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Effects of Epidermal Growth Factor on Deoxyribonucleic Acid Synthesis and Stomach Weight in Hypophysectomized Adult Mice

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SCHEVING, L. A., L. E. SCHEVING, T. H. TSAI, R. LOPEZ AND J. E. PAULY. *Effects of epidermal growth factor on deoxyribonucleic acid synthesis and stomach weight in hypophysectomized adult mice.* PEPTIDES 5(5) 945–951, 1984.—Epidermal growth factor (EGF) or saline was administered intraperitonally to hypophysectomized adult male $CD2F_1$ mice or intact controls at 0700 hr. Subgroups of mice were killed at 4, 8, or 12 hr after injection. EGF was shown to stimulate [^{3}H]TdR incorporation into DNA into several organs as previously reported. The response to EGF was found to be enhanced in both hypophysectomized and fasted mice. Differences in [^{3}H]TdR incorporation into DNA, corneal epithelium mitotic index, RNA in pancreas and kidney of hypophysectomized and intact mice are reported. EGF was shown to result in stomach enlargement due to increased luminal contents in both hypophysectomized and intact mice.

Epidermal growth factor Hypophysectomy Stomach weight DNA Corneal epithelium Mitotic index Circadian

EPIDERMAL growth factor (EGF) is a 6045 dalton polypeptide hormone that appears to play an important role in the growth and differentiation of a number of different tissues [6]. Recently, we have studied the effects of EGF on [^{3}H]TdR incorporation into DNA in over 20 organs of the intact adult mouse [22, 23, 33].

Hypophysectomy has been shown to result in dramatic reduction in the level of submandibular gland EGF [13], a reduction in the ability of serum to promote *in vitro* growth of several cell lines [31], and atrophic changes in a number of EGF target organs, including the corneal epithelium [24], epidermis [5], and several regions of the alimentary tract [18,32]. Because of these changes, we investigated the *in vivo* effect of EGF on [^{3}H]TdR incorporation into DNA in various tissues of hypophysectomized mice. We also examined the effects of EGF on total RNA in some organs, on the corneal epithelium mitotic index, and finally on stomach size. Moreover, since EGF has been shown to stimulate the synthesis and secretion of prolactin by a pituitary cell line [12,25], this study was done partially to exclude the possibility that some of the effects observed previously by us were mediated by pituitary hormone release.

Finally, since fasting has recently been shown by us to result in dramatic reduction in DNA synthesis [20], particularly in a number of EGF target organs, we examined the response of the esophagus to EGF at 12 hr after injection.

METHOD

Six week old male $CD2F_1$ mice were obtained from Simonsen (Gilroy, CA). The mice underwent hypophysectomy 7 weeks prior to sacrifice by Dr. Les Barnes (Altech Lab, Madison, WI). Intact and hypophysectomized mice were killed at 13 weeks of age. One month prior to sacrifice, the animals were subdivided into groups of 6 mice per cage, and 4 or 5 such cages were placed in sound-attenuated temperature-regulated isolation chambers. Each chamber was illuminated from 0600–1800 daily. Food and water was available *ad lib*. The cages were replaced with clean ones each week. At the time of sacrifice, completeness of hypophysectomy was assessed by testicular regression [27].

Epidermal growth factor (receptor grade) was obtained from Bethesda Research Labs, Inc. (Bethesda, MD). EGF was dissolved in 0.9% saline and injected intraperitonally at a dosage of 1 μg/g body weight into 18 intact and 18 hypophysectomized mice at 0700 hr. The carrier without EGF served as the control vehicle and was injected at the same time into 18 intact and 18 hypophysectomized mice. Mice were sacrificed at 4, 8, or 12 hr after injection by rapid cervical dislocation.

