SG and thoracic ganglia play only a negligible and little understood role[5,7].

We can conclude that release of the prothoracicotropic hormone occurs in response to integration of stimuli transmitted largely via the nerve cord. For example, immobilization of wandering larvae caused that only 22% of the insects pupated in 17.8±3.3 days, but when the connectives between brain and SG were cut, 50% of the insects pupated in 11.3±3.6 days. Significance of individual ganglia changes during the development. The complex of brain and SG is indispensable for the food intake and its damage before the larvae finish obligatory feeding is lethal[4]. Removal of SG or cut between brain and SG were lethal also when performed at the end of the feeding period in the penultimate instar (table 2). We have no explanation for this effect. Transection of the nerve cord between T_3 and A_1 inflicted during the penultimate or at the start of the last larval instar prolonged the respective interecdysial period less than when inflicted to wandering larvae. Larvae subjected to this operation in the penultimate instar mostly underwent a supernumerary larval ecdysis, apparently as a result of suppressed body growth; starvation readily induces extra larval moults in *Galleria*[8]. High importance of the integrity of the last thoracic and the 1st few abdominal ganglia in wandering larvae may be related to the fuse of these ganglia during the larval-pupal transformation[9].

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Lack of relationship between Langerhans cells, epidermal cell proliferation and epidermal G1 chalone

J. Schweizer* and F. Marks

German Cancer Research Center, Institute of Biochemistry, Im Neuenheimerfeld 280, D-69 Heidelberg (Federal Republic of Germany), 16 March 1978

Summary. Mouse tail interscale epidermis contains numerous Langerhans cells, whereas the adjacent scale regions are devoid of these cells. No difference in a) proliferative activity and b) inhibitory effect of the epidermal G1 chalone can be demonstrated in both regions. A direct relationship between Langerhans cells and growth control may be excluded.

Investigations in the past years have adequately demonstrated that the high-level dendritic Langerhans cells in the epidermis constitute an active, self-maintaining cell population[1], which is not related to the pigmentary system[2,3] but whose biological significance is not yet entirely understood. Since there seems to be an inverse relationship between the frequency of Langerhans cells and the proliferative rate of the corresponding tissue[4-7], they have been supposed to be involved in the control of proliferation or tissue homoestasis in keratinizing epithelia[3].

A similar function has been attributed to a family of endogenous growth inhibitors, the chalones[8]. It has been suggested, therefore, that Langerhans cells may synthesize and secrete chalonelike epidermal growth inhibitors[5,9]. In this context, especially the Langerhans cell granule has been supposed to be the site of production of those inhibitors, since the changes in their frequency per cell could be correlated to the kinetics of cell proliferation during wound healing in epidermis[10]. According to the original chalone concept, the increased proliferative activity in the vicinity of wounds is thought to be due to a local depression of the chalone level[8]. Also, after wounding the relative frequency of Langerhans cells is transiently decreased[10] and the normal frequency is attained only when the epidermal continuity has been reestablished[11]. Furthermore, both Langerhans cells and the chalones are encountered only in keratinizing epithelia[12,13]. Mature, granule-containing Langerhans cells are located predominantly in the suprabasal layers of keratinizing tissues, and the epidermal G1 chalone which controls the G1-S transition is apparantly concentrated in the same region[14].

These considerations seemed sufficiently plausible to investigate whether a direct relationship between Langerhans cells and the epidermal G1 chalone which is thought to be the physiologically more important of the 2 epidermal chalones[8], could be proved. Since it is not possible to separate Langerhans cells from keratinocytes to check their possible chalone content, a more indirect approach had to be made. Recently, we were able to show that adult mouse tail epidermis consists of a regularly alternating pattern of parakeratotic scale resions without Langerhans cells and orthokeratotic interscale regions in which Langerhans cells are abundant[6] (figures 1-3). Provided there is any relationship between Langerhans cells and growth control, especially the chalone mechanism, one should predict a measur-

