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ARTICLE

Higher Bioaccessibility of Iron and Zinc from Food Grains in the Presence of Garlic and Onion

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Bioavailability of micronutrients iron and zinc is particularly low from plant foods. Hence there is a need to evolve a food-based strategy to improve the same to combat widespread deficiencies of these minerals in a population dependent on plant foods. Dietary sulfur-containing amino acids have been reported to improve the mineral status of experimental animals. Our objective was to examine whether sulfur compound-rich *Allium* spices have a similar potential of beneficially modulating the mineral bioavailability. In this context, we examined the influence of exogenously added garlic and onion on the bioaccessibility of iron and zinc from food grains. Two representative cereals and pulses each were studied in both raw and cooked condition employing two levels of garlic (0.25 and 0.5 g/10 g of grain) and onion (1.5 and 3 g/10 g of grain). The enhancing effect of these two spices on iron bioaccessibility was generally evidenced in the case of both the cereals (9.4−65.9% increase) and pulses (9.9−73.3% increase) in both raw and cooked conditions. The two spices similarly enhanced the bioaccessibility of zinc from the food grains, the extent of increase in cereals ranging from 10.4% to 159.4% and in pulses from 9.8% to 49.8%. Thus, both garlic and onion were evidenced here to have a promoting influence on the bioaccessibility of iron and zinc from food grains. This novel information has the potential application in evolving a food-based strategy to improve the bioavailability of trace minerals and hence contributes to the human health benefit.

KEYWORDS: ***Allium*** **spices; bioaccessibility; trace minerals**

INTRODUCTION

Cereals and legumes are the major sources of minerals such as iron and zinc for the population dependent on plant foods. However, the bioavailability of iron and zinc from these food grains is reported to be poor (*1*, *2*). Bioavailability of dietary iron (non-heme) and zinc is greatly influenced by both inhibitors (phytate, polyphenols, dietary fiber, oxalate, and calcium) and enhancers (organic acids) of mineral availability also present in the diet (*1*−*3*).

We have recently reported the beneficial influence of β-carotene or its potential vegetable sources on iron and zinc bioaccessibility from food grains (*4*). There is growing evidence that dietary sulfur amino acids influence the status of zinc. Renal metallothionine content has been reported to be higher in rats fed diets supplemented with cystine, suggesting improved zinc status (*5*). Higher tissue zinc and zinc excretion was evidenced in rats fed diets supplemented with excessive cysteine (15 g/kg) (*6*, *7*). Increased absorption of zinc was apparent when rats were fed a cysteine-supplemented lactalbumin diet compared with an unsupplemented diet (*8*). Garlic (*Allium sativa*) and onion (*Allium cepa*), which are liberally consumed in Indian diets, are rich sources of sulfur compounds. In the context of previous reports on the influence of dietary sulfur-containing amino acids on iron and zinc status in animals, it would be highly relevant to evaluate these two *Allium* spices for any influence on the mineral bioavailability. The objective of this investigation was therefore to examine the influence of exogenously added garlic and onion on the bioaccessibility of iron and zinc from food grains consumed in India. For this purpose, two representative cereals and pulses each were studied in both raw and cooked condition employing two levels of garlic (0.25 and 0.5 g/10 g of grain) and onion (1.5 and 3 g/10 g of grain). The information thus generated would make advances in human nutrition and is likely to help to evolve a food-based strategy to combat micronutrient malnutrition in a population dependent on plant foods.

MATERIALS AND METHODS

Materials. Two cereals, rice (*Oryza sativa*) and sorghum (*Sorghum vulgare*), and two pulses, whole green gram (*Phaseolus aureus*) and chickpea (*Cicer arietinum*), were procured locally, cleaned, and used. Fresh bulbs of garlic and onion were procured from the local market and cleaned, and the edible portions were used for the experiment. Pepsin (porcine), pancreatin (porcine), and bile salt (1:1 mixture of sodium cholate and sodium deoxycholate), iron and zinc standards for atomic absorption spectroscopy, were obtained from Sigma-Aldrich. All other chemicals used were of analytical grade. Triply distilled water and acid-washed glassware were used throughout the study.

