Epidermal growth factor (EGF) accelerates the maturation of fetal mouse intestinal mucosa in utero[1]

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Summary. Pregnant Swiss ICR mice were injected i.p. with 0.5 µg of epidermal growth factor (EGF) per g b.wt at 15, 16 and 17 days of gestation and fetuses were removed at 18 days of gestation. EGF treatment had no effect on the weight of the fetuses and on the length of the small intestine. No modification of the protein and DNA contents was noted. However brush border alkaline phosphatase and trehalase activities were significantly increased as well as endoplasmic reticulum membrane-bound glucose-6-phosphatase.

Epidermal growth factor (EGF) is a small polypeptide mitogen that has been isolated from the male mouse submaxillary gland[2] and from human urine[3]. The presence of EGF in human Brunner's gland, shown by Elder et al.[4], suggests that EGF may have a role in mucosal growth and control of gastrointestinal secretion. In the adult male mouse Scheving et al.[5,6] have demonstrated that EGF rapidly stimulates DNA synthesis in some regions of the digestive tract. In the newborn mouse EGF stimulates ornithine decarboxylase activity in the stomach and duodenum[7] and accelerates the maturation of the intestinal mucosa[8]. Using an organ culture method of fetal mouse small intestine, we have observed that EGF induces the differentiation of the rough endoplasmic reticulum in absorptive cells after 72 h of culture[9].

The aim of this work was to determine the effect of EGF on the fetal mouse small intestine in utero by measuring the protein and DNA contents as well as enzymatic activities of the brush border and endoplasmic reticulum.

Materials and methods. Timed pregnant Swiss ICR mice were injected i.p. at 15, 16 and 17 days of gestation with either 0.5 µg of EGF per g b.wt or 0.5 ml of distilled water. EGF was purchase from Collaborative Research Inc. (Waltham, Mass) or from Sigma Chemical Co. (St-Lous, Mo). Results were comparable with both products. Fetuses were removed at 18 days of gestation.

The entire small intestine of the fetuses was removed, measured and cut into 3 equal parts. For each assay, the intestinal thirds of 5 fetuses were pooled, weighed and homogenized in 49 vol. of ice-cold redistilled water in an Omnimixer during 2 min at full speed. The homogenates were used immediately for enzymatic determinations.

Protein was measured by the method of Lowry et al.[10] with bovine serum albumine as standard and DNA content was determined by the method of Burton[11] as modified by Giles and Myers[12]. Protein and DNA values were expressed by µg/cm of intestine. Lactase (β-D-galactoside galactohydrolase, EC3.2.1.23) and trehalase (α,α-trehalase, EC3.2.1.28) activities were assayed according to a modification by Lloyd and Whelan[13] of Dahlqvist's method[14]. Alkaline phosphatase (EC3.1.3.1) activity was assayed according to the method of Eichholz[15]. Enzymatic activities were expressed as micromoles of substrate hydrolyzed per min per cm of intestine. The phosphohydrolase activity of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC3.1.3.9) was assayed in the proximal intestinal thirds as reported by Ménard and Malo[16]. Activity was expressed as µmoles of phosphorus liberated per min per cm of small intestine. Significance of the difference of means was evaluated by using Student's t-test.

Results and discussion. The effects of daily injections of EGF for 3 days beginning at 15 days of gestation have been studied in the small intestine of fetuses at 18 days of gestation. As shown in table 1 the weight of the EGF-treated fetuses, the length of their small intestine and the weight of the different intestinal segments are comparable to that of the controls. Furthermore no modification of the protein and DNA contents is noted along the small intestine. The development of some brush border membrane hydrolytic activities has been investigated. Significant increases of alkaline phosphatase and trehalase activities are recorded along the entire length of the small intestine following EGF treatment (table 2), except for trehalase activity in the distal thirds where the increase is non-significant. It is interesting to note that even though alkaline phosphatase and trehalase activities increase following EGF treatment, the gradient of activity previously established along the fetal small intestine is maintained[17]. On the other hand lactase activity remains at the control levels (table 2). The fact that the different hydrolytic activities of the brush border membrane do not respond in a similar fashion to EGF has also been observed in the neonate for EGF[8] as well as for other hormones[18,19].

Table 1. Effect of EGF on the proximal (P) middle (M) and distal (D) thirds of the fetal intestinal mucosa[a]

		Controls	EGF
Weight of fetuses (g)		1.30 ± 0.02[b] (35)[c]	1.31 ± 0.01 (55)
Mean length of the small intestine (cm)		8.25 ± 0.18 (7)[c]	8.37 ± 0.09 (11)
Weight of the small intestine (mg/cm)	P	5.66 ± 0.17 (15)[c]	5.42 ± 0.17 (23)
	M	4.15 ± 0.21 (7)	3.78 ± 0.13 (11)
	D	2.29 ± 0.10 (7)	2.18 ± 0.07 (11)
Protein (µg/cm)	P	490.7 ± 31.0 (17)[d]	536.4 ± 7.6 (23)
	M	498.0 ± 56.0 (7)	397.0 ± 18.4 (11)
	D	230.0 ± 5.8 (7)	218.6 ± 10.6 (11)
DNA (µg/cm)	P	51.4 ± 2.8 (7)[d]	46.6 ± 2.2 (11)
	M	38.5 ± 2.5 (7)	36.9 ± 1.5 (11)
	D	24.2 ± 2.1 (7)	24.8 ± 0.9 (11)

[a]Mothers were injected with 0.5 µg/g b.wt of EGF at 15, 16 and 17 days of gestation and embryos removed at 18 days. [b]Mean ± SEM. No statistical difference was observed between control and experimental group for each parameter. [c]Number of mice used. [d]Number of assays.

