# PROLIFERATION OF THE INTESTINAL EPITHELIUM AND OF THE REGENERATING LIVER OF RATS WITH EPIDERMAL GROWTH FACTOR DEFICIENCY

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Epidermal growth factor (EGF), a polypeptide with mol. wt. of 6045 daltons, is a powerful mitogen for various types of cells both *in vitro* and *in vivo* [3]. The main source of EGC in rodents is the submandibular salivary glands, which secrete it mainly exogenously, into the saliva. On entering the gastrointestinal tract with the saliva, EGF can inhibit hydrochloric acid secretion, can have a marked cytoprotective action on the mucous membrane of the stomach and duodenum [9], and can stimulate proliferation of the epithelium of the gastric glands [7], the crypts of the duodenum, ileum, and jejunum in rats, and crypts of the large intestine also in mice [4]. EGF stimulates DNA synthesis in hepatocytes *in vitro* [14]. There are no data on the effect of EGC on postresection proliferation of the intestinal and hepatic epithelium, although many investigations have been undertaken to study possible stimulators of regeneration of these organs [11, 12].

The presence of specific receptors for EGC in hepatocytes and enterocytes, changes in their number during the period of postresection regeneration of the liver [13], and also the inexplicably high concentrations of this powerful growth factor in the saliva determined the main purpose of the present investigation, which was to study the effect of EGF deficiency, produced by submandibular sialadenectomy, on proliferation of the intestinal and hepatic epithelium during postresection regeneration of these organs.

## EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 150-200 g, bred at the R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR. The animals were divided into six groups: group 1) resection of two-thirds of the small intestine (n = 15), group 2) resection of two-thirds of the small intestine and submandibular sialadenectomy (n = 15), group 3) sialadenectomy alone (n = 15), group 4) sialadenectomy and partial hepatectomy (n = 30), group 5) partial hepatectomy alone (n = 30), and group 6 (control) — laparotomy alone (n = 15). The small intestine was resected 5 cm distally to Treitz' ligament and 15 cm proximally to the ileocecal angle. During hepatectomy two-thirds of the liver was removed. All animals of groups 1, 2, and 3 were decapitated on the 6th day after resection of the intestine. Rats of groups 4 and 5 were decapitated 3-5 at a time under ether anesthesia at different times after partial hepatectomy. The rats received an intraperitoneal injection of $^3$H-thymidine in a dose of $37 \cdot 10^7$ Bq/g body weight 1 h before decapitation. Histoautoradiographs of sections through the liver and inestine were prepared as described previously [2]. The number of labeled cells was counted in 50-100 longitudinally sectioned crypts or among 1000-2000 hepatocytes from each animal, and the index of DNA-synthesizing cells (the labeling index — LI) was calculated in percent. The significance of the differences was determined by Student's test.

EGF was obtained from the submandibular salivary glands of male mice by the method described previously [1]. Antibodies to EGF were obtained by triple immunization of rabbits with EGF (500 μg in Freund's complete adjuvant each time). The EGF was iodized by the chloramine

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T–bisulfite method [6]. The specific activity of $^{125}$I-EGF was 12,000 cpm/ng. The EGC concentration in the rats' blood serum, saliva, and urine was determined by radioimmunoassay, using anti-EGF antiserum in a final dilution of 1:18.000. Bound $^{125}$I-EGF was precipitated with the aid of a precipitating complex from INC (USA). The EGC concentration in the rats' blood was determined by extraction of EGF from the serum with acetone and redissolving in extract in analysis buffer (five times the concentration); the urine for analysis was diluted 25 times and the saliva 10 times.