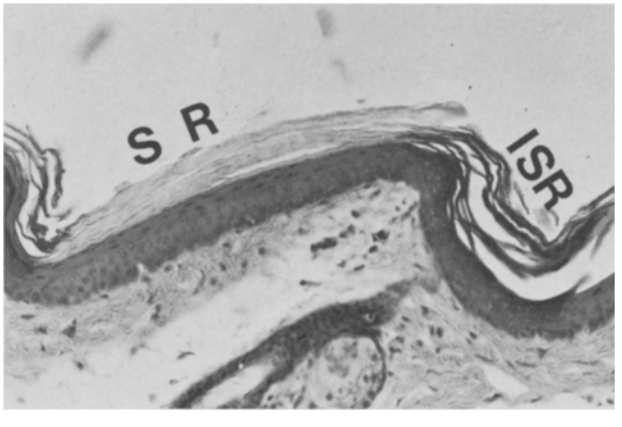

Fig. 1. Vertical section through adult mouse tail skin (H&E; × 110). SR: Langerhans cell-free parakeratotic scale region. ISR: Langerhans cell-containing orthokeratotic interscale region. Note the clear-cut transition between the 2 areas, which can be followed up to the keratin layer.

able difference in the proliferative activity in both regions as well as possibly a difference with respect to the response to exogenous chalone.

As shown in the table, the labelling index (LI) in the scale regions seems to be slightly elevated when compared with the total LI and the interscale LI. However, statistical analysis showed the difference not to be significant ($p<0.05$). It may, therefore, be concluded that the turnover rate in both compartments is essentially the same. To evaluate the effect of the epidermal G1 chalone, DNA-labelling was performed 6.5 h after i.p. injection of chalone preparations from pig skin and mouse epidermis, i.e. at a time when chalone-dependent inhibition of epidermal DNA synthesis in tail epidermis reaches its maximum[15]. Regardless of the source of the chalone fraction used, an overall depression of about 40–45% was observed (table). This value is consistent with that determined on the basis of specific radioactivity (table and Bertsch et al.[15]). Contrary to the situation in mouse back epidermis[15], the depression could not be increased by raising the chalone concentration, and could tentatively be interpreted as a selective inhibition of only distinct parts of the tail epidermis. However, separate determination of LI in the scale and interscale regions clearly revealed no difference in the inhibitory effect of the G1 chalone in the Langerhans cell containing interscale and the Langerhans cell-free scale regions (table).

Based on these results, it may be concluded that Langerhans cells are neither actively engaged in the synthesis of the epidermal G1 chalone nor in an overall control of epidermal proliferation. Even a supply of the scale regions with the possibly diffusible chalone from the Langerhans cell-containing interscale regions seems to be improbable, since no gradient in proliferative activity could be observed from the edges to the centre of the scales. Our conclusions are further substantiated by the fact that an active G1 inhibitor can be extracted from chick epidermis (S. Bertsch and F. Marks, unpublished). Langerhans cells, however, have not yet been demonstrated in avian epidermis[2]. The apparent parallelism in the decrease of proliferative activity and the appearance of Langerhans cells during embryonic development of mouse skin can hardly be explained in

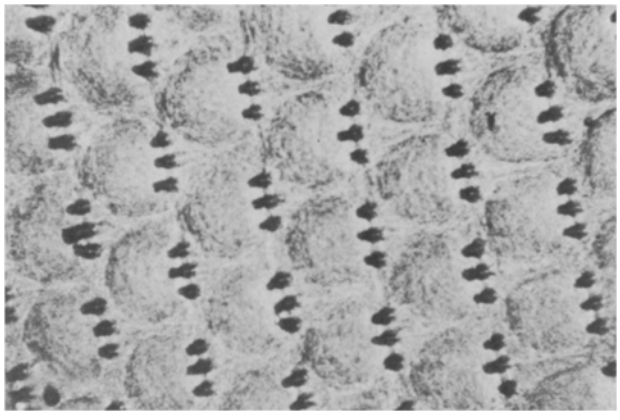

Fig. 2. Whole mount of separated tail epidermis of adult mouse (H; × 40). Epidermis was separated from dermis by means of 1% acetic acid (15 h/4 °C) and stained free floating. Parallel rows of clearly outlined scales, separated by interscale areas are visible. Follicular triads are located under each scale.

