Plasma Glucagon and Insulin Responses to Various Sugars in Gastrectomized and Normal Subjects[1] (36294)

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(Introduced by Piero P. Foà)

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It has been reported that gut glucagon represents a large fraction of the plasma immunoreactive glucagon (IRG) in the fasting state (1–3) and that it contributes most of the rise in plasma IRG observed after the ingestion of glucose (4, 5). Gut IRG is one of several enteric hormones believed to play a significant role in the control of insulin secretion (6–9). Although the factors regulating the secretion of pancreatic glucagon have been studied intensively (10–16), little is known about the secretion of gut glucagon. The purpose of this study was to study the plasma IRG and immunoreactive insulin (IRI) responses to various sugars, administered orally or intravenously, in normal and gastrectomized subjects.

Subjects and Methods. The observations were carried out in 9 male and 2 female healthy, nonobese, 32- to 56-year-old volunteers, and in 21 patients (29 to 70 years old), who had been gastrectomized for gastric or duodenal ulcers 8 months to 18 years previously. All subjects were given 100 g of glucose orally after an overnight fast. In addition, several days later, some of them received oral loads of galactose (100 g), fructose (100 g), glucose (50 g), or an intravenous injection of glucose (25 g). All subjects were instructed to consume a diet containing at least 300 g carbohydrate for 3 days preceding the tests. Blood was drawn from the antecubital vein into a syringe containing a small amount of heparin. The specimens were placed immediately in an ice bath and were centrifuged within 30 min. The plasma was removed, Trasylol was added (4000 KIU/ml of sample) to prevent the proteolytic destruction of glucagon and the mixture was stored at −20°.

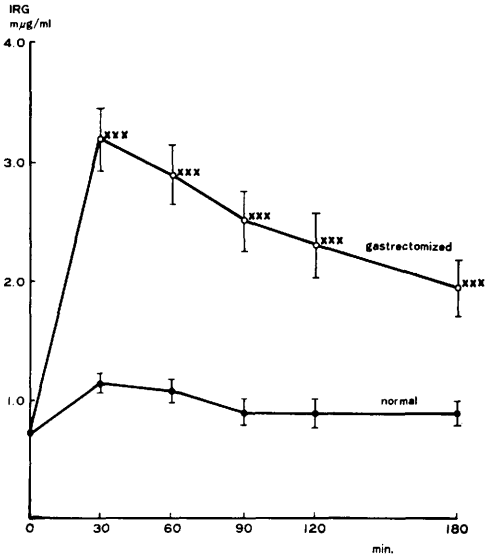

FIG. 1. Mean plasma IRG responses to oral glucose in 21 gastrectomized and 11 normal subjects: The Student's t test was used for statistical analysis; (xxx) $p < .005$.

IRG was measured according to a method described previously (17). IRI was determined according to the method of Hales and Randle (18); and glucose was measured enzymatically (glucose oxidase) and/or with the method of Somogyi-Nelson.

Results. Figure 1 shows the plasma IRG responses to oral glucose in normal and in gastrectomized subjects. The mean fasting IRG levels of 11 healthy volunteers and of 21 gastrectomized subjects were about the same (0.71 ± 0.09 and 0.70 ± 0.05 mμg/ml, respectively). Thirty minutes after

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TABLE I. Effect of Oral Glucose on Plasma IRG, IRI, and Glucose in Normal and Gastrectomized Subjects.

		After loading (min)					
		0	30	60	90	120	180
IRG	(mμg/ml)						
normal	(11)	0.71[a]	1.15	1.08	0.89	0.89	0.89
		±0.09	±0.08	±0.09	±0.11	±0.13	±0.11
gastrectomized	(21)	0.70	3.19	2.89	2.55	2.35	1.95
		±0.05	±0.26	±0.25	±0.25	±0.27	±0.24
p value			<.005	<.005	<.005	<.005	<.005
IRI	(μU/ml)						
normal	(11)	17.9	87.9	104.4	90.6	76.2	50.0
		±1.2	±6.9	±12.3	±10.4	±8.7	±9.9
gastrectomized	(19)	13.6	135.2	185.7	116.9	68.7	21.5
		±1.2	±31.0	±27.4	±29.0	±23.3	±7.9
p value				<.025			<.05
BS	(mg/100 ml)						
normal	(11)	81.3	140.8	126.4	107.0	97.3	93.3
		±4.3	±5.7	±7.7	±5.4	±6.0	±6.1
gastrectomized	(19)	89.1	191.6	183.6	121.0	82.5	65.1
		±3.9	±11.9	±16.1	±12.3	±9.0	±3.1
p value			<.005	<.005			<.005