## EXPERIMENTAL RESULTS

Proliferation of hepatocytes in the regenerating rat liver was strictly coordinated in time and characterized by two distinct peaks of DNA synthesis (Fig. 1), reflecting the entry of different hepatocyte populations into the S phase of the mitotic cycle (MC). Hepatocytes located in the periportal zone of the hepatic lobule entered the phase of DNA replication first, with a relatively high degree of synchronization. After sialadenectomy and subsequent partial hepatectomy, two peaks of DNA synthesis and two corresponding rises of mitotic activity also were observed in the regenerating liver (Fig. 1),but the degree of synchronization of proliferation of hepatocytes in the periportal zone of the lobules was significantly reduced. This was shown by an increase in the duration of the first wave of DNA synthesis by 6 h (compared with the control) and by some delay of entry of the hepatocytes of the intermediate and central lobule into the S-phase. According to existing data [13], supraphysiological doses of $^{125}$I-EGF, if injected into the portal vein, are virtually completely taken up by hepatocytes of the periportal zone, and the concentration of the labeled hormone falls sharply toward the center of the lobule. This may perhaps be because of the large number of receptors for EGF on the plasma membrane of the periportal hepatocytes, which is responsible for their rapid entry into MC in response to stimulation of proliferation. Direct proof of the role of EGF in stimulating resting hepatocytes to divide was obtained in experiments *in vitro* on isolated liver cells [2, 14]. Hepatocytes also take part in the transport of EGF from the blood stream into the bile [13]. Removal of the main source of EGF, namely the salivary glands, possibly leads to a disturbance of certain hepatocyte functions that are controlled by EGF [13], and this is manifested as a change in the cytokinetics of proliferating cells of the regenerating liver.

Marked activation of new crypt formation and hyperplasia of some of the pre-existing crypts and villi were observed in the rats 5-6 days after resection of two-thirds of the small intestine. Although determination of LI for the enterocyte population of the crypts as a whole in the residual small intestine revealed only moderate stimulation of proliferation (Table 1), individual "giant" crypts up to 80-90 cell positions high (normal 35-45 cell positions), and with a greatly widened zone of proliferation, in which LI reached 60-70%, were observed. The processes of crypt formation, like those observed in the period of early postnatal development of the intestine, were characterized by active colony formation in the deep layers of the mucous membrane, longitudional splitting of some crypts, and the formation of new crypts by lateral budding, taking place simultaneously. After sialadenectomy a decrease in the intensity of proliferation was observed in the small intestine of animals of groups 2 and 3 (Table 1). However, sialadenectomy had virtually no effect on the morphogenetic processes of new crypt formation, taking place actively in the course of postresection compensatory hyperplasia of the mucous membrane: All the varieties of crypt formation described above were observed.

It will be clear from Fig. 2 that by means of a calibration curve the EGF concentration can be determined reliably within the range from 125 to 9000 pmoles/liter. The dissociation constant ($K_d$) for antibodies obtained to EGF (calculated from a Scatchard plot, see Fig. 2b) was $0.4 \cdot 10^{-9}$ M. The EGF concentration in the blood serum after sialadenectomy was unchanged on the first day after the operation, but by the 5th day it was statistically significantly reduced. The EGF concentration in the saliva fell by 75% after submandibular sialadenectomy (Table 2), whereas according to data in the literature [10] it fell almost tenfold. The EGF concentration in the urine was significantly higher than in the blood, and it did not fall significantly after removal of the salivary glands (Table 2). Thus it is impossible to achieve a significant EGF deficiency in the body simply by removal of the submandibular salivary glands, for other as yet unidentified sources of EGF also exist.

Considering that a sharp increase in the blood level of EGF in intact animals, arising under the influence of stress, was not observed in sialadenectomized animals [8], and the writers' observations showing a fall in the blood EDG concentration after removal of the salivary glands, it can be concluded that the salivary glands secrete EGF not only exogenously