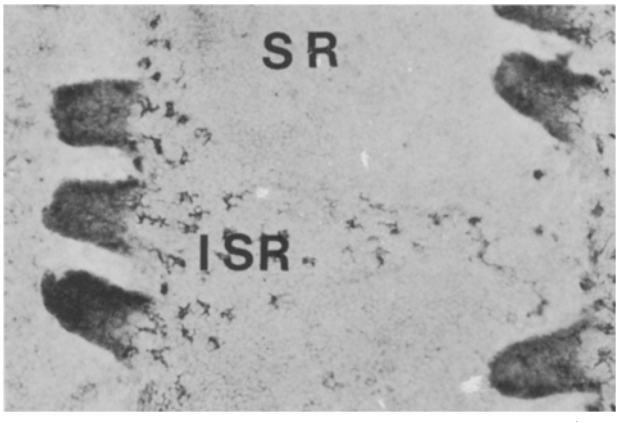

Fig. 3. Whole mount of separated tail epidermis of adult mouse (ATPase-staining; × 110). For ATPase-staining technique, see Schweizer and Marks[6]. ATPase-positive dendritic Langerhans cells are exclusively located in the interscale regions.

DNA-labelling and effect of epidermal G1 chalone in mouse tail epidermis in vivo

Epidermal region	Control	Pig skin chalone	Inhibition (%)	Mouse epidermis chalone	Inhibition (%)
Total tail epidermis	83.99 ± 16.4	47.12 ± 12.23	44	45.70 ± 14.47	46
Tail scale epidermis	94.83 ± 12.84	58.57 ± 8.43	38	54.17 ± 10.39	43
Tail interscale epidermis	78.99 ± 9.73	44.56 ± 7.19	44	48.02 ± 11.71	39
Specific radioactivity of isolated DNA (cpm/μg)					
Total tail epidermis	52.84 ± 5.85	33.17 ± 4.61	37	37.48 ± 6.72	39

3 groups of 5 mice each were used (strain NMRI, females, 7–8 weeks old). The chalone preparation used was a 50–72% EtOH precipitate of an aqueous extract from pig skin (code number CH 1332; kindly supplied by Dr W. Hondius Boldingh, N.V. Organon, Oss. Holland) which had been shown to be enriched in G1 chalone activity[8]. Alternatively, a G1 chalone preparation from pure mouse tail epidermis was employed (PM 10-ultrafiltratet 105,000 × g supernatant of an aqueous extract from isolated tail epidermis). 1 mg of this fraction depressed DNA-labelling in mouse back skin in vivo to approximately 50% (J. Schweizer and F. Marks, unpublished). Both chalone preparations were dissolved in saline and doses of 0.9 mg (pig skin chalone) and 2 mg (mouse tail chalone) per animal, respectively, were injected i.p. at 09.00 h. Control animals received only saline. At 15.30 h 50 μCi (methyl-^{3}H)-thymidine (specific activity 6.7 Ci/mmole, New England Nuclear, Boston, Mass.) were i.p. injected. 1 h later (16.30 h) the animals were killed and pieces of tail skin of about 1 cm^2 were fixed in 10% formalin, containing 0.1% of unlabelled thymidine. Labelling index (LI) was determined in 3–5 μm sections by the stripping film method using Kodak AR 10 plates. Samples were exposed for 2 weeks, developed and stained with heamatoxylin-eosin.

LI was expressed as the number of labelled cells per 1000 basal cells. A cell was considered to be labelled if the grain count over the nucleus was 5 or more. LI in total, scale and interscale epidermis of each experimental group was determined independently in the same sections. Interscale regions in which the plane of section had passed through a hair follicle were excluded from counting.

LI values indicated represent the mean values of counts in 20 sections (4 sections/animal/group).

Simultaneously, 3 further groups were included in which DNA labelling was measured by means of specific radioactivity of isolated epidermal DNA as described elsewhere[15]. Experimental conditions were the same exept that the mice received only 30 μCi of radioactive thymidine.

terms of the chalone hypothesis, since fetal and newborn epidermis, although rich in Langerhans cells[6] and active G1 inhibitor, does not respond at all to the G1 chalone[16]. Finally, there is a large body of evidence that Langerhans cells originate from the mesenchyme rather than from ectodermal tissue; and this is certainly not in accordance with the concept that chalones are growth regulators which are synthesized by the same tissue they act upon.