Bioaccessibility of Iron and Zinc. The bioaccessibility of iron and zinc was determined using 10 g of the grain sample in the absence or presence of garlic/onion added at the specific proportion indicated below.

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Thus, the various food samples evaluated for zinc and iron bioaccessibility were (1) food grain alone (10 g), (2) food grain (10 g) + garlic (0.25 g), (3) food grain (10 g) + garlic (0.50 g), (4) food grain (10 g) + onion (1.5 g), and (5) food grain (10 g) + onion (3.0 g). Grain samples were finely powdered in a grinder with stainless steel blade assembly (Braun, Germany) and sifted through an 85-mesh sieve (180 μm opening); garlic or onion was finely chopped and ground into a smooth paste before addition to the powdered grain sample. The two levels of spices examined here correspond to the range of their normal intake by the Indian population (*9*).

Bioaccessibility of iron and zinc from the above food samples was determined by using an *in vitro* method (*10*), involving a simulated gastrointestinal digestion procedure with suitable modifications (*11*). The samples were subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) at 37 °C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in presence of the pancreatin−bile extract mixture (4 g of pancreatin + 25 g of bile salt + 0.1 M sodium bicarbonate). Titratable acidity was defined as the amount of 0.2 M sodium hydroxide required to attain a pH of 7.5.

To simulate intestinal digestion, segments of dialysis tubing (molecular mass cutoff, 10 kDa) containing 25 mL of sodium bicarbonate solution, being equivalent in moles to the sodium hydroxide needed to neutralize the gastric digest (titratable acidity) determined as above, were placed in Erlenmeyer flasks containing the gastric digest and incubated at 37 °C with shaking for 45 min or longer until the pH of the digest reached 5.0. Pancreatin−bile salt mixture (5 mL) was then added, and incubation was continued for 3 h or longer until the pH of the digest reached 7.0. At the end of simulated gastrointestinal digestion, iron and zinc present in the dialyzate, which represent the bioaccessible fraction, were analyzed by atomic absorption spectrometry after acidification with HNO_3 (5%) and centrifugation (10000*g*) and filtered through Whatman No. 42 filter paper.

Bioaccessibility (%) was calculated as follows: bioaccessibility (%) = $100(Y/Z)$, where Y is the bioaccessible fraction of the mineral (mg/100 g of grain), and Z is the total mineral (iron or zinc) content (mg/100 g of grain) (*3*).

Determination of Total Zinc and Iron Content. Grain samples were ground finely and ashed in a muffle furnace at 550 °C for 6 h for pulses and 10 h for cereals, and the ash was dissolved in concentrated HCl (analytical grade). Zinc and iron content was determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of the mineral measurements was performed using iron and zinc standards and appropriate acid blanks. All measurements were carried out with standard flame-operating conditions as recommended by the manufacturer.

Heat Processing of Food Grains. All food samples were pressure cooked (15 psi) in 30 mL of triply distilled water for 15 min. The cooked samples were homogenized in a stainless steel blender and used for the determination of mineral bioaccessibility as described above.

Statistical Analysis. All determinations were carried out in six replicates, and the average values are reported. Statistical analysis of analytical data was done employing *t*-test by using the software Origin (Origin Lab, Northampton, MA).

RESULTS AND DISCUSSION

Effect of Garlic on the Bioaccessibility of Iron. Panels **A** and **B** of **Figure 1** present the effect of garlic on the bioaccessibility of iron from rice, sorghum, green gram, and chickpea. Garlic exerted a promoting effect on the bioaccessibility of iron from all of the four food grains examined. The percent increase in the bioaccessibility of iron from raw rice was 12.6 at the higher level of garlic, while the same in cooked rice was 13.5 and 16.2 at the two levels of garlic, respectively. In case of sorghum the positive effect of garlic was observed only in raw grains, the percent increase in iron bioaccessibility being13.9 at the lower level, while it was 37.6 at the higher level.

