0013-7227/82/1111-0308$02.00/0
Endocrinology

Vol. 111, No. 1
Printed in U.S.A.

Circadian Stage-Dependent Effects of Insulin and Glucagon on Incorporation of [^{3}H]Thymidine into Deoxyribonucleic Acid in the Esophagus, Stomach, Duodenum, Jejunum, Ileum, Caecum, Colon, Rectum, and Spleen of the Adult Female Mouse*

L. A. SCHEVING, L. E. SCHEVING, T. H. TSAI, AND J. E. PAULY

Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

ABSTRACT. Insulin and glucagon were injected ip at four different circadian stages into separate subgroups of female adult BALB/Cann mice that had been standardized to 12 h of light alternating with 12 h of darkness. Comparable control subgroups were injected with saline. Four, 8, 12, and 18 h after each of the four injections, subgroups of seven mice that had been injected 30 min earlier with tritiated thymidine ([^{3}H]TdR) were killed; a total of 336 mice were used. The incorporation of [^{3}H]TdR into DNA of the esophagus, stomach, duodenum, jejunum, caecum, colon, rectum, and spleen was subsequently determined.

The results demonstrate for the first time that both hormones affect the incorporation of [^{3}H]TdR into DNA in all of the examined organs but in different ways and at different circadian stages. The effects of these hormones were complex, but several generalizations emerged. 1) Insulin tended to increase the incorporation of [^{3}H]TdR into DNA in the examined organs, whereas glucagon tended to decrease it. 2) Insulin was more effective in stimulating the incorporation of [^{3}H]TdR into DNA when injected either at the end of the dark span or the beginning of the light span, as opposed to the end of the light span or the beginning of the dark span. 3) Insulin had its greatest effect on [^{3}H]TdR incorporation into DNA in the glandular stomach and rectum, whereas glucagon had its greatest effect on the colon and spleen. 4) The effects of both insulin and glucagon were different from those of epidermal growth factor, as revealed in a similar study done by us. Our results suggest that insulin, glucagon, and epidermal growth factor play important roles in the control of growth of various endodermally derived organs. (*Endocrinology* **111**: 308, 1982)

RECENT evidence suggests that insulin and glucagon may play a role in the growth control of various entodermally derived organs. Both hormones stimulate DNA synthesis of a human colonic cancer cell line (1, 2) as well as potentiate the stimulatory effect of epidermal growth factor (EGF) on DNA synthesis in the regenerating liver (3, 4). Insulin has been shown to stimulate DNA synthesis in a duodenal cell line (5), to accelerate the enzymatic maturation of the rodent small intestine (6), and to stimulate rodent gastric mucosal DNA synthesis (7). Furthermore, a surprisingly high concentration of mucosal insulin receptors relative to classic insulin target organs has been demonstrated, supporting a hitherto unemphasized site of action of this hormone (8, 9). Glucagon has been reported to stimulate colonic DNA synthesis (10), and a patient bearing a renal glucagon-secretory tumor exhibited marked hypertrophy of the small intestine (11); however, others have reported that glucagon is ineffective in stimulating the growth of rodent small bowel mucosa (12).

In spite of the above evidence, a comprehensive study of the effects of these hormones on DNA synthesis in different regions of the gastrointestinal tract in the intact animal has yet to be done. In this study, we begin this work by examining their effects on DNA synthesis, as judged by tritiated thymidine ([^{3}H]TdR) incorporation into the esophagus, glandular stomach, duodenum, jejunum, caecum, colon, rectum, and spleen, and comparing them to the effects of EGF found in earlier work (13–15).

Materials and Methods

Six-week-old female BALB/Cann mice were received from Charles River Breeding Laboratories, Inc. (Wilmington, MA), on May 14, 1980. The animals were assigned randomly, with stratification by body weight, into 4 groups of 84 animals each. The 84 animals were then randomly subdivided into subgroups

Received April 13, 1981.

Address requests for reprints to: Dr. L. E. Scheving, Department of Anatomy, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, Arkansas 72205.

* This work was supported in part by Grant OH-00952 from the National Institute of Occupational Safety and Health.

of 7/cage, and groups of 4 cages were placed in sound-attenuated, temperature-regulated isolation chambers. Each chamber was illuminated with 12 h of light alternating with 12 h of dark (LD 12:12); lights were regulated automatically. The light-dark (LD) schedules were staggered so that 1 group of 3 isolation chambers (84 mice) was illuminated from 1200–2400 h daily; in a second group, the LD schedule was the exact reverse; in the third group, the lights were on from 2000–0800 h daily; and in the fourth group, lights were on from 0800–2000 h daily (Fig. 1). Food and water were available *ad libitum*. The mice remained standardized under these conditions for 6 weeks before the experiment and were not intentionally disturbed (except for the perturbation caused by replacing cages with clean ones once a week and replenishing the food supplies). The technique of automatically turning lights on and off at different times in the different chambers (staggered lighting) made it possible to inject all of the animals with insulin, glucagon, or saline within a short span of time (0900–0945); presumably, this was the equivalent to injecting at 4 different mouse circadian stages. In relation to the LD cycle, this represented 1 and 9 h after lights on (1 HALO and 9 HALO, respectively) or 1 or 9 h after lights off (1 HALOff and 9 HALOff, respectively).

