Changes in glycogen content in the eggs and oxygen permeability of the chorion

Incubation temperature		Days after oviposition				
		<1	30–35	60–65	75–80	95–100
5 °C	Glycogen content	38.5	9.7	11.5	21.4	27.1
	Oxygen permeability	0.53*	0.52	0.43	0.47	0.37
25 °C	Glycogen content	38.5	10.3	8.6	8.5	9.2
	Oxygen permeability	0.53*	0.45*	0.47	0.51	0.40

One group of the eggs destined to diapause was transferred to 5 °C on the 2nd day after oviposition at 25 °C, and another group was kept at 25 °C without chilling. Glycogen content and oxygen permeability of the chorion were expressed as mg/g of eggs, and μmole/mm^2 chorion/h, respectively. * Values are based on Sonobe et al[9].

was measured (table). The glycogen content of the eggs being kept at 5 °C reached the lowest level at 30–35 days of chilling. After that the glycogen content began to increase gradually and reached about 70% of the initial level (eggs within 1 day after oviposition) after 95–100 days of chilling. When the eggs kept at 5 °C for more than 75 days were incubated again at 25 °C, all the larvae hatched completely after 14–15 days of incubation. When the diapause eggs were incubated at 25 °C without chilling, glycogen content did not increase but maintained a low level throughout the incubation period. These results are consistent with Chino's results showing that glycogen in the eggs begins to be resynthesized gradually from about 60 days under chilling, and the resynthesis of glycogen coincides with the termination of diapause in the silkworm eggs[3,4].

We next examined whether the prolonged chilling brings about the increase in oxygen permeability of the chorion. As shown in the table, oxygen permeability of the eggs being kept at 5 °C more than 2 months was almost constant, although they were freed from diapause during the long chilling. No significant difference was detected in the oxygen permeability of the chorions between the eggs being kept at 5 °C and the control eggs being kept at 25 °C without chilling. These results suggest that the termination of the diapause and the resynthesis of glycogen from 2 polyols occur without a change in the oxygen permeability of the chorion. It is conceivable therefore that factor(s) other than the oxygen permeability of the chorion may be involved in the regulatory mechanisms of carbohydrate metabolism accompanying diapause termination.

In conclusion, it is substantiated from our present and previous experiments[9] that changes in the oxygen permeability of the chorion are not indispensable for the induction of the diapause[9], and for the termination of the diapause.

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Neogenesis of functional hair follicles in adult mouse skin selectively induced by tumour-promoting phorbol esters

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Summary. Neogenesis of functional hair follicles in the tail skin of adult mice can quantitatively be demonstrated after long-term treatment with tumour-promoting phorbol esters. The ability to induce the formation of new hair follicles correlates with the hyperplasiogenic and tumour-promoting capacity of the phorbol esters. Hyperplasiogenic but nonpromoting phorbol esters do not lead to the formation of new hair follicles.

We have recently been able to demonstrate that the DMBA/TPA-mediated 2-stage carcinogenesis experiment, when carried out in mouse tail skin, causes, in addition to tumour formation, the neogenesis of new functional hair follicles in the treated skin area[2].

Evidences for hair neoformation after treatment with TPA have first been described in mouse back skin[2,3]; however, tail skin is especially suited for the study of such processes since it exhibits a highly regular arrangement of hairs, allowing exact quantification. In tail epidermis, parallel rows of parakeratotic scale rings sharply alternate with orthokeratotic interscale regions, and almost exclusively groups of 3 hairs are located under each scale (figure 1).

Evidence for TPA-induced hair neoformation is given by the increased occurrence of sequences of 4 and more hairs associated with 1 scale (figure 2). A mechanism of hair neoformation starting at the vicinity of existing follicles has been described in detail[2], and the different stages of development of a new follicle can easily be seen in 1 sample of separated tail epidermis.

Since TPA produces a pronounced hyperplasia of the tail epidermis, and since initiation by carcinogenic hydrocarbons was found not to be a prerequisite for hair neogenesis[2], the question whether the stimulation of new hair follicle growth is unique to tumour promoters or only a consequence of the concomitant hyperplasia had to be clarified.

