Effects of Growth Hormone and Insulin-Like Growth Factor I on Endocrine Function of Human Fetal Islet-Like Cell Clusters During Long-Term Tissue Culture

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The effects of recombinant human growth hormone (GH, 1 μg/ml) and insulin-like growth factor I (IGF-I, 200 ng/ml) on the production of insulin and glucagon by human fetal islet-like cell clusters (ICCs) were studied in tissue culture. ICCs were derived after collagenase digestion and culture of pancreases from 16 fetuses (mean gestational age 15.6 wk). The ICCs were cultured with or without GH or IGF-I for 7 or 31 days. Basal rates of insulin and glucagon production were not altered by GH during the first 17 days of culture, but the release of both hormones was increasingly augmented by GH during the last 2 wk of culture (131% increase in insulin and 85% in glucagon compared with controls). ICCs cultured for 7 days in the presence of GH secreted more insulin when incubated for 120 min in 20 mM than in 2 mM glucose (2.1-fold response, $P < .05$), whereas ICCs maintained in basal medium did not respond to glucose. GH had no effect on DNA and insulin content or insulin biosynthesis. Exogenous IGF-I caused a 28% suppression of insulin release ($P < .05$) between days 4 and 10 of culture but induced a 49% increase in the mean secretion rate during the last week (days 25–31, $P < .01$). Glucagon release was not affected by exogenous IGF-I. In contrast to GH, exogenous IGF-I induced a twofold increase in the DNA content of the 7-day–cultured ICCs. However, insulin biosynthesis and release were markedly suppressed. We conclude that GH influences the functional maturation of human fetal islet cells in vitro. The effects of GH could not be reproduced by the addition of IGF-I, suggesting a direct rather than a somatomedin-mediated action for GH. *Diabetes* 37:1678–83, 1988

Insulin produced by the fetal pancreas has a central role in the growth of the fetus (1). Factors controlling the growth and functional maturation of the human endocrine pancreas during fetal development are still poorly understood. In animal experiments, glucose is the primary stimulus for β-cell proliferation and insulin production of postnatal islets, but amino acids may be more important during fetal development (2,3). Several hormones are also capable of stimulating the islet cells directly (3). Growth hormone (GH) has an insulinotropic action in addition to its peripheral diabetogenic effects (4,5). In vitro studies have confirmed a stimulatory effect on β-cell replication and insulin production in fetal (6), neonatal (7), and adult (8,9) rat islets. In the fetal rat, the stimulatory effects of GH may largely be mediated via local production of insulin-like growth factor I (IGF-I) (10), as in several other tissues (11). However, evidence has also been presented for a direct effect of GH on the β-cells (12).

Sandler et al. (13) showed that GH increased the formation of islet-like cell clusters (ICCs) in cultures of human fetal pancreas. The amount of insulin released by the cell clusters in response to theophylline plus glucose was low but was slightly enhanced by GH. Swenne et al. (14) used larger fragments of human fetal pancreas to study the ability of GH and human placental lactogen to modulate the in vitro production of insulin and IGF-I. GH had no effects on the release or content of insulin.

To further clarify the role and mechanism of GH in the functional maturation of human fetal endocrine pancreatic cells, we studied the effects of GH on the insulin and glucagon production of ICCs in long-term (31 days) culture. The sensitivity of insulin release and biosynthesis to glucose were examined at 7 days. To elucidate the role of IGF-I in the GH-induced changes, the effects of exogenous addition of this growth factor were also studied.

MATERIALS AND METHODS

Fetal material and handling of tissues. Pancreases of 16 human fetuses were included in the study. Fetuses were obtained after legally approved abortions performed either