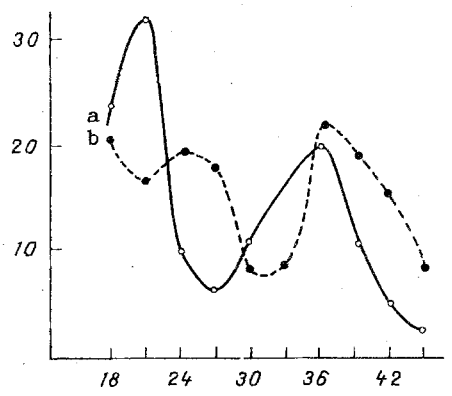


Fig. 1

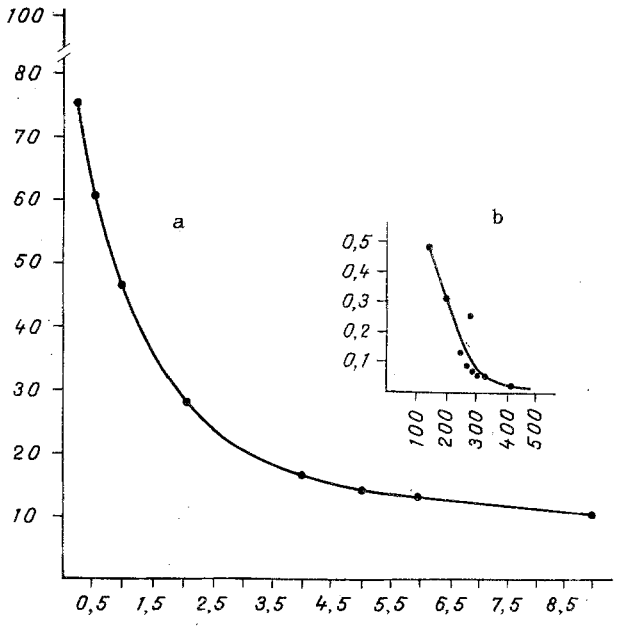


Fig. 2

Fig. 1. LI (in %) of hepatocytes of regenerating rat liver: a) control (group 5); b) sialadenectomy and partial hepatectomy. Abscissa, time after hepatectomy (in h).

Fig. 2. Calibration curve for radioimmunoassay of EGF concentration (a) and Scatchard plot of binding of $^{125}$I-EGF with antibodies against EGF (dilution of antiserum 1:18,000) (b). In a: abscissa, concentration of ECG standards (in nmoles/liter); ordinate, ratio of radioactivity of specifically bound fraction obtained in presence of competitor to activity of fraction bound in absence of competitor; in b: abscissa, quantity of specifically bound ligand (in pmoles/liter); ordinate, ratio of specifically bound to free ligand.

TABLE 1. Index (in %) of DNA-Synthesizing Cells in Epithelium of Different Parts of the Rat Intestine (M ± m)

| Part of intestine | Control | Sialadenectomy | Resection of two-thirds of intestine | Resection of intestine + sialadenectomy |
|---|---|---|---|---|
| Duodenum | 33,8±1,7 | 30,8±2,5 | 29,8±3,2 | 20,6±1,5** |
| Jejunum | 34,1±1,3 | 30,8±1,2* | 45,5±4,1 | 35,0±1,8** |
| Ileum | 30,2±1,1 | 22,8±1,5* | 34,8±1,7 | 28,0±1,1** |
| Ascending part of large intestine | 9,6±1,7 | 5,7±0,8* | 9,1±0,1 | 8,2±0,5 |
| Descending part of large intestine | 10,6±2,5 | 9,5±1,3 | 7,6±0,2 | 6,9±0,3 |

Legend. *P < 0.05 compared with control, **P < 0.05 compared with previous group. Number of experiments 10-15.

(into the saliva), but also endogenously (into the blood). The results of the present investigation indicate that EGF is a regulatory factor which modifies proliferation, and whose effect is particularly marked in the mucous membrane of the small intestine, where renewal of the epithelium takes place most intensively. EGF probably plays an essential, though not the main, role in the regulation of these processes. The reduction in proliferative activity observed in the intestine after sialadenectomy was most probably associated with a sharp fall in the EGF concentration in the intestinal contents, for among more than 25 biologically active substances produced by the salivary glands and excreted into the saliva [5], only EGF is present in concentrations capable of inducing a mitogenic effect *in vivo*. Meanwhile our data showing inhibition of entry of hepatocytes into MC after removal of the salivary glands suggest that the presence of EGF in the blood is evidently an essential condition for stimulation of hepatocyte proliferation *in vivo*, a conclusion which is in agreement with existing data on the powerful mitogenic action of EGF on primary cultures of hepatocytes [14].

TABLE 2. EGF Concentration (in moles·$10^9$/liter) in Rats' Blood Serum, Saliva, and Urine

| Test object | Control | Sialadenectomy | |
|---|---|---|---|
| | | 1st day | 5th day |
| Blood serum | 0,12±0,03 | 0,13±0,04 | 0,05±0,01* |
| Saliva | 6,66±1,36 | 1,25±0,5* | 2,21±0,54* |
| Urine | 6,25±1,85 | 5,95±2,53 | 5,2±2,41 |

Legend. EGF concentration determined by radioimmunoassay using a calibration curve (Fig. 2). *$P < 0.05$ compared with control.

To obtain a deeper understanding of the role of EGF in the renewal and differentiation of the epithelium of the gastrointestinal tract, more penetrating investigations are needed of the distribution of receptors for this polypeptide hormone on different types of cells, and the quantitative characteristics of the EGF-receptor apparatus must be established under the influence of various factors and, in particular, of other growth-stimulating factors.

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