To this end, the influence of phorbol esters of varying promoting and hyperplasiogenic activity [12-O-tetradecanoylphorbol-13-acetate, (TPA); phorbol-12,13-di(2,4-decanoate), (PDD-dien); phorbol-12,13-didecanoate, (PDD)][4,5] as well as non-promoting hyperplasiogens [4-O-methyl-12-O-tetradecanoylphorbol-13-acetate, (4-O-methyl-TPA); ethyl-phenylpropiolate, (EPP)][5,6] on the hair pattern in non-initiated tail skin was investigated. In comparison with back skin, tail skin is less sensitive to hyperplasiogenic

Group	Substance	Number of samples	Mean values of scales associated with ≦3 hairs	> 3 hairs	Epidermal thickness (μm)	Promoting activity in back skin
I	Acetone	20	979	21	30.68±0.219	−
II	PDD	16	977	23	37.45±0.305	+[a]
III	PDD-dien	16	961*	39*	40.15±0.414	+[b]
IV	4-O-Methyl-TPA	23	976	24	43.76±0.430	−[a]
V	TPA	24	933*	67*	51.79±0.313	++[a]
VI	Acetone	7	982	18	31.29±0.198	−
VII	EPP	6	981	19	26.67±0.270	−[c]

Female NMRI-mice, 7 weeks old, were used in the experiments. Animals were kept under an artificial day-night-rhythm (light from 18.00–06.00 h), maintained in Macrolon type III cages, and fed with Altromin R 10 Standard (Lage/Lippe, Germany) with water available ad libitum. 5 groups, each consisting of 28 animals, were composed by random distribution and each animal of the individual groups was treated twice weekly with a 100-μl acetone solution of the following substances: group II: 40 nmoles phorbol-12,13-didecanoate (PDD); group III: 40 nmoles phorbol-12,13-di (2,4-decanoate) (PDD-dien); group IV: 400 nmoles 4-O-methyl-12-O-tetradecanoyl-phorbol-13-acetate (4-O-Methyl-TPA); group V: 20 nmoles 12-O-tetradecanoyl-phorbol-13-acetate (TPA). (The phorbol esters were kindly provided by Dr. E. Hecker and his group). Group I served as control group and received only the solvent.
The substances were applied on the whole tail skin, starting 1 cm caudal to the termination of the dorsal pelage, over a period of 25 weeks. The animals were killed by cervical dislocation and the tails cut off. All tails had been depilated prior to sacrifice. The whole tail skin was removed, soaked overnight in 1% acetic acid at 4 °C and the epidermis separated from dermis with fine foreceps. 1 circular section (r = 0.5 cm) was punched out from each epidermal sheet, stained with hematoxylin and mounted flat in glycerin. Scales per circular section were counted under a microscope and the number of scales with 2, 3, 4, 5 and 6 hair follicles was determined. The number of punch sections per group suitable for counting is indicated in column 3. Since the absolute number of scales per section differed from section to section, the mean values of hair sequences per section were calculated for 1000 scales. Statistical significance of the differences in hair distribution was calculated by the Westerkamp median test ($p = 0.005$). Values indicated by* are statistically different from the control. In addition, animals of 2 further groups (10 animals/group) were treated according to the scheme of groups I–V either with acetone (group VI) or with 0.04 mmoles ethyl-phenylpropiolate (EPP) (group VII). The analysis of hair distribution in these groups was carried out as described above. Prior to incubation in acetic acid, a tissue sample was excised from tail skin of 4 animals/group and vertical sections (5 μm) were prepared by routine histology. These sections were used for histological examination and determination of epidermal thickness. Thickness was measured from the basal lamina to the transition of the cornified layer in the midscale region of epidermis. Values indicated represent mean values of 80 measurements. [a]Hecker[5]. [b]E. Hecker, personal communication. [c]Raick and Burdzy[6].