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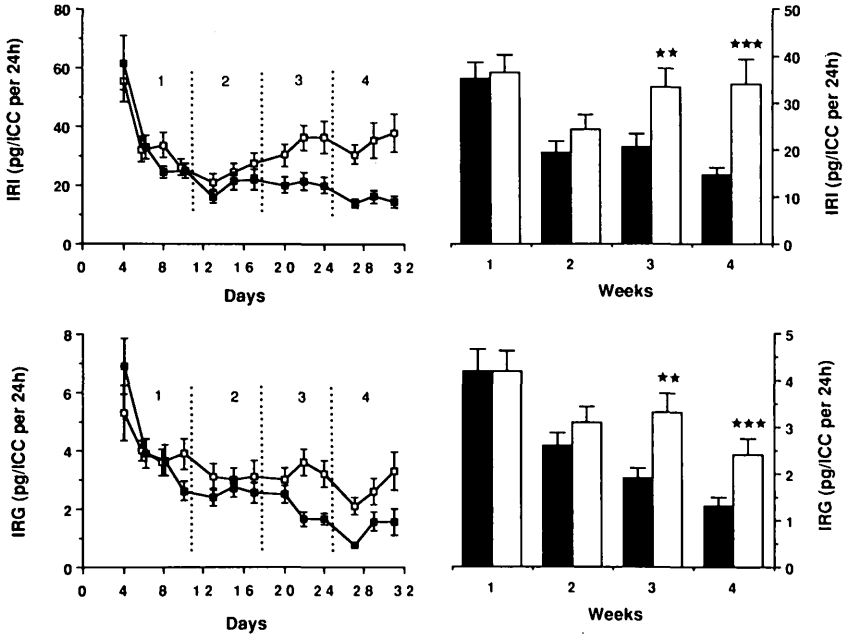

FIG. 1. Effect of growth hormone (GH) on release of immunoreactive insulin (IRI; *top panel*) and glucagon (IRG; *bottom panel*) into culture medium during 31 days in culture. Islet-like cell clusters (ICCs) were obtained from 5 fetuses (gestational age 14–18 wk) and cultured in groups of 30–50 in either control basal medium (RPMI-1640/10% human serum; ■ and *solid bars*) or medium supplemented with 1 μg/ml recombinant GH (□ and *open bars*). Samples from cultures of 3 fetuses were also analyzed for glucagon content. Data are means ± SE for 28 (IRI) or 18 (IRG) culture wells. *Left panel* shows original data; *right panel* shows mean weekly secretion rates. Analysis of variance for repeated measures showed that IRI and IRG release rates differ between GH and control ($P < .01$). **$P < 0.01$ and ***$P < .001$, significant differences (Student's *t* test for unpaired data).

by dilation and extraction ($n = 14$) or by prostaglandin induction ($n = 2$). The use of the fetal organs in this study was approved by the ethics committees of the respective hospitals. The mean gestational age was 15.6 wk (range 14–20) as estimated by measurement of fetal foot length (15). The tissues were treated as described previously (16). The pancreases were dissected within 15 min after the completion of the abortion after dilation and extraction and within 90 min after prostaglandin. After mincing, the pancreases were incubated with collagenase (2.5 mg/ml; Cooper, Malvern, PA) in a shaking water bath at +37°C for 20–40 min. The digested tissue was divided into two petri dishes that do not allow cell attachment (Sterilin, Feltham, UK). The culture medium was RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10% pooled human serum, type AB (Finnish Red Cross, Helsinki, Finland). The cultures were kept in an incubator at +37°C in 5% CO_2. On the 3rd day of culture, numerous rounded free-floating ICCs had appeared. The medium was changed daily during the first 4 days and every 2–3 days thereafter.

Long-term hormone release. From the beginning, half of the ICCs were cultured in the basal medium and half in the experimental medium containing either recombinant human methionyl GH (1.0 μg/ml; Somatonorm, Kabi, Sweden) or recombinant human IGF-I (200 ng/ml; CGP 35126/lot 1-285), kindly donated by E. Froesch of Zurich, Switzerland. In the first part of the study, groups of 30–50 equal-sized ICCs were transformed to tissue culture wells with 0.5 ml of medium. The ICCs were placed on filters (pore size 5 μm; Nuclepore, Pleasanton, CA) in plastic specimen-processing capsules with a net-like bottom wall (Agar Aids, Stansted, UK). Medium was drawn from outside the capsule, thus preventing accidental loss of the nonattached ICCs (16). Cultures were continued for 31 days. From day 4 on, the media were analyzed for their contents of immunoreactive insulin (IRI) and glucagon (IRG). Reagents for the insulin radioimmunoassay (RIA) were kindly donated by Farmos Diagnostica (Oulunsalo, Finland). The detection limit was 38 pg/ml, and intra- and interassay coefficients of variation were 6 and 14%, respectively. IGF-I (0.5 μg/ml) did not cross-react in the insulin assay. Glucagon was determined after ethanol extraction (17) of the media samples by use of K5563 antibody (Novo, Bagsvaerd, Denmark). The detection limit of the glucagon RIA was 10 pg/ml, and the intra- and interassay coefficients of variation were 6 and 16%, respectively.

