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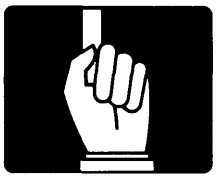

Insulin Treatment for the Early 80s: Facts and Questions About Old and New Insulins and Their Usage

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Recent data now emphasize the importance of blood glucose control as a means of forestalling diabetic microvascular disease. As a result, attempts are being made to optimize conventional insulin therapy, and new modes of insulin delivery (e.g., pumps) are being adopted. Also, improvements in manufacturing technology have resulted in the commercial availability of porcine insulin, which is highly effective in preventing the complications of insulin therapy in those patients receiving only this material. Human insulin, produced biosynthetically in bacteria using recombinant DNA technology, is now being tested. While these developments in insulin and its administration offer great promise to the diabetic patient, many more studies will be needed to determine their absolute clinical benefits. DIABETES CARE 3: 615–622, SEPTEMBER–OCTOBER 1980.

Since its first clinical use in 1922 therapeutic insulin has undergone important pharmaceutical modifications.[1–7] While there has been an exponential increase in basic information on human metabolism and diabetes, only recently has this knowledge been translated into major advances in treatment. Consequently, patients as well as physicians, fired by the expectations of modern technology and eager for therapeutic progress, are accepting new approaches to insulin therapy, often before they have been adequately evaluated. It is therefore appropriate to review the present state of the art of insulin therapy, which has resulted from new information on the absorption and effect of injected insulin and from increased purity of commercial insulin preparations.

The important recent developments in insulin treatment are based on two assumptions: (1) normalization of blood glucose will reduce the rate of development of diabetic complications[8–10] and (2) distinct benefits, some yet to be elucidated, will result from the use of the purest insulin with a structure most like that of human.[3,11–13] An increased awareness of the shortcomings of traditional insulin treatment, particularly as indicated by glycosylated hemoglobin measurements, and the possibility that diabetic microvascular disease can be prevented or treated by normalization of plasma glucose and other parameters, has resulted in attempts to improve insulin therapy and to find other methods to improve the way insulin is administered. These efforts have led to an increased use of multidose regimens and pump systems that deliver insulin subcutaneously. The second assumption—namely, that the best insulin is that which is the purest and has a chemical structure most like human insulin—has resulted in a marked shift of insulin usage from beef-containing insulins to monospecies pork. It has been an important impetus to the research and development that has led to the production of human insulin using recombinant technology.[14–16a]

CONVENTIONAL INSULIN THERAPY

The optimal use of insulin requires a knowledge of the availability of preparations and an awareness of factors that modify their effects. The insulins now available in the United States and their important characteristics are listed by manufacturer in Table 1. Factors influencing the effect of insulin are listed in Table 2. Information concerning the times of onset and duration of PZI (protamine, zinc, and insulin), NPH (neutral protamine Hagedorn), and R (regular) insulins) is based on data generated at the Lilly Clinic between 1935 and 1965[26] and by extramural investigators[27–29] using patients with both insulin-dependent (type I) and adult-onset (type II) diabetes. The individuals were given doses of insulin in excess of their estimated needs, and the blood glucose was measured at regular intervals for 24–48 h. Food was given every 4–6 h. These curves, representing responses to pharmacologic and not therapeutic doses of insulin, can only approximate the expected response of patients to therapeutic

TABLE 1
Insulins available in the United States (listed by manufacturer)

Purity/species and type of action	Lilly products	Novo products	Nordisk products	Squibb products
Purified pork (<10 ppm)				
Rapid acting	Regular	Actrapid (reg.)	Velosulin Quick (reg.)	
		Semitard (semilente)	Mixtard (30% reg/70% NPH)	
Intermediate acting	NPH			
	Lente	Monotard (lente)	Insulatard (NPH)	
Long acting	PZI			
Purified beef (<10 ppm)				
Rapid acting	Regular			
Intermediate acting	NPH			
	Lente			
Long acting	PZI	Ultratard (ultralente)		
Purified beef-pork (<10 ppm), intermediate acting		Lentard (lente)		
Improved single peak (USP beef-pork <50 ppm)				
Rapid acting	Regular			
	Semilente			
Intermediate acting	NPH			
	Lente			
Long acting	PZI			
	Ultralente			
USP beef-pork (conventional <10,000 ppm)				
Rapid acting				Regular
Intermediate acting				NPH
				Globin
Long acting				PZI
USP beef (conventional <10,000 ppm)				
Rapid acting				Semilente
Intermediate acting				Lente
Long acting				Ultralente

doses of insulin. Recent studies in diabetic subjects, using both radiolabeled[18,30] and unlabeled materials,[31,32] have confirmed these earlier reports and have provided new information on NPH and regular insulin mixtures. Data from our studies[31,33] of the various insulins in normal fasting subjects are presented in Figures 1A and 1B.