agents of any kind (unpublished results); thus relatively high doses of the phorbol esters were used.
The hyperplasiogenic effect of the 3 cocarcinogenic phorbol esters tested on mouse tail epidermis correlated with their promoting activity in back skin[4,5]. This correlation has also been shown to exist when both parameters were determined in back skin[4]. A similar, although less clear-cut relationship seems to exist with regard to the hyperplasiogenic and hair inducing capacity of the 3 tumour promoters (table).
In contrast, and despite the pronounced hyperplasia, the non-promoting 4-O-methyl-TPA did not produce significant alterations of hair distribution per scale (table). Moreover, the 4-O-methyl-TPA-treated epidermis only seldom showed the accumulation of basophilic cells and buds in the neck region of existing follicles which, according to the proposed mechanism[2], are typical signs of the beginning hair neogenesis. On the other hand, 'budding' was frequently recognizable in epidermis treated with the promoter PDD (figure 3). Obviously the hair follicle-inducing capacity of PDD is not sufficient to 'promote' the complete outgrowth of new hairs, so that no definite alteration in terminal hair frequency occurs. Rather surprisingly, EPP, which is a potent hyperplasiogen in back skin[6], failed to produce thickening of the tail epidermis; instead, the longterm application of the substance led to a significant reduction of epidermal thickness without affecting the hair distribution (table).
From these results the conclusion may be drawn that the ability to induce the formation of new functional hair follicles in adult animals reflects a specific property of tumour promoters. Apparently, both phorbol ester-mediated tumour promotion and hair neogenesis depend on a permanent state of increased proliferative activity in the epidermis; however, like tumour promotion[7], hyperplasia per se is not automatically sufficient to bring about the development of new hair follicles.

It is generally believed that the primordia of all hair follicles in mammals are determined before birth or soon after birth, and that neoformation of a functional pilosebaceous unit does not occur after the adult complement has been established[8]. The hair neogenesis observed in tail skin may therefore be explained by a dedifferentiating effect of tumour-promoting phorbol esters, eventually enabling cells which have more or less lost their commitment to either mimic an otherwise embryonic process or – if the appropriate 'initiation' has occurred before treatment with promoters – to produce neoplastic growth[9]. Indeed, dedifferentiation or delay of differentiation by tumour-promoting phorbol esters has repeatedly been described as occurring in several cell and tissue culture systems[10], and ultrastructural alterations in TPA-treated epidermis that resemble morphological characteristics of embryonic tissue have been observed in vivo[11].
As far as skin is concerned, a reprogramming of tissue differentiation has best been demonstrated by another tumour-promoting stimulus, namely wounding[12]. It is well-known that upon wounding sebaceous gland cells rapidly undergo squamous metaplasia[8] and that large skin wounds are re-epithelialized by cells of the outer root sheath of follicular remnants[9]. Accordingly, most of the evidences of adult hair neogenesis stem from observations in healing wounds[2,8]. Since the pilosebaceous unit represents the developmentally last differentiation step of a non-committed epidermal stem cell, it is not surprising that dedifferentiation and metaplasia take place preferentially at this labile boundary region of the integument.
The occurrence of scales associated with more than 3 hairs in untreated adult animals (table) may also be traced back to wounding. Embryonic and very young animals do not show sequences with more than 3 hairs per scale[2], and the parallel order of the scale rings is almost never disturbed. In contrast, perturbations of the scale rings are relatively frequent in adult animals, and it is predominantly at those