Beginning at 0900 h on June 26, 1980, 112 mice (now approximately 12 weeks of age), consisting of 28 from each of the 4 lighting schedules, were injected ip with 1.6 μg/g pork insulin (Eli Lilly Co., Indianapolis, IN) contained in 0.2 ml saline. A second group of 112 mice was similarly injected with 1.0 μg/g glucagon (Eli Lilly), and a third group of 112 mice was injected with 0.2 ml saline/20 g mouse. The amounts of insulin and glucagon injected were approximately equimolar. All injections were accomplished within 45 min.

Subgroups of 7 mice each from the insulin, glucagon, and control groups from the 4 different lighting schedules were killed by rapid cervical dislocation 4 h after injection; this was repeated 8, 12, and 18 h after injection (total of 336 mice; Fig. 1). Thirty minutes before killing, all mice were injected ip with 25 μCi [^{3}H]TdR (17 Ci/mmol). After killing, the thoracoabdominal cavity was opened, and the carcasses were fixed in 100 μl/ml buffered formalin solution. After fixation for 2 weeks, pieces

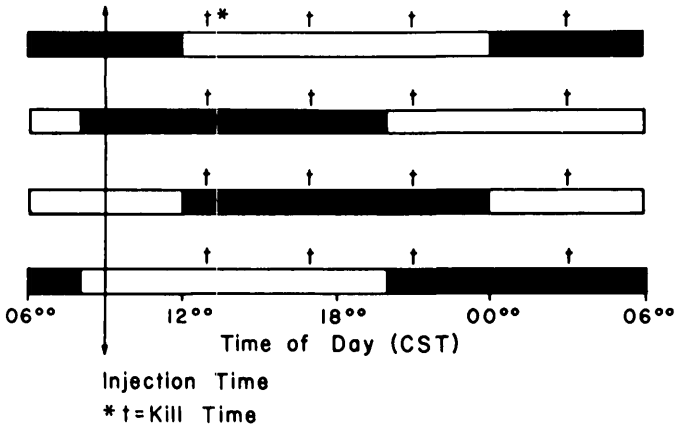

FIG. 1. *Horizontal bars* represent the four staggered LD schedules used to standardize mice so that sampling could be performed at four different circadian stages in mice injected at one time span (between 0900 and 0945 h), as indicated by the *vertical line*. †, Postinjection kill times.

of the tissues studied were removed. The tissues of the insulin- and glucagon-injected animals being reported on in this paper are: esophagus, the glandular part of the stomach, duodenum, jejunum, caecum, colon, rectum, and spleen. 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 h. Details of this modified procedure have been previously reported (17). We believe, from much previous data obtained and from comparison with circadian mitotic index rhythms, that this technique measures DNA synthesis *in vivo*, but we also are aware of the potential pitfalls or problems associated with it, especially in *in vitro* studies (18). Future labeling index studies will be helpful in confirming the findings of this study.

An analysis of variance (ANOVA) was used to test statistical significance among different time points in each set of time series data illustrated in Fig. 2. Student's *t* test was used to compare differences in DNA incorporation between experimental and control data at specific hours. In addition two- and three-way ANOVA and a cosinor analysis (19) were carried out on the data.

A dose-response curve for insulin was determined, with injections beginning at two different circadian stages before the study being reported and with the glandular stomach and caecum as the target organs. The variation in DNA synthesis response with the different doses varied as a function of the circadian stage injected. The dosages for this study (1.6 μg/g for insulin and 1.0 μg/g for glucagon) were selected because they elicited many strong responses in our initial dose-response studies and because these doses frequently appear in the literature, especially for insulin in rodent experiments involving liver regeneration (4). The glucagon dosage was also selected based on its usage in a recent study demonstrating an inhibitory effect of glucagon on erythropoiesis (20).

Results

General remarks

The staggered LD system is a valid technique only when the animals are given time to adapt to their new LD regimen. The shifts in DNA synthesis rhythms are not immediate, but require a period of time to phase shift. Although the different groups of animals were on different LD regimens, Fig. 2 illustrates that after 6 weeks, DNA synthesis in the spleen, esophagus, and glandular stomach had been resynchronized in all groups to the LD cycle, as opposed to local clock time. This conclusion can be drawn because of the similarity of he phasing and waveform of incorporation of [^{3}H]TdR incorporation into DNA rhythms to those one would expect if all groups of mice had been subjected to the same LD schedule.