TABLE 2
Factors that influence the effect of insulin[17]

1. Site of injection: After a dose of regular insulin, peak concentrations are most quickly achieved with abdominal injection and are slowest to occur when injected in the anterior thigh. Peak concentrations are highest in the deltoid area and lowest after buttock injection. Exercise increases insulin absorption from a given extremity.[18]
2. Depth of injection: The deeper insulin is injected, the quicker is its onset and the higher is its peak.[19,20] However, the clinical significance of these differences may be minimal.
3. Concentration of insulin in the range of 40–100 U/ml is not a significant factor in insulin bioavailability.[21]
4. There is significant day-to-day variation in serum insulin and/or blood glucose response of normal and diabetic individuals given the same dose of insulin twice or more.[19,22]
5. Insulin antibodies bind injected insulin for variable periods of time, thereby delaying its onset and duration of effect.[23–25]
6. The most important factor(s) in insulin effect may be the dynamics of its interaction with insulin receptors.[19]

The insulin therapist is frequently called upon to make a choice between NPH and lente insulin. The published literature is replete with favorable reports on both NPH[34,35] and lente[36] insulin. Until recently, the author viewed NPH and lente insulins as comparable in most respects. However, when our studies in normal fasting subjects disclosed that the separate effects of regular insulin could be better maintained when used with NPH rather than lente insulin,[31] we began using NPH insulin for our new patients and for patients with problems in insulin therapy. The mixability of NPH and of lente insulin with regular insulin and other differences between NPH and lente insulins are listed in Table 3.

In using combinations of intermediate and regular insulin, one may encounter patients in whom regular insulin has a long duration of action, in excess of 8 h.[23] As a result, correction of before-supper hypoglycemia may be more readily accomplished by reducing the morning dose of regular insulin rather than the morning dose of NPH insulin.[41] Conversely, presupper hyperglycemia will respond to an increase in the before-breakfast dose of regular insulin.

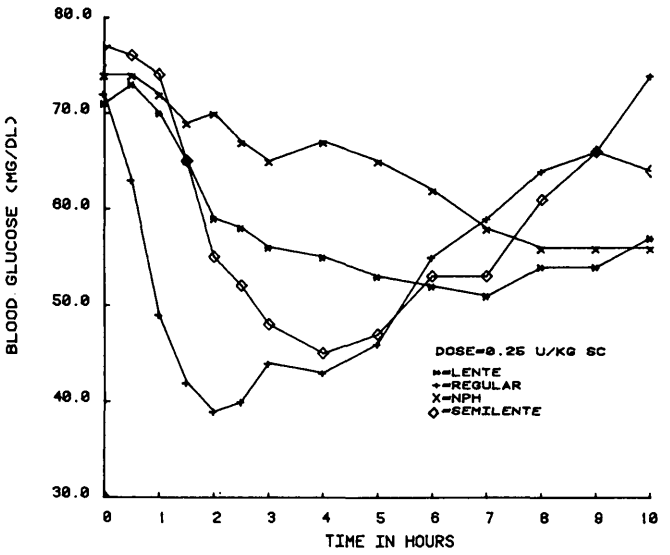

FIG. 1a. The blood glucose response of normal, fasting subjects to lente, regular, NPH, and semilente insulins. The onset of action is compatible with the general clinical perception of the insulins shown.

