# A viscous fibre (methylcellulose) lowers blood glucose and plasma triacylglycerols and increases liver glycogen independently of volatile fatty acid production in the rat

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1. Adult male rats were maintained on diets containing 80 g methylcellulose/kg of low (25 cP), medium (400 cP) and high (1500 cP) viscosity.

2. After 10 d, the viscosity of stomach and caecal contents was found to have increased in proportion to that of the dietary fibre. Concentrations of volatile fatty acids in caecal digesta were lowest with the high-viscosity fibre but acetate was the major acid present with all three diets. Acetate was the only acid found in significant quantities in hepatic portal venous plasma and concentrations of this acid were unaffected by diet.

3. Concentrations of glucose in arterial blood were low with the medium- and high-viscosity diets while the content of liver glycogen was high. These effects of fibre were not directly on glucose absorption as the intestines were net removers of the hexose at the time of sampling.

4. Hepatic lipogenesis and plasma triacylglycerol concentrations were both higher in rats fed on the low-viscosity fibre. Plasma cholesterol concentrations, hepatic cholesterol synthesis and faecal bile acid excretion were not altered by dietary fibre viscosity.

5. We conclude that the effects of dietary fibre on carbohydrate absorption and storage and fatty acid synthesis are a function of the viscosity of the fibre in solution, high viscosity slowing the digestion and absorption of nutrients in the small intestine. Large-bowel microbial fermentation is not of direct significance to these events. In contrast, effects of fibre polysaccharides on sterol metabolism seem not to be related to their rheological properties.

An increased dietary intake of plant fibre is currently being recommended for a variety of reasons. These include the lowering of plasma lipids in persons with hyperlipidaemia and improving the control of blood glucose in subjects with impaired tolerance (Anderson, 1981). Several fibre preparations have been shown to confer these benefits when consumed either as integral components of common foodstuffs or as isolated fractions. For example, guar gum has been shown to lower plasma cholesterol in hyperlipidaemics (Simons *et al.* 1982) and to lower the peak glucose response to a test meal in normal humans and in diabetics (Jenkins *et al.* 1977; Munoz *et al.* 1979). Similarly, oat bran (which is high in a $\beta$-glucan hemicellulose commonly called oat gum) has been shown to lower plasma cholesterol (Kirby *et al.* 1982) and improve blood glucose control in diabetics (Chen *et al.* 1984). The effects of oat bran on plasma cholesterol have been replicated when isolated gum (Chen *et al.* 1981) was fed, but with some fibre fractions (such as pectin) the effects were only observed when the fibre was given in the isolated form and were lost when the whole foods were consumed (Stasse-Wolthuis, 1981).

The way in which these fibre fractions exert their effects remains uncertain but two mechanisms have been proposed. (1) The fibres that are demonstrably effective are plant polysaccharides which form viscous solutions in water, suggesting that it is this property which mediates their effects on plasma lipids and blood glucose (Jenkins *et al.* 1978; Judd & Truswell, 1985). The inclusion of these viscous fibres in the diet is believed to slow transit from the stomach to the small intestine and to delay the digestion and absorption of

nutrients (Jenkins *et al.* 1978). (2) These polysaccharides also are fermented extensively by the large-bowel microflora giving rise to volatile fatty acids (VFA) in significant quantities (Illman & Topping, 1985). From this it has been argued (Chen *et al.* 1984) that one of these VFA (propionate) directly mediates the effects of dietary fibre through altered hepatic carbohydrate and lipid metabolism. To distinguish between these two mechanisms we have attempted to determine whether fibre viscosity *per se* altered lipid and carbohydrate metabolism in the rat in the absence of major changes in the production of VFA. This was done by providing diets containing methylcelluloses of one of three grades of viscosity: low, medium and high. Such cellulose esters also are likely to be resistant to fermentation by large-bowel bacteria (Topping & Illman, 1986).