[a] Mean ± SEM.

the oral administration of glucose, the mean IRG level rose to a peak of 3.19 ± 0.26 mμg/ml in the gastrectomized group, a value significantly higher than 1.15 ± 0.08 mμg/ml, observed in the normal subjects ($p < .005$). At the end of the experiment, the average IRG concentration approached base line levels in the normal subjects, but remained significantly elevated in the gastrectomized patients. The peak values of plasma IRI and glucose concentrations were also higher in the gastrectomized subjects than in the normal volunteers (Table I). Figure 2 shows that, in contrast to the oral administration, the intravenous injection of glucose caused no elevation of plasma IRG in the gastrectomized subjects. We have reported similar results in normal subjects (17). Figure 3 shows that in 4 gastrectomized subjects, 100 g of glucose caused a more marked increase in the plasma IRG than 50 g of glucose. Figure 4 shows that in the normal subjects, the IRG response to oral galactose was not significantly different from the response to oral glucose. However, in 7 gastrectomized subjects, the mean concentration of plasma IRG was significantly lower following oral galactose than following oral glucose. Slight increases of plasma IRI and of glucose were noted after oral galactose (Fig. 5 and Table II). Figure 6 shows that in 8 gastrectomized subjects, the average IRG level rose from 0.80 ± 0.06 mμg/ml to a peak of 1.28 ± 0.25 mμg/ml 30 min after the oral administration of fructose. This response was statistically different from the patients' own response to glucose. After the ingestion of fructose, the average IRI concentrations were not increased as much as could have been expected from the observed elevation in plasma glucose (Table III). No direct comparison between the effects of the 3 sugars was possible because not all patients received all 3 of them. However, Fig. 7 shows that the plasma IRG responses were significantly different from the response to oral glucose and from each other. In gastrectomized subjects, glucose was the most potent stimulus to IRG

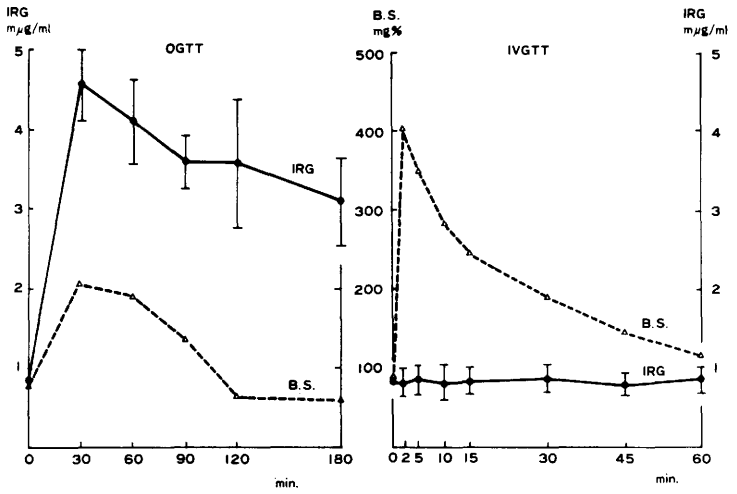

FIG. 2. Mean plasma IRG and glucose responses to iv glucose in 5 gastrectomized subjects.

