
VOLUME 62 2002

SOME EFFECTS ON FOOD INTAKE OF INFUSIONS OF AMINO ACID-CONTAINING MATERIALS

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SUMMARY

Some effects induced by infusing casein, casein hydrolysate and an amino acid mixture to sheep fed a low-quality roughage are reported. Infusions were generally for an 8 hr period and were delivered into the duodenum and the venous circulation.

A change of food intake was just one of the effects induced; others included changes in water intake, electrolyte and faecal DM excretion, blood composition and gut motility. The effects on food intake were variable, ranging from a marked increase to an equally marked decrease and were generally confined to the period of infusion. They were modified by dose rate, composition of vehicle, a preliminary infusion of water, nature of material administered, route of infusion, and animal variation.

It is considered that the effects of the infusions were mediated by several mechanisms located at various sites within the body.

THE CONSUMPTION of low-quality roughages by sheep and cattle is often improved by dietary additions of nitrogen. The effect is generally attributed to an increase in the rate of cellulose digestion in, and disappearance of digesta from the reticulorumen (Clark and Quin, 1951; Campling *et al.*, 1962; Coombe and Tribe, 1963). The resulting reduction of gut distension is thought to facilitate the continued ingestion of food.

Egan and Moir (1965) and Egan (1965a) reported that the consumption of low-quality roughage by sheep was increased by the intraduodenal (i.d.) infusion of casein. The effect was not entirely explicable in terms of a distension hypothesis. Egan proposed that a form of metabolic regulation was involved and suggested that roughage intake was dependent on the ability of the animal to utilize energy (Egan, 1965c; 1966).

The work described here and in greater detail by Bryant (1968) had three objectives:

(1) To define better the effects of i.d. infusions of casein on food intake.

(2) To identify factors influencing those effects.

(3) To establish the sites of action and the mechanisms involved.

MATERIALS AND METHODS

SHEEP

The sheep used were Romney ewes 16 to 18 months of age fitted with duodenal cannulae. They were housed indoors in individual stands which allowed the separate collection of faeces and urine.

FEED AND FEEDING

Their diet was chaffed, threshed ryegrass straw containing 0.9 to 1.05% nitrogen on a dry matter (DM) basis and a DM digestibility of approximately 45%. An amount of weighed food approximately 20% in excess of expected intake was offered at 8.30-9.00 a.m. Residues were collected and weighed at the end of a 24 hr feeding period. On occasions when rate of food intake was required, residues were weighed at intermediate times. Water intake was measured by determining the residue after 24 hr or when food residues were weighed after 4 l had been offered at the time of presentation of fresh feed. Samples of feed, feed residues and faeces for assessing DM content were taken daily and dried at 90 to 92° C.

SOLUTIONS AND THEIR INFUSION

Except where stated, all infusions were made over an 8 hr period commencing with the presentation of fresh feed. Infusion rate ranged from 1.3 to 3.2 ml/min depending on the experiment.

Casein for i.d. infusion was a water-soluble calcium salt of milk protein ("Casilan", Glaxo Labs. (N.Z.) Ltd.) containing 13.5% nitrogen.

Saline. Except where stated, the casein vehicle was a salt solution as described by Blaxter and Martin (1962), isotonic with duodenal contents, and hereafter referred to as "Saline".

Casein hydrolysate was a sterile 6% solution of amino acids and peptides derived from casein by acid hydrolysis ("Parenamine", F. Stearns and Co., Sydney). This was used both as an i.d. infusion and an i.v. infusion.

Isotonic NaCl (Sodium chloride injection U.S.P.; Abbott Labs. (N.Z.) Ltd.) served as a control for the i.v. infusion of casein hydrolysate.

Amino acid mixture which was infused i.d. in one experiment was prepared from "Aminofusin forte" (J. Pfrimmer and Co., Erlangen, W. Germany) a sterile solution containing 100 g/l of crystalline amino acids. Immediately prior to the start of an infusion, 500 ml "Aminofusin forte" were diluted to 1,500 ml with distilled water. The resulting solution is referred to as the "amino acid mixture".

Salt solution. Inorganic salts and sorbitol contained in 500 ml "Aminofusin forte" were made to 1,500 ml with distilled water and the pH adjusted to 5.5 with N/1 HCl. The resulting solution, used as a control for the i.d. infusion of the amino acid mixture, is referred to as the "Salt solution".