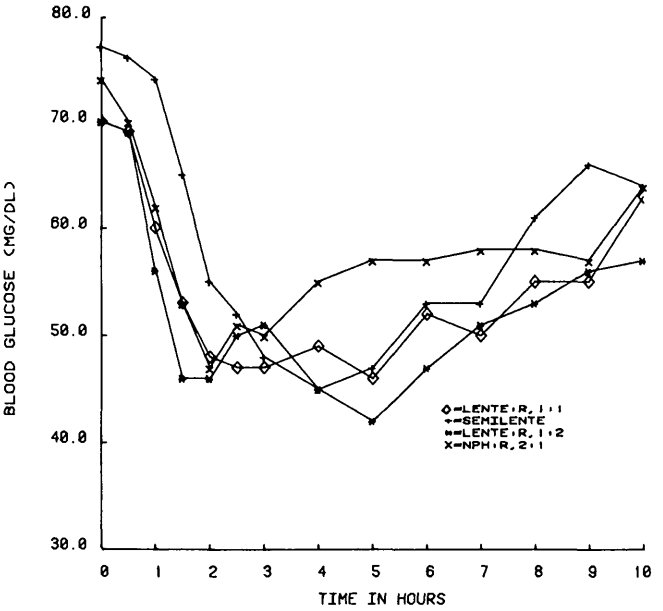

FIG. 1b. The blood glucose response of normal, fasting subjects to NPH:regular (R) 2:1, lente:R 1:1 and 1:2, and semilente. There is no statistical significant difference between lente:R combinations in spite of the wide range in the proportion of lente:R used.

ALTERNATE METHODS OF INSULIN DELIVERY

Since conventional insulin therapy rarely normalizes plasma glucose concentrations, there have been many attempts to improve insulin delivery using various infusion systems and routes of administration (subcutaneously, intravenously, and intraperitoneally).[42–44] In the United States the "pump era" was launched by the report of Tamborlane et al.,[45] who, using the AutoSyringe (AutoSyringe, Hookset, New Hampshire), remarkably improved the plasma glucose control of 17 type I diabetic subjects. Although most physicians consider the administration of insulin by "open-loop" automatic pump systems to be research tools,[43,44,46] the encouraging preliminary results have led to the widespread use of this method. The advantages of the open-loop pump systems are (1) flexibility of meal-dose administration, (2) prevention of fasting hyperglycemia by a midsleep burst of insulin to offset overnight secretion of anti-insulin hormones,*[47] and (3) normalization of not only plasma glucose but other parameters.[48,49] On the other hand, Phillips et al. have reported comparable blood glucose control in patients who have used injections of regular insulin before meals with a single dose of long-acting in-

*This feature is not present in the AutoSyringe but undoubtedly will be included in pumps that are eventually developed.

TABLE 3
Advantages and disadvantages of lente and NPH insulins

	Advantages	Disadvantages
NPH		
	1. Can be mixed in the same syringe with regular over a wide range of ratios.	1. Contains protamine, which may simulate and/or augment insulin allergy.[37] Rarely, it may sensitize patients to the extent that massive allergic responses may follow the administration of protamine when used to neutralize heparin anticoagulation.[38,39]
	2. Has a time action that meets the needs of a number of diabetic patients on a once-daily dose regimen.	
Lente		
	1. Has a time action that meets the needs of a number of diabetic patients on a once-daily dose regimen.	1. In lente:regular mixtures the effect of the regular may be blunted, possibly because of binding by the excess zinc in the lente. (However, this limitation does not necessarily preclude the use of lente:regular combinations.)
	2. Contains no foreign protein.	2. The preparation of lente may result in polymer formation, which augments the immunogenicity.
	3. Can be mixed with ultralente and semilente in the same syringe or vial to meet individual patient needs.	3. High zinc content may exacerbate allergy to zinc in a minority of patients.[40]

sulin.[50] Using a combination of regular and intermediate-acting insulin and intensive patient self-monitoring of blood glucose, Peterson et al. have achieved the same results.[51] For optimal results, regardless of whether patients use infusion pumps or multiple doses of regular insulin administered in a syringe, strict dietary adherence and home glucose monitoring are essential. Prospective controlled studies will be required to establish whether insulin administered by infusion pumps is superior to multiple doses of regular insulin in terms of metabolic control, morbidity, mortality, and patient acceptance.