When the control data of the examined tissues were fit to a 24-h cosine curve, they were all found to be significant at least at the 95% level, except for the spleen. When, however, an ANOVA analysis for the effect of time was performed on the data from the spleen, it was

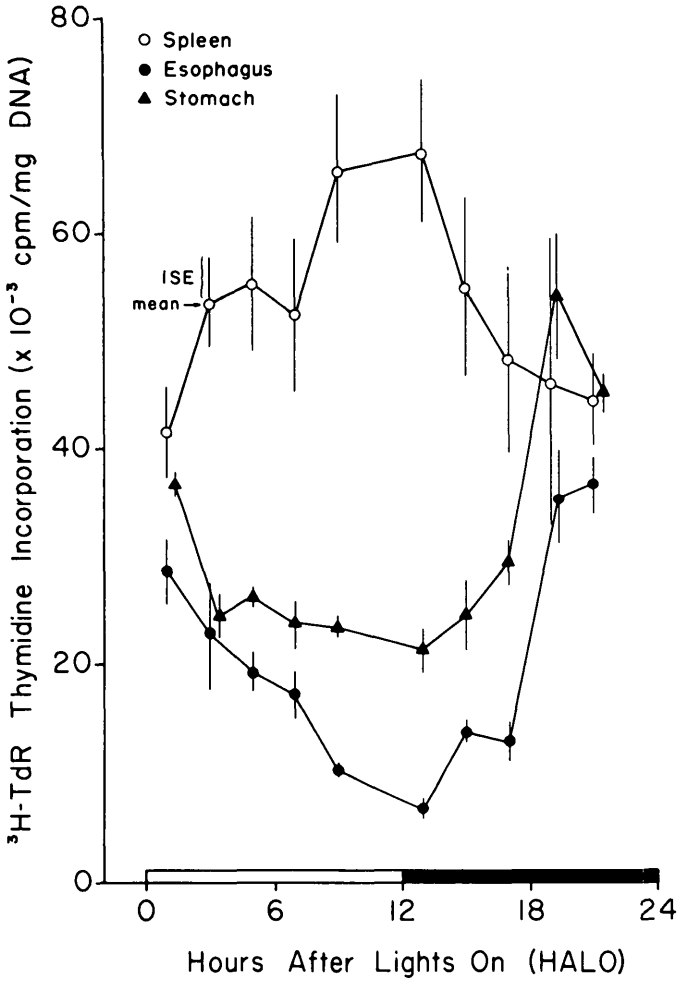

FIG. 2. Rhythmic patterns of [^{3}H]TdR incorporation into DNA in the spleen, esophagus, and glandular stomach of saline-treated female BALB/C mice. The *abscissa* (as in all figures) represents the LD cycle under which the mice were standardized. An ANOVA test revealed a highly statistically significant variation among the 10 time points for each tissue ($P < 0.01$ in all cases). These data serve as a marker to show that the rhythms in DNA synthesis in each of the tissues had phase shifted during the 6-week standardization period to each of the four LD schedules, because the pattern was similar to that one would expect with conventional lighting (12 h of light alternating with 12 h of darkness, with sampling at about 2- to 3-h intervals around the clock).

highly statistically significant ($P < 0.001$). As we have described previously for the gut, there was an oral to anal gradient in the computer-determined time of acrophase (peak) for the esophagus and stomach preceding that of the rectum by several hours (14). The same was clearly evident from this study. The acrophase for the data from the esophagus was −352° (−328 to 18), whereas in the rectum it was −54° (−354 to −84); this represents approximately a 4-h delay in the acrophase (peak) of incorporation of [^{3}H]TdR into the DNA of the rectum (19) relative to the esophagus.

Figure 2 also illustrates two significant points. 1) The phasing of rhythms in different organs were not necessarily identical. The increase in DNA incorporation in the spleen appeared to increase or peak during the light period, whereas in the esophagus and glandular stomach, it appeared to increase and peak during the dark period. 2) Two organs may have similar DNA incorporation rhythms and yet respond differently to different mitogenic proteins. For example, as described below, the responses of the esophagus to insulin and EGF were quite different from those of the glandular stomach, although the phasing of their DNA incorporation rhythms were similar.

Both insulin (Table 1 and Fig. 3) and glucagon (Table 2 and Fig. 4) significantly affected [^{3}H]TdR incorporation into DNA in the examined organs. Thirty-eight percent of all of the sets of data obtained were statistically significant ($P < 0.05$; usually far less) for the insulin-injected mice, whereas 24% were significant for the glucagon-injected mice (Tables 1 and 2).

Effect of insulin on [^{3}H]TdR incorporation into DNA

Insulin affected the various regions of the gastrointestinal tract and the spleen differently. In the insulin-treated mice, statistically significant sets of data were obtained from the examined tissues in the following order: glandular stomach (69%), rectum (50%), caecum (44%), jejunum (38%), and finally the esophagus, duodenum, colon, and spleen (25% each; Table 1).