Insulin release and biosynthesis in response to glucose. In the second part of the study, ICCs were cultured for 7 days. On the 8th day in culture, groups of 30 ICCs were washed with Hanks' balanced salt solution supplemented with 0.2% human serum albumin (HSA) and 20 mM HEPES (Gibco), pH 7.30, and transferred to microwells (Flow, McLean, VA) in 200 μl Gey & Gey bicarbonate buffer containing 0.2% HSA, 20 mM HEPES, pH 7.30, and 50 μCi/ml [4,5-^{3}H]-L-leucine (sp act 146 Ci/mmol, Amersham, UK). No GH or IGF-I was added to the buffer. The glucose concentration of the buffer was either 2 or 20 mM. The ICCs were then incubated at 37°C in an atmosphere of 5% CO_2 in humidified air for 120 min. Samples were drawn from the buffer for determination of insulin release. The ICCs were washed three times in 10 ml PBS containing 5 mM cold L-leucine. After the final wash, the ICCs were disrupted ultrasonically in 300 μl of 50 mM glycine buffer, pH 8.0. Samples were taken from the sonicate for analysis of DNA content after addition of (final concentrations) 100 mM NaCl, 10 mM EDTA, and 0.1% Triton X-100. DNA was measured fluorometrically with the fluorochrome Hoechst 33258 (Bisbenzimide, Sigma, St. Louis, MO) (18,19). The remaining tissue homogenate was used for measurement of insulin content by RIA and (pro)insulin and total protein biosynthesis by a previously described immunoprecipitation method (20). Duplicate samples were incubated with anti-bovine insulin antiserum made in guinea pig (Miles, Naperville, IL) or normal pooled guinea pig serum. Immunocomplexes were then precipitated with protein A–Sepharose (Pharmacia, Uppsala, Sweden). Total protein synthesis was measured after trichloroacetic acid (TCA) precipitation of the sonicate. For expression of the

TABLE 1
Effects of growth hormone (GH) on insulin release, content, and biosynthesis and DNA content of human fetal islet-like cell clusters (ICCs) after 7 days in culture

	Basal medium		Basal medium plus GH	
	2 mM glucose	20 mM glucose	2 mM glucose	20 mM glucose
DNA (ng/ICC)	40 ± 3	59 ± 13	35 ± 3	53 ± 14
Insulin release (ng/μg DNA)	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	1.2 ± 0.3*
Insulin content (ng/μg DNA)	7.9 ± 1.8	8.0 ± 1.1	7.7 ± 1.2	7.8 ± 1.2
(Pro)insulin biosynthesis (cpm/μg DNA × 10^{-3})	22.8 ± 3.0	22.1 ± 1.5	24.3 ± 2.9	26.3 ± 3.3
Total protein biosynthesis (cpm/μg DNA × 10^{-3})	240 ± 42	230 ± 29	281 ± 52	293 ± 41
Insulin/total protein biosynthesis (%)	11.3 ± 1.5	11.3 ± 0.8	11.9 ± 1.2	10.2 ± 0.9

ICCs were derived from 6 fetuses (mean gestational age 16.1 wk, range 14.5–20) and divided among the 4 experimental groups. After being cultured in basal medium (RPMI-1640/10% human serum) with or without GH, ICCs were incubated for 120 min in bicarbonate buffer with 2 or 20 mM glucose (no GH added) in presence of [^{3}H]leucine. Values are means ± SE of 19–25 groups of 30 ICCs.
*$P < .05$ compared with incubation in 2 mM glucose but similar culture conditions.

results, nonspecifically bound radioactivity (35 ± 12%, mean ± SD) was subtracted from the radioactivity precipitated by the anti-insulin serum.