Thirty min before killing, each mouse was injected IP with 1 μCi/g body wt of [^{3}H]TdR (25 Ci/mmol). After killing, the thoracoabdominal cavity was opened and the carcasses

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TABLE 1

EFFECT OF HYPOPHYSECTOMY OR EGF ON DNA SYNTHESIS, MITOTIC INDEX, RNA, AND STOMACH WEIGHT

	(H)vs.(S)			(HE)vs.(H)			(E)vs.(S)		
	4 hr	8 hr	12 hr	4 hr	8 hr	12 hr	4 hr	8 hr	12 hr
Tongue	113	59		−23	81	344	61	50	257
Esophagus	−26			86	340	900		27	364
Non-gland stomach	−34	−48		102	120	263			143
Gland. stom.		26			48	74	−20	36	133
Duodenum	23								
Jejunum						32			−15
Ileum	46		67						
Colon					106	191		82	86
Rectum	−22			38	88	143			79
Spleen									
Thymus					−25				
Bone marrow	−25	−39					24		
Liver				−20				−19	
Kidney		−64	−61	−59		56		−39	
Pancreas	−61	−15		−31		67		36	
Pancreas Total RNA/DNA		−44	−34	−31					
Kidney Total RNA/DNA	−54	−57	−45				7	7	30
Stomach wt.	−31	−24	−55	81		92	26		
Mitosis in corneal epithelium	−31			53					

In this table, we compare the percent increase or decrease of [^{3}H]TdR incorporation into DNA, the ratio of pancreatic RNA/DNA, total RNA in the left kidney, stomach weight, and corneal epithelium mitotic index in (1) hypophysectomized, (H) vs. intact (S) male CD2F$_1$ mice; (2) EGF-injected hypophysectomized (HE) vs. saline injected hypophysectomized (H); and (3) EGF-injected intact (E) vs. saline injected intact (S) mice. In each case, the percent increase or decrease is referenced to the second group. For example, in the H vs. comparison at 4 hr after injection, [^{3}H]TdR incorporation into DNA in the tongue is 113% greater in the hypophysectomized (H) mice compared to the intact ones (S), whereas at the same time, in the esophagus, it is decreased by 26% in the H group compared to the S group. Only statistically significant differences between compared groups are presented. The level of statistical significance is at least at the $p<0.05$. The absolute levels are not presented since the observed levels are similar to ones previously published by us [3, 4, 5] for male CD2F$_1$ mice of similar age. The kill times after injection are represented by 4, 8, and 12 hr which represent 1100, 1500 and 1900 clock hrs, respectively. Treatment time was at 0700.

were fixed in phosphate buffered solution. After fixation, pieces of the tissues to be studied were removed. The stomach, including luminal contents, and left kidney were weighed. The DNA was then extracted from each piece of tissue by the method of Ogur and Rosen [16], with the modification that the RNA hydrolysis was carried out in a 1 N NaOH solution at 60°C for 18 hr. Details of this procedure have been previously reported [3]. Total RNA in the kidney and pancreas were determined in the supernatant by the Orcinol method [15]. The mitotic index of the corneal epithelium was also determined [24].

In a second study, to assess the effects of EGF on DNA synthesis in the esophagus of fasted mice, the mice were treated as described above, except that they were 8 weeks old at the time of sacrifice. Food was removed from the cages at 0600, the mice were injected with EGF or saline at 1100, and they were sacrificed at 2100. Water was provided *ad lib* to both groups.

Student's *t*-test was used to compare differences in [^{3}H]TdR incorporation into DNA, total RNA, and mitotic index between experimental and control data. Since the absolute level of [^{3}H]TdR incorporation into DNA, total RNA and mitotic index in the corneal epithelium was similar to that reported earlier [3, 22–24, 33], this data is not presented in Table 1.

RESULTS

Hypophysectomy vs. Sham

Table 1 compares the percent increase or decrease in DNA synthesis in several organs of hypophysectomized mice relative to intact ones. At 1100 hr, [^{3}H]TdR incorporation into DNA decreased in the former relative to the latter in rectum, bone marrow (Fig. 1), esophagus (Fig. 2), non-glandular stomach, and pancreas by 22, 25, 26, 34, and 61%, respectively. At the same time, it was increased in hypophysectomized mice in the tongue, ileum, and duodenum by 113%, 46% and 23%, respectively (Table 1).