In general, the effects of insulin on [^{3}H]TdR incorporation into DNA in the above tissues were stimulatory. The grandular stomach and rectum, which showed the greatest frequency of response to insulin, also showed the greatest magnitude of response. They were the only tissues having increases in DNA synthesis exceeding 100%. Preliminary dose-response studies involving insulin and the glandular stomach and caecum suggest that at certain circadian stages insulin is capable of stimulating DNA synthesis at a dose as low as one tenth of that used in this study. The resolution of the effect of different doses in relation to temporal organization of the mouse awaits further study.

Only 17% of the statistically significant sets of data represented inhibitory effects of insulin. These inhibitory effects had several common features. First, 50% of the inhibitory effects occurred in the group of mice injected late in the light period. Secondly, all of the inhibitory effects were localized to regions of the gut proximal to the glandular stomach and distal to the small intestine (Table 1 and Fig. 3). In fact, only in the esophagus and colon did insulin inhibit [^{3}H]TdR incorporation into DNA as many times as it stimulated it (Table 1). Finally, all but one of the inhibitory effects of insulin occurred in mice killed either 4 or 8 h after injection, as opposed to 12 or 18 h.

TABLE 1. Percent increase (↑) or decrease (↓) in the incorporation of [^{3}H]TdR into DNA 4, 8, 12, and 18 h after the injection of insulin at four different circadian stages

Injection time: Kill time (h after injection):	1 HALO (early light)				9 HALO (late light)				1 HALOff (early dark)				9 HALOff (late dark)				Total responses out of 16	
	4	8	12	18	4	8	12	18	4	8	12	18	4	8	12	18	↑	↓
Esophagus	76↑	90↑							NS	NS	30↓			25↓			2	2
Glandular stomach		81↑		59↑		83↑	88↑	68↑	107↑	62↑			73↑	50↑	41↑	111↑	11	0
Duodenum											24↑		33↑		44↑	20↑	4	0
Jejunum	39↑	37↑	43↑								17↑		40↑	35↑			6	0
Caecum		54↑	40↑		29↓	27↑							46↑	39↑		27↓	5	2
Colon	41↓	34↑			51↓								49↑				2	2
Rectum		75↑	141↑	58↑	32↓			100↑	98↑	106↑			49↑				7	1
Spleen	85↑		74↑	36↑	50↓												3	1

HALO, Hours after lights on; HALOff, hours after lights off. A blank space indicates that any change seen was not statistically significant. NS, Specimens lost.

The effect of insulin was relatively rapid. Thirty-three percent of the statistically significant sets of data were recorded 4 h after injection, 29% were recorded at 8 h, 21% were recorded at 12 h, and 17% were recorded at 18 h (Table 1 and Fig. 3).

The effects of insulin clearly depended on the time (circadian stage) of injection. Insulin was more likely to affect [^{3}H]TdR incorporation into DNA when injected at either the end of the light span or the beginning of the dark span (Fig. 3). For example, statistically significant sets of data were obtained 47% and 53% of the times for mice injected in the late dark or early light phase, respectively, as compared to only 28% and 22% for mice injected in the late light or early dark phase (Table 1). However, the two tissues that responded most positively to insulin, namely the glandular stomach and rectum, were least affected by changing the injection time. They both responded strongly to insulin, even during the periods when the other tissues responded weakly if at all (Table 1 and Fig. 3).

Effect of glucagon on [^{3}H]TdR incorporation into DNA

The effect of glucagon on [^{3}H]TdR incorporation into DNA in the examined organs tended to be less dramatic, slower, and more inhibitory than that of insulin. Statistically significant sets of data were obtained 29%, 29%, 19%, and 22% of the times when glucagon was injected in the early dark, late dark, early light, and late dark phases, respectively. Thus, glucagon had a somewhat greater effect on DNA synthesis when injected in the dark period than in the light period (Fig. 4 and Table 2).

Thirty-nine percent of the statistically significant responses in the glucagon-injected mice occurred 4 h after injection, 19% occurred at 8 h, 13% occurred at 12 h, and 29% occurred at 18 h. When compared to insulin, the effect of glucagon on DNA synthesis was greater at 4 and 18 h and less at 8 and 12 h.

The effect of glucagon at this particular dosage also tended to be more inhibitory in nature than that of insulin. For example, glucagon inhibited DNA synthesis in 58% of the statistically significant sets of data, whereas insulin inhibited it only 17% of the time. However, the effect of glucagon did depend on the time of injection (circadian stage). When glucagon was injected late in the dark period, it only inhibited [^{3}H]TdR incorporation into DNA. When it was injected in the early dark period, 77% of its statistically significant effects were stimulatory ones (Fig. 4 and Table 1).

Finally, the tissues differed in their responses to insulin or glucagon. The colon and spleen were the tissues most affected by glucagon, which generally inhibited DNA synthesis (six of the seven statistically significant sets of data for the colon and five of seven sets of data for the spleen). The duodenum, jejunum, and rectum were the only tissues in which glucagon stimulated DNA synthesis more times than it inhibited it.