EXPERIMENTAL AND RESULTS

Some Effects of Daily Infusions of Casein

The initial experiments were concerned with the effects of daily 8 hr i.d. infusions of casein on food intake. The effects were variable. On occasions, food intake was increased to about 140% of that when Saline was infused. On others, no effect was apparent even though nitrogen balance measurements indicated that about half the infused nitrogen was retained in the body. The increase of intake was often transitory and could not always be confirmed by a subsequent period of infusion into the same animal.

It was during experiments of this nature that an effect on food intake associated with changes in the casein vehicle was observed (Fig. 1).

Of interest is the increase in DM intake on the first day of casein infusion (Day 19, Fig. 1A, B, C), when the casein vehicle was changed to water for one day (Day 30, Fig. 1B; Day 32, Fig. 1C), and on the day following the infusion of water (Day 36, Fig. 1B; Day 37, Fig. 1C).

These effects suggested that the diet was not the primary cause of the lack of response. They did suggest that the increase in food intake was dependent on short-term changes occurring within the animal, induced by changes

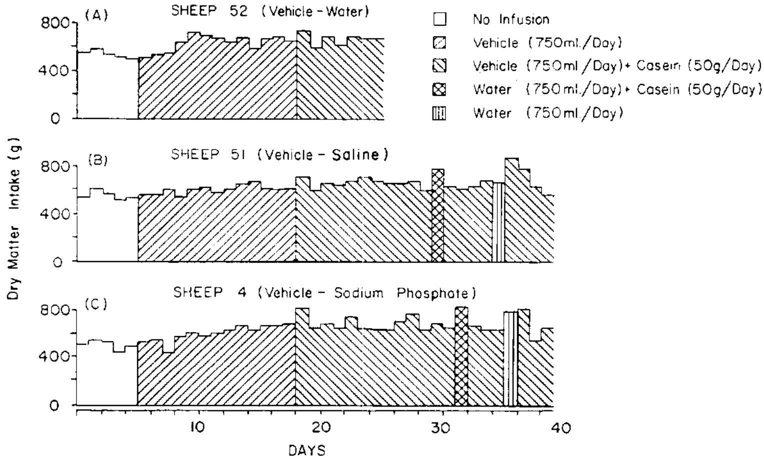

FIG. 1: *An effect on food intake associated with a change in casein vehicle.*

in the nature of the infusion. For these reasons, subsequent work was confined to examining the effects of single 8 hr infusions administered at intervals of 5 to 7 days.

SOME EFFECTS OF SINGLE INTRADUODENAL INFUSIONS OF CASEIN

An experiment in which the i.d. infusion of casein increased food intake is shown in Fig. 2. The increase was confined to the day of infusion and was accompanied by an increase in the amount of water drunk. In this experiment there was a marked increase of food intake. In some others, however, the effects were more variable, ranging from a marked increase to an equally marked decrease.

FACTORS THAT MODIFIED THE EFFECT ON FOOD INTAKE

(a) *Casein dose rate* was examined in two experiments. In one, food intake was reduced by 75 g casein infusion but was increased by 50 g. In the other (Table 1), the infusion of casein at the rate of 4 g/kg body weight resulted in lesser increases of food intake than did infusions at the rate of 1 and 2 g casein/kg B.W.

(b) *The influence of water* was demonstrated in an experiment in which the same quantities of the same solutes were administered in different amounts of water. The solutes consisted of casein (2 g/per day kg B.W.) plus the

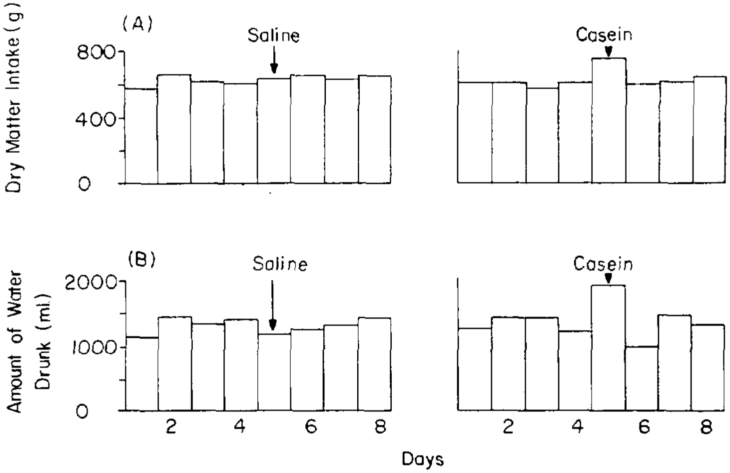

FIG. 2: *Effect of i.d. infusions of 750 ml Saline alone and containing 50 g casein on food and water intake of 4 sheep.*

