prostatic tissues, and that analogous determinations should be made on the epithelial cells of other normal glandular structures.

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Effects of anti-EGF serum on newborn mice

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Summary. Administration of anti-EGF serum to newborn mice led to delay of eyelid opening and incisor tooth eruption, acceleration of hair growth and delay of weight gain. These results indicate that in the first week after birth EGF still has a physiological function, which can be abrogated by anti-EGF serum.
Key words. EGF; anti-EGF serum; newborn mice.

Epidermal growth factor (EGF) has been generally suggested to play a role in growth and differentiation of epithelial tissues of the epidermis, the cornea, the respiratory and intestinal tracts and the mammary gland[1–3], in spermatocyte maturation[4], and also in skin wound repair processes[5]. It has been found, moreover, to act as a powerful cocarcinogen[6]. The biological significance of a number of further data, largely obtained in vitro, has however remained questionable and needs clarification. No clinical symptoms associated with or attributable to failure or dysfunction of EGF have been discovered to occur spontaneously in man or animals. Surgical removal of the submandibular glands, the major site of EGF biosynthesis in mice, is capable of influencing proliferative processes like, for example, the development of the lactating mammary gland[3] or tumor incidence and growth[6], but contradictory data on its effect on serum EGF levels in adult animals (mice, hamsters) have been reported[4,7,8]. Therefore the existence of further sites of EGF biosynthesis is highly probable[9,10].

In order to stimulate a syndrome of systemic loss of EGF function, newborn mice were treated with high-titre anti-EGF serum and observed for the emergence of biological effects.

Material and methods. Mouse EGF was prepared from submandibular glands as described by Savage and Cohen[11]. Purity was assessed by SDS gel electrophoresis in 15% polyacrylamide gels[12] following reduction and denaturation. A single band of M_r = 6000 could be demonstrated. The EGF preparation was subjected to partial amino acid sequence analysis and the specificity, in addition, confirmed by competition in a quantitative ^{125}-I-EGF binding assay to cell membranes[13].

Antisera to mouse EGF were prepared in rabbits according to Rizzino et al.[14] by immunization with the EGF prepared and purified as referred to above. The specificity of the antisera was assessed by neutralization of EGF-dependent enhancement of colony formation of NRK cells in soft agar (unpublished results) and by an enzyme-linked immunoassay according to Engvall and Perlmann[15] with EGF and NGF (Wellcome, Beckenham) as antigens.

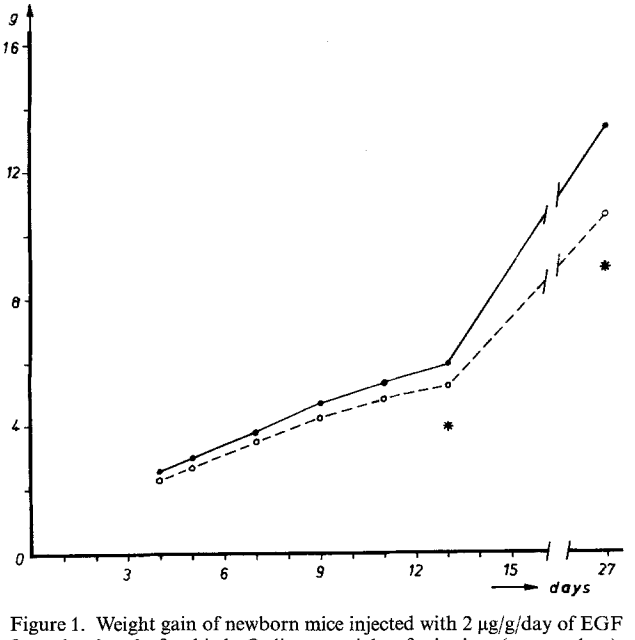

Figure 1. Weight gain of newborn mice injected with 2 µg/g/day of EGF from day 0 to 9 after birth. Ordinate: weight of mice in g (mean values), ●—● PBS (n = 9), o—o EGF (n = 8). Asterisks indicate statistically significant differences (Student's t-test, $p < 0.01$).

The experiments were performed with R17 inbred mice, using mothers with litters of 5–7 animals. Each newborn mouse in the experimental group received 0.1 ml by s.c. injection twice or three times during the first week, beginning at the day of birth (day 0). Control mice received pre-immune serum or anti-goat Ig serum of rabbits. Ear and eyelid opening, incisor tooth eruption, hair growth and weight gain until the days indicated in figure 1 and 2 were recorded.

EGF was dissolved in phosphate buffered saline (PBS). 2 µg/g/day were injected s.c. from day 0 to day 9 after birth.