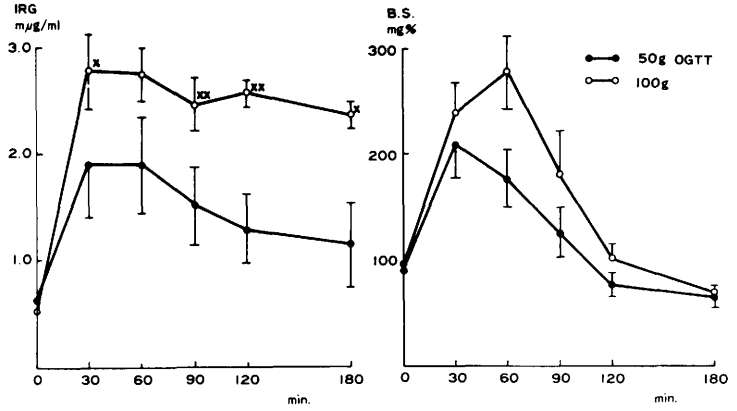

FIG. 3. Mean plasma IRG and glucose responses to 50 and 100 g of oral glucose in 4 gastrectomized subjects: statistical analysis based on paired comparisons (x) $p < 05$; (xx) $p < .025$.

release, followed by galactose and fructose, in that order.

Discussion. The radioimmunoassay used in this work does not differentiate pancreatic from gut IRG (Fig. 8). However, we believe that the observed rise in total plasma IRG following the oral administration of various sugars was due primarily to changes in gut glucagon, for the following reasons: the oral administration of glucose causes significant increase in serum IRG, while the intravenous injection of glucose is followed by a slight decline or no change (17); the release of glucagon from isolated islets is suppressed when the glucose concentration in the incubation medium is increased (13, 19); simultaneous measurements of IRG in the plasma of pancreaticoduodenal, mesenteric, and caval blood shows that after intraduodenal glucose loading, the earliest and greatest IRG rise occurs in the plasma of the mesenteric blood (5); a rise in serum IRG after intrajejunal glucose loading occurs also in totally depancreatized dogs (5, 20). Our gastrectomized patients, whose intestine received a bolus of relatively concentrated glucose solu-

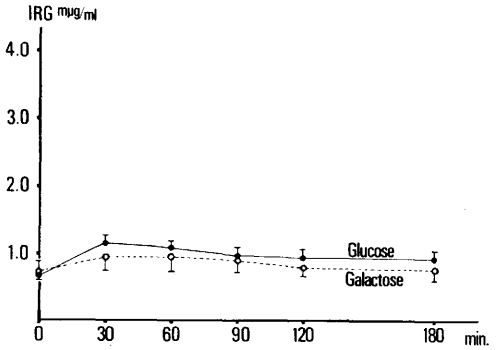

FIG. 4. Mean plasma IRG responses to oral glucose and galactose in 5 normal volunteers.

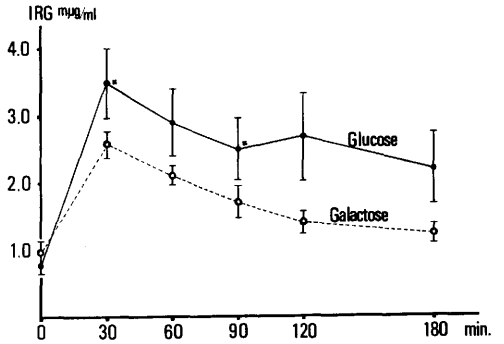

FIG. 5. Mean plasma IRG responses to oral glucose and galactose in 7 gastrectomized subjects: statistical analysis based on paired comparisons, (x) $p < .05$.

tion, had a plasma IRG response greater than that noted of the normal subjects. This response increased with the amount of glucose solution ingested, suggesting that mechanical or osmotic stimuli to the gut may have contributed to the release of enteric IRG. Of course, the possibility that the intestinal absorption of glucose *per se* may stimulate the release of enteric IRG cannot be excluded because the hyperglucagonemia observed in our study was always associated with hyperglycemia. Additional studies are needed to elucidate this problem. Significant differences between the effects of 3 different sugars were noted: glucose appeared to be the most potent stimulus, followed by galactose and by

TABLE II. Effect of Oral Glucose and Galactose on Plasma IRG, IRI, and Glucose in the Same Gastrectomized Subjects.