Statistics. Data are presented as means ± SE. For the statistical analysis of long-term hormone release data, the time in culture was divided into four 7-day periods (days 4–10, 11–17, 18–24, and 25–31). Differences between the mean rates of hormone release by the controls and the experimental cultures during the four periods were tested with analysis of variance for repeated measures in two groups (BMDP program 2V, BMDP Statistical Software, Los Angeles, CA) and Student's *t* test for unpaired data (program 3D). If repeated measurements were not included, one-way analysis of variance and *t* test for unpaired data or the Mann-Whitney *U* test were applied (programs 7D and 3D).

RESULTS

Effects of GH. The release of both insulin and glucagon into the culture medium declined rapidly during the 1st wk in culture, but then stabilized for the rest of the 31 days. GH had no effect on the hormone levels during the first 17 days, but significantly augmented the release of both insulin and glucagon during the rest of the culture period (Fig. 1). Between days 18 and 31, the mean daily rates of hormone production by GH-supplemented cultures were 32.9 pg insulin and 2.8 pg glucagon/ICC, compared to 17.7 and 1.6 pg/ICC, respectively, for the controls ($P < .01$ for both insulin and glucagon).

The DNA and insulin contents of the ICCs were not altered by GH at 7 days of culture (Table 1). After 31 days, the insulin content was about half the content of the 7-day-cultured ICCs and was not significantly affected by GH (GH: 4.7 ± 1.1 ng insulin/μg DNA, control: 2.9 ± 0.6; mean ± SE of 22 culture wells from 4 fetuses; NS, Mann-Whitney *U* test). The DNA content was also unaffected (1.8 ± 0.1 vs. 2.0 ± 0.3 μg/dish for GH and control, respectively; NS).

ICCs cultured in the presence of GH for 7 days secreted more insulin ($P < .05$) when incubated for 2 h in 20 mM compared with 2 mM glucose (2.1-fold response). Control ICCs obtained from the same fetuses responded with a 1.3-

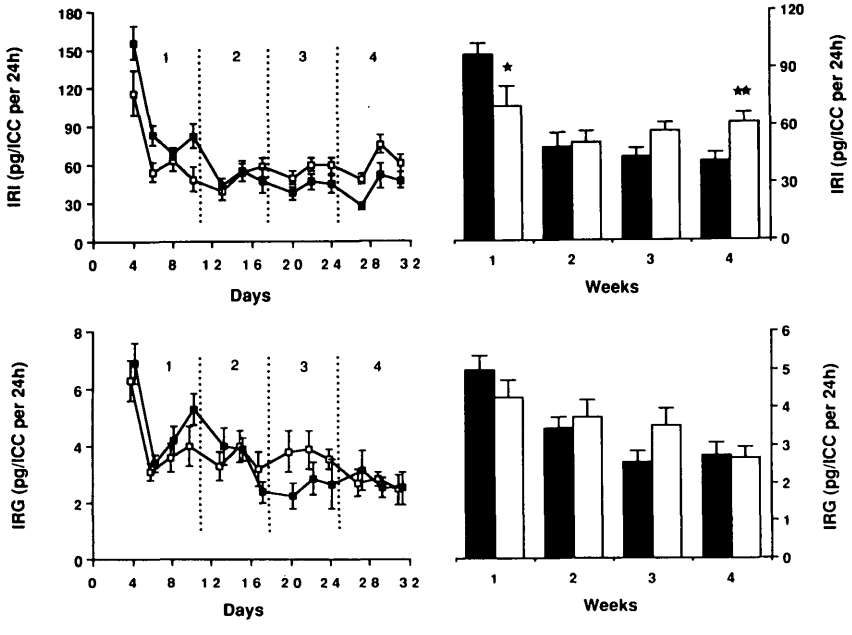

FIG. 2. Effect of exogenous insulin-like growth factor I (IGF-I) on release of immunoreactive insulin (IRI, *top panel*) and glucagon (IRG, *bottom panel*) during 31 days in culture. Islet-like cell clusters (ICCs) were derived from 2 fetuses (gestational ages 14.5 and 15.5 wk) and cultured either in control basal medium (■ and *solid bars*) or in basal medium supplemented with 200 ng/ml of recombinant IGF-I (□ and *open bars*). Data are presented as means ± SE for 16 culture wells. Analysis of variance for repeated measures showed that mean weekly IRI release rates differ between IGF-I and control ($P < .001$), whereas IRG release is not affected. *$P < 0.05$ and **$P < 0.01$, significant differences (Student's *t* test for unpaired data).