salts contained in 750 ml Saline. A comparison was made of the effects of an infusion of 750 ml of the "isotonic" casein preparation with

(i) the same treatment following an i.d. infusion of 1,000 ml of water during the preceding 16 hr ("water priming") and

(ii) infusions of "hypertonic" and "hypotonic" casein preparations made by dissolving the solutes in 400 and 1,500 ml water, respectively. The treatments differed in their effect on food intake (Table 2).

TABLE 1: EFFECTS OF CASEIN DOSE RATE ON FOOD INTAKE. MEAN OF 3 OBSERVATIONS (g DM)

Casein Dose Rate (g/kg B.W.)	*Mean of 3 Days Prior to Infusion*	*Day of Infusion*	*Mean of 3 Days After Infusion*
		SHEEP 42	
1	638	755	615
2	648	708	600
4	615	632	632
S.E.	± 21	± 37	± 21
		SHEEP 56	
1	582	671	588
2	576	695	581
4	598	627	578
S.E.	± 11	± 19	± 11

The greatest increases, which occurred mainly during the 8 hr infusion period, were induced by the hypotonic and water priming treatments. The trend towards increased food intake with decreasing vehicle tonicity (or increasing vehicle volume) was significant ($P = 0.024$). Though various confounded factors may have contributed to these effects, it is clear that, in the water priming treatment, the preliminary infusion of water facilitated an increase of food intake during and shortly after the subsequent infusion of casein.

(c) *Route of infusion.* When casein hydrolysate (0.28 g N/kg B.W.) was administered as an intraduodenal infusion, an increase of food intake of the order of 10% was obtained. This effect was in sharp contrast with that observed when infused at the same rate into the jugular vein (Table 3). Its effects on food intake were apparent as a marked reduction during the period of infusion, partly compensated for by an increase during the subsequent 16 hr. It is noteworthy that in this experiment the i.d.

TABLE 2: EFFECT ON FOOD INTAKE WHEN i.d. INFUSIONS OF CASEIN (2 g/kg/B.W.) WERE EITHER VARIED IN VOLUME AND SOLUTE CONCENTRATION OR PRECEDED BY AN i.d. INFUSION OF WATER. MEAN OF 4 SHEEP

Treatment	*Intake on Day of Infusion less Control† Intake (g DM)* 0-24 hr	0-8 hr	8-16 hr
Hypertonic	+ 29	+ 33	— 4
Isotonic	+ 48*	+ 64*	— 16
Hypotonic	+ 98**	+ 70*	+ 28
Water priming	+ 96**	+ 62*	+ 34
S.E.	± 19	± 27	± 19
Mean control† intake	666	540	126

†The mean intake during 3 days prior to and 3 days following infusion.
*Significantly different from control intake $P < 0.05$, $^{**}P < 0.01$

infusion of casein (0.28 g N/kg B.W.) reduced intake. This contrasts with the data of Fig. 2 where a similar infusion resulted in an increase of food intake. Kymographic recordings of jaw movements indicated that in this experiment the casein had no marked effect on eating or rumination patterns. In contrast, time spent eating was greatly reduced during the period 2 to 8 hr after the start of the i.v. casein hydrolysate infusion. No rumination occurred during or in the hour following infusion.