Since it might be argued that some of the statistically significant sets of data described above were simply generated by a rhythm alteration, such as a phase shift or amplitude reduction or increase, the data were subjected to additional analyses; these included two- and three-way ANOVA as well as the cosinor technique (19). For the latter test, some of the sets of data showed a highly statistically significant fit to a 24-h cosine curve, whereas others did not; this was not unexpected, since for this type of test the data were limited in that there were only four time points along the 24-h time scale. To present this mass of data would contribute little to the main theme. Moreover, the analysis of variance for the main effects of drug treatment time and interval to kill time was highly statistically significant, with $P = 0.001$

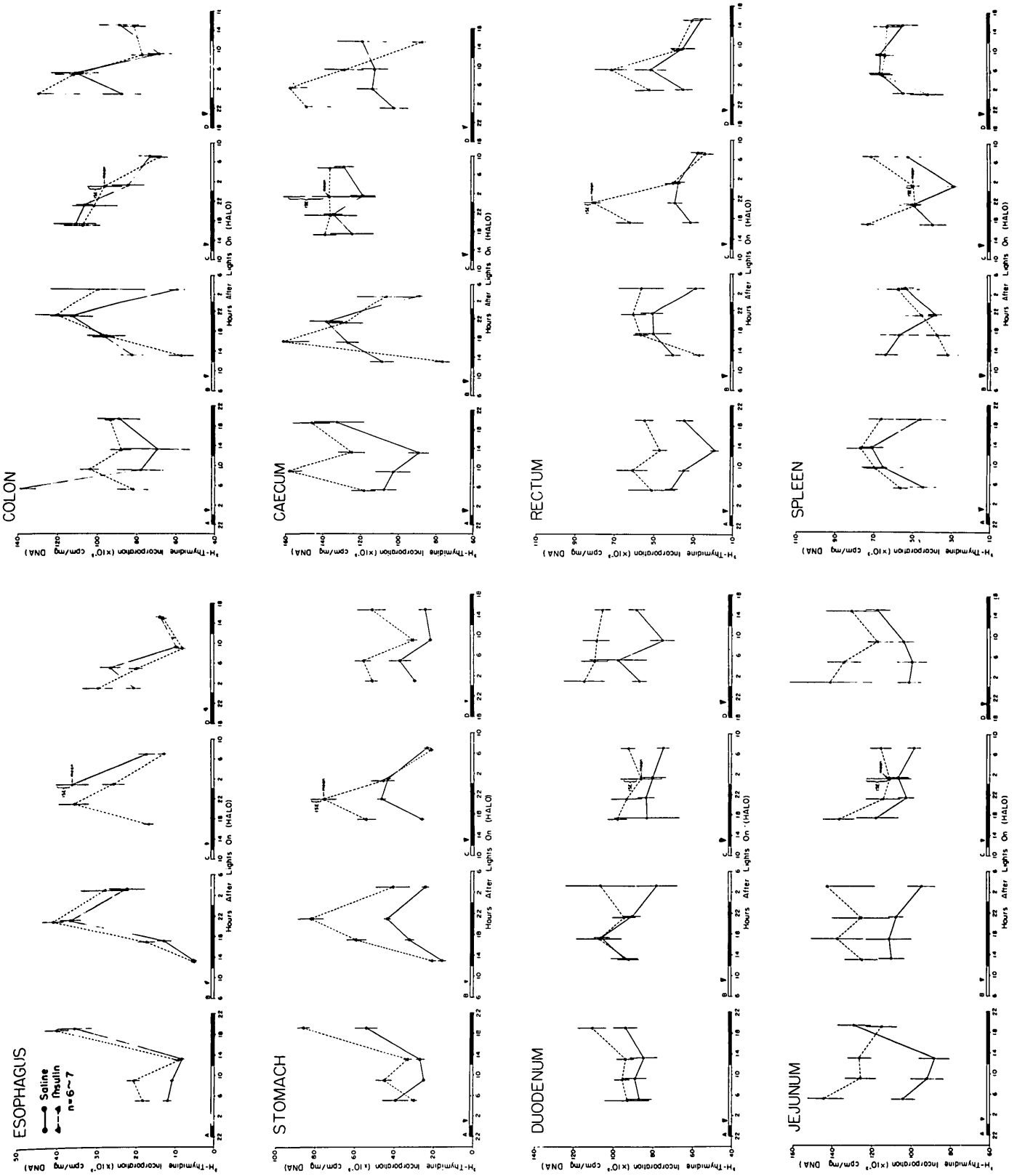

FIG. 3. Pattern of response of each tissue studied 4, 8, 12, and 18 h postinjection of insulin. The injections were made 1 h after lights on (HALO), 9 HALO, 13 HALO, and 21 HALO (indicated by *arrows*). Each time point represents seven animals (occasionally six).