TABLE 2
Effects of insulin-like growth factor I (IGF-I) on insulin release, content, and biosynthesis and DNA content of human fetal islet-like cell clusters (ICCs) after 7 days in culture

	Basal medium		Basal medium plus IGF-I	
	2 mM glucose	20 mM glucose	2 mM glucose	20 mM glucose
DNA (ng/ICC)	18 ± 2	17 ± 2	32 ± 4*	32 ± 3†
Insulin release (ng/μg DNA)	3.9 ± 0.8	3.9 ± 0.6	1.4 ± 0.2*	1.3 ± 0.2†
Insulin content (ng/μg DNA)	7.5 ± 1.2	7.4 ± 1.5	3.9 ± 0.7*	4.0 ± 0.7*
(Pro)insulin biosynthesis (cpm/μg DNA × 10^{-3})	29.2 ± 3.1	26.0 ± 3.1	18.5 ± 2.1*	16.5 ± 1.4‡
Total protein biosynthesis (cpm/μg DNA × 10^{-3})	225 ± 38	182 ± 29	142 ± 23	125 ± 15
Insulin/total protein biosynthesis (%)	15.5 ± 2.0	16.5 ± 2.2	15.5 ± 2.1	14.4 ± 1.4

ICCs were derived from 3 fetuses (mean gestational age 14.7 wk, range 14.5–15) divided among the 4 experimental groups. After being cultured in basal medium (RPMI-1640/10% human serum) with or without IGF-I, ICCs were incubated for 120 min in bicarbonate buffer with 2 or 20 mM glucose in presence of [^{3}H]leucine (no IGF-I added). Values are means ± SE of 10–11 groups of 30 ICCs.
*$P < .01$, †$P < .001$, and ‡$P < .05$, vs. control ICCs incubated in the same glucose concentration.

fold (nonsignificant) increase in insulin release (Table 1). The rates of [^{3}H]leucine incorporation into (pro)insulin and total (TCA-precipitable) proteins were not altered by GH or glucose (Table 1). Proinsulin accounted for 11% of the total protein synthesis.

Effects of IGF-I. When the culture medium contained 200 ng/ml IGF-I, the release of insulin was suppressed during the first 10 days of culture (70.5 ± 10.2 vs. 97.4 ± 6.0 pg/ICC per 24 h for IGF-I and control, respectively; $P < .05$; Fig. 2). However, during the last week (days 25–31), the mean rate of insulin production by ICCs cultured in the presence of IGF-I was higher than in controls (61.8 ± 5.3 vs. 41.5 ± 4.3 pg/ICC per 24 h, $P < .01$). The levels of immunoreactive glucagon were not altered by IGF-I at any time (Fig. 2). As a whole, the delayed stimulatory effect of GH on insulin release was more pronounced than that of IGF-I (131% increase by GH and 49% by IGF-I during the 4th week).

In contrast to GH, the DNA content of the ICCs was increased almost 2-fold by exogenous IGF-I after 7 days in culture (Table 2). The insulin content per unit of DNA was decreased, whereas the insulin content per ICC remained unchanged.

The ICCs cultured in the presence of IGF-I did not show a secretory response to glucose (Table 2). The levels of insulin release from these ICCs were significantly lower than in controls when expressed in relation to DNA content ($P < .001$) or per ICC ($P < .05$). The rate of (pro)insulin synthesis was also significantly lower in the IGF-I–supplemented cultures than in controls when related to the DNA content and did not respond to glucose. There were no significant differences in the rates of total protein biosynthesis (Table 2).

DISCUSSION

This study indicates that GH stimulates the total insulin production of cultured human fetal pancreatic cells. The effect is delayed, appearing after 2 wk in culture, and is not β-cell specific, because glucagon production is also stimulated. In previous studies with human fetal pancreas, the explants were exposed to GH for only 3 (14) or 7 (13) days, which may not be sufficient to detect the delayed effect. Sandler et al. (13) found that GH stimulated the formation of ICCs and increased the total insulin content of the cultures. We were not able to detect any effect on the insulin content, possibly because the total number of ICCs formed was not recorded. In long-term culture of mouse islets, GH prevented the progressive decrease in the rate of insulin release in the presence of 0.5% serum, an effect resembling our findings (8).