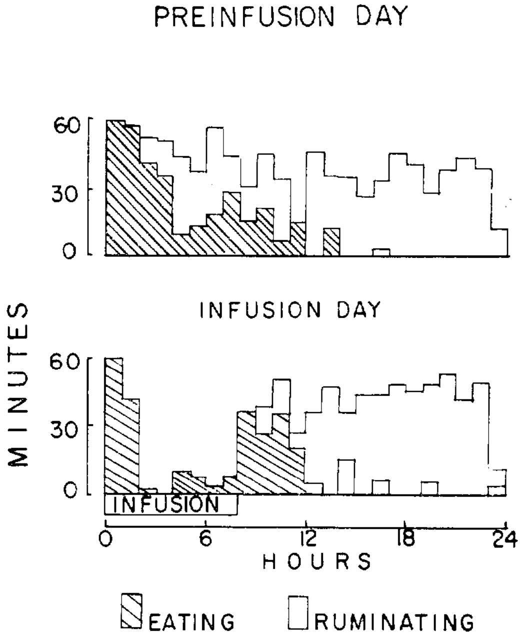

FIG. 3: *Effect of i.v. infusions of casein hydrolysate on the feeding behaviour of 3 sheep.*

(d) *Material administered.* In addition to casein and casein hydrolysate, the effects of i.d. infusions of an amino acid mixture were examined. The mixture, and, as a control, a Salt Solution were administered to four sheep. The infusions were repeated at five-day intervals so that each sheep received each treatment twice. The amino acid mixture caused a marked reduction of food intake on the day of infusion and to a lesser extent on the following day. Similar effects on faecal DM output were apparent. In contrast, the Salt Solution increased food intake on the day of infusion.

THE TIME RELATIONSHIP BETWEEN CASEIN INFUSION AND THE INCREASE OF FOOD INTAKE

This was examined in greater detail in an experiment in which three treatments involving i.d. infusions of casein at different times during the day were administered to three sheep. The effects are illustrated by the data obtained with one of the treatments (Table 5). It consisted of 50 g casein in 500 ml Saline administered i.d. in equal portions

TABLE 3: EFFECT ON FOOD INTAKE OF i.d. INFUSIONS OF CASEIN AND i.v. INFUSIONS OF CASEIN HYDROLYSATE. MEAN OF 4 SHEEP

Treatment	*Intake on Day of Infusion less Control Intake (g DM)* 0-24 hr	0-8 hr	8-16 hr
Saline (i.d.)	— 28	— 25	— 3
Casein (i.d.)	— 24	— 64*	+ 40
Isotonic NaCl (i.v.)	+ 13	+ 22	— 9
Casein hydrolysate (i.v.)	— 72**	— 195**	+ 124**
S.E.	± 17	± 26	± 20
Mean control intake	597	386	111

*$P < 0.05$ **$P < 0.01$

TABLE 4: EFFECT ON FOOD INTAKE AND FAECAL DM OUTPUT OF i.d. INFUSIONS OF AN AMINO ACID MIXTURE

Treatment	*Mean Control Intake†*	*Day of Infusion*	*Day after Infusion*
Feed intake (g DM/24 hr)			
Salt Solution	710	812**	698
Amino acid mixture	680	438**	573**
S.E.		± 28	± 17
Faecal DM output g DM/24 hr			
Salt Solution	405	401	405
Amino acid mixture	392	189**	359**
S.E.		± 6	± 6

†Mean of 2 days preceding infusion and 2 days following infusion.
**Significantly different from control $P < 0.01$.

TABLE 5: RELATIONSHIP BETWEEN THE TIME OF CASEIN INFUSION AND THE INCREASE OF FOOD INTAKE SHOWN BY ONE OF THREE TREATMENTS

Sheep	*Intake during the Hours of Casein Infusion (g DM)* *Mean Control†*	*Day of Infusion less Control*	*Intake during Remainder of Day (g DM)* *Mean Control*	*Day of Infusion less Control*
4 (a)*	223	+ 226	479	+ 19
(b)	223	+ 48	479	+ 43
24	230	+ 14	406	+ 3
51	282	+ 137	572	— 4

†Mean of 5 days preceding infusion.
*Indicates two observations on same sheep.

during the periods 3-6 hr and 9-12 hr after the start of feeding. In addition, each casein infusion was preceded by an i.d. infusion of 250 ml water during the preceding 3 hr. The results of the three treatments indicated that when an increase of food intake occurred, it occurred during the time of casein infusion, even though, as in the treatment described, the infusion was of only 3 hr duration. The amount of water drunk was similarly affected; in the case of the treatment described, a consistent increase of approximately twice the normal intake occurred during the casein infusion in spite of the preceding infusion of water.