		After loading (min)					
		0	30	60	90	120	180
IRG	(mμg/ml)						
glucose	(7)	0.77[a]	3.49	2.90	2.50	2.70	2.20
		±0.14	±0.51	±0.50	±0.47	±0.64	±0.55
galactose	(7)	0.99	2.57	2.10	1.69	1.40	1.23
		±0.17	±0.22	±0.14	±0.25	±0.16	±0.14
p value			<.05		<.05		
IRI	(μU/ml)						
glucose	(7)	14.0	178.4	195.0	45.4	23.5	16.0
		±1.7	±70.1	±56.5	±8.5	±2.8	±2.3
galactose		14.9	29.9	18.8	19.6	18.8	16.8
		±1.6	±4.2	±2.1	±2.2	±2.7	±2.7
BS	(mg/100 ml)						
glucose	(7)	85.4	186.9	157.1	82.6	58.4	60.9
		±3.7	±20.7	±22.9	±12.1	±7.1	±3.5
galactose	(7)	93.4	186.4	198.4	171.6	127.6	93.2
		±7.4	±23.5	±17.2	±18.9	±15.5	±9.8
		75.3[b]	90.4[b]	85.1[b]	89.3[b]	84.4[b]	81.9[b]
		±6.4	±6.3	±5.6	±3.0	±3.8	±4.0

[a] Mean ± SEM.

[b] Measured by the glucose oxidase method.

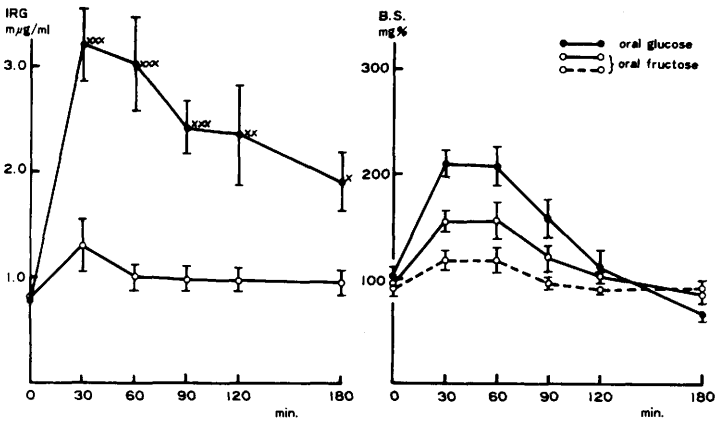

FIG. 6. Mean plasma IRG and glucose responses to oral glucose and fructose in 8 gastrectomized subjects: statistical analysis based on paired comparisons: (x) $p = .05$; (xx) $p < .025$; (xxx) $p < .005$; in blood sugar section: (O) measured with the method of Somogyi-Nelson; (O- - -) measured with the glucose oxidase method.

fructose. These results suggest that the secretion of enteric IRG may be regulated also by the chemical characteristics of the stimulus.

The significance of the plasma IRG rise after oral carbohydrate is not known, but it may reflect a role in the relationship between the hormones of the gastrointestinal tract and those of the islets of Langerhans (9, 21, 23).

In gastrectomized subjects, an oral glucose

TABLE III. Effect of Oral Glucose and Fructose on Plasma IRG, IRI, and Glucose in the Same Gastrectomized Subjects.

		After loading (min)					
		0	30	60	90	120	180
IRG	(mμg/ml)						
glucose	(8)	0.76[a]	3.20	3.04	2.41	2.37	1.91
		±0.05	±0.35	±0.45	±0.24	±0.48	±0.41
fructose	(8)	0.80	1.28	1.00	0.99	0.96	0.95
		±0.06	±0.25	±0.11	±0.12	±0.11	±0.11
p value			<.005	<.005	<.005	<.025	<.05
IRI	(μU/ml)						
glucose	(7)	14.5	132.9	187.5	75.0	42.2	20.6
		±2.6	±58.0	±86.1	±22.9	±7.3	±3.5
fructose	(7)	13.2	19.4	23.7	18.1	16.9	13.1
		±2.8	±3.8	±4.3	±3.2	±3.3	±2.3
BS	(mg/100 ml)						
glucose	(8)	100.1	209.5	207.0	158.0	110.1	68.7
		±7.1	±12.4	±18.8	±18.5	±14.9	±7.3
fructose	(8)	95.0	153.8	154.7	121.7	102.8	90.0
		±8.2	±10.9	±17.7	±12.7	±6.0	±9.7
		90.5[b]	118.7[b]	117.4[b]	95.1[b]	91.7[b]	90.6[b]
		±5.6	±11.4	±12.9	±5.7	±4.1	±4.4

[a] Mean ± SEM.