* Present address: German Cancer Research Center, Institute of Experimental Pathology, Im Neuenheimer Feld 280, D-69 Heidelberg.

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Topographic localisation of insulinogenic and insulinoprival areas in the hypothalamus

S.K. Garg[1], G.S. Chhina and Baldev Singh

Department of Physiology, All India Institute of Medical Sciences, New Delhi - 110016 (India), 17 January 1978

Summary. Electrical stimulation of hypothalamic areas through stereotaxically implanted electrodes were carried out in conscious male rhesus monkeys. There was a significant increase in immunoreactive insulin (IRI) following lateral hypothalamus (LHA) stimulation. An opposite response was obtained from ventromedial hypothalamus (VMH). Thus, insulinogenic and insulinoprival responses were obtained from feeding and satiety, suggesting a significant role of insulin in the regulation of food intake.

Hypothalamic stimulation produces changes both in food intake and endocrinal-metabolic activity. The feeding and satiety behaviours are influenced by LHA and VMH[2]. The earlier studies have either been done to determine hypothalamo-hypophyseal interactions or hypothalamo-metabolic regulations[3-11]. The present study was undertaken to investigate the serum insulin (IRI) response following electrical stimulation of these as well as other areas of the brain.

Materials and methods. 8 male rhesus monkeys *(Macaca mulatta)* weighing between 5 and 7 kg were observed for 15 days in an air-conditioned laboratory maintained at a temperature of 26±1 °C. The monkeys were given a synthetic diet supplied by Hind Lever Ltd, India, which essentially has a carbohydrate content of 73.6%, protein 11.3% and fat 9.0%. The animals were restrained daily in a primate chair for a period of 1 h till they ceased to display signs of struggle or uneasiness. Subsequently, the blood samples were drawn after an overnight fast on alternate days for the determination of serum insulin.

Bipolar varnish insulated stainless steel electrodes made from 26-gauge were implanted stereotaxically in different parts of hypothalamus and cerebral cortex; the methodology has been discussed earlier[7]. The animals took 4-5 days to recover from the effects of surgery. Subsequently, the estimations of fasting serum insulin were obtained to confirm that these were in the preoperative range.

Electrical stimulation of VMH, LHA, preoptic (Po) and cerebral cortex (CC) was carried out on alternate days and changes in serum insulin were investigated. The stimulation was carried out for 30 min, using square wave pulses of 5.5-7.0 V, 0.5 msec duration and a frequency of 75 cycles/sec. The blood samples were drawn by putting an indwelling catheter in the saphenous vein before, during, immediately after and 120 and 210 min following the termination of electrical stimulation. Serum insulin was measured by radioimmunoassay[12], using dextran coated charcoal for separation of free and bound hormone. After the completion of the experiment, electrolytic lesions were produced

Effect of electrical stimulation of different hypothalamic areas and cerebral cortex on serum levels of insulin (μU/ml) in normoglycaemic monkeys

Time in min	0	10	20	30	120	210
Lateral hypothalamus	21.65±6.0 (8)	26.2±8.17 (6)	26.0±8.0 (6)	33.37±5.77** (8)	46.36±12.0* (8)	24.2±4.5 (8)
Ventromedial hypothalamus	38.7 ±5.2 (7)	32.0±2.0 (6)	33.6±7.2 (6)	29.5 ±4.8 (7)	26.1 ± 5.2* (7)	24.0±5.0* (7)
Preoptic	27.6 ±3.8 (6)	23.9±5.6 (5)	21.8±5.3 (5)	20.6 ±4.5 (6)	21.6 ± 5.2 (6)	21.8±5.2 (6)
Cerebral cortex	19.6 ±2.3 (5)	20.8±1.4 (5)	18.4±1.7 (5)	22.1 ±2.2 (5)	16.0 ± 2.0 (5)	20.8±2.5 (5)

Values are mean ± SE. Number of observations in parentheses. * $p<0.05$; ** $p<0.01$. Paired t-test was done for the log values between basal level (0 min) and subsequent time intervals taking only those animals for which observations at both these levels were available.