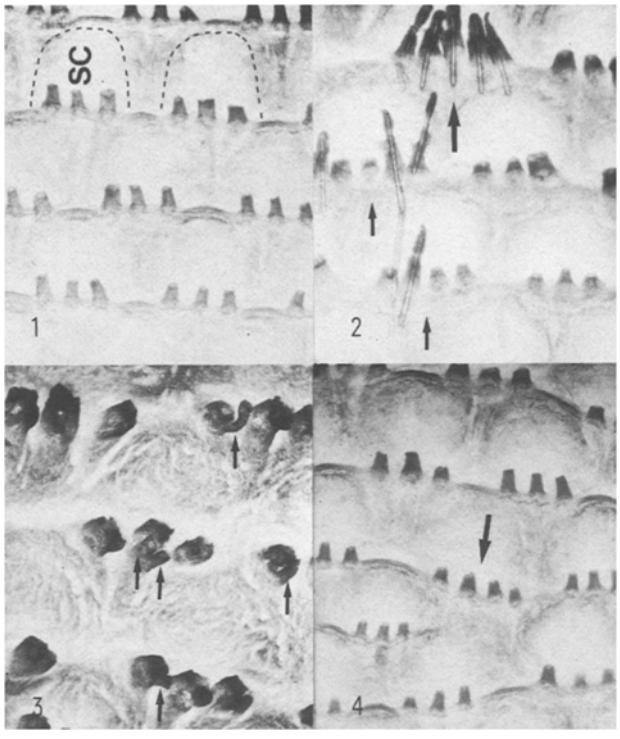

Fig. 1. Whole mount of depilated tail epidermis of an untreated adult mouse, showing triads of hair follicles associated with each scale (SC). During the separation of the epidermis from dermis, hair follicles are ruptured at the level of the sebaceous glands, so that only follicular necks can be seen. Note the absence of sequences with more than 3 hairs per scale. Hematoxylin; × 50.

Fig. 2. TPA-treated mouse, showing 1 sequence with 4 follicles and 2 sequences with 5 follicles per scale (arrows). Despite depilation, 1 set of 5 follicles has retained the hairs (large arrow). × 50.

Fig. 3. PDD-treated mouse, showing numerous buds and rudimentary follicular outgrowths (arrows) in the vicinity of parent follicles. × 75.

Fig. 4. Untreated mouse. Note the occurrence of a sequence with 4 follicles (arrow) at the site of disturbance of the parallel order of the scale rings. × 50.

sites that the rare sequences with more than 3 hairs are encountered (figure 4). It is only reasonable to assume that these disorders are the consequences of wounding.

Both promotion of epithelial tumour growth[13] and hair neoformation[8] in adult animals after wounding are dependent on concomitant dermal injury. On the other hand, normal hair development in mammals also requires the symbiotic action of both embryonic dermis and epidermis[8]. It may therefore be speculated that tumour promotion and hair neogenesis in adult animals is accompanied by specific dedifferentiation processes not only in the epidermis but also in the dermis, regardless of the nature of the promoting stimulus. The present knowledge of dermal alterations after treatment of skin with a tumour promoter is more or less restricted to the descriptive level of rather drastic events (i.e. edema, leucocyte infiltration), and in general alterations of this kind are produced also by non-promoting irritants. It is, however, noteworthy that even in long-term tests the strongly hyperplasiogenic non-promoter 4-O-methyl-TPA, which does not induce formation of new hairs, leaves a morphologically essentially unchanged dermis[14].

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Sexual dimorphism of mouse fetal brain lesions after X-irradiation prior to gonadal differentiation

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Summary. Fractionated X-irradiation of gestational days 11–13 in the mouse, with doses between 3 × 1.05 and 3 × 1.33 Gy resulted in rosette-like clusters of primitive ependym-resembling cells dispersed within the cortex walls. Quantification of these abnormalities showed a general prevalence in the female fetuses, especially due to the larger number of rosettes in the females than in the males. It was concluded that X-irradiation acts on sex-specific differentiation steps, which are fully developed at the beginning of the fetal period. At is was recently speculated that these are linked to an early divergence of gene expression between the sexes, we suggest that X-chromosome damage may be involved in the pathogenesis of the dimorphic lesion pattern. While, in principle, this will be valid for any fetal tissue, it only becames evident in the forebrain because of the outstanding relationship between cell necrosis and rosette development in this specific organ.

In a previous report[2] we presented quantitative data calculating the severity of pathological aberrations in mouse neocortex after a fractionated X-irradiation insult on days 11–13 of gestation. The main findings were rosette-like clusters of primitive neuroblasts dispersed throughout the telencephalic cortex. The incidence of these rosettes showed a clear dose dependency from 3 × 1.05 Gy upwards, and also increased in number linearly with a decrease of