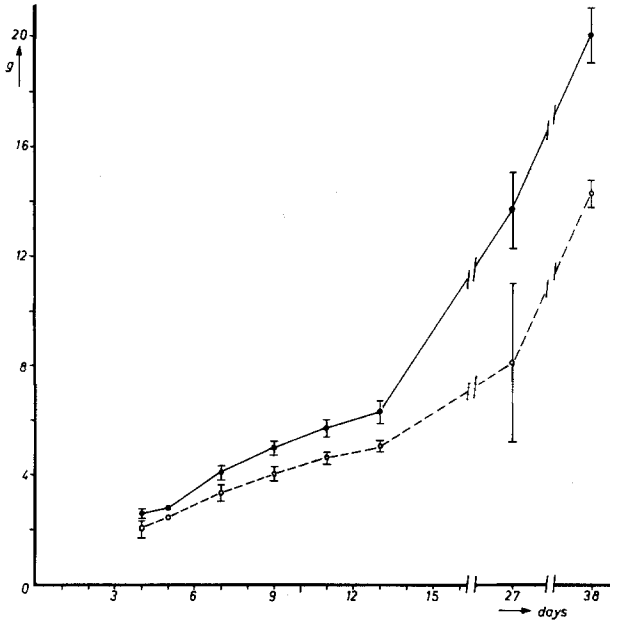

Figure 2. Weight gain of newborn mice (mean values and standard error) treated three times with 0.1 ml of anti-EGF serum s.c. at days 0, 3 and 6 after birth (experiment B). Ordinate: weight of mice in g, ●—● control serum (n = 11), o—o anti-EGF serum (n = 12). The differences at all days are statistically significant (Student's t-test, $p < 0.01$).

Table 1. Enzyme-linked immunoassay according to Engvall and Perlmann[15] for confirmation of specificity of the anti-EGF serum employed. Briefly, tablet blisters of the pharmaceutical industry were coated with EGF (80 ng/0.2 ml) or NGF (25 units/0.2 ml). After incubation and washing, anti-EGF or pre-immune rabbit serum (1:1000) were added. Finally, bound antibodies were detected with peroxidase-conjugated goat anti-rabbit IgG and o-phenylendiamine. Absorbance values were measured with a Spekol (VEB Carl Zeiss, Jena) at 492 mm. – The detection limit of the assay was about 0.8 ng EGF/0.2 ml.

Antigen	Pre-immune serum	Anti-EGF serum
EGF	0.020/0.020	1.900/1.900
NGF	0.018/0.022	0.020/0.022

Biological parameters were recorded as described above. Control groups received PBS alone.

Results. As shown in table 1, the anti-serum was specific for EGF. No cross-reactivity against NGF, also obtained from mouse submandibular glands, could be observed.

Daily administration of EGF from day 0–9 led to acceleration of eyelid opening and incisor tooth eruption and delay of hair growth as reported in the literature, whereas the time of ear opening was not affected (table 2). Furthermore, a slight but significant reduction of body weight gain was noted from the 2nd to the 4th week after birth (fig. 1).

In contrast to these results, both eyelid and ear opening as well as incisor tooth eruption were delayed by 3 injections of anti-EGF antisera given during the first week after birth, as demonstrated in two representative experiments (table 2). Beginning of hair growth, on the other hand, was unequivocally found to be accelerated (table 2). Growth of the mice was increasingly stunted as demonstrated by reduction in body weight gain till day 38. The results of one representative experiment are given in figure 2. The majority of the animals exhibited signs of ruffling between days 20–30 after birth, albeit no restraints on general behavior and motility were evident.

Discussion. The results demonstrate that epidermal differentiation processes in newborn mice as 1) ear and eyelid opening and incisor tooth eruption as well as 2) hair growth which are accelerated and delayed, respectively, by pharmacological doses of EGF, can be adversely affected by anti-EGF serum treatment during the first week after birth. This observation indicates that EGF may in fact be involved physiologically in the regulation of epithelial growth and differentiation.

The antiserum-induced delay in weight gain seems more complex and difficult to interpret. EGF is found to stunt growth in newborn mice, as observed by Moore et al.[16] and Tam[17] and corroborated in our experiments, but it increased weight gain in adult Syrian hamsters[8]. This points to age-dependent rather than species-dependent differences in its action. Similar conclusions have been drawn with regard to differentiation effects of EGF on intestinal mucosa[2]. The early administration of anti-EGF serum may be reasonably suggested to lead to at least partial neutralization of EGF and its action. The time-course of the weight increase of treated mice indicates that shortly after birth EGF still carries out functions whose abolition has long-term biological consequences, which are only compensated for several weeks after birth. The target points of this early EGF activity may be multifaceted, as indicated by its known in vitro effects[1]. Histological studies will elucidate which factors are involved in the antiserum-induced delay of weight gain. The results nevertheless point to the possibility that deficiency of EGF or abolition of its action can be attained by injection of specific antisera and might be of nosological significance, at least in newborn animals.

Table 2. Ear and eyelid opening, incisor tooth eruption and beginning of hair growth (days) after treatment of newborn mice with EGF or anti-EGF serum as compared to controls. EGF was administered daily from days 0–9, anti-EGF serum three times at days 0, 3 and 6 after birth. All differences, except for ear opening in experiment A, are statistically significant (U-test according to Mann and Whitney, $p < 0.01$). The moment of eyelid opening and incisor eruption were determined according to Carpenter and Cohen[1], that of ear opening by visibility of the external acoustic duct, and hair growth by appearance of the down hair over back and neck.