A similar positive influence of garlic on iron bioaccessibility was also evident in the pulses examined. Garlic enhanced iron bioaccessibility from raw green gram by 15.6% and 20.5% at the two levels, respectively. This positive effect of garlic was much higher in the case of cooked green gram, the percent increase

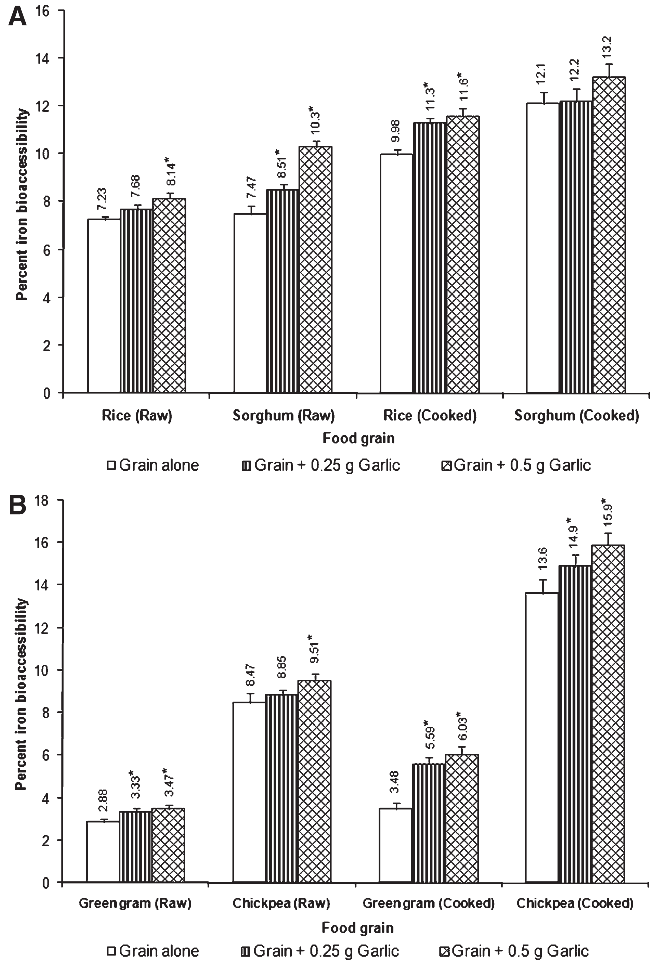

Figure 1. (**A**) Effect of garlic on the bioaccessibility of iron from raw and cooked cereal grains. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$). (**B**) Effect of garlic on the bioaccessibility of iron from raw and cooked pulses. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$).

being 60.6 and 73.3 at the two levels, respectively. In the case of chickpea, garlic enhanced the bioaccessibility of iron by about 12.3% (higher level) in the raw grain and 10% and 17.2% at the two levels, respectively, in the cooked grains.

Effect of Garlic on the Bioaccessibility of Zinc. The effect of garlic on the bioaccessibility of zinc from the four food grains is presented in **Figure 2**. Garlic significantly enhanced the bioaccessibility of zinc only in cooked rice, the percent increase being 61 and 71 at 0.25 and 0.5 g levels, respectively. Similar increases in the bioaccessibility of zinc were evidenced in sorghum, where garlic brought about 32.5% and 54.5% increase in zinc bioaccessibility in the raw grain, and in cooked grain, the percent increase was 157 and 159 at the two levels of garlic. In the case of raw green gram, garlic enhanced the bioaccessibility of zinc to an extent of 9.8% at the higher level, while in cooked green gram the percent increase in zinc bioaccessibility was 14.5 and 19.1 at the two levels, respectively. Thus, the increase in zinc bioaccessibility by exogenous garlic was more evident in cooked grains.