At 1500 hr, DNA synthesis was decreased in the hypophysectomized mice in the pancreas, bone marrow, non-glandular stomach, and kidney by 15, 39, 48, and 64%, but increased by 59% and 26% in the tongue and glandular

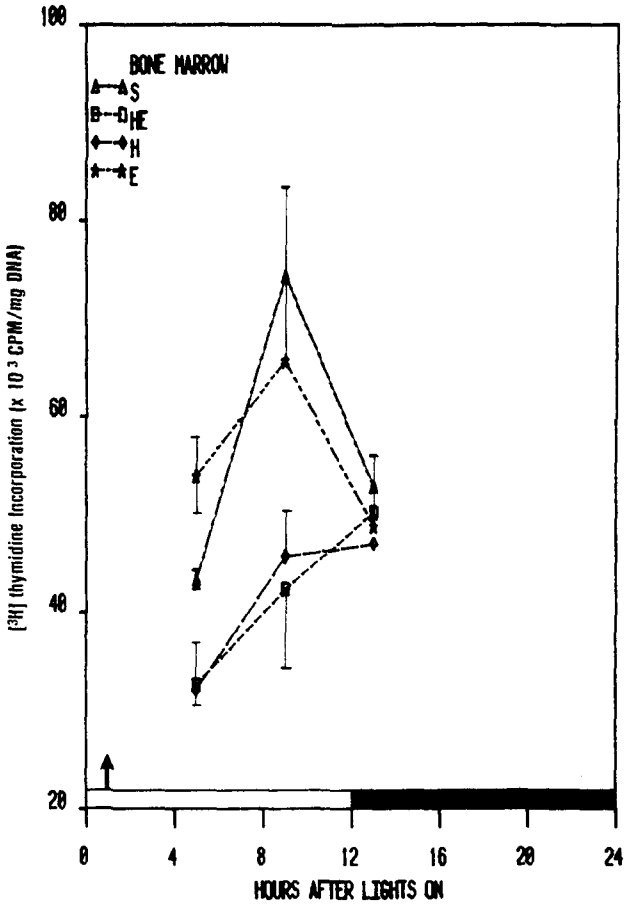

FIG. 1. A comparison of the mean and standard error of [^{3}H]dR incorporation into DNA of the bone marrow of the different experimental groups. S=sham. HE=hypophysectomized epidermal growth factor (EGF) treated hypophysectomized animals, H=hypophysectomized untreated mice and E=EGF treated normal animals. N=6 mice/point. The ↑ indicates time EGF was administered. The kill times were 1100, 1500, and 1900 CST; or 4, 18 and 12 hr after treatment. The mice were standardized to 12 hr of light (0600–1800) alternating with 12 hr of darkness.