INSULIN MANUFACTURE, PURITY, AND COMPLICATIONS OF INSULIN THERAPY AND OF DIABETES MELLITUS

The conventional methods for manufacturing insulin consist of acid-ethanol extraction of insulin from beef and pork pancreata followed by a series of isoelectric precipitations and recrystallizations. This process results in conventional or USP insulin. The finding and identification by Steiner and Oyer[52] and by Chance et al.[53] in 1968 of proinsulin in commercial insulin preparations marked the beginning of a new era in insulin purification technology. Over the past 10 years, two chromatographic methods have been developed and used alone or in combination for the additional purification of insulin: gel filtration chromatography, which acts as a molecular sieve and separates proteins according to molecular weight, and ion exchange chromatography, which separates proteins according to ionic charge. Because proinsulin elutes near insulin in these chromatographic procedures and is easily measured by a sensitive radioimmunoassay, this precursor molecule has become a useful index of purity in marketed insulins. The proinsulin contents of various insulin preparations available in the United States are indicated in Tables 1 and 4. Immunoassays for glucagon, pancreatic polypeptide (PP), vasoactive intestinal peptide (VIP), and somatostatin are also used to monitor insulin purity.

The clinical significance of the improvements in insulin purity has been the subject of much research and some controversy[11,13,54,55] for several years. No controlled clinical trials have been reported using insulins of standardized levels of purity and species sources (beef, pork, and mixed). However, certain facts are worth noting. First, insulin lipodystrophy (atrophy and hypertrophy) and allergy (local and systemic) have not been reported in patients who have received only purified pork insulin.[56,57] Second, virtually all patients with insulin lipoatrophy improve when treated with the purified pork insulin.[58,59] Third, serum antibody formation has been reported to be minimal and frequently undetectable in the majority of patients receiving only purified pork insulin.[60–62] The development of antibodies to insulin and other substances present in commerical insulin may be related to many factors, including (1) the subject's genetic make-up,[63] (2) the pattern of exposure—interrupted insulin therapy is a frequent finding in patients with systemic allergy[11] and immunologic resistance,[25] (3) the species source of the insulin (beef being more immunogenic than pork),[62,64] and (4) pharmaceutical form (lente insulin is more immunogenic than regular insulin).[62]

There are conflicting data on the clinical importance of antibodies to insulin and other substances in commercial insulin preparations. Clearly, antibodies delay the hypoglycemic effect of injected regular insulin.[23,41,65] Some workers perceive that insulin antibodies may act as buffers to protect patients from the immediate hypoglycemic effect of injected regular insulin and reduce the lability of the blood glucose.[66] Others have reported that patients with the highest degree of insulin binding have the lowest concentrations of "free" insulin.[67] However, the physiologic significance of "free" insulin has not been established. In a study of insulin-treated diabetes there was no correlation between metabolic control and association constants of high and low affinity antibodies, total insulin antibody titer, or insulin binding capacity.[68]

On the other hand, there are data[69] to support the possibility that insulin antibodies do reduce by some mechanism the secretion or effect of endogenous insulin. For instance, in type I diabetes, increased serum titers of antibodies to insulin have been reported to be associated with unmeasurable fasting concentrations of human C-peptide, suggesting deleterious influence of the insulin antibodies on beta-cell function.[69] Another factor in the preservation of endogenous insulin secretion in type I diabetes apparently is the vigor of initial insulin treatment. Thus, in a report in which purified pork insulin was administered in conventional and intensive dose regimens, remissions of minimal glycosuria occurred with significantly greater frequency in the intensively treated group.[70]

Insulin dose reductions and decreases in serum insulin antibody titers have been reported in patients switched from conventional beef or mixed beef-pork to purified pork insulins. For example, Andreani et al.[71] reported a 26% decrease in daily insulin requirement after 2 yr. Similarly, Mustaffa et al.[72] observed a 12% decrease, and Asplin et al.[73] reported 26% and 20% decreases in two separate groups of patients. In a study of 211 insulin-dependent patients[74] (Figure 2) followed for 3 mo on mixed beef-pork "single peak" insulin and

TABLE 4
Proinsulin contents of insulins available in the United States

Insulin	Proinsulin (parts per million)
Conventional USP	10,000–40,000
"Single Peak"	300–3,000
"Improved Single Peak"	<50
"Purified"*	<10†

* Novo pork and mixed insulins, Nordisk pork, Lilly pork (Iletin II pork), and Lilly "purified" beef (Iletin II Beef) to be introduced in September 1980.