## MATERIALS AND METHODS
### Care of animals

Adult male rats of the Hooded Wistar strain (200–230 g body-weight) were used. They were housed in groups of five in stainless-steel cages, with wire-mesh bottoms to minimize coprophagy, in a room of controlled heating and lighting and of low background noise (Cheng *et al.* 1987). They were given free access to water and to a sucrose-based diet which contained methylcellulose (80 g/kg) as the fibre source (Topping *et al.* 1985*b*). The methylcellulose was obtained in three different viscosities: low (25 cP; LV), medium (400 cP; MV) and high (1500 cP; HV) from the Sigma Chemical Co., St Louis, Mo. The formulation of the diets and the subsequent experimental procedures used were all formally approved by the Animal Care and Ethics Committee of the Division of Human Nutrition.

### Sampling procedures

After 10 d of adaptation to the three diets, five rats from each group were lightly anaesthetized with diethyl either at 10.00 hours and blood drawn from the hepatic portal vein (2 ml) and systemic aorta (5 ml) and collected into ice-cold tubes containing EDTA as anticoagulant. Concentrations of glucose in whole blood and VFA in plasma were determined in samples from both blood vessels while plasma lipids were measured only on aortic samples. A portion of the liver was quickly excised, blotted dry and weighed and then frozen in liquid nitrogen for subsequent determination of glycogen content. The remainder of the liver was similarly excised, blotted dry, weighed and frozen for measurement of liver triacylglycerols. Whole caeca were also removed and the contents extruded for measurement of weight, viscosity, pH, VFA and bile acids.

On the next day further groups of five animals were given 5 mCi $^3H_2O$ in 0·15 M-sodium chloride by intraperitoneal injection. After 70 min the rats were anaesthetized with diethyl ether and blood drawn from the systemic aorta for determination of $^3H_2O$ specific radioactivity, and the liver excised and frozen for measurement of $^3H$ incorporation into fatty acids and cholesterol. Whole caeca and stomachs were excised for determination of the viscosity of digesta.

### Analytical procedures

The pH and water content of the caecal digesta were measured after diluting the contents with approximately 5 ml water (Cheng *et al.* 1987). Because of the relatively high viscosity and rather hydrophobic nature of the digesta it was found necessary to carry out this dilution using a Polytron homogenizer (30 s). Concentrations of VFA in the slurry of caecal digesta and also in plasma were determined by gas–liquid chromatography as described previously (Cheng *et al.* 1987), except that oenanthic acid was used as an internal standard

for quantification rather than caproate. This change was necessary because under some conditions significant quantities of caproate were found in samples, particularly those from gut contents and the hepatic portal vein. The unpredictable presence of this acid could give errors as high as 14% in the determination of total and individual VFA. Concentrations of blood glucose and of plasma triacylglycerols and cholesterol, and liver concentrations of glycogen and triacylglycerols were determined by procedures that have been described previously (Topping *et al.* 1985*b*). Portions of liver were extracted with chloroform–methanol (2:1, v/v) and the solvent partitioned with 40% volume of 0·03 M-hydrochloric acid and the chloroform extract hydrolysed for measurement of the incorporation of $^3H_2O$ into cholesterol and fatty acids (Illman & Topping, 1985). The specific radioactivity of body water was obtained by counting portions of the supernatant fraction remaining after deproteinization of blood for determination of glucose. Rates of fatty acid and cholesterol synthesis were calculated using the factors and assumptions of Windmueller & Spaeth (1966).

### Viscosity measurements

All viscosities were measured with an Epprecht Rheometer (Contraves AG, Zurich) at 20°. The methylcellulose solutions and digesta samples containing that fibre were always non-newtonian liquids and apparent viscosities were obtained at several different rates of shear. For purposes of comparison, values were obtained by interpolation for a fixed rate of shear (100/s for methylcellulose solutions and diets made into a slurry with water; 0/s for the more viscous stomach and caecal contents). Viscosities of caecal contents were determined on pooled collections of the diluted slurries used for VFA determination. The viscosities of the three diets were measured on slurries prepared by homogenizing 10 g diet with 30 ml water.

### Statistical methods

All values are represented as means with their standard errors for the numbers of observations indicated. Statistical evaluation was by analysis of variance and a value $P < 0·05$ was taken as the criterion of significance.

## RESULTS
### Food intake and body-weight gain

Daily food intake did not differ between any of the groups and averaged over 23·6 g/rat for all groups combined. Body-weight gain was correspondingly similar with all three treatments with final mean weights (*n* 10) of 250 (SE 5), 252 (SE 5) and 252 (SE 4) for the LV, MV and HV diets respectively.