Table 2. Effect of EGF of the activity on brush border hydrolytic activities in the proximal (P) middle (M) and distal (D) intestinal thirds[a]

		Controls	EGF	p-Value
Lactase	P	26.6 ± 2.1[b] (7)[c]	22.8 ± 1.1[b] (11)[c]	NS[d]
	M	16.6 ± 1.8 (7)	14.9 ± 0.9 (11)	NS
	D	3.1 ± 0.6 (7)	3.5 ± 0.4 (11)	NS
Trehalase	P	0.20 ± 0.03 (9)	0.38 ± 0.03 (19)	<0.0005
	M	0.08 ± 0.01 (7)	0.12 ± 0.02 (11)	<0.05
	D	0.05 ± 0.02 (7)	0.08 ± 0.02 (11)	NS
AlPase	P	146.0 ± 8.6 (13)	198.0 ± 11.0 (23)	<0.0005
	M	119.0 ± 11.0 (7)	149.0 ± 13.0 (11)	<0.05
	D	36.0 ± 6.0 (7)	55.0 ± 5.0 (11)	<0.0125

[a]Mothers were injected with 0.5 µg/g b.wt of EGF at 15, 16 and 17 days of gestation and embryos removed at 18 days. Enzymatic activities were expressed as $IU \times 10^{-3}$/cm of intestine. [b]Mean ± SEM. [c]Number of assays. [d]NS: non significant.

The involvement of hormones in the regulatory mechanism of enzymatic development of intestinal brush border membrane has been established especially in the neonate[20]. Recently sucrase[21] maltase[22] and enterokinase[23] activities were induced precociously in rat embryos by administration to the mother of cortisone and/or thyroxine. In cultured chick embryo intestine, thyroxine is able to enhance the early accumulation of AlPase in the explants[24]. The present data show that EGF treatment increases AlPase and trehalase activities in fetal mouse small intestine in utero.

We have previously shown that endoplasmic reticulum membrane-bound glucose-6-phosphatase (G-6-Pase) activity develops late during the gestational period[25]. The daily administration of EGF during 3 days beginning at 15 days of gestation induces a significant increase of G-6-Pase activity in the proximal intestinal thirds of the fetuses at 18 days of gestation (controls: $(3.43 \pm 0.24)10^{-3}$; EGF-treated: $(5.10 \pm 0.40)10^{-3}$; 10 assays each; $p < 0.0025$). This observation is in agreement with the ability of EGF to induce in vitro the differentiation of the rough endoplasmic reticulum of fetal mouse absorptive cells[9]. This effect of EGF on intestinal G-6-Pase activity seems to be particular to the fetal period since it has no effect on this activity during the postnatal period when the endoplasmic reticulum is well developed[8].

In conclusion the present observations suggest that EGF may play a role in the overall maturation of absorptive cells in mouse fetus, as it does in the differentiation of the lung in the fetal rabbit[26]. EGF accelerates the differentiation of the endoplasmic reticulum in absorptive cells and induces in increase in some specific brush border enzyme activities.

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Two cell types in monoamine-containing 'liquor contacting' neuron system of the frog brain

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Summary. By means of an immunofluorescent technique, 'liquor contacting' neurons, serotonergic in nature were demonstrated in the paraventricular organ and in the nucleus infundibularis dorsalis, and neurons catecholaminergic in nature were noted in the preoptic recess organ and in the caudal part of the 4th ventricle.

The distribution of monoaminergic neurons in the amphibian brain has been demonstrated by means of formaldehyde-induced fluorescence methods[2-9]; catecholaminergic and serotonergic neuron systems were localized within specific areas of the CNS. In the amphibian brain, very strong monoamine fluorescence was found in the cells of 'circumventricular organs' such as the preoptic recess organ (PRO), paraventricular organ (PVO) and the lateral infundibular region or nucleus infundibularis dorsalis (NID). These cells were termed 'liquor contacting neurons' because of their intense contact with the cerebrospinal fluid[2]. These circumventricular organs have been known to contain green fluorescent neurons and a few yellow or greenish-yellow ones[6-8]. With the technique of microspectrofluorimetry, the fluorescence in PVO or NID was thought to be due to catecholamine (CA), probably dopamine, and serotonin (5-HT), and that of PRO to CA probably dopamine only[8].

We have now performed an immunofluorescent study in frogs using antibodies to CA-synthesizing enzymes, tyrosine hydroxylase (TH), and to 5-HT, in order to corraborate the findings obtained by the histofluorescence method and to provide an 'immunohistochemical map' of monoaminergic neuron systems with special reference to the liquor contacting neuron system.

Materials and methods. Antiserum to TH was produced in rabbits and tested for specificity as described previously[10]. Rabbit antiserum to 5-HT was purchased from RIA (UK). Frogs (*Rana catesbiana*; b.wt 300–450 g) were anesthetized with ether and perfused via the arterial trunk with Zamboni's solution (2% paraformaldehyde-0.2% picric acid in 0.1 M phosphate buffer, pH 7.2). The brains were removed and postfixed with the same fixative for 6-17 h. Subse-