizophrenia could not be excluded.

It is interesting that with as few as 4 exposured to ECS the response of the sympatho-adrenal medullary system to such an intensive stimulus was diminished. In animal experiments a diminished response of plasma catecholamines to repeated immobilization was observed in blood collected by chronically indwelling catheters[8] but in rats that had been decapitated[9], which represented a new stimulus for the animals, a significant increase of plasma catecholamines was found. The capacity of the adrenal medulla of repeatedly stressed rats is greatly increased[10] but plasma catecholamine levels are reduced suggesting that the adaptation to a given stimulus is not a question of the adrenal medullary responsiveness but might be mediated by a decreased brain or spinal cord activity. Moreover, ECS might also be expected to increase the availability of NA at the synapse and could initiate a neurochemical adaptation at the receptor level[11].

On the basis of our experimental data a statement could be made that repeated ECS induces a decrease in plasma catecholamine response in man. The mechanism of this adaptation is not clear yet.

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Molting processes and hormonal control: an in vitro model

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Summary. In vitro cuticular deposition by larval epidermic material depends on hormonal conditions. Quantitative and qualitative variations are reported according to defined stimulations. Possible specific actions of each hormone are discussed.

We have investigated the in vitro development of the abdominal integument of the 4th larval instar of the locust *Schistocerca gregaria.* 3 principal events occur under our experimental conditions[1-3]: 1. cuticular deposition, 2. cellular alterations, 3. cellular differentiation. This paper summarizes the different secretory activities and the intensity of cellular alterations obtained with epidermic material under defined hormonal conditions.

Material and methods. Abdominal explants are excised aseptically and cultured in a hormone-free medium[4] or in medium with synthetic hormones (alpha and betaecdysone, juvenile hormones (C 18 JH 1, C 17 JH 2 and C 16 JH 3). Co-cultures of integument and endocrine glands (prothoracic glands and corpora allata) are also performed. All the experiments including. JHs are performed in vessels coated with 1% siliclad to avoid adsorption on the walls. The explants are cultured for a week and then fixed for ultrastructural study.

Results and discussion. Cultures in a hormone-free medium (figure 1). In a hormone-free medium[1,5], after a critical period (CP) 2 types of cuticular secretion are displayed. This CP lasts until between the 4th and the 5th of the 7 days of the 4th larval instar. Before the end of this CP, epidermic cells deposit a procuticular material but never new epicuticle that would prove that a new cuticular cycle has started. If cells adhere to the old cuticle, the material proves to have a lamellar organization; otherwise, it becomes less abundant and cells deposit a fibrillary material which is randomly disposed. These results are in accordance with those of Miciarelli et al.[6]. Every alteration produced by hormonal stimulation is determined after comparison with a 2-day-old explant which is considered as a control.

Actions of synthetic ecdysteroids. Alpha and bete-ecdysone have been applied in concentrations increasing from 0.001 to 15 µg/ml[7]. With such stimulation the epidermis can overcome the CP and deposit a new cuticle composed of epicuticular layers and under-lying procuticular material. In the constant experimental time of a week, the different secretory events and alteration intensities are shown in figure 2. With alpha ecdysone, alterations are already at a maximum with 0.001 µg/ml and remain in such a state as the dose increases. The CP is overcome with doses of 2–5 µg/ml. With beta ecdysone, partial alterations take place with doses from 0.001 to 0.5 µg/ml and decrease as doses increase. The CP is overcome with only 0.1–0.5 µg/ml. With doses above 1 µg/ml the CP is repeatedly overcome and the epidermis deposits several cuticles.

At physiological concentrations, alpha ecdysone is particularly effective in inducing the preparatory processes of molting. Beta ecdysone displays a qualitatively similar but slower and less active response. In addition, beta ecdysone increases the quantity of secreted material and allows the epidermis to initiate a new cuticular secretion cycle. Such events are not noticed with alpha ecdysone.

Actions of juvenile hormones (C 18 JH 1, C 17 JH 2 and C 18 JH 3). The range of concentrations studied is 0.001–0.100 µg/ml. Doses above 0.100 µg/ml provoke an immediate destruction of cells. JH 2 and JH 3 have an inhibitory effect on cuticular deposition and on the processes of cellular alteration. Contrary to this, JH 1 mainly stimulates the procuticular secretion activity at a dose of 0.050 µg/ml. At the same time, significant detachments occur and the epidermis shows the same alterations as in a pre-apolysis stage in vivo.