TABLE 2. Percent increase (↑) or decrease (↓) in the incorporation of [^{3}H]TdR into DNA 4, 8, 12, and 18 h after the injection of glucagon at four different circadian stages

Injection time:	1 HALO (early light)				9 HALO (late light)				1 HALOff (early dark)				9 HALOff (late dark)				Total Responses out of 16	
Kill time (h after injection):	4	8	12	18	4	8	12	18	4	8	12	18	4	8	12	18	↑	↓
Esophagus	76↑					51↑						29↓				32↓	2	2
Glandular stomach	40↓								38↑					39↓			1	2
Duodenum												22↑					1	0
Jejunum			32↑									18↑					2	0
Caecum		43↑			24↓											33↓	1	2
Colon	54↓				52↓	25↓		45↑	24↓					25↓		48↓	1	6
Rectum			64↑		77↓				58↑			54↑					3	1
Spleen					34↓				50↑		47↑		22↓	24↓	24↓	35↓	2	5

HALO, Hours after lights on; HALOff, hours after lights off. A blank space indicates that any change seen was not statistically significant.

for all the tissues studied. In general, these additional analyses support the conclusion that there was a time of treatment effect as well as a hormonal effect which were not due to rhythm alteration.

Discussion

Recent evidence suggests that insulin and glucagon play a role in the growth control of entodermally derived organs (1–10). In this paper, we have demonstrated that both of these hormones are capable of affecting the incorporation of [^{3}H]TdR into DNA in several entodermally derived organs in the intact mouse within 18 h after injection. In general, the effects of the two hormones on DNA incorporation differ. The effects of insulin tend to be rapid, stimulatory, widespread, and greatest when the injection time occurs late in the dark span and early in the light span. In contrast, the effects of glucagon tend to be more inhibitory, localized, and less specific with respect to the time course and injection time. Whether these effects represent a direct effect on the target cells or an indirect one, mediated by any of a number of other hormonal or metabolic factors, such as hypoglycemia-induced exfoliation, remains to be determined; however, the evidence summarized previously supports a direct physiological role for insulin and glucagon.

The effects of these hormones on [^{3}H]TdR incorporation into DNA in the examined tissues contrasted with those of EGF, revealed in a similar study done by us (13–15). Each hormone affected a different set of organs, and where these sets overlapped, there were frequently qualitative and quantitative differences in the effects. For example, EGF dramatically stimulated [^{3}H]TdR incorporation into DNA in the esophagus and colon, insulin had little effect on them, and finally, glucagon primarily inhibited colonic DNA synthesis. In contrast to glucagon, insulin and EGF also stimulated DNA synthesis in the glandular stomach and rectum. Additionally, insulin had a much greater stimulatory effect on DNA synthesis in both the small intestine and spleen than either glucagon or EGF. Occasionally, EGF inhibited [^{3}H]TdR incorporation into DNA in the proximal small bowel, whereas both EGF and glucagon frequently inhibited it in the spleen. These preliminary comparisons await more extensive dose-response studies, tempered by the realization that on a molar basis, 1.7 times as much insulin and glucagon were injected as EGF.

The studies also suggest that the optimal response times to inject EGF, glucagon, and insulin differ. Although the examined organs show considerable variation in their temporal responses to each hormone, in general, EGF had its greatest overall effect on DNA synthesis when injected toward the end of the light span, when DNA synthesis in most of the examined organs tends to be either falling or at its lowest levels. In contrast, insulin had its greatest effect when injected in the late dark or early light period, when DNA synthesis and serum insulin tend to be at the highest and lowest levels, respectively (21). Glucagon was most inhibitory when injected during the late dark period. Moreover, EGF and insulin both had their least stimulatory effects on [^{3}H]TdR incorporation into DNA when injected in the early dark period, which corresponds to the time when DNA synthesis in most of the examined organs begins to increase. This is also the time when the generally inhibitory glucagon stimulated [^{3}H]TdR incorporation into DNA more times than it inhibited it. Whether these complex circadian variations in the effects of these hormones on DNA synthesis depend on variations in the local concentration and cell cycle role of each hormone, on the presence of other hormones, on the feeding schedule, or on cellular response requires further study.

Innate regional and temporal differences characterize various growth-related parameters in the gut. For ex-