Some Other Effects of Casein Infusions

(a) *Blood composition.* The i.d. infusion of casein was accompanied by marked changes in blood composition. This is illustrated by the changes in packed cell volume, blood

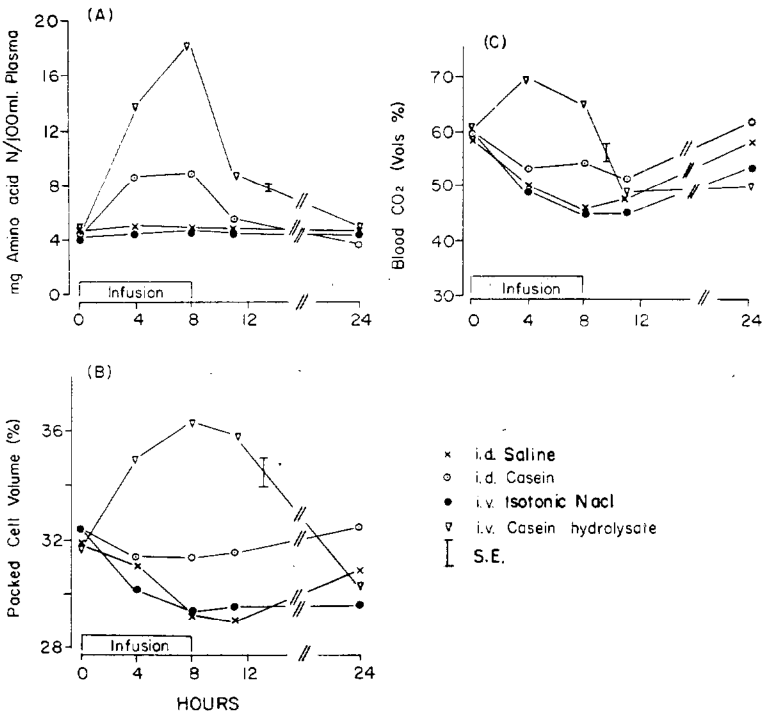

FIG. 4: *Effect of infusions of casein and casein hydrolysate on some blood characteristics of 4 sheep.*

amino acid and total CO_2 content observed in the experiment comparing this treatment with the i.v. infusion of casein hydrolysate (Fig. 4). Compared with the control infusions, both treatments resulted in marked increases of all three parameters. On this occasion, food intake was decreased. Similar changes were apparent, however, when the casein infusion caused an increase of food intake.

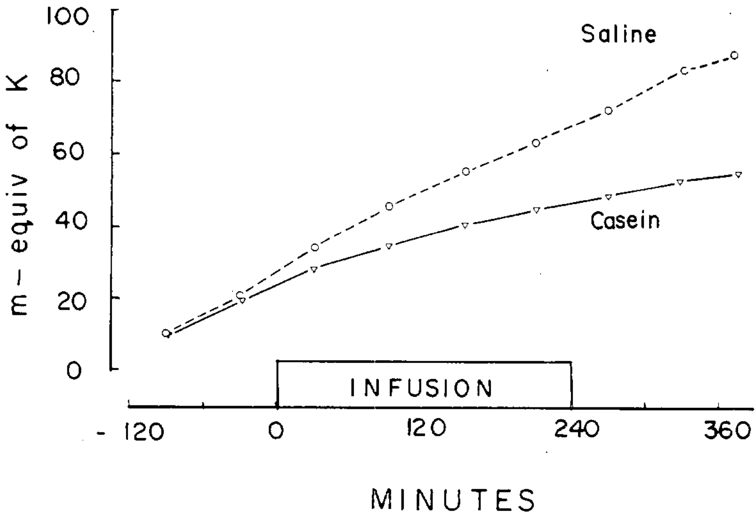

FIG. 5: *The cumulative excretion of potassium in the urine. Mean of 4 sheep. Mid-points of 60 min collection periods plotted.*

(b) *Electrolyte excretion.* This was examined using four fasted sheep. They were given i.d. infusion over 4 hr of Saline (750 ml) and Saline containing 40 g casein. Urine was collected during 30 min intervals for 8.5 hr commencing 90 min prior to the start of the infusions. Marked effects on both sodium and potassium excretion were observed. The urinary excretion of potassium decreased on infusing casein (Fig. 5) whereas that of sodium increased. The net effect on potassium excretion as at the end of the collection period may be regarded as under-estimating the total effect for its rate of excretion was either at a minimum or still declining when collection terminated. The apparent retention of potassium is even more striking when considered in relation to the fact that only 4 m equiv. was contained in each infusion.