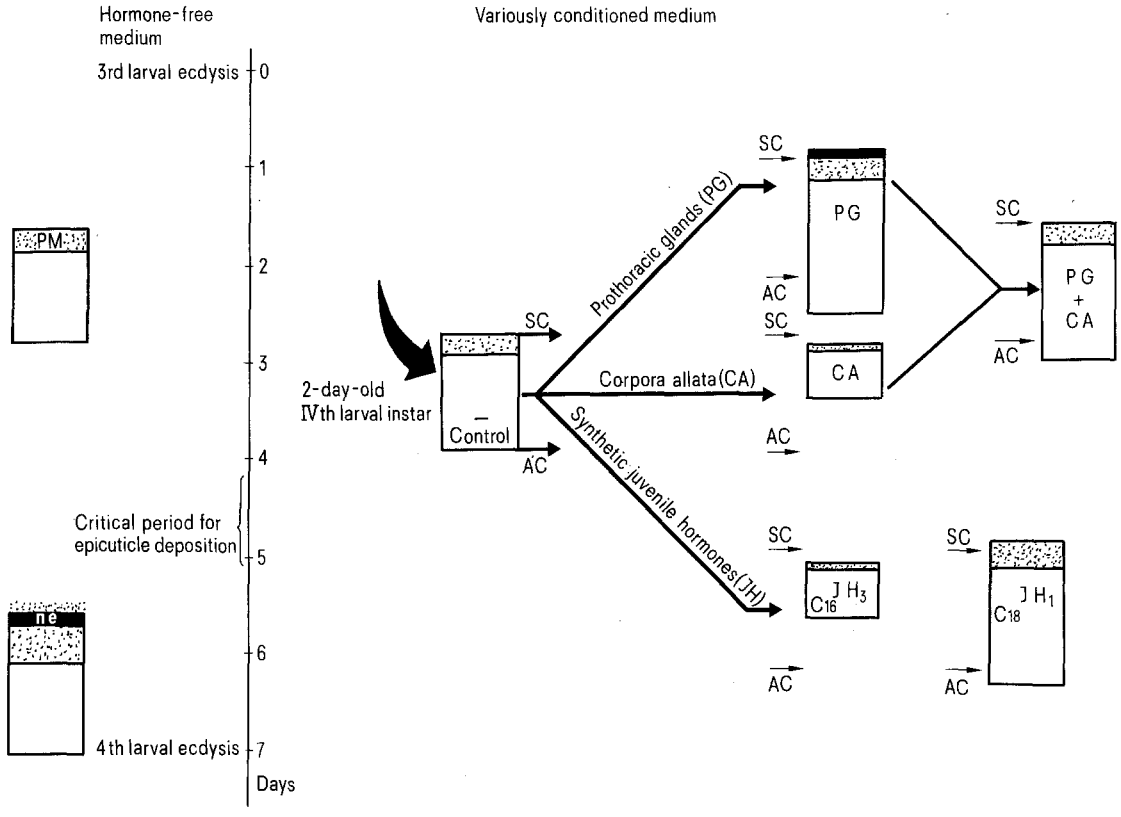

Fig. 1. Culture of larval epidermis of the 4th larval instar of *Schistocerca gregaria*. In a hormone-free medium, epidermis deposits only a procuticular material (PM) before the end of a critical period (CP). Later on, a new epicuticle (ne) is inserted into the procuticular material. A 2-day-old epidermis (before CP) is used as a control and permits us to appreciate the alterations obtained in conditioned medium. 2 parameters are studied with respect to control of cuticular secretion and cellular alterations of the epidermis. SC (secretion control) and AC (alterations control) are reported in each experiment. PG = prothoracic glands; CA = corpora allata; JH 1 and JH 3 = C 18 and C 16 juvenile hormone.

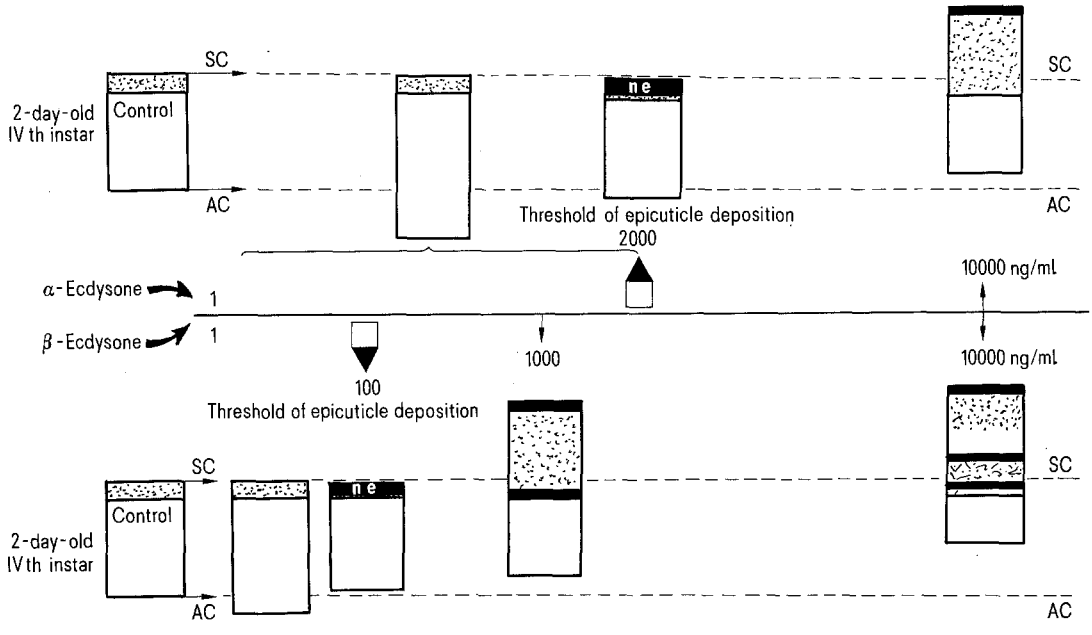

Fig. 2. Actions of alpha and beta ecdysone on the cuticular secretion and cellular alterations. Still with a 2-day-old epidermis control, this diagram shows the alterations obtained with a range of various concentrations of alpha and beta ecdysone. The critical period is overcome with 2000 ng/ml of alpha ecdysone and with 100 ng/ml of beta ecdysone. The height of cells and secretions allows a comparison of hormonal stimulation effects with AC and SC.