Effect of Onion on the Bioaccessibility of Iron. Panels **A** and **B** of **Figure 3** present the effect of onion on the bioaccessibility of iron from food grains. Onion had a significant positive effect on the bioaccessibility of iron from all of the four food grains examined. The positive effect of onion on iron bioaccessibility was higher in raw rice, the percent increase in the same being 37.5 and 41.2 at the

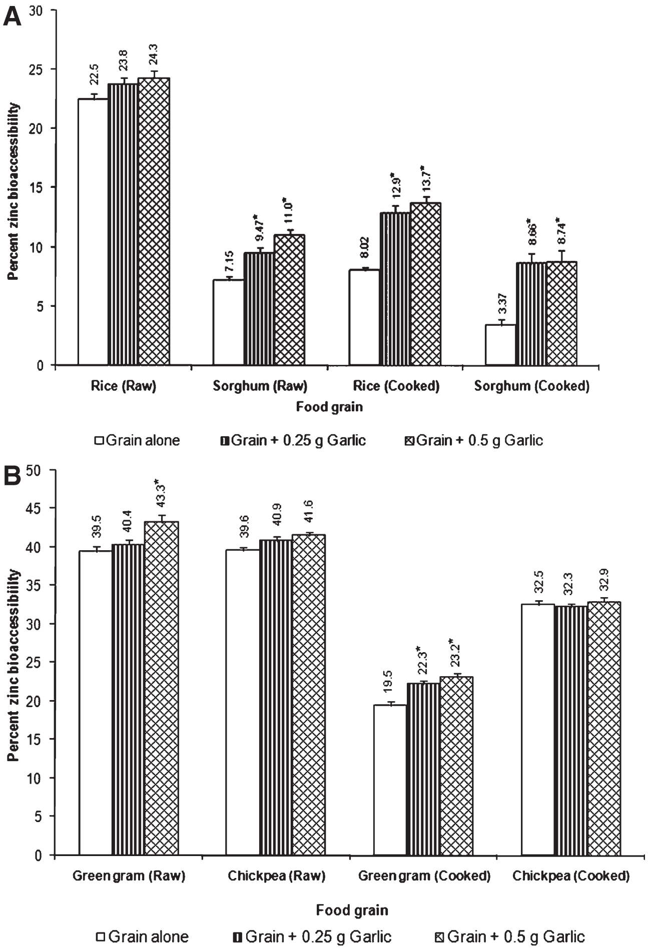

Figure 2. (**A**) Effect of garlic on the bioaccessibility of zinc from raw and cooked cereal grains. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$). (**B**) Effect of garlic on the bioaccessibility of zinc from raw and cooked pulses. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$).

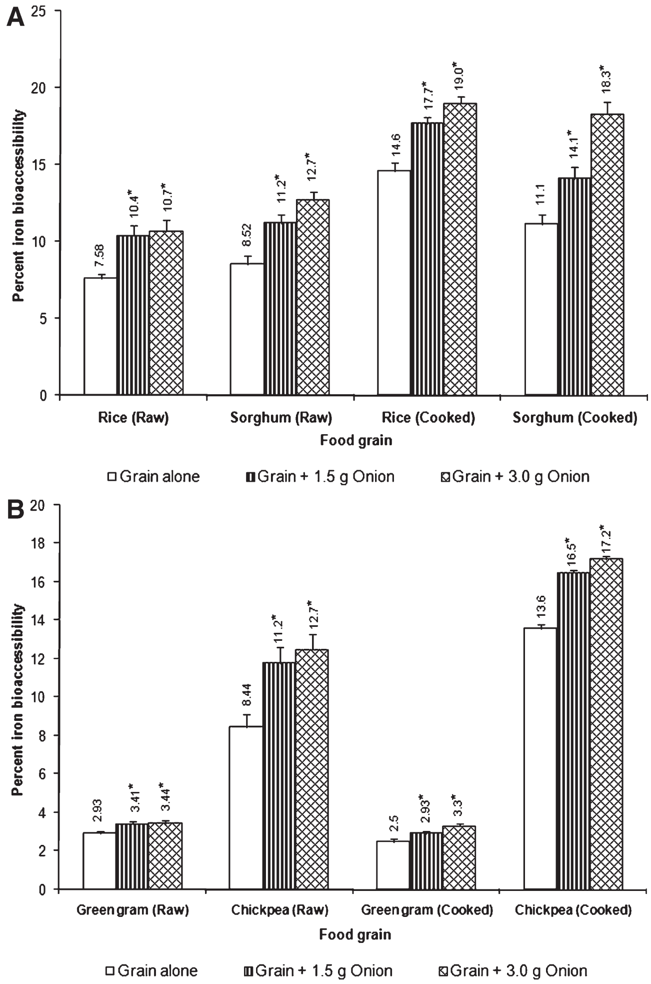

Figure 3. (**A**) Effect of onion on the bioaccessibility of iron from raw and cooked cereal grains. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$). (**B**) Effect of onion on the bioaccessibility of iron from raw amd cooked pulses. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$).