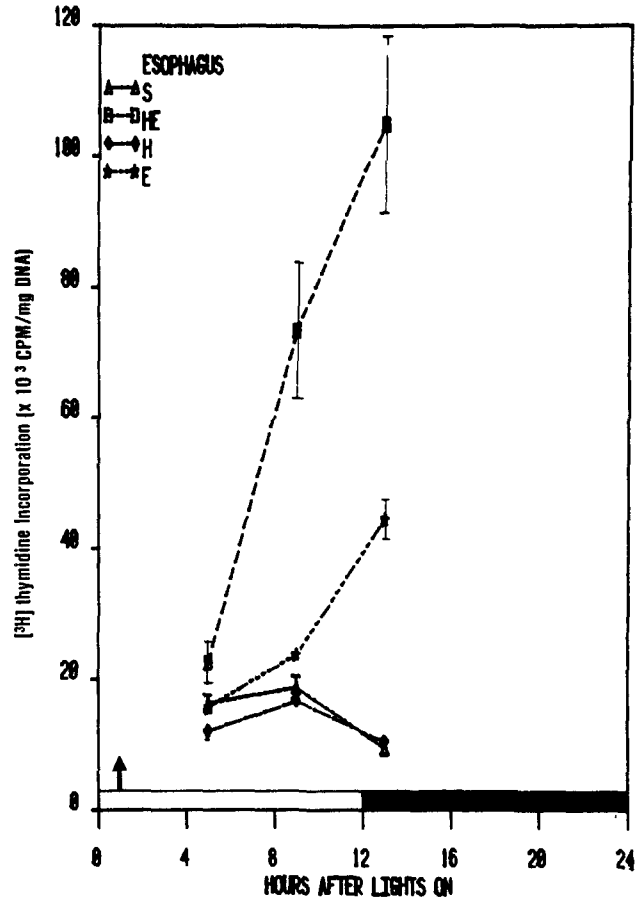

FIG. 2. Data from esophagus. See explanation in Fig. 1 above.

stomach, respectively. At 1900 hr, it was decreased in the kidney by 61% and increased in the ileum by 67%. Thus, 10 of the 16 statistically significant differences between hypophysectomized and intact mice in [^{3}H]TdR incorporation into DNA in the 15 tissues studied represent decreases in the former compared to the latter.

The mitotic index of the corneal epithelium (Fig. 3) was decreased at 1100 hr in the hypophysectomized mice by 31%, but not at 1500 or 1900 hr (Table 1).

Because the comparison of [^{3}H]TdR incorporation into DNA and corneal epithelium mitotic index covers only the latter part of the light span and early dark span, one cannot make any overall generalizations concerning the average level of DNA synthesis between the 2 groups. However, it is obvious that some of the expected circadian changes persisted in the hypophysectomized mice, such as the decline in corneal epithelium mitotic index during the light span (Fig. 3) and that the most consistent effects of hypophysectomy on DNA synthesis are to elevate it in the tongue and ileum and depress it in the non-glandular stomach, bone marrow (Fig. 1), kidney and pancreas.

RNA was determined in 2 tissues, namely the pancreas (Fig. 4) and kidney (Fig. 5). In hypophysectomized mice, the ratio of total RNA to total DNA was depressed by 44% at 1500 hr and 34% at 1900 hr, whereas total RNA per kidney was depressed by 54% at 1100 hr, 57% at 1500 hr, and 45% at 1900 hr (Table 1).

Total body weight of the hypophysectomized mice at the time of sacrifice was 28.2±0.8 g compared to 32.2±0.4 g in intact mice, representing a 13% reduction in the former compared to the latter.

Response of the Hypophysectomized and Intact Mice to EGF

As shown in Table 1, EGF exerted its greatest effects on DNA synthesis in the esophagus (Fig. 2), colon, and rectum (Fig. 6). Moreover, in these organs, with the exception of the glandular stomach, the magnitude and rapidity of response to EGF was greater in the hypophysectomized mice compared to intact ones. For example, in the esophagus (Fig. 2), EGF stimulated DNA synthesis by 86, 340, and 900% at 4, 8, and