### Viscosity of diets and gut contents

The viscosity of aqueous solutions of three grades of methylcellulose compared with guar gum (all at a rate of shear of 100/s) are shown in Fig. 1. All three grades of methylcellulose behaved as their designations would indicate with only a small effect on viscosity from the 25 cP material. The HV methylcellulose behaved very similarly to guar gum under the conditions employed.

We also tested the viscosity of the diets (made into a slurry with water using a Waring blender). The proportion of water to diet was 1·5:1 in each case and was designed to give with the LV diet a mixture corresponding approximately to normal stomach contents. As expected, guar gum and the HV diet produced mixtures of much greater viscosity, although in each case there was shear thinning (Fig. 2). The guar-gum-based diet had the highest
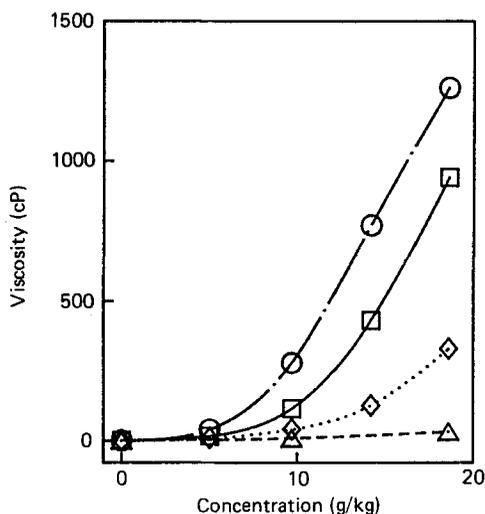
Fig. 1. Viscosity of aqeous solutions of methylcellulose of low (25 cP; LV) (△----△), middle (400 cP; MV) (◇....◇) and high (1500 cP; HV) (□——□) viscosity compared with that of guar gum (○--○). The values are for a shear rate of 100/s obtained by interpolation of 20°. For details of procedures see pp. 22–23.



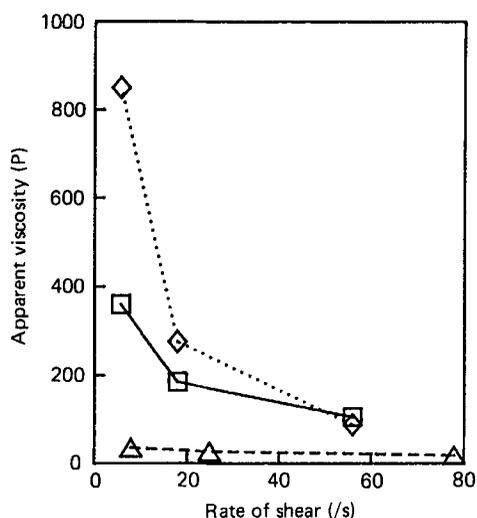Fig. 2. Viscosity of aqueous slurry of diets containing methylcellulose of low (25 cP; LV) (△---△), and high (1500 cP; HV) (□——□) viscosity compared with that of guar gum (◇....◇). The values are for a shear rate of 100/s obtained by interpolation at 20°. For details of procedures see pp. 22–23.

viscosity at the low rates of shear but approached that of the HV diet as the rate increased.

The effects of the three grades of methylcellulose on the viscosities of pooled samples of stomach and caecal contents are shown in Fig. 3. The undiluted caecal digesta had the appearance and consistency of plasticine and maintained the full shape of the organ when the wall was dissected away. Because of the relative solidity of the caecal samples, they were diluted twofold with water. Viscosity in each case was closely related to that of the diet with the highest value being found in the HV group.
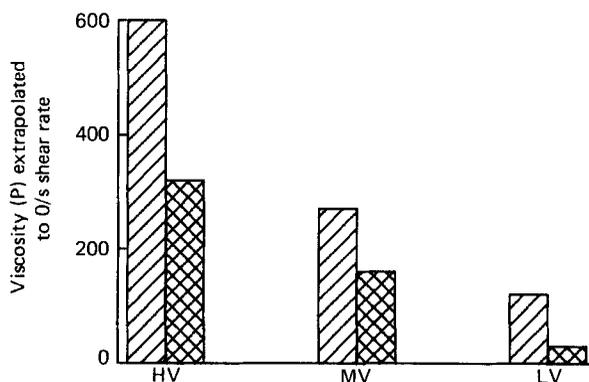
Fig. 3. Effects of diets containing methylcellulose of low (25 cP; LV), middle (400 cP; MV) and high (1500 cP; HV) viscosity on the viscosity of stomach (▨) and caecal (⊠) contents of rats. The values are for a shear rate of O/s (obtained by extrapolation) and measurements were made at 20°. For details of procedures see pp. 22–23.

### Mass, pH, moisture content and VFA of caecal digesta

The mass of caecal material was similar in rats fed on the LV and MV diets and was lower (but not significantly) in animals in the HV group (Table 1). Although moisture content was significantly lower in the LV group, this difference was small, being less than 2% of the total. On the other hand, pH rose significantly from 7·18 in caecal contents of rats fed on the LV diet to 7·42 in animals fed on the HV diet. This latter increase presumably reflected the differences in VFA concentration between the groups. Total VFA were similar in rats fed on the MV and HV diets and significantly lower than those in the LV group. The difference between the LV rats and those fed on the other two diets was largely in the concentrations of acetate and propionate; butyrate was present in equal (and low) amounts in all groups. Acetate was the major acid present in rats fed on the three diets and represented > 650 mmol/mol total VFA. Minor acids (iso-butyrate, valerate, iso-valerate and caproate) were unaffected by diet and in all animals were present to > 50 mmol/mol total caecal VFA.

### Plasma VFA and blood glucose

Concentrations of VFA in hepatic portal venous plasma did not differ between any of the three dietary treatments. The mean values (all groups combined; $n$ 13) were acetate 0·83 (SE 0·01), propionate 0·05 (SE 0·00), butyrate 0·03 (SE 0·00) $\mu$mol/ml.

Blood glucose was determined in the hepatic portal vein and systemic aorta and in all groups a negative arterio-hepatic portal venous concentration difference was found, indicating net intestinal glucose utilization (Table 2). Concentrations in hepatic portal venous plasma were significantly lower in the MV and HV rats than in those fed on the LV diet. The same situation was found in arterial blood with lower glucose concentrations in animals fed on the MV and HV diets than in the LV animals. Liver glycogen was also affected by the viscosity of the dietary fibre. Glycogen content was highest in rats fed on the HV diet and fell with decreasing viscosity (Table 2). It may be calculated that total hepatic storage of glucose was nearly 100% higher in rats fed on the HV diet than in animals given the LV diet.

Table 1. *Mass, moisture content, pH and volatile fatty acid (VFA) concentrations of caecal digesta of rats fed on diets containing methylcellulose of low (LV), middle (MV) or high (HV) viscosity\**

(Mean values with their standard errors for five observations per group)

| Diet | Mass (g) | | Moisture (g/kg) | | pH | | VFA concentrations ($\mu$mol/ml) | | | | | | | |
| | | | | | | | Acetate | | Propionate | | Butyrate | | Total | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| LV | 2·69[a] | 0·31 | 68·1[a] | 0·5 | 7·18[a] | 0·06 | 42[a] | 3 | 13[a] | 1 | 7[a] | 1 | 64[a] | 3 |
| MV | 2·58[a] | 0·18 | 69·3[b] | 0·2 | 7·26[a] | 0·07 | 35[a,b] | 3 | 9[b] | 1 | 5[a] | 1 | 52[b] | 4 |
| HV | 2·20[a] | 0·33 | 69·6[b] | 0·2 | 7·42[b] | 0·02 | 30[b] | 2 | 8[b] | 0 | 6[a] | 0 | 46[b] | 2 |

\* For details see pp. 22–23.

[a,b] Values in any vertical column not sharing the same superscript letter were significantly different ($P < 0.05$).

Table 2. *Concentrations of glucose in arterial and hepatic portal venous blood and glycogen in livers of rats fed on diets containing methylcellulose of low (LV), middle (MV) or high (HV) viscosity\**

(Mean values with their standard errors for five observations per group)

| | Blood glucose ($\mu$mol/ml) | | | | Liver glycogen ($\mu$mol/g liver) | |
| | Arterial | | Hepatic portal venous | | | |
| Diet | Mean | SE | Mean | SE | Mean | SE |
| --- | --- | --- | --- | --- | --- | --- |
| LV | 9·2[a] | 0·2 | 7·9[a] | 0·1 | 74[a] | 9 |
| MV | 8·0[b] | 0·3 | 6·8[b] | 0·2 | 106[a, b] | 25 |
| HV | 8·0[b] | 0·1 | 7·3[b] | 0·2 | 146[b] | 14 |

\* For details see pp. 22–23.
[a, b] Values in any vertical column not sharing the same superscript letter were significantly different ($P < 0.05$).

### Plasma and liver lipids and hepatic fatty acid and cholesterol synthesis

Plasma triacylglycerol concentrations did not differ between the MV and HV groups and both were significantly lower than those in arterial samples from rats fed on the LV diet (Table 3). In contrast, plasma cholesterol was unaffected by the viscosity of the dietary fibre and averaged 3·31 $\mu$mol/ml for all groups, combined. Liver triacylglycerols and cholesterol were also unaffected by the dietary treatment (Table 3). Fatty acid synthesis was lowest in livers from rats fed on the HV diet with a rate of only 42 % of that seen in animals given the LV diet. Lipogenesis was intermediate in the MV group with a rate that did not differ from either of the other two treatments. Cholesterol synthesis also was unaffected by fibre viscosity (Table 3). Samples of fresh faeces were also taken from the animals for 3 d before death, pooled for each treatment and analysed for bile acids. No differences were found between groups, with a combined mean of 3·19 mg bile acids/g dry faecal matter.

### DISCUSSION

The present study was designed to examine the effects of dietary fibre of different viscosity on a number of metabolic variables in the rat in the absence of the possible confounding effects of large-bowel bacterial fermentation. For this reason we based the experimental diets on methylcellulose as it is a modified polysaccharide resistant to microbial metabolism and is available in viscosities which are similar to those of fibres known to lower plasma cholesterol and improve glycaemic control. This choice of fibre also enabled us to avoid using antibiotics which might, of themselves, modify gastrointestinal function. In keeping with the apparent resistance of methylcellulose to fermentation, concentrations of VFA in large-bowel contents and hepatic portal venous plasma were low and in the range we have found previously in rats subjected to restricted feeding (Illman *et al.* 1986). As in those earlier experiments, acetate was the major acid found in both compartments and concentrations of propionate and butyrate were low. This finding is consistent with the limited availability of fermentable material as the concentrations of the latter two acids rise when bacterial metabolism of fibre is increased by feeding polysaccharides that are particularly susceptible to fermentation. The concentrations of acetate and propionate in caecal contents fell with increasing viscosity of the diet, an effect which may relate to the availability of dietary starch and sucrose trapped in the methylcellulose matrix (Kim *et al.*

Table 3. *Rates of hepatic fatty acid and cholesterol synthesis and plasma and liver concentrations of triacylglycerols (TAG) and cholesterol in rats fed on diets containing methylcellulose of low (LV), middle (MV) or high (HV) viscosity*\*

(Mean values with their standard errors for five observations per group)

| | Synthesis (μmol/g liver per h) of | | | | Plasma (μmol/ml) | | | | Liver (μmol/g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fatty acids | | Cholesterol | | TAG | | Cholesterol | | TAG | | Cholesterol | |
| Diet | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| LV | 3·06[a] | 0·52 | 0·35[a] | 0·05 | 1·58[a] | 0·10 | 3·37[a] | 0·11 | 15·2[a] | 1·3 | 6·5[a] | 0·2 |
| MV | 2·39[a] | 0·47 | 0·38[a] | 0·06 | 1·13[b] | 0·07 | 3·25[a] | 0·06 | 13·1[a] | 0·9 | 6·7[a] | 0·3 |
| HV | 1·39[a] | 0·27 | 0·37[a] | 0·06 | 1·24[b] | 0·13 | 3·32[a] | 0·10 | 13·6[a] | 0·9 | 6·3[a] | 0·2 |

\* For details see pp. 22–23.

[a,b] Values in any vertical column not sharing the same superscript letter were significantly different ($P < 0.05$).

1978; Topping *et al.* 1985*a*). However, in the hepatic portal vein there were no such variations and concentrations of propionate and butyrate were so low as to be negligible ($< 0.08\ \mu$mol/ml). Thus, we can say with some confidence, that the observed effects of the three diets were unrelated to the production of VFA or to the presence of variable quantities of individual or total VFA in the hepatic portal vein. This apparent lack of fibre fermentation is supported also by the fact that the viscosities of the caecal contents reflected that of the parent fibre so that the methylcelluloses must have retained much of their original structure. Therefore, we conclude that viscosity was responsible for the lower blood glucose concentrations seen in rats fed on the MV and HV material. This lowering effect is similar to the lower glycaemic response reported with fibre polysaccharides such as pectin and guar gum (Jenkins *et al.* 1977; Munoz *et al.* 1979) and it is of interest that the viscosity of the 1500 cP (HV) methylcellulose used in the present experiment is similar to guar gum under comparable conditions. Fibres, such as guar gum, are known to slow the absorptive process and Wahren *et al.* (1982) have presented evidence that less monosaccharide is absorbed also. However, it should be pointed out that we found that the lower glycaemia with increasing fibre viscosity was after the period of active glucose absorption and it was accompanied also by an increase in the glycogen content of the liver. While it is possible that the highly viscous digesta may have affected a circulating glycogenolytic factor, it seems rather more likely that the changes simply reflected enhanced hepatic storage of glucose. Studies in vivo (Muratoglou *et al.* 1986) and in vitro (Topping *et al.* 1984) have shown that the fractional extraction of glucose by the liver is relatively low ($< 20\%$) and that the glucose not taken up escapes hepatic storage and enters the peripheral circulation. By slowing the absorptive process, the MV and HV methylcellulose would allow the liver to store more of the absorbed carbohydrate.

This hypothesis is also supported by the rates of hepatic lipogenesis in each of the three groups. Fatty acid synthesis (measured in postabsorptive animals) was highest in rats fed on the LV (25 cP) diet and fell with increasing viscosity of the methylcellulose. This difference is analogous to the difference between meal-fed and nibbling rats. The former show much higher rates of *de novo* fatty acid synthesis than animals allowed free access to their daily ration (Baker & Huebotter, 1973), presumably because of the more rapid entry of metabolizable substrate into the circulation. This situation could reasonably be likened to that in rats fed on the LV diet compared with those fed on the HV (1500 cP) diet. The higher viscosity methylcellulose would spread the absorption across a longer time-span. The tendency towards a higher mass of caecal material in rats fed on the LV diet supports this concept of slower transit with increased viscosity of the feed.

Lipogenesis is an important determinant of the hepatic secretion of very-low-density lipoprotein triacylglycerols and the latter is increased under conditions where fatty acid synthesis is also enchanced (Kannan *et al*). 1981; Kazumi *et al.* 1985). Although rates of lipogenesis were highest with the LV diet, plasma triacylglycerols were raised by only 29% above those of rats given the other two diets and liver concentrations did not differ between the three groups. This may be a consequence of the choice of dietary fibre as a similar diet containing cellulose or oat gum gave rather lower concentrations of triacylglycerols in liver but higher values in plasma (Illman & Topping, 1985), suggesting perferential export. On the other hand, we have to recognize that plasma concentrations are a balance between secretion and clearance. Kazumi *et al.* (1986) have recently reported that the net in vivo secretion rate is not always accurately reflected by steady-state plasma triacylglycerol concentrations although, in general, higher concentrations indicate greater secretion.

While it is true to say that dietary fibre viscosity had a significant effect on hepatic fatty acid synthesis as well as carbohydrate metabolism, there was no effect on cholesterol. Nor did plasma cholesterol, hepatic cholesterol synthesis and faecal excretion of bile acids differ

between the three diets. While fibre apparently can lower blood glucose and plasma triacylglycerols through its rheological properties, these properties do not influence sterol metabolism. Thus, the hypocholesterolaemic effects of certain plant polysaccharides (Topping & Illman, 1986) would seem to be effected through another mechanism besides their high viscosity in aqueous solution.

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