Exp.	Treatment	No. of mice	Ear opening	Eyelid opening	Incisor eruption	Hair growth
A	PBS	9	2–3	10–12	9–10	5–7
	EGF	8	2–3	8–9	7–8	7–9
B	Control serum	11	2	10–11	9–10	5–7
	Anti-EGF serum	12	3–4	12–13	10–12	3–5
C	Control serum	32		9–12	9–10	5–7
	Anti-EGF serum	16		12–14	10–12	3–6

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Kinetic arguments for the existence of a single form of intestinal ornithine decarboxylase during the postnatal development of normal and sparse-fur mutant mice and after EGF treatment

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Summary. The K_m for ornithine is remarkably constant during the course of postnatal development in both normal and spf mutant mice even if a large but transient increase in ornithine decarboxylase (ODC) activity is noted. Four hours after EGF injection (4 μg/g b.wt) to 17-day-old normal and spf mice, a marked stimulation of ODC activity is observed but K_m remains unaffected. These data argue against the existence of multiple forms of ODC in the intestinal mucosa of mice.
Key words. Ornithine decarboxylase; small intestine; postnatal development; kinetic properties; EGF treatment; sparse-fur mutant mice.

Ornithine decarboxylase (ODC, EC 4.1.1.17), the first enzyme in the pathway leading to polyamine biosynthesis, catalyses the conversion of ornithine to putrescine. There is increasing amount of experimental evidences indicating that ODC plays a key role during the differentiation and proliferation of a variety of tissues and cells [2–4]. In the mouse [5] and rat [6] small intestine, ODC activity increases during the course of the normal postnatal development as well as following mucosal injury, jejunectomy and during lactation [7]. We have recently demonstrated that the intestinal ODC activity is lower in suckling sparse-fur (spf) mutant mice as compared to normal animals [5]. This strain of mouse exhibits X-linked ornithine transcarbamylase (OTC, EC 2.1.3.3) deficiency and thus represents a useful model to study the effects of an impaired ornithine metabolism on polyamine biosynthesis during the course of postnatal development.

In rat heart [8] and liver [9, 10] as well as in mouse kidney [10, 11], the existence of multiple forms of ODC has been documented. In rat heart, a change in the affinity for ornithine has been observed after hormonal, neuronal and ontogenic stimuli [8] and a heat-sensitive form seems to be preferentially induced after androgen stimulation in mouse kidney [11]. In adult rat ileum [12], ODC activity was found to be stimulated by epidermal growth factor (EGF) and glucagon while duodenal ODC has been shown to increase after EGF injection to 8-day-old suckling mice [13]. One control mechanism proposed for the rapid increase of ODC activity lies in the existence of multiple forms of the enzyme [8–11] with different affinities for L-ornithine [8, 9]. However, this hypothesis has never been tested on intestinal ODC during the course of normal postnatal development nor after hormonal induction. In the present study, we have determined the kinetic parameters of the enzyme in the intestinal mucosa from both normal and spf mice and after EGF treatment. There was no change in the affinity for L-ornithine in either situations, which suggests the presence of a single form of ODC in the intestinal mucosa of mice.

Materials and methods. Sparse-fur hemizygous male mice (spf/Y) were used as experimental animals and normal Swiss ICR male mice as controls. Mutant spf male mice were kindly provided by Dr Ijaz A. Qureshi, form Ste-Justine Hospital where inbreeding was done as previously described [5]. Post-weaning animals were fed ad libitum on mouse Purina chow (Ralston Purina). 17-day-old normal and spf mice were injected s.c. on the dorsal surface with 4 μg of EGF/g b.wt or equivalent volume of water for the control animals, as established previously [14]. The mice were then returned to their mother for the next 4 h. 13-, 17-, 21-, 25-day-old and 8-week-old normal and spf mice were killed by decapitation without being fasted. Controls and EGF-treated animals were sacrificed 4 h after injection. The first 15 cm of the small intestine were removed and rinsed with cold saline. The mucosa was scrapped with a spatula, weighed and used immediately for the determination of ODC activity. Since there was no difference between 17-day-old normal and control animals, they were all combined and included as controls in the table.

The tissues were homogenized in 5 vols of 0.1 M Tris-HCl (pH 7.4) containing 0.1 mM EDTA, 5 mM dithiothreitol and 0.3 mM pyridoxal 5'-phosphate and then centrifuged at 100,000 × g for 60 min. ODC activity was determined in the supernatant by measuring the rate of formation of $^{14}CO_2$ from L-[1-^{14}C] ornithine as previously described [5]. Samples of the supernatant were incubated in the presence of 1 μCi L-[1-^{14}C] ornithine (spec. act. 57 mCi/mmol, Amersham, Oakville, Ontario, Canada) and various concentrations of cold L-ornithine (0.05–3 mM). Blanks were incubated with 10 mM difluoromethyl-ornithine (DFMO) (a gift from Dr P. McCann, Merrell Research Center), a specific inhibitor of ODC. Radioactivity was counted in Aquasol II using a Minaxi Tri-Carb Series 4000, model 4450 scintillation counter