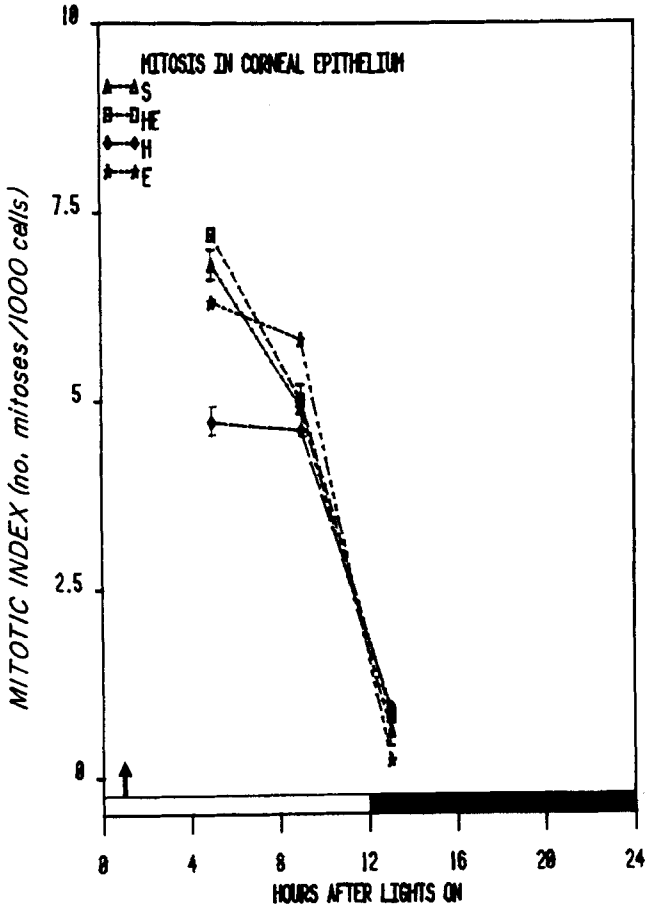

FIG. 3. Mitotic index of corneal epithelium. See explanation in Fig. 1 above.

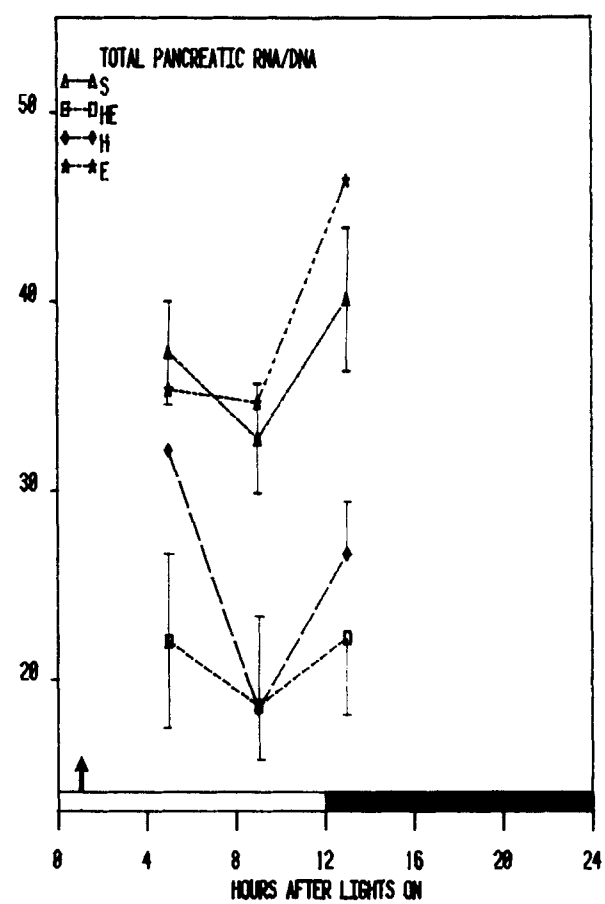

FIG. 4. Ratio of total pancreatic RNA/DNA. See explanation in Fig. 1 above.

12 hr, respectively, after injection in the EGF-treated hypophysectomized mice compared to saline-treated hypophysectomized mice. In the same organ at 8 and 12 hr, EGF stimulated DNA synthesis by 27% and 364% in EGF-treated intact mice compared to saline-treated intact mice (Fig. 2). EGF had less effect on DNA synthesis in the other organs studied. Table 1 also demonstrates that EGF stimulated corneal epithelium mitotic index by 53% in hypophysectomized mice at 1100 hr, restoring it to levels seen in saline-treated intact mice (Fig. 3).

Finally, EGF treatment increased total RNA levels in the kidney of intact, but not hypophysectomized mice, by 7%, 7%, and 30%, at 4, 8, and 12 hr after injection, respectively.