† Internal specifications for manufacturers vary. Lilly assays indicate that all three producers are marketing products with about <1–5 ppm proinsulin.

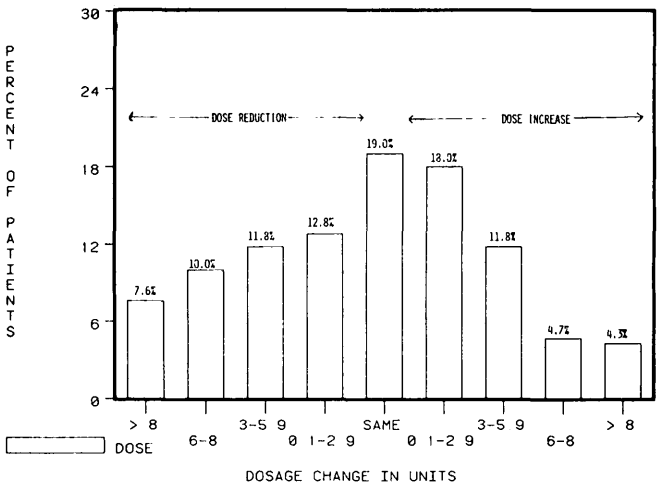

FIG. 2. Distribution of changes from mean baseline doses in 211 insulin-dependent diabetic subjects 4 mo after being switched from beef-pork single-peak insulin (BP) to purified pork (PP) insulin.[74] *The median change with pork insulin was −1.7%. In 19% there was no dose change; in 43% a dose decrease occurred; and there was a dose increase in 38%. Conclusion: The switch from single-peak BP to PP is not associated with a consistent change in insulin dose.*

then switched to purified pork insulin for 4 mo, there was no dose change in 19%, a dose decrease in 43%, and a dose increase in 38% of the patients. After the switch to purified pork insulin, a fall in serum insulin antibody titers was the rule. We are unable to explain the dichotomy between dose change and insulin antibody titers in this study.

A relationship between serum insulin antibody titers and the development of the complications of diabetes has been suggested, but not established. In a retrospective study[75] the frequency of retinopathy was greater in the patients with increased antibody titers than those without. On the other hand, Deckert et al., studying a smaller group of patients, found no correlation between elevation of insulin antibody titers and increased capillary basement membrane thickening and proliferative retinopathy.[76] A study in which rabbits were immunized with "a" and "b" components from a conventional commerical insulin demonstrated circulating antibodies to insulin. Also, material was deposited in the kidneys that contained insulin: insulin antibody complexes associated with lesions indistinguishable from those of diabetic intercapillary glomerulosclerosis.[77] Animals given saline or Monocomponent pork insulin had neither measurable titers of insulin antibodies nor kidney lesions. Concern about this latter finding is vitiated by the fact that diabetic intercapillary glomerulosclerosis occurs in patients who have never received insulin.[78]

The importance of antibodies to substances other than insulin is even less well understood than that of antibodies to insulin. Clearly, in some patients antibodies to glucagon develop, which may impair the action of endogenous glucagon secreted in response to hypoglycemia.[79] Antibodies to other pancreatic hormones, such as PP, VIP, and somatostatin, occur in patients treated with unchromatographed insulin and probably single-peak insulins,[80] but not in patients receiving the purified pork insulins. Immunocytochemical examinations have disclosed that plasmas from diabetic patients with increased titers of these substances (PP, VIP, and somatostatin) react to their respective hormone-producing cells.[80,81]

While the benefits of purified pork insulin are being elucidated, the demand is increasing. There are conflicting views on the adequacy of worldwide supplies of insulin from animal sources, particularly porcine, to meet projected needs.[82] Chromatographic[83] and immunologic[61] data indicate that the insulin from manufacturers who utilize pork pancreata from vendors who also supply beef glands will contain small amounts of beef insulin. For this reason, Lilly uses pork-only vendors as the source for Iletin II Pork (purified pork insulin, Lilly). While this procedure reduces the beef contamination to virtually zero, the pool of pork pancreas is decreased by 50%.

Because of many unanswered questions concerning the importance of purity and species source on the complications of insulin therapy, including immunogenicity and preservation of beta-cell function, Eli Lilly and Company has embarked upon a double-blind study (Table 5).