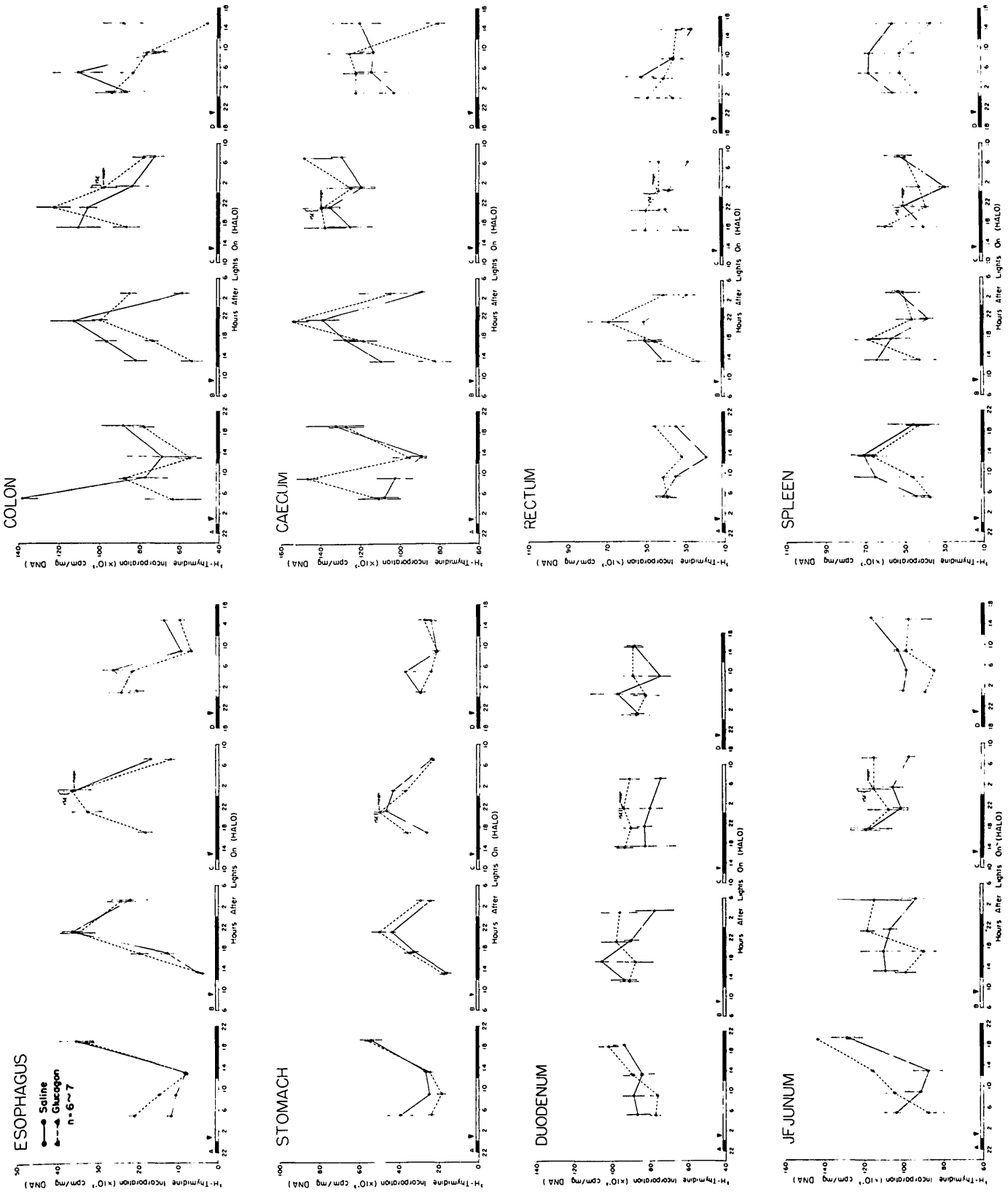

FIG. 4. Pattern of response of each tissue studied 4, 8, 12, and 18 h postinjection of glucagon. The injections were made 1 h after lights on (HALO), 9 HALO, 13 HALO, and 21 HALO (indicated by *arrows*). Each time point represents seven animals (occasionally six).

ample, there is a linear gradient in villus size in the small intestine of the rodent, decreasing from a maximal size in the duodenum to less than half this in the terminal ileum (22). Furthermore, within any region of the small intestine there is a circadian variation of considerable magnitude in villus size (23). The presence of various stimulatory hormones, such as EGF, insulin, and glucagon, or the absence of inhibitory ones, such as corticosteroids, at any particular place in the small intestine at any particular time doubtless plays some role in the maintenance of these regional and temporal variations in villus height (13, 14).

It has been demonstrated by surgical ablation and transposition techniques that villus-enlarging factors reach the small intestine from the stomach and proximal intestine (24), on the one hand, and from the duodenal papillae (23), on the other. In the second case, the data suggest that this enlarging factor emanates from the pancreas. Our results, which have demonstrated that EGF, insulin, and glucagon are each capable of stimulating [^{3}H]TdR incorporation into DNA in the small intestine, suggest that these hormones should be considered as candidates for the putative villus-enlarging factors implied by surgical studies. The exocrine secretion of large amounts of EGF into the saliva (25, 26), the presence of EGF in the duodenal glands (Brunner's glands), and the recent demonstration that high concentrations of insulin and glucagon are in fact present in exocrine pancreatic fluid (27) lend additional support to such a consideration. Studies to test the effects of various dosages of the above hormones singly and in combination when given at various circadian stages, the utilization of their monoclonal antibodies to inhibit DNA synthesis, and the active attempt to isolate and characterize additional growth-promoting or -inhibiting factors should prove fruitful in resolving the hormonal requirements for growth control of the gastrointestinal tract.