DISCUSSION

The outstanding conclusion that emerges from this work is that the effects induced by, and the mechanisms involved in, supplementation with amino acid containing materials are exceedingly complex. This is illustrated by the multiplicity of effects induced, the influence of modifying factors, the variability, rapid onset and short duration of the effects.

A marked difference is apparent between the results of Egan and Moir (1965), Egan (1965a, b) and those reported here. Egan apparently found no difficulty in either maintaining or repeating an increase of food intake by prolonged periods of daily infusions of casein. He observed a direct relationship between casein dose rate and food intake. Further, he found that the increase that accompanied a single infusion of casein occurred not during the period of infusion but during the succeeding 40 hr. That two apparently similar investigations should produce such divergent results again emphasizes the complexity of the situation.

It seems unlikely that a single mechanism could account for all the features involved. More probably a number of mechanisms were operative, mechanisms involving receptors located at various sites in the body and responding to a range of stimuli.

The effects of a single infusion of casein appeared to depend on the summation of inhibitory and excitatory stimuli. At times, inhibitory stimuli predominated and a reduction of food intake was observed; at others excitatory stimuli appeared more important with a resulting increase of food intake. The action of the modifying factors may have been to favour one or other of these contrasting stimuli.

Reduced faecal DM output on the day of treatment was a feature common to all nitrogen-containing infusions irrespective of their effects on food intake. This suggests that a reduction of gut motility may have been one of the inhibitory components, present even when an increase of food intake was observed. This belief is supported by the observation that some of the procedures resulted in a marked reduction of rumen motility (Bryant, 1968).

The effects on food intake of i.v. infusions of casein hydrolysate were predominantly one of inhibition. This contrasts with the mildly excitatory effects observed when the material was infused into the duodenum. These differing effects suggest that the i.v. route enabled the hydrolysate to act at sites or on mechanisms that were not accessible when the material was delivered into the gut.

The i.d. infusions of the amino acid mixture were clearly more effective in stimulating inhibitory mechanisms than were casein or casein hydrolysate infused by the same route. This was so even though the nitrogen dose rate, volume and tonicity of the vehicle were those that facilitated the excitatory effects of casein, and infusions of the vehicle alone increased food intake. It may be that the amino acid mixture lacked the properties, possibly physical, that enhanced food intake. Alternatively, the effects may have arisen from the more effective stimulation of extra-intestinal mechanisms capable of inhibiting intake than when less readily absorbed materials such as casein or casein hydrolysate were infused into the duodenum.

Receptors involved in the excitation of food intake may be located in or associated with the intestinal wall or portal vascular field. The triggering stimuli for these receptors may be events associated with the presence of the infused material in the intestines. The time course of the increase in food intake is consistent with this view.

There was no clear relationship between changes in blood composition and food intake. Although marked changes occurred in plasma amino acids, total CO_2 and packed cell volume when food intake was decreased, similar changes were apparent when food intake was increased. In addition, when feeding recommenced at the completion of i.v. infusions of casein hydrolysate, the magnitude of the changes in blood composition were at or near their maximum.

The effect on food intake may have been mediated by an osmometric mechanism. It has been suggested that, in monogastric animals, changes in the extent of hydration of certain tissues may elicit stimuli that are involved in the regulation of food intake (Schwartzbaum and Ward, 1958; Smith, 1966). That changes occurred in the distribution of water accompanied by changes in acid-base status is suggested by the changes seen in water intake, blood composition and electrolyte excretion.

It has been the specific intention of this paper to demonstrate the complexity of effects induced and mechanisms involved in amino acid supplementation. It appears unlikely that the effects of such a procedure on food intake or any other process of ruminant production, including perhaps wool growth, will be simple. A unitary hypothesis which claims the effects are due to a correction of an amino acid deficiency or imbalance may be of more than doubtful validity.

ACKNOWLEDGEMENTS

This work was undertaken at Massey University and Plant Chemistry Division, DSIR, while one of us (A.M.B.) was seconded from Ruakura Animal Research Station.

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