two levels of onion, while in cooked rice the percent increase in iron bioaccessibility was 21.5 and 30.3 at the two levels of onion, respectively. Onion exerted a similar enhancing influence on the bioaccessibility of iron from sorghum. The percent increase in iron bioaccessibility was 31.9 and 49.4 in the raw, and 27.2 and 65.9 in the cooked, grain at the two levels of onion, respectively. In the case of raw green gram onion enhanced the bioaccessibility of iron to an extent of about 17% at both of the levels, while in cooked green gram, the same was 17.2% and 32% at the two levels of onion, respectively. In the case of chickpea, the positive effect of onion on iron bioaccessibility was higher in the raw grains, the percent increase being 39.3 and 48.1 at the lower and higher levels, respectively. Onion increased the bioaccessibility of iron from cooked chickpea to an extent of 21.2% and 26.3% at the two levels, respectively.

Effect of Onion on the Bioaccessibility of Zinc. The effect of onion on the bioaccessibility of zinc from food grains is presented in **Figure 4**. The effect of onion on zinc bioaccessibility (10.4% increase) from raw rice was evident only at the higher level of onion. This positive effect of onion was more pronounced in the case of cooked rice, the percent increase in zinc bioaccessibility being 47.0 and 58.4 at the two levels of onion. In the case of raw sorghum, onion increased the bioaccessibility of zinc by 26.4% and 35.2%, while an increase of 14.5% was seen with a higher level of onion in the case of the cooked grain. Onion marginally increased the bioaccessibility of zinc from cooked green gram, the percent increase being 13.6 and 19.2 at the two levels, respectively. The bioaccessibility of zinc from raw green gram in the presence of onion was comparable to that from the grain alone. About 49% increase in bioaccessibility of zinc was seen from raw chickpea in the presence of either of the levels of onion, while the percent increase in zinc bioaccessibility was around 12% in the case of cooked chickpea. Thus, the promotive effect of onion on the bioaccessibility of zinc was generally higher in the raw grains as compared to the cooked grains.

The *Allium* spices garlic and onion, common ingredients of the Indian diet, are characterized by a rich content of thiosulfinates, sulfides, polysulfides, mercaptans, and other odoriferous sulfur compounds. The sulfur content of garlic is 3.3 mg/g fresh weight, four times the level in onion (*12*). Onion is used both as a spice and as a vegetable. As much as 500 g or more of fresh onion may be consumed per adult per day when used as a vegetable, either raw or cooked. Fe and Zn content of garlic and onion has been measured and taken into consideration while calculating the percent bioaccessibility of these minerals from grain samples to which these were added. Additional amounts of zinc contributed by these exogenous *Allium* spices at the higher level ranged from 2.8% to 8% of the amount intrinsically present in the grains tested. Similarly, these *Allium* spices contributed iron to the extent of 2.8−8.3% when added at the higher level.