Effect of EGF on Stomach Size

The weight of the stomach with luminal contents was decreased in hypophysectomized mice relative to intact ones by 31%, 24%, and 55% at 4, 8, and 12 hr after saline injection, respectively (Fig. 7). EGF treatment resulted in an 81% and 92% increase in stomach weight at 4 and 12 hr after injection in hypophysectomized mice, but only a 26% increase at 4 hr after injection in intact mice (Table 1).

Effect of EGF on Esophageal DNA Synthesis in Fasted Mice

[^{3}H]TdR incorporation into esophageal DNA was 3.7±0.7 cpm/μg DNA in the fasted mice, compared to 7.9±0.7 cpm/μg DNA in ad lib fed mice at 2100 hr. At 10 hr after EGF injection, [^{3}H]TdR incorporation into DNA of the EGF-treated fasted mice was 34.3±6.8 cpm/μg DNA, but only 21.1±5.3 cpm/μg DNA in the EGF-treated ad lib fed mice. Thus, EGF treatment increased DNA synthesis by 11-fold in fasted mice, but only 3-fold in the ad lib fed mice.

DISCUSSION

In this paper, we have (1) confirmed and enhanced earlier work demonstrating the differential effects of EGF on the incorporation of [^{3}H]dR incorporation into DNA [22, 23, 33] in many different organs of the mouse. In our earlier papers we discussed in some detail these findings, but still we do not know why EGF enhances [^{3}H]dR incorporation in some tissues whereas in some it brings about a decrease. (2) We have studied the effect of EGF on DNA synthesis in several organs, corneal epithelium mitotic index, and gross stomach weight of intact and hypophysectomized adult male mice. (3) We have also examined the effect of EGF on esophageal DNA synthesis in ad lib fed and fasted mice.

The effects of EGF have been shown to persist in both hypophysectomized and fasted mice and are in fact enhanced in both cases. Others have reported an enhanced response of the adrenal gland to ACTH [14] and of the liver to growth hormone [17] in hypophysectomized mice as well as of the small intestine to exogenous EGF in fasted mice [4].

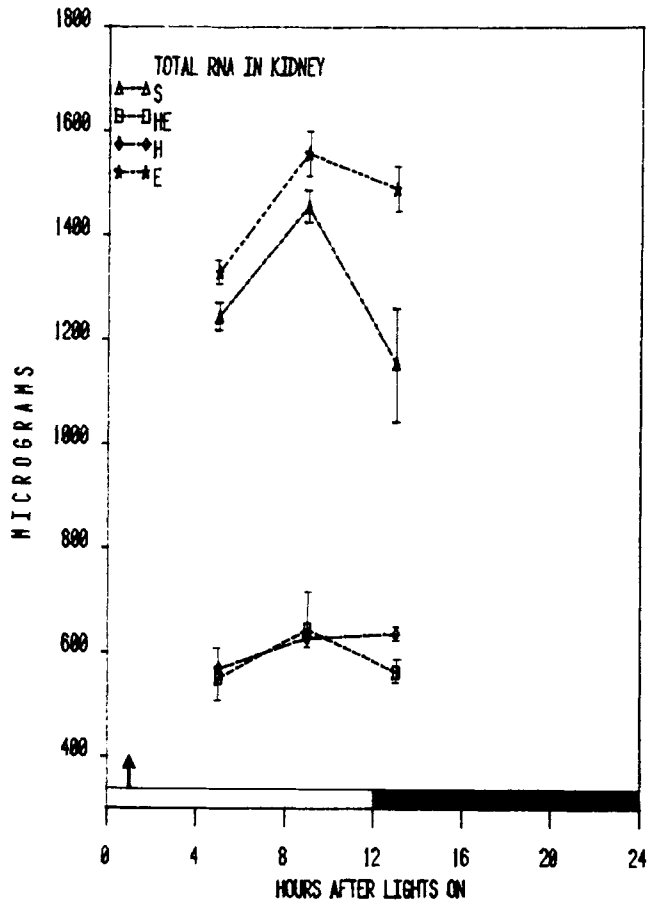

FIG. 5. Total RNA in kidney. See explanation in Fig. 1 above.