[b] Measured by the glucose oxidase method.

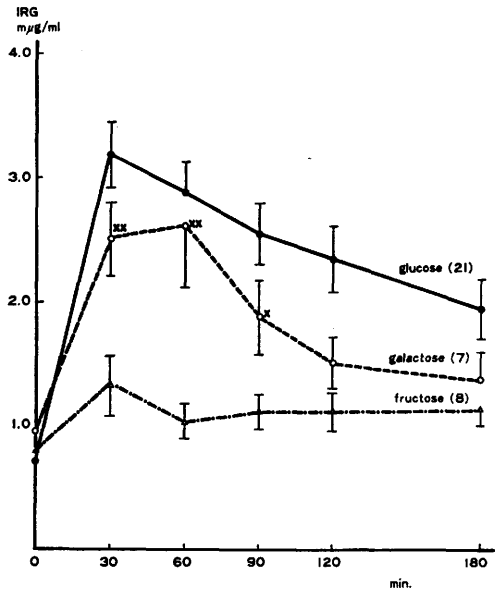

FIG. 7. Relative concentrations of plasma IRG after galactose and fructose, calculated as ratios to the values obtained after oral glucose: (x) $p < .05$; after galactose vs after fructose: (xx) $p < .025$.

load caused a marked rise in plasma IRI and in the insulinogenic index (insulin to glucose ratio; data not shown) greater than that caused by intravenous glucose. In addition oral glucose caused an increase in IRG greater than that observed in normal subjects (17). Since gut IRG has an insulinogenic activity similar to that of pancreatic glucagon (24, 25), our findings suggest that enteric glucagon, released following the sudden entry of carbohydrate into the gut, may have contributed to the augmented insulin response to oral glucose observed most dramatically in the gastrectomized subjects. By enhancing the IRI response to blood glucose, gut IRI level may contribute to the reactive hypoglycemia observed a couple of hours after a meal in gastrectomized patients with the dumping syndrome, as proposed by Rehfeld and Heding (26).

It is possible that, by enhancing the response of the B cells to glucose, enteric glucagon may play a permissive rather than a direct role in the stimulation of insulin secretion. This would explain why 2 and 3 hr after oral glucose or galactose, when the concentration of blood glucose was no longer elevated, the serum IRI level returned to normal despite persistent hyperglucagonemia. Gut glucagon may share this role with secretin and pancreozymin, which are also secreted in response to the ingestion of glucose or amino acid (27, 28), and by stimulating insulin secretion would prevent postprandial hyperglycemia.

Summary. Plasma immunoreactive glucagon (IRG) and immunoreactive insulin (IRI) were measured in 21 gastrectomized and 11 normal subjects during a 3 hr, 100 g oral glucose tolerance test. Plasma IRG and IRI responses to oral glucose (50 g), oral galactose (100 g), oral fructose (100 g), and to intravenous glucose (25 g) were also investigated in some individuals.

Although no difference in the fasting IRG concentration between the two groups was found, 30 min after ingesting 100 g of glucose, the plasma IRG concentration of the gastrectomized subjects was significantly higher than that of the normal subjects (3.19 ± 0.26 vs 1.15 ± 0.08 mμg/ml; $p < .005$).

In gastrectomized patients, oral glucose caused hyperglucagonemia, also a marked rise in plasma IRI; whereas intravenous glucose load caused only a moderate increase in plasma IRI, with no changes in IRG concentration.

In the gastrectomized patients, a 50 g oral glucose load caused a significantly smaller plasma IRG response than a 100 g glucose load. The increment in plasma IRG level

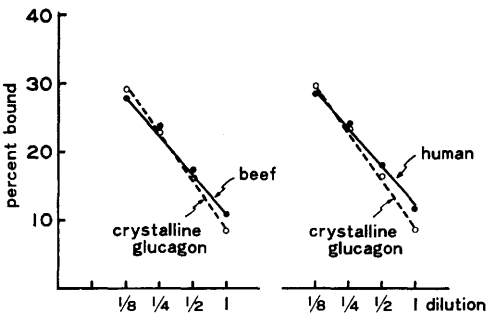

FIG. 8. Dilution slopes of crystalline beef–pork glucagon and acid–alcohol extract of the beef and human jejunum.

following galactose was smaller than that following glucose in both groups. However, the peak value of plasma IRG was significantly higher in the gastrectomized subjects than in the normals. After oral fructose administration, the gastrectomized subjects showed a small, but significant, increase in plasma IRG concentration. Glucose was the most potent stimulus for plasma IRG; and fructose was the weakest of the three sugars tested orally.