Actions of medium conditioned by the secretion of endocrine glands (figure 1.) - Prothoracic glands (PG). PGs excised from 5- or 6-day-old 5th instar larvae induce a fast and intense development of cellular alterations, and a new epicuticle is deposited on a fibrillary procuticular material. The titre of ecdysteroids released into the medium never exceeds 50 ng/ml with 2 pairs of PGs[8]. However, 50 ng/ml of synthetic alpha ecdysone is not capable of inducing a new cuticular cycle. The whole process suggests another role for PGs involved in cuticular secretory mechanisms.
- Corpora allata (CA). A medium conditioned by CA from mature males or larvae[11] does not initiate secretory activity or cellular alterations. The result of the presence of corpora allata is an inhibition of all the events observed in a hormone free medium.
- Corpora allata and prothoracic glands. This association reduces the intensity of the cellular alterations induced with PGs alone. The secretory activity is not completely inhibited as it is with CA alone. A sparse fibrillary material is deposited after detachment of the old cuticle.
The epidermal cell development in vitro suggests that a defined hormonal context induces various secretory activities (with qualitative and quantitative variations) and various intensities of cellular alterations. The in vitro model appears to be a good tool for studying the mechanisms involved in molting processes. Alpha ecdysone and beta ecdysone have specific effects at physiological doses: - alpha ecdysone induces cell alterations, - beta ecdysone induces cuticular secretion.
PG which release alpha ecdysone[9,10] turns out to be as favorable as possible for total performance of preparatory alterations. Another role of PG is possible with regard to cuticular secretion in spite of low doses of alpha ecdysone released. Corpora allata which release C16 JH3[11] have inhibitory effects.
Preliminary results show that JH 1 and JH 3 have specific effects upon cuticular activity. They alter the threshold of response to alpha and beta ecdysone. With a low concentration of beta ecdysone, JH 1 reduces the threshold of secretion (0.050 µg/ml) and JH 3 enhances it (200–500 µg/ml). The numerous epicuticles induced by high doses of beta ecdysone are regulated by JH 3 so that the epidermis deposits only one perfect cuticle.

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PRO EXPERIMENTIS

FITC-dextrans as fluorescence and electron microscopic tracers in studies on capillary and cell permeability of the CNS

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Summary. FITC-dextrans can be detected at the ultrastructural level as round electron dense particles, with no sign of distortion or aggregation. Because this tracer is a stable, non-toxic polymer of biological origin obtainable in a wide range of molecular weights, it may be useful for further studies of tissue permeability.

Fluorescein isothiocyanate labelled dextrans (FITC-dextrans[2]) have been used for direct observation of vascular permeability in living animals. Svensjö et al.[3] examined the microvasculature in hamster cheek pouches and Nakamura and Wayland[4] studied the macromolecular transport in the cat mesentary.

Investigations of vascular permeability in the nervous system are usually carried out by injecting a tracer i.v., for instance fluorochrome labelled albumin[5]. For electron microscopic observations other tracers have been used, such as ferritin[6] (diameter ~ 100 Å) or peroxidase. The presence of the peroxidase can be detected by histochemical reactions and the deposition of an electron dense reaction product[7]. The variability in molecular size and chemical structure reduces the usefulness of these tracers.

Non-labelled dextrans were used in electron microscopic studies of the intestine and kidney[8-10]. Olsson et al.[11] and Tervo et al.[12] reported on light microscopic studies in the nervous system with FITC-labelled dextrans. FITC-dextrans are stable[12], non-toxic polymers obtainable in a wide range of molecular weights. It would be advantageous if this tracer could be utilized for both light microscopy and electron microscopy.

Material and methods. 10 adult, randomly-bred NMRI mice were used. A 10% FITC-dextran 150 (Pharmacia Fine Chemicals, Sweden) solution was applied under anaesthesia either into the tail vein (125 mg/kg) or by a stereotactic injection (David Kopf Instruments) into the left telencephalic ventricle (50 mg/kg), near the foramen interventriculare.
5 or 30 min after injection, the animals were sacrificed by a perfusion of formalin solution 1:9 for fluorescence microscopy (FM) or with phosphate-buffered 1% glutaraldehyde for electron microscopy (EM). The samples of brain for FM were excised immediately and frozen in liquid nitrogen, then sectioned in a cryostat. The 6-µm-thick sections were mounted in 50% glycerin in water and viewed under a Leitz fluorescence microscope with a high pressure mercury lamp (filters BG 12 and K 510).