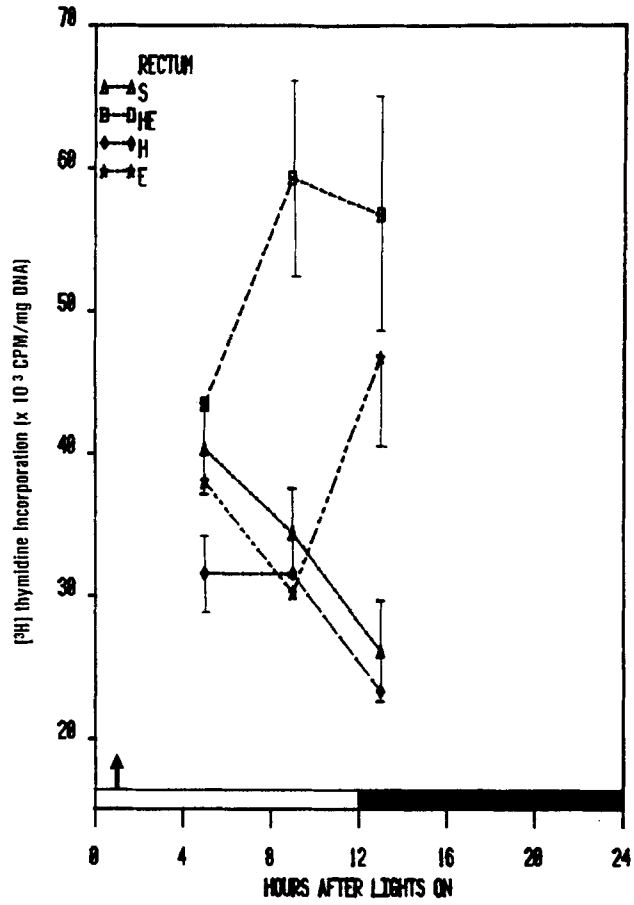

FIG. 6. Data from rectum. See explanation in Fig. 1 above.

Since we have found in a companion study that EGF stimulates guanylate cyclase in several tissues to a greater extent in hypophysectomized mice than in intact mice both *in vivo* and *in vitro* [19], it is unlikely that the results presented herein on DNA synthesis are due to delayed excretion or altered metabolism of EGF.

The reason for the enhanced response in hypophysectomized and fasted mice is unknown. Decreased feeding could be involved in the former. In both cases, the enhanced response could be mediated at the level of the receptor. Hypophysectomy has been reported to dramatically decrease the level of the submandibular gland EGF [13], presumably due to decreased levels of circulating thyroxine [30] and testosterone [13], which positively regulate EGF synthesis. Inanition has been reported to decrease the protease content of the EGF-producing glandular convoluted tubular cells in the submandibular gland [28] as well as the serum level of EGF [4]. Although it is not known whether or not hypophysectomy or fasting have similar effects on other sites of EGF secretion, such as the duodenal glands [9] and pancreas [10], the aforementioned results imply that the levels of EGF in the saliva and foregut lumen are decreased.

If local levels of EGF are decreased, then the atrophy observed in both conditions may ensue as a result of increased quiescence of mucosal stem cells. These cells, as a result of EGF depletion, might in turn adapt by increasing the number of EGF cell surface receptors or receptor affinity for EGF, leading to increased sensitivity to endogenous or exogenous EGF. Hypophysectomy has been reported to modulate the receptor number for several hormones, increasing it for the growth hormone receptor in the liver [17] and decreasing it for human choriogonadotropin in the ovary [26] and follicle stimulating hormone in the testes [7].