References

1. Cezard JP, Forgue-Lafitte ME 1980 Biological activity and binding of insulin on two human intestinal cancer cell lines in culture. Gastroenterology 78:1110 (Abstract)
2. Murakami H, Masui H 1980 Hormonal control of human colon carcinoma cell growth in serum-free medium. Proc Natl Acad Sci USA 77:3464
3. Bucher NLR, Patel U, Cohen S 1977 Hormonal factors and liver growth. Adv Enzyme Regul 16:205
4. Leffert HL, Koch KS, Moran T, Rubalcava B 1979 Hormonal control of rat liver regeneration. Gastroenterology 70:1470
5. Reinprecht J 1980 Receptors for insulin on small intestinal epithelial cells. Gastroenterology 78:1331 (Abstract)
6. Menard D, Malo C, Calvert R 1981 Insulin accelerates the development of intestinal brush border hydrolytic activities of suckling mice. Dev Biol 85:150
7. Scheving LE, Pauly JE, Tsai TH, Scheving LA 1981 Effect of insulin on DNA labelling in gastric mucosa depends on circadian stage of mouse when drug is administered. Anat Rec 199:277 (Abstract)
8. Bergeron JJM, Rachubinski R, Searle N, Barts D, Sikstrom R, Posner BI 1980 Polypeptide hormone receptors *in vivo*. J Histochem Cytochem 28:824
9. Forgue-Lafitte ME, Marescot MR, Chamblier MC, Rosselin G 1980 Evidence for presence of insulin binding sites in isolated rat intestinal epithelial cells. Diabetologia 19:373
10. Johnson LR 1977 New aspects of the trophic action of gastrointestinal hormones. Gastroenterology 72:788
11. Gleeson MH, Bloom SR, Polak RM, Henry K, Dowling RH 1971 Endocrine tumor in kidney affecting small bowel structure, motility, and absorptive function. Gut 12:773
12. Bell DM, Weser E 1979 Intravenous glucagon has no effect on mucosal growth in rats nourished by total parenteral nutrition. Gastroenterology 76:1098 (Abstract)
13. Scheving LA, Yeh YC, Tsai TH, Scheving LE 1979 Circadian phase-dependent stimulatory effects of epidermal growth factor on deoxyribonucleic acid synthesis in the tongue, esophagus, and stomach of the adult male mouse. Endocrinology 105:1475
14. Scheving LA, Yeh YC, Tsai TH, Scheving LE 1980 Circadian phase dependent stimulatory effects of epidermal growth factor on deoxyribonucleic acid synthesis in the duodenum, jejunum, ileum, caecum, colon and rectum of the adult male mouse. Endocrinology 106:1498
15. Yeh YC, Scheving LA, Tsai TH, Scheving LE 1981 Circadian stage-dependent effects of epidermal growth factor on deoxyribonucleic acid synthesis in ten different organs of the adult male mouse. Endocrinology 109:644
16. Ogur M, Rosen G 1970 The nucleic acids of plant tissues. I. The nucleic acid. Arch Biochem 25:262
17. Burns ER, Scheving LE, Pauly JE, Tsai TH 1976 Effect of altered lighting regimens, time-limited feeding, the presence of Ehrlich ascites carcinoma on the circadian rhythm in DNA synthesis of mouse spleen. Cancer Res 36:1538
18. Maurer HR 1981 Potential pitfalls of ^{3}H-thymidine techniques to measure cell proliferation. Cell Tissue Kinet 14:111
19. Halberg F, Johnson EA, Nelson W, Sothern R 1972 Autorhythmometry procedures for physiological self-measurements and their analysis. Physiol Teacher 1:1
20. Naets JP, Guns M 1980 Inhibitory effect of glucagon on erythropoiesis. Blood 55:997
21. Gagliardino JJ, Hernandez RE 1971 Circadian variation of the serum glucose and immunoreactive insulin levels. Endocrinology 88:1529
22. Altmann GG 1971 Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am J Anat 132:167
23. Stevenson NR, Day SE, Sitren H 1979 Circadian rhythmicity in rat intestinal villus length and cell number. Int J Chronobiol 6:1
24. Altmann GG, Leblond CP 1970 Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. Am J Anat 127:15
25. Murphy RA, Pantazis NJ, Papastavros M 1979 Epidermal growth factor and nerve growth factor in mouse saliva. Dev Biol 71:356
26. Hirata Y, Orth DN 1979 Epidermal growth factor (urogastrone) in human tissues. J Clin Endocrinol Metab 48:667
27. Prinz RA, Kirsteins L, Connick E, Paloyan E, Lawrence AMK 1980 Insulin and glucagon in human pancreatic exocrine fluid. Horm Metab Res 12:38