Until the results of the Lilly or other studies are known, or until alternate sources of pork or human insulin become widely available, one should assume that mixed beef-pork improved single-peak insulin is adequate to meet the needs of the majority of diabetic patients, and the use of purified pork insulin should be limited to the indications listed in Table 6. If patients are treated with purified pork insulin for

TABLE 5
A clinical study sponsored by Eli Lilly and Company to ascertain the importance of insulin purity and species source and purity on the complications of insulin therapy and on plasma glucose control

Patients:	550–600 of all ages with all types of diabetes* who have never received insulin
Design:	Completely randomized, double-blind
Insulins:	1. "Purified pork 78"—collected from suppliers who deal in both cattle and pigs, beef insulin content up to 0.5% (proinsulin <10 ppm) 2. "Purified pork 79"—collected from pork only suppliers, virtually no measurable beef insulin (proinsulin <10 ppm) 3. "Improved single peak"—mixed beef-pork insulin (<50 ppm proinsulin)
Parameters:	The usual clinical ones with emphasis on evidence of blood glucose control and complications of insulin therapy as indicated by: 1. Fasting and postprandial blood glucose levels 2. A_{1c} hemoglobin 3. Findings of atrophy, hypertrophy, allergy, and serum antibody formation a. Insulin and proinsulin antibody titers b. Total and free insulin c. Fasting and stimulated plasma human C-peptide

* HLA typing to be done toward the end of the study.

TABLE 6
Indications for purified pork insulin*

1. Insulin allergy, local and/or systemic†
2. Immunoresistance (requirements greater than 100 U daily)
3. Insulin-induced lipodystrophy—lipoatrophy and lipohypertrophy
4. Temporary insulin administration
   a. Surgery or major stress in type II diabetes
   b. Gestational diabetes

* There are no conclusive data demonstrating unique efficacy of purified pork insulin in labile diabetes or in preventing the complications of diabetes.
† Patients with systemic allergy usually require desensitization before the administration of therapeutic doses.[11]

reasons not substantiated by available clinical data, then therapeutic expectations may go unfulfilled. It appears presently that normalization of blood glucose is a far more important factor in detering the complications of diabetes than insulin purity or species source.

BIOSYNTHETIC HUMAN INSULIN[16,16a]

The projection of a possible insulin shortage in the 1990s,[81] as well as concerns about the adequacy of supplies of pork, has stimulated extensive research and development leading to the production of human insulin in *Escherichia coli* using recombinant DNA technology.[14,15,16a] Briefly, the process involves linking synthetic A- and B-chain genes to a common *E. coli* plasmid gene (e.g., beta-galactosidase) via a methionine codon. (The latter facilitates the eventual cyanogen bromide cleavage of the appropriate polypeptide chain from the chimeric protein translation products.) After an appropriate fermentation period under very highly controlled conditions, the A-chain or B-chain plasmid-bearing *E. coli* are killed and then extracted to obtain the chimeric protein (e.g., beta-galactosidase-methionine-A-chain and beta-galactosidase-methionine-B-chain). Cyanogen bromide is then used to cleave the A- and B-chains from the beta-galactosidase. The A- and B-chains are converted to S-sulfonate derivatives and then subjected to initial purification, combined, and the combination product (insulin) further purified. Amino-acid analyses disclose that biosynthetic human insulin is the same as pancreatic human insulin. The activity of biosynthetic human insulin is comparable to that of pancreatic pork and human insulin in radioimmunoassays, radioreceptor assays, and in lowering blood glucose in rabbits. The resulting insulin is of "purified" quality and contains no pancreatic proteins. The reactivity of biosynthetic human insulin in tests for pyrogenicity and endotoxins is equal to or less than that found from conventionally produced insulins. The results of extensive clinical testing, now in progress, will be needed before the benefits to patients of using a homologous insulin are known. Clearly, all the questions that have been asked concerning purified pork insulin will be applied to biosynthetic human insulin. Additionally, in spite of the remarkable purity of the biosynthetic product and its apparent freedom from bacterial contaminants, attention will be directd to immunologic evidence of *E. coli* protein contamination in the serum of patients treated with biosynthetic human insulin prepared from Coliform sources. These issues notwithstanding, the production of insulin in bacteria effectively precludes any likelihood of an insulin shortage.

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