Epidermal growth factor is produced by the GCT cells in the murine submandibular salivary gland [6]. These cells also produce nerve growth factor, amylase, renin, and a number of esteropeptidases [1]. These hormones appear to be positively regulated in a similar, but not identical, manner by a number of hormones, such as testosterone and thyroxine [11], which are in turns regulated by the pituitary hormones. Inspection of the genetic constitution of human chromosome I reveals the curious localization of the genes for NGF, amylase, and renin to the same region of the p arm of this chromosome, with linkage between NGF and amylase [11]. Although it is not known whether or not the EGF gene is similarly localized, if so, it will be of interest to determine whether or not the localization of genes is related to regulatory similarities involving pituitary-dependent hormones.

This study also documents that exogenous EGF causes luminal accumulation of food within the stomach in both intact and hypophysectomized mice within 12 hr after injection (Fig. 7). This has been previously observed by us not only in mice injected IP with either EGF or sympathomimetic-elicited saliva, but also in mice injected

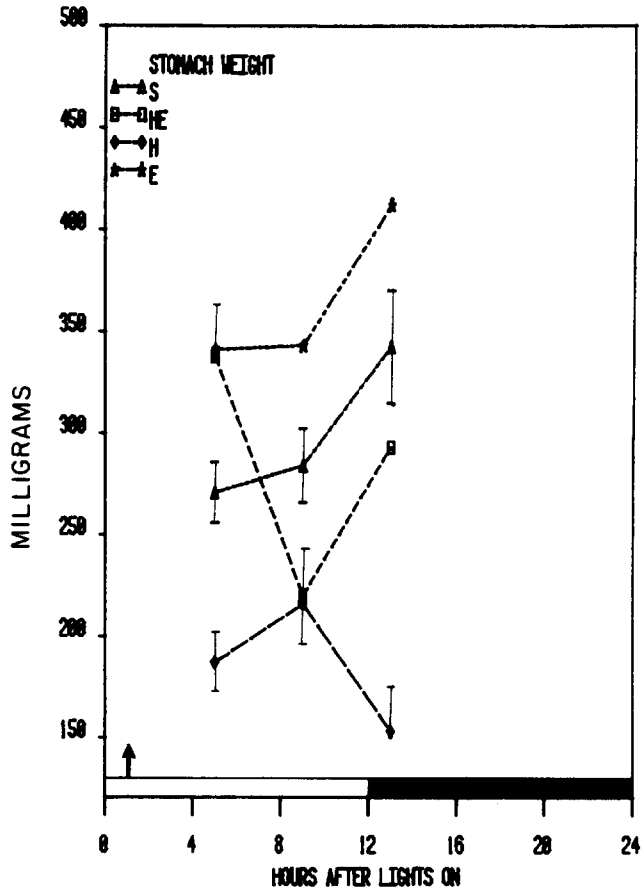

FIG. 7. Comparison of stomach weights among the different groups. See explanation in Fig. 1 above.

with norepinephrine or isoproterenol (100 μg/kg) directly under the fibrous sheath of the submandibular gland [21].

Although these effect may be pharmacologic, they can be related to the antisecretory effects of EGF on parietal cell acid secretion [2] and the anti-cholinergic effects of EGF *in vitro* on intestinal smooth muscle motility [29]. Moreover, since stimulatory effects of EGF on DNA synthesis can be demonstrated in fasted as well as fed mice, this rules out our concern that luminal accumulation in bulk forming organs in EGF-treated mice, rather than EGF itself, leads to stimulation of DNA synthesis as a result of mucosal shearing and resultant physiologic renewal.

In summary, it has been demonstrated that EGF is capable of stimulating DNA synthesis, corneal epithelium mitotic index, and stomach enlargement in hypophysectomized as well as intact mice. Furthermore, we have found an enhanced response to exogenous EGF in fasted and hypophysectomized mice and speculated that the greater synchronization of response may be mediated at the receptor level.

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