Our results also suggest that GH promotes functional maturation of the human fetal β-cells by inducing glucose sensitivity of insulin release. This is in line with the previous finding that GH enhances the stimulation of insulin release by glucose plus theophylline (13). In most of the previous studies, insulin release from human fetal pancreas in vitro has not responded to glucose alone in short-term static incubation, although a modest stimulatory effect exists in perifusion experiments (21). It has been suggested that cultured human fetal β-cells become responsive to glucose when the absolute age (gestational age plus time in culture) reaches 25 wk (22). In this study, the absolute age of the cultured cells remained <21 wk. The responsiveness of fetal rat islets to glucose was not enhanced by GH (23).

The stimulatory effect of GH on pancreatic β-cells has been suggested to be mainly direct (7,24), possibly through specific receptors on the β-cell membrane (12). However, neonatal (25) and fetal (10) rat islet cells are capable of IGF-I synthesis, and it was recently shown that human fetal β-cells also contain and release IGF-I in culture (26). In the fetal rat islets, the production of this growth factor was stimulated by GH, and the effect of GH on DNA synthesis was partially blocked by a somatomedin antibody, suggesting that GH exerts at least some of its effect by increasing the local production of somatomedins (IGFs) (10).

Addition of exogenous IGF-I to the medium led to a two-fold increase in DNA content after 7 days, suggesting a mitogenic effect, which has previously been demonstrated in several fetal tissues (11,27), including fetal rat β-cells (10). However, it is difficult to determine which cells proliferated, because the ICCs contain only a minority of β-cells (16,28). The specialized functions of the β-cells were simultaneously markedly suppressed. There are at least two possible explanations for this finding. First, active β-cell replication could

have led to channeling of protein synthesis to proteins needed for replication at the expense of specific products. Some examples are available of inhibitory effects of growth factors on the specialized functions of endocrine cells (29,30). Second, the observed inhibitory effects could be explained by a direct receptor-mediated action exerted through specific high-affinity IGF receptors, which have recently been identified on pancreatic α- and β-cells (31).

When exposure to IGF-I was continued for 31 days, a weak stimulatory effect on the insulin levels appeared, resembling the effect of GH. However, the effect of IGF-I seemed to be more specific to β-cells, because the glucagon levels were unchanged. Accordingly, it is unlikely that the GH-stimulated enhancement of glucagon release is mediated by locally synthesized IGF-I.

Variation between cultures obtained from different fetuses is a constant problem with primary culture of human fetal tissues. The variation is due to technical factors, e.g., the method of abortion, the ischemia time (16), and biological factors, especially the developmental stage of the fetus (32,33). Digestion of the fetal pancreas with collagenase may further increase the variation of the results if the level of tissue disintegration is not constant. In our experience, it is difficult to standardize the digestion of fetal glands of different developmental stages. This difficulty is illustrated in this study. The ICCs used for the IGF experiments were of younger gestational age, were smaller (lower DNA content), and released a higher proportion of their insulin content than those used in the GH experiments (Tables 1 and 2). Consequently, we have always divided ICCs from a single fetus among all of the treatment groups in one type of experiment.

GH is structurally related to the lactogen hormones human placental lactogen and prolactin. Several lines of evidence suggest that human placental lactogen has analogous effects to GH but is more important during fetal development (34,35). Human placental lactogen increased the insulin content and release of human fetal pancreatic explants in the presence of a high glucose concentration, whereas GH was ineffective (14). It was recently shown that prolactin can also directly modulate the function of neonatal rat islets, including sensitization of glucose-induced insulin release (36). Consequently, the possibility exists that the effects of the high concentration of GH used in this study may have been exerted by cross-reactivity with lactogen receptors.

In summary, our study indicates that GH stimulates the hormone production and influences the functional maturation of cultured human fetal islet cells. In contrast to GH, exogenous IGF-I increased the DNA content of the ICCs, suggesting a mitogenic effect. However, IGF-I suppressed insulin production at the outset of the culture period and had only a weak stimulatory effect after prolonged exposure. Thus, the effects of GH do not appear to be somatomedin mediated.

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