These results suggest that glucagon, probably of gastrointestinal origin, is released in response to chemical, as well as mechanical or osmotic, stimuli. The physiologic significance of enteric IRG is discussed.

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1. Samols, E., Tyler, J., Megyesi, C., and Marks, V., Lancet **2**, 727 (1966).

2. Unger, R. H., Ketterer, H., and Eisentraut, A. M., Metabolism **15**, 863 (1966).

3. Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R., and Unger, R. H., Diabetes **19**, 614 (1970).

4. Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R., and Unger, R. H., Diabetes **19**, 624 (1970).

5. Unger, R. H., Ohneda, A., Valverde, I., Eisentraut, A. M., and Exton, J., J. Clin. Invest. **47**, 48 (1968).

6. Dupre, J., Curtis, J. D., Unger, R. H., Waddell, R. W., and Beck, J. C., J. Clin. Invest. **48**, 745 (1969).

7. Kraegen, E. W., Chisholm, D. J., Young, J. D., and Lazarus, L., J. Clin. Invest. **49**, 524 (1970).

8. Kahil, M. E., McIlhaney, G. R., and Jordan, P. H., Jr., Metabolism **18**, 50 (1970).

9. Creutzfeldt, W., Feurle, G., and Ketterer, H., N. Engl. J. Med. **282**, 1139 (1970).

10. Buchanan, K. D., Vance, J. E., and Williams, R. H., Diabetes **18**, 381 (1969).

11. Edwards, J. C., Howell, S. L., and Taylor, K. W., Nature (London) **224**, 809 (1969).

12. Chesney, T. M., and Schofield, J. G., Diabetes **18**, 627 (1969).

13. Nonaka, K., and Foà, P. P., Proc. Soc. Exp. Biol. Med. **130**, 330 (1969).

14. Luyckx, A. S., and Lefebvre, P. J., Proc. Soc. Exp. Biol. Med. **133**, 524 (1970).

15. Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H., Diabetes **18**, 717 (1969).

16. Unger, R. H., Aguillar-Parada, E., Müller, W. A., and Eisentraut, A. M., J. Clin. Invest. **49**, 837 (1970).

17. Shima, K., and Foà, P. P., Clin. Chim. Acta **22**, 511 (1968).

18. Hales, C. N., and Randle, P. J., Biochem. J. **88**, 137 (1963).

19. Vance, J. E., Buchanan, K. D., Challoner, D. R., and Williams, R. H., Diabetes **17**, 187 (1968).

20. Buchanan, K. D., Vance, J. E., Aoki, T., and Williams, R. H., Proc. Soc. Exp. Biol. Med. **126**, 813 (1967).

21. Haddad, J. G., Jr., and Owen, J. A., Jr., Metabolism **18**, 71 (1969).

22. Penhos, J. C., Wu, C.-H., Basabe, J. C., Lopez, N, and Wolff, F. W., Diabetes **18**, 733 (1969).

23. Veverbrants, E., Olsen, W., and Arky, R. A., Metabolism **18**, 6 (1969).

24. Moody, A. J., Markussen, J., Schaich-Fries, A., Steenstrup, C., Sundby, F., Malaisse, W., and Malaisse-Lagae, F., Diabetologia **6**, 135 (1970).

25. Samols, E., Marri, G., and Marks, V., Lancet **2**, 415 (1965).

26. Rehfeld, J. F., and Heding, L. G., Brit. Med. J. **2**, 706 (1970).

27. Chisholm, D. J., Young, J. D., and Lazarus, L., J. Clin. Invest. **48**, 1453 (1969).

28. Wang, C. C., and Grossman, M. I., Amer. J. Physiol. **164**, 527 (1951).

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