Effects of AcD and CAF administration in pregnant rats on day 3 or 4 of gestation

Groups	Treatment	Number pregnant females	Number blastocysts recovered	Mean number blastocysts per female ± SD	Mean number cells per blastocyst ± SD
A	AcD 300 µg/kg day 3	13	64	4.9 ± 3.40*	29.41 ± 6.60*
B	AcD 300 µg/kg day 4	7	35	5.0 ± 2.94*	36.45 ± 4.80
C	CAF 250 mg/kg day 3	7	59	8.4 ± 2.81	29.38 ± 3.87*
D	CAF 250 mg/kg day 4	7	62	8.8 ± 2.03	34.98 ± 5.90
E	Saline 1 ml/kg days 3 and 4	7	60	8.6 ± 2.99	36.95 ± 5.60

* Significantly different from controls at $p < 0.05$.

pendently from the day of treatment. However the CAF administered on day 3 of pregnancy reduces significantly the average number of blastomeres in the rat blastocyst. The same effect is detected also in the blastocysts from females treated with AcD, but this drug induces a remarkable embryolethality, as can be seen by the dramatic decrease of the collected blastocysts in the A and B groups. The antibiotics used in this experiment show different effects on the preimplantation embryo: the AcD induces embryolethality and embryotoxicity, while CAF shows exclusively a remarkable embryotoxic effect revealed by the reduction of the mean blastomeres number when administered on the day 3 of gestation.

Our results agree with those obtained by Wilson[8], who described, in rats treated with 300 µg/kg on the day 4 of pregnancy, a remarkable preimplantation loss, deduced by a low number of implantation sites at term; and with results obtained by Fritz and Hess[6], who did not find decrease of implantation rate in females treated with CAF from day 1 to day 6 of gestation.

The reduced number of blastomeres observed in our investigation is a sign of a harmful effect of the drugs on the conceptus and, therefore, could be used as an index of a probable teratogenic effect.

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Oxygen permeability of the chorion in relation to diapause termination in *Bombyx* eggs[1]

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Summary. Oxygen permeability of the chorion of the silkworm, *Bombyx mori*, was measured in relation to embryonic diapause. It did not change appreciably when the eggs were freed from diapause by being kept under long chilling. This finding suggests that the increase in oxygen permeability of the chorion is not a pre-requisite for the termination of the diapause and resynthesis of glycogen from 2 polyols.

Chino[3,4] has shown that almost all glycogen present in the eggs of *Bombyx* silkworm is rapidly converted to 2 polyols (i.e. sorbitol and glycerol) at the onset of the diapause, and glycogen is resynthesized from these polyols at the termination of the diapause. It has also been demonstrated that the conversion of glycogen to 2 polyols resulted from anaerobic metabolism in the non-diapause eggs[5–9]. From the experiments of dechorionation and measurement of water loss from the silkworm eggs, Okada[5] has proposed a hypothesis that the formation of an oxygen barrier in the chorion causes an oxygen-deficiency, which induces diapause, and recovery of oxygen permeability of the chorion to the initial level after long chilling causes the termination of diapause and resynthesis of glycogen from 2 polyols. However, our recent result with the direct determination of the oxygen permeability of the chorion does not support Okada's hypothesis, at least as to the formation of an oxygen barrier in the chorion at the onset of the diapause[9]. The purpose of the present study is to test whether or not the oxygen permeability of the chorion changes at the termination of the diapause after long chilling.

Materials and methods. Eggs of the bivoltine race (Nichi 106 × Daizo) of the silkworm, *Bombyx mori*, were used. For the purpose of artificially terminating diapause, diapause eggs kept at 25 °C for 2 days after oviposition were placed at 5 °C for about 100 days. Glycogen content was determined using the anthrone method[10]. For measurement of oxygen permeability of the chorion, the apparatus especially designed for this purpose[9] was used with minor modifications. The quantity of oxygen was measured using high speed gas chromatography (Yanagimoto HSG-1). A stainless steel micro column, 60 cm long and 1 mm inner diameter packed with Molecular Sieve 5 A, 200/250 mesh (Gasukuro Kogyo Co.) was used. The column was operated at 40 °C. The pressure of hydrogen gas as a carrier gas was 1.5 kg/cm^2.

Results and discussion. To know the period of termination of the diapause by chilling, the glycogen content of the eggs

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