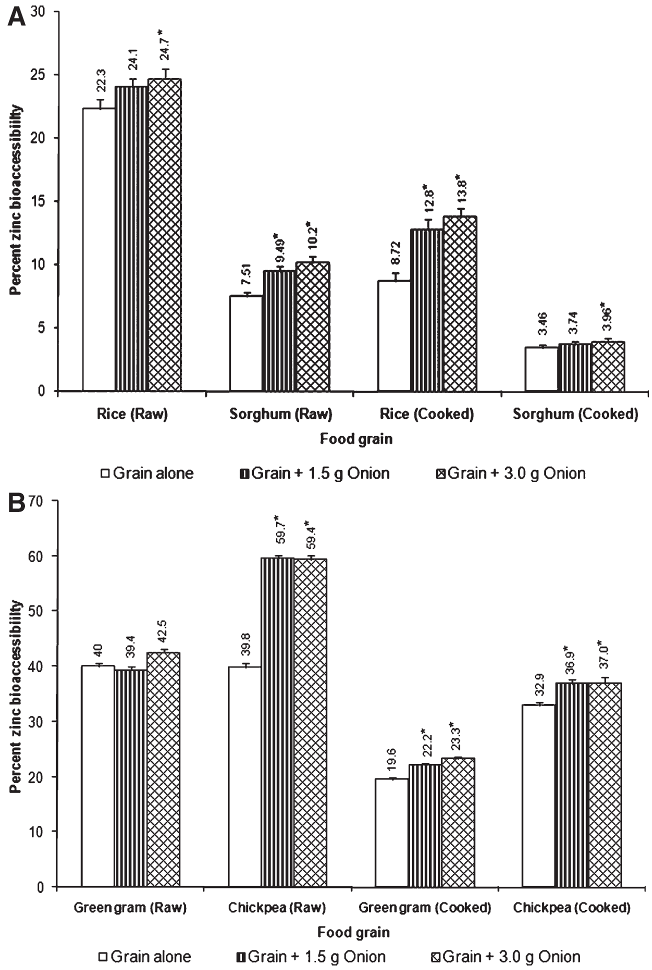

Figure 4. (**A**) Effect of onion on the bioaccessibility of zinc from raw and cooked cereal grains. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$). (**B**) Effect of onion on the bioaccessibility of zinc from raw and cooked pulses. Values represent mean ± SEM of six independent determinations. *, significant increase ($p < 0.05$).

Supplemental sulfur amino acids, particularly cysteine, have been shown by several workers to enhance zinc status in experimental animals (*5*, *7*, *8*). This has been attributed to a possible promotion of zinc absorption from the diet by the sulfur amino acids, which, however, has not been quantitated and merits further investigation. Such a possible beneficial effect of sulfur amino acids on zinc absorption may also be exerted by other natural sulfur compounds such as those present in plant foods. Garlic and onion, being abundant in sulfur compounds, qualify for evaluation of their influence on mineral absorption.

In this direction, the present study has examined the influence of these two *Allium* spices on the bioaccessibility of iron and zinc from representative cereals and pulses. The results of this study have evidenced a positive influence of both these spices on the bioaccessibility of both iron and zinc, when included at levels normally encountered in the Indian diet. Such bioaccessibility values may provide an estimate of the amounts of the minerals likely to be absorbed.

The highest effect of added *Allium* spices on mineral bioaccessibility was that of garlic, which produced more than 150% increase in bioaccessibility of zinc from cooked sorghum. The promotive effect of garlic on the bioaccessibility of zinc was generally higher in the cooked grains as compared to the raw grains, while that of onion on the same was generally higher in the raw grains. The increased bioaccessibility of the two minerals observed here as a result of addition of *Allium* spices is not attributable to increase in the content of these minerals alone, since the increase in percent bioaccessibility far exceeded the increase in the mineral content. For example, the iron content of the four grains was increased by 2.8−5.5% as a result of exogenous garlic at the higher level, while the percent increase in bioaccessible iron ranged from 10 to 73 in these grains. In the case of zinc, the content of this mineral of only sorghum was increased by 8% and 5.5% by the addition of onion and garlic, respectively, at the higher level, while the percent increase in bioaccessible zinc was 55 in the raw and 159 in the cooker grain, by the addition of garlic. Similarly, onion enhanced zinc bioaccessibility by 35% and 16% in the raw and cooked sorghum, respectively.

Among the two levels of the spices examined for an effect on mineral bioaccessibility, the higher level, which was double the lower level, did not generally produce a proportionately higher quantum of the positive effect. This observation was true in the case of both garlic and onion, irrespective of the mineral in all of the food grains tested. This could be because the positive effect might have reached/approached saturation at the lower dose itself.

The information generated in this study on the promotive influence of natural sources of sulfur compounds on mineral bioaccessibility from food grains is novel and has a promising application in evolving a food-based strategy for alleviating deficiencies of these